Development of Methods to Improve Lactose Recovery from Permeate and Drying Characteristics of Greek Acid Whey

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South Dakota State University

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DEVELOPMENT OF METHODS TO IMPROVE LACTOSE RECOVERY FROM PERMEATE AND DRYING CHARACTERISTICS OF GREEK ACID WHEY

BY

VENKATESWARLU SUNKESULA

A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

Major in Biological Sciences

Specialization in Dairy Science

South Dakota State University

2020
This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation 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.
This dissertation is dedicated to

All those from whom I learned, have been learning and will learn
ACKNOWLEDGEMENTS

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Microstructure

Morphological analysis
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<tr>
<td>BM</td>
<td>Bound moisture</td>
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<tr>
<td>BOD</td>
<td>Biological oxygen demand</td>
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<tr>
<td>Ca</td>
<td>Calcium</td>
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<tr>
<td>CAGR</td>
<td>Compound annual growth</td>
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<tr>
<td>CDS</td>
<td>Crystal size distribution</td>
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<tr>
<td>CED</td>
<td>Circular equivalent diameter</td>
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<td>CP</td>
<td>Concentrated permeate</td>
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<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
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<td>FM</td>
<td>Free moisture</td>
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<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
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<td>GAW</td>
<td>Greek yogurt acid whey</td>
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<td>GAW-NFR</td>
<td>GAW nanofiltration retentate</td>
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<td>GAW-NFR-SSPS</td>
<td>GAW nanofiltration retentate with SSPS addition</td>
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<td>GAW-SSPS</td>
<td>GAW with SSPS addition</td>
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<tr>
<td>LA</td>
<td>Lactic acid</td>
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<td>MPC</td>
<td>Milk protein concentrate</td>
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<td>MPI</td>
<td>Milk protein isolate</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>MWCO</td>
<td>Molecular weight cut off</td>
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<td>NF</td>
<td>Nanofiltration</td>
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<td>NFR</td>
<td>Nanofiltration retentate</td>
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<tr>
<td>NPN</td>
<td>Non-protein nitrogen</td>
</tr>
<tr>
<td>RH</td>
<td>Relative humidity</td>
</tr>
<tr>
<td>RO</td>
<td>Reverse osmosis</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SSPS</td>
<td>Soluble soybean polysaccharide</td>
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<tr>
<td>$T_g$</td>
<td>Glass transition temperature</td>
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<tr>
<td>TM</td>
<td>Total moisture</td>
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<tr>
<td>TN</td>
<td>Total nitrogen</td>
</tr>
<tr>
<td>TP</td>
<td>True protein</td>
</tr>
<tr>
<td>TS</td>
<td>Total solids</td>
</tr>
<tr>
<td>UF</td>
<td>Ultrafiltration</td>
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<tr>
<td>VCF</td>
<td>Volume concentration factor</td>
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<tr>
<td>WPC</td>
<td>Whey protein concentrate</td>
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<td>WPI</td>
<td>Whey protein isolate</td>
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ABSTRACT

DEVELOPMENT OF METHODS TO IMPROVE LACTOSE FROM PERMEATE AND DRYING CHARACTERISTICS OF GREEK ACID WHEY

VENKATESWARLU SUNKESULA

2020

Growth of higher protein dairy products and ingredients such as cheese, Greek yogurt, whey protein concentrates, and milk protein concentrates is limited by the amount of co-product generated and the ability to process them into value added ingredients. Finding value for all the co-products will help dairy industry to meet the growing demand for dairy protein products and ingredients. Commercial scale evaluation of new technologies for improving lactose crystallization and drying ability of Greek yogurt acid whey will help dairy industry to better utilize different dairy co-products.

Commercially, lactose is manufactured from concentrated permeate by cooling and separating α-lactose monohydrate crystals. Factors that impact crystallization of lactose during cooling will influence the yield of lactose. Caking and powder stickiness are major challenges during drying of Greek yogurt acid whey or Greek acid whey (GAW), because of high acidity, high mineral concentration, and relatively low protein content in it.

The first objective of this study was to evaluate commercial feasibility of using Soluble Soybean Polysaccharide (SSPS) to improve lactose recovery during manufacture of lactose from permeate. Previous research has demonstrated that lactose crystallization
could be modified by using SSPS in pure lactose solutions. However, commercial lactose is typically manufactured by crystallization of concentrated permeate (CP). A laboratory scale crystallization set up with parallel crystallizers was utilized to conduct control and treatment experiments simultaneously. Lactose recovery with 0.1% SSPS addition was significantly (P < 0.05) higher (76.31%) as compared to the control (71.33%). Out of the total SSPS added to the treatment solution, 79.82% was recovered into the wash water. The findings of this study suggest potential feasibility of SSPS for enhancing lactose crystallization during lactose manufacture from concentrated permeate.

The objective of the second study was to evaluate commercial feasibility of using SSPS to improve the drying ability and powder characteristics of GAW. The composition of GAW is considerably different from that of cheese whey making it difficult to process into powder ingredients. The high lactic acid and mineral content in GAW delays lactose crystallization which causes stickiness during spray drying and caking during storage of the powder. The first study had shown that soluble soybean polysaccharide (SSPS) can enhance lactose crystallization in concentrated permeate. However, the effect of SSPS on the crystallization of lactose in concentrated GAW has not been evaluated. GAW obtained from a Greek yogurt manufacturer was vacuum concentrated to 56% total solids (30% Lactose) and crystallized using a laboratory scale crystallization tank. After crystallization, the concentrate was spray dried using a pilot scale NIRO dryer. GAW powder yield (51.87%) with 0.1% SSPS addition was significantly (P < 0.05) higher compared to that of GAW without SSPS (44.51%) and observed to be less sticky on the dryer surface. The findings of this study indicate that SSPS can enhance lactose crystallization in concentrated GAW during crystallization, reduce the sticking of the
powder in the dryer and improve the drying characteristics such as hygroscopicity and caking of the GAW powder.

The objective of the third study was to evaluate the feasibility of partial demineralization and deacidification of GAW by nanofiltration (NF) to improve the drying ability and powder characteristics of GAW. Greek yogurt acid whey (GAW) contains high concentrations of lactic acid (LA) and minerals as compared to cheese whey. The LA and the minerals, particularly the Calcium (Ca) in GAW cause stickiness during spray drying, thus limiting the processing and utilization of GAW. Nanofiltration (NF) has been in use to for partial removal of minerals from cheese whey and milk to produce high value-added dairy ingredients. Similarly, NF can potentially be applied for partial demineralization and deacidification of GAW to improve spray drying and powder properties. By NF processing, the lactic acid and total ash concentrations were reduced significantly (P < 0.05) by 34.3±0.2 and 37.8±0.7, respectively. The reduction of monovalent ions, i.e., sodium and potassium were observed to be higher (66% and 62%) than that of calcium (41%). GAW-NFR powder yield (56.35%) was significantly (P < 0.05) higher compared to that of GAW (44.51%) and observed to be less sticky on the dryer surface. The findings of this study indicate that partial demineralization and deacidification of GAW using NF can improve the spray drying characteristics of the GAW powder.

The objective of the final study was to evaluate the effect of addition of SSPS to NFR-GAW on the drying characteristics of the GAW. In this study, the yield, and drying characteristics of GAW with and without 0.1% SSPS were compared. There was no significant (P > 0.05) difference observed between the yields of GAW-NFR (56.35%)
and GAW-NFR with SSPS addition (58.24%). However, the yield of NFR-GAW (56.35%) was significantly (P < 0.05) higher than that of GAW with SSPS addition (51.87%). From this study it can be concluded that there may not be any additional advantage in addition of SSPS to NFR-GAW in improving the spray drying characteristics of GAW.

Chapter I

Introduction and Project overview

The demand for global cheese production is growing at a 3% linear increase and 23.2 M tons was produced in 2017 (OECD/FAO, 2018) corresponding to 208.6 M tons of cheese whey. The simultaneous increase in demand for high protein dairy ingredients such as whey proteins concentrate (WPC), whey protein isolates (WPI) helped industry to utilize large volumes of sweet whey produced leaving behind a co-product, rich in lactose referred as permeate or deproteinized whey (DPW). Additionally, milk permeate is generated during manufacture of milk protein concentrate (MPC) and milk protein isolate (MPI). Greek yogurt is another high protein dairy product that registered a tremendous market growth in the U.S, from 1 to 2% of the total yogurt market in 2004 to nearly 40% of the U.S. yogurt market in 2015 (USDA, 2017). Every pound of Greek yogurt produced results in 2 to 3 pounds of co-product, referred as Greek yogurt whey or Greek acid whey (GAW). GAW is compositionally different from sweet whey making it difficult to process into value-added ingredients and currently underutilized. The ability to better utilize these co-products may limit the growth of mentioned high protein dairy products and ingredients. Thus, development of new technologies for improving lactose
crystallization in permeate and drying ability of Greek yogurt acid whey will address these problems and help dairy industry to better utilize the discussed dairy co-product streams.

Soluble soybean polysaccharides (SSPS) is a water-soluble polysaccharide extracted and refined from soybean. SSPS is a non-gelling polysaccharide soluble in both cold and hot water and shows a relatively low viscosity compared to the viscosity of other gums or stabilizers (Maeda and Namamura, 2009). SSPS has been finding food applications as a stabilizer, emulsifier, thickener, anti-caking agent in food systems like drinking yogurt, baking goods, noodles, frozen foods, jelly, and confectionery. Combined with low viscosity, the ability of SSPS to hold moisture is the matter of relevance for this project.

Nanofiltration (NF) technology is a pressure driven membrane process that falls between ultrafiltration (UF) and reverse osmosis (RO) and mainly used for the removal of divalent ions and larger monovalent ions like heavy metals. In dairy processing industries, NF is mainly used for demineralization of whey and milk ultrafiltration permeate to produce low mineral and free flowing powders (Janet et al., 2000; Lifran et al., 2010; Suarez et al., 2006; Xu and Lebrun, 1999). Nanofiltration can be potentially used for partial removal of minerals and lactic acid from Greek yogurt whey to improve the dryability and quality of the powder.

The overall goal of this project is to utilize the SSPS and NF technology in developing methods to improve lactose recovery from permeate and drying ability and powder characteristics of Greek yogurt whey. The following chapters describe the objectives of the experiments conducted in this project, focused on evaluating new
technologies to improve lactose crystallization in permeate and drying characteristics of GAW.
Objectives

- Chapter 3 - Evaluate commercial feasibility of using Soluble Soybean Polysaccharide (SSPS) to improve lactose recovery during manufacture of lactose from permeate.

- Chapter 4 - Evaluate commercial feasibility of using SSPS to improve the drying ability and powder characteristics of GAW.

- Chapter 5 - Evaluate the feasibility of partial demineralization and deacidification of GAW by nanofiltration (NF) to improve the drying ability and powder characteristics of GAW.

- Chapter 6 - Evaluate the effect of addition of SSPS to partially demineralized and deacidified GAW (GAW-NFR) on the drying characteristics of the GAW.
REFERENCES


Chapter II
Review of literature

1. Lactose

Lactose universally described as “milk sugar” is the principal carbohydrate in the milk of most mammals, with the known exceptions of Californian sea lion and other Pacific pinnipeds. While a substantial amount of lactose is found in mammalian milk, small amounts are occasionally present in a complex form in oligosaccharides of plants and Sapotacea fruit (Reithel and Venkataraman, 1956). The concentration of lactose in cow’s milk is around 4.5% wt./vol (up to 40% of the total solids in whole milk). For the first time in 1633, Bartoletius isolated lactose from whey by evaporation (Holsinger, 1998). Thereafter several researchers conducted numerous studies to develop an understanding of the physical and chemical properties of lactose.

1.1. Characteristics of lactose.

Lactose is a disaccharide (4-0-β-D-glactopyranosyl-D-glucopyranose) with a molecular formula of C12H22O11, synthesized in the mammary gland from galactose and glucose linked by β-1,4 glycosidic bond (Schaafsma, 2008). Because of the anomeric center at the C1 carbon of the glucose, it exists as α and β forms (Figure 2.1) and is converted to each other by mutarotation (Figure 2.2).

1.1.1. Solubility of lactose.

Lactose is the least soluble compared to other sugars such as fructose, fructose, and sucrose (Andreeta, 2012) as shown in Figure 2.3. The two anomers have very different properties, particularly solubility and crystallization. At 20°C, solubility of α-
and β-lactose in water are 70 and 500 g/L, respectively. In aqueous solutions, α-lactose dissolves rapidly followed by conversion of some of α-lactose into β-lactose and this process continues until the equilibrium between the two forms is reached. The ratio of α-:β-lactose is approximately 37:63 when equilibrating at 20°C, equivalent to an overall lactose solubility of 180 g/L (Fox, 2009), which is much lower than the solubility of sucrose (2000 g/L). As illustrated in Figure 2.4, solubility of α-lactose is more temperature-dependent than β-lactose. α-lactose is less soluble than β-lactose at a temperature below 93.5°C, and the opposite is true above 93.5°C (Fox, 2009; Walstra et al., 2006). Therefore, crystals formed below 93.5°C are composed of mostly α-lactose, in the monohydrate form, while β-lactose, in the anhydrate form, is present in crystals formed above 93.5°C.

1.1.2. States of lactose.

Based on the molecular arrangement, the state of lactose can be categorized as crystalline and amorphous. When the molecules are arranged in an orderly manner with the space lattice, lactose exists in a crystalline state and if the molecules are not organized, it exists in an amorphous state (Mullin, 2001; Carpin et al., 2016). The temperature and water content of the system influence the transition of the lactose state between crystalline and amorphous. The known forms of crystalline and amorphous lactose are presented in Table 2.1. Amorphous lactose is formed when concentrated lactose without crystallization is spray dried or freeze-dried rapidly and exists as a mixture of α- and β-lactose (Carpin et al., 2016). The α-lactose monohydrate is the most stable form with one molecule of water attached to the lactose molecule account to 5% of
the crystal mass (Mimouni et al., 2005). The availability of a few hydrophilic groups in
the α-lactose monohydrate crystal form makes it non-hygroscopic and less soluble
(Zadow, 1984). The pre crystallization step during the manufacturing of high lactose
containing dairy powders promotes the formation of α-lactose monohydrate and increases
the storage stability of such dairy powders. Because of its stability, α-lactose
monohydrate is the targeted form during lactose crystallization at temperatures <93.5°C.
Anhydrous β-lactose is recovered by crystallization of lactose at temperatures above
93.5°C. It is characterized by uneven sided diamond shape which is stable and less
hygroscopic compared to anhydrous forms of α-lactose (Gänzle et al., 2008).

1.2. Lactose production.

1.2.1. Industrial sources to produce lactose.

The annual milk production of United States during 2017 was 215 billion pounds
(107.5 million tons), of this approximately 59.1% of the milk was used in cheese
production. Thus, producing 12.7 billion pounds (6.35 million tons, 3.9% above 2016
production) of total cheese, excluding cottage cheeses and around 114.3 billion pounds
(57.2 million tons) of cheese whey, the coproduct during manufacturing of cheese
(USDA, 2017). Similarly, during 2017, global cheese whey production was 208 million
tons and growing at a 3% annual linear rate for the last two decades (OECD, 2018).
Lactose contributes to approximately two-thirds of the dry weight of the whey, minerals
and protein contribute to most of the remaining one-third (Hobman, 1984). Generated at
such a large volume and containing a high concentration of lactose (approximately 5 %
w/w), cheese whey (94% of the total whey) becomes a major industrial source of lactose.
A relatively small amount of whey (6% of total whey) is also generated from the
production of caseinates, cottage cheese, and strained yogurt (Affertsholt-Allen, 2007). Additionally, two other growing sources of whey are production of Milk Protein Concentrate (MPC) and Milk Protein Isolate (MPI) and traditional Greek yogurt, such whey is generally referred as “native whey” and “acid whey” respectively. Until the introduction of ultrafiltration in dairy processing in the 1970s, sweet whey had been considered as waste and disposal on farms and in sewerage. But the tighter environmental regulations, advancements in membrane technology, increased consumer awareness, and demand for functional foods have significantly changed the way whey is utilized leading to production and marketing of WPC, WPI, and functional peptides. This process leaves behind the next level of dairy co-product stream rich in lactose, up to 85% of the total solids. Because of the increasing environmental concerns related to biological oxidation demand (BOD), there is a noticeable shift towards recovering lactose from permeate compared to whey (Patel and Murthy, 2012).

About 1124 million pounds of lactose was recovered from the cheese whey produced in the US during 2017 (2.2% up over the previous year) corresponding to just 20% of the total lactose produced. The global market value for pharmaceutical and conventional lactose along with permeate powders is forecasted to grow by 4% CAGR (Affertsholt and Pedersen, 2017). But the decreasing demand for whey powder and increasing demand for high-end whey ingredients particularly in the US deliver increasing amounts of lactose more than current lactose utilization. This indicates an opportunity and needs for new lactose utilization technologies.

1.2.2. Lactose production process.
Industrial production of lactose has started a century ago (Dryden, 1992). Western Europe typically uses whole whey to produce lactose, although, in the US, permeate is typically used (Figure 2.5). There are three general methods proposed for the commercial production of lactose. Crystallization and separation from a supersaturated solution is the most common method, and this method is economically favorable at a large scale. Steffen process of lactose production involves precipitation of sugars by alkaline earth minerals (Nickerson, 1979). The third process utilized solvents like methanol to decrease the solubility of lactose, but this method is not used commercially because of high cost of solvents and low market price of lactose.

In a standard industrial method of lactose production from cheese, whey starts with the removal of casein fines and whey cream by separator followed by whey proteins using ultrafiltration. The permeate is concentrated to 58-62% total solids (TS) using falling film evaporators. Some plants are using reverse osmosis to preconcentrate the permeate to 12-15% solids before concentrating to final solids using evaporators. Crystallization of lactose in the super saturated solution is initiated either by spontaneous crystal formation or by the addition of a small amount of lactose crystals, often referred as seeding. The crystals are separated using decanting centrifuge with simultaneous washing by the introduction of wash water into the centrifuge. The addition of wash water to remove mineral impurities is an essential step to produce high-quality lactose. The washed crystal slurry is then spray dried and bagged. Commercial lactose production processes are tailored with additional steps like re-dissolving and recrystallization to improve purity and treatment with active carbon to remove minor impurities, depending
on different end uses (Paterson, 2009; Sinelnikov, 2007; Kramtsov, 2011; Paterson, 2016).

1.3. Crystallization principles.

Crystallization is the main process step in the manufacture of lactose. The important stages in crystallization include nucleation, growth of nucleus and growth of the crystal (Hartel, 2001).

1.3.1. Nucleation.

The nucleation mechanism occurs in two stages, primary nucleation, and secondary nucleation. Further primary nucleation can be distinguished as homogeneous and heterogeneous (Hartel, 2001; Myerson and Gindle, 2001) as shown in Figure 2.6.

Primary nucleation is considered a classical example of the formation of nuclei and it rarely occurs in practical solutions (Hartel, 2001; Dirksen and Ring, 1991). During primary nucleation, solute molecules in a solution come together to form a primary cluster. Formation and dissociation of these clusters is a continuous phenomenon, depending on the conditions of the system. At suitable supersaturation and temperature conditions, the rate of association supersedes the rate of dissociation and a stable cluster of critical size is formed (Hartel, 2001; Myerson and Gindle, 2001). Heterogeneous nucleation occurs in the presence of impurities and prior crystals formed in the solutions. These impurities include dust or dirt particles, rough surfaces of the process vessel or crystallizer wall, and agitator surfaces in case of industrial crystallization.

Primary nucleation is typically dominant only during the star-up phase of a non-seeded crystallization process. Once enough crystals have formed and supersaturation
drops with the meta-stable zone, (Figure 2.7), secondary nucleation becomes the dominant mechanism for generation of new nuclei.

Neither growth nor nucleation can take place in the undersaturated zone. The growth of crystals can take place in both the metastable and labile zones. Crystallization can only take place in the metastable zone by seeding with lactose, or with substances containing lactose, or with any substances that are isomorphous (of similar form) to lactose. In a seeded crystallization process, the metastable zone can be further categorized as the upper and lower metastable limits separated by the forced crystallization line. The upper region is the area between the solubility and the forced crystallization lines. In this area, the growth of seeded crystals is promoted, while the formation of small nuclei via secondary nucleation is minimized. The lower metastable area is the region between the forced crystallization and the super-solubility. During the cooling crystallization process, mass crystallization (the formation of a multitude of small crystal centers) is enhanced when the seed crystal is added at the beginning of the forced crystallization line, and further cooling is maintained in the lower metastable area.

During secondary nucleation, the nuclei are formed because of the presence of existing crystals in the solution and it is the dominant nucleation mechanism in many commercial crystallization processes (Jone, 2002). Secondary nucleation occurs at relatively lower levels of supersaturation compared to primary nucleation (Mcleaod, 2007). Secondary nucleation can be forced by adding seed crystals since the added crystals are larger than the critical nuclei size, they act as nucleation sites. The small broken crystals formed by agitation and collision with crystallizer walls and with each
other act as nucleation sites and trigger secondary nucleation (Pandalaneni and Amamcharla, 2016; Myerson and Gindle, 2001).

1.3.2. Factors influencing the nucleation.

The rate of nucleation defined as the number of the nuclei formed per unit volume per unit time and the induction time described as the time required for nuclei to form determine the effectiveness of the nucleation process. The other factors that affect the nucleation process include temperature, supersaturation, rate of cooling, rate of agitation, pH, seeding, and presence of additives or impurities (Hartel and Shastry, 1991). Temperature and supersaturation are interrelated phenomena and are the major factors influencing the homogeneous nucleation in the system (Hartel and Shastry, 1991). When some critical supersaturation is reached, it gives a driving force for the beginning of nucleation and leads to an increase of nucleation rates (Hartel, 2001). Decreasing the temperature of the solution results in increasing the rate of crystallization because of increased supersaturation (Livney et al., 1995). On the contrary, the increased nucleation rate is caused by an increase in temperature (Nickerson, 1956; Mcleaoed et al., 2011). Additionally, lowering the temperature and increasing concentration leads to increased viscosity which eventually results in decreased nucleation rate (Hartel, 2001; Livney et al., 1995). Several other influencing factors of nucleation and their effects are summarized in Table 2.2.

1.3.3. Crystal growth.

After the formation of stable nuclei, the onset of crystal growth occurs by the diffusion of lactose in the supersaturated solution into the crystal lattice formed during
the nucleation step. Temperature and supersaturation conditions are two important
driving forces for crystal growth (Hourigan et al., 2013). Crystal growth continues till all
available solute in the supersaturated solution is consumed and the equilibrium phase of
the solution is reached (Hartel, 2001). Like the nucleation process, the crystal growth
process also includes three important steps: 1) mass transportation, 2) surface integration,
and 3) heat transportation (Wong and Hartel, 2014). The first step involves the
transportation of mass from liquid phase to the solid phase. The driving force for this
transportation step is the difference in concentrations between the bulk solution and
interfacial concentration (Hartel and Shastry, 1919). After the molecules have arrived at
the surface of the growing crystal, in the second step the molecules find a suitable
incorporation site and integrate into the crystal lattice structure. Based on the growth
conditions, lactose molecules can choose different sites for surface integration. Under the
slow growth conditions, molecules choose energetically favorable sites for incorporation
leading to crystals with smooth surfaces. Whereas in rapid growth conditions, molecules
prefer less energetically favorable sites resulting in formation of crystals with
imperfections (Pisponen, 2017). Crystal growth is an exothermic process, and the third
step of crystal growth involves transportation of heat away from the growing crystal. In
some systems, latent heat transfer away from the growing crystals may limit crystal
growth rate (Hartel, 2001), but in case of lactose crystallization, the growth rate is
controlled by surface interactions (Schmitt et al., 1999).

1.3.4. Crystal shape.

The environmental conditions under which the crystal was formed determine the
crystal morphology (Hartel, 2001). Lactose crystal is found in many shapes varying from
needle-shaped to well-developed tomahawk crystals (Herrington, 1934b; Arellano et al., 2004; Dincer et al., 1999; Pandalaneni and Amamcharla, 2016; Paraladevi and Srinivasan, 2014a; Paraladevi and Srinivasan, 2014b). Typically, lactose crystals grow in three directions (Figure 2.8) and each face grows at a different speed (Dincer et al., 2014) leading to a different growth rate (Shi et al., 1989). Among different crystalline shapes (Figure 2.9) of α-lactose monohydrate, tomahawk shaped crystals mainly grow in -b direction and have large (0ī1) and (0ī0) faces and a small (0ī0) face (Kreveld & Michaels, 1965). Plate-shaped crystals occur because of fast growth of (0ī0) face and moderate growth of (0ī1), (0ī0) and (010) faces (Arellano et al., 2004). The β-lactose plays an important role in the formation of different crystal forms. β-molecules incorporate into the lattice of apex faces and block their growth. Lactose molecules in this direction (-b) are situated with their glucose moieties, so they might form bonds with the galactose moiety of β-molecules. Once incorporated, β-lactose can inhibit further growth of that site. Respectively, in faces (010), (110) and (011) α-lactose molecules are situated with their galactose moieties, so they are least affected by β-lactose (Visser & Bennema, 1983). Needle-shaped crystals grow in aqueous solutions with low β-lactose content. These crystals typically have large (010), (0 10) and (100) and small (0 11) faces (Visser & Bennema, 1983).

### 1.3.5. Factors affecting crystal growth.

Several factors that affect the crystal growth include the concentration of the solution, degree of supersaturation, rate of cooling, final cooling temperature, pH and viscosity of the solution, rate of agitation during crystallization, and presence of impurities and additives. Supersaturation and rate of cooling are two important
interdependent driving forces of crystallization process and largely determine the crystal growth (Jelen and Coulter, 1973a). Supersaturation generally promotes crystal growth, however excessive supersaturation may limit the mobility of molecules (the mass transfer step of crystal growth) because of changes in viscosity of solution and results in decreased crystal growth (Shi et al., 1989). Similarly, cooling to the lowest possible temperatures theoretically should result in increased crystal growth, however, lowering the crystallization temperatures below 20°C will decrease the viscosity of the solution and interferes with the mobility of the molecules and decreases the crystal growth (Whittier and Gould, 1930). The ideal cooling curve should quickly cool the solution to the point, where primary nucleation starts and held there while the nucleation occurs, and growth starts. Beyond this point, the cooling curve should be adjusted to maintain a supersaturation just below the secondary nucleation threshold required for secondary nucleation to occur (Shi et al., 2006, Wong and Hartel, 2014). Another reason for decreased crystal growth at lower temperatures is slower mutarotation rates (Tan, 2009). At extremely low temperatures the mutarotation rate falls below that of α-lactose crystallization thus, limiting further crystallization process (Livney et al., 1995).

Numerous studies were conducted on the influence of presence of additives and impurities and process conditions on the crystal growth. Table 2.3. summarizes some of the studies with a brief description of the results.

In addition to studies on additives and impurities, in recent years, there is a growing interest in different processing conditions during crystallization such as sonication and gas addition. Ultrasonication of the solution during nucleation step was observed to enhance primary nucleation, and the produced lactose crystals showed a
closer size distribution (Guo et al., 2015; Bund and Pundit, 2007). Adhikari et al., 2018 reported that addition of gases such as CO₂ and N₂ to the supersaturated lactose solution resulted in a better yield of lactose crystals.


Lactose has several applications in infant formulae, processed food, confectionery, bakery, and pharmaceutical industries. Affertsholt-Allen (2007) elaborated on the contemporary utilization pattern in the USA and Europe. Commodities (lactose and permeate powder) make up the most significant part (approximately 50%) of the lactose utilization. Majority of the lactose produced in the US is used in infant formulae (66%) followed by confectionery (16%) and nutraceuticals and pharmaceuticals (5%) industries. Whereas in EU 60% of the total lactose utilization is shared equally between processed food and pharmaceuticals followed by infant formulae (18%) and chocolate confectionery (16%) industry (Affertsholt-Allen, 2007). The physical and chemical properties of lactose affect its use in different applications. Several food applications of lactose with respect to its functionality are summarized in Table 2.4.

1.4.1. Infant formulations.

Lactose is a major source of energy in milk, including that of humans. The prebiotic functionality and low glycemic index that supplies consistent energy release make lactose, the perfect source of energy for infants. The level of lactose in human milk is about 7% compared to 4.8% in cows’ milk and this was recognized by Henri Nestle who enriched milk powder made from cows’ milk with lactose to produce the first infant formulae in 1867, Since then it has been one of the major uses of lactose. Although
maltose, sucrose, maltodextrins, glucose syrup or dried glucose syrup, pre-cooked starch naturally free of gluten and gelatinized starch naturally free of gluten may be used as a carbohydrate source in an infant formulation (Nasirpour et al., 2006) lactose is the most important energy source in an infant formula with around 35-50% in most of the commercial formulations. Lactose also plays an important role in the absorption of calcium, magnesium, and manganese in infant (Ziegler and Fomon, 1983).

1.4.2. Confectionery applications.

Lactose is only 30-35% sweet as sucrose. This reduced sweetness, coupled with aroma fortification, improved color binding, texture and mouthfeel make lactose best candidate for chocolate, sugar, and flour confectionery applications. Lactose also delays the crystallization of sucrose in concentrated solutions. In most confectionery systems, lactose can be used (up to 10-20%) to partially replace sucrose without affecting textural properties (Lee and Szilagyi, 2012). Lactose is a reducing disaccharide and depending on the type of protein present in caramel and toffee formulations, it provides typical brown color to the product because of Millard reaction (Bouzas, 1999). Using too much lactose results in lactose crystallization causing a sandy texture and affects the product acceptability. Cost benefit is another motive for using lactose in most of the confectionery products. The primary purpose of lactose use in backing industry is to provide color and flavor through browning reaction. However, lactose also helps with increased loaf volume, external appearance, and shelf life.

1.4.3. Pharmaceutical applications.
Pharmaceutical grade lactose has been in use by the pharmaceutical industry for many years and is more pure than edible lactose. Lactose can provide a wide range of granular distribution, free flowability and bulk density to various pharmaceutical products (Pritzwald-Stegmann, 1986). Lactose is principally used as an extender, filler, diluent, and drug carrier. The drug-binding ability of lactose facilitates tablet compression and pill molding. Lactose is also used as a coating for some pills (Call, 1958).

1.4.4. Other applications.

Lactose is also used to reduce the sweetness of toppings and icings by replacing up to 20% of the sucrose. The addition of 6 to 8% of lactose to pie formulations, gives a characteristic flakier crust with a golden-brown color. More uniform golden color can be obtained by dipping the potato chips and french-fried potatoes in a lactose solution before cooking in fat. The addition of a small amount of lactose to chocolate drinks enhances the flavor. In the dairy products such as frozen desserts and ice creams, lactose offers a price advantage over other sweetening agents.

1.5. Soluble soybean polysaccharide (SSPS)

Soluble Soybean polysaccharide (SSPS) is a water-soluble polysaccharide obtained and refined from “okara”, the byproduct of soybean protein extraction process during manufacture of soya protein isolate. SSPS has been shown to be functional as a dispersing agent, stabilizer, emulsifier, and for its adhesion properties (Maeda, 1992).

1.5.1. Composition, structure, and properties.
Soyafibe-S is the most popular variety of SSPS and typically contains 60-77% soluble fiber, 4.8-13.9% crude protein, and 6.0-8.6% ash. The predominant sugar components are galactose, arabinose, and galacturonic acid, but many other sugars such as rhamnose, fucose, xylose and glucose are also present. The composition is like that of pectin, acidic polysaccharides found in abundance in the peel of various fruits (e.g., citrus, apple). However, the neutral monosaccharides content in SSPS is much higher than in pectin (Maeda and Nakamura, 2009).

The SSPS backbone consists of long-chain rhamnogalacturonan and short-chain homogalacturonan, while citrus pectin, for example, consists of short-chain rhamnogalacturonan and long-chain homogalacturonan. The neutral sugar side chains of 1,4-galactans (degree of polymerization = 43±47), branched with fucose and arabinose residues, and 1,3- or 1,5-arabinans are linked to the C-4 side of rhamnose residues in the rhamnogalacturonan. SSPS is a non-gelling polysaccharide soluble in both cold and hot water, and shows a relatively low viscosity compared to the viscosity of other gums or stabilizers such as guar gum, allowing a highly concentrated (more than 30%) solution to be produced (Figure 2.10). Furthermore, the viscosity of the solution is not significantly affected by heating, the addition of acid or salts (Figure 2.11 and Figure 2.12). These physical properties can be explained by the globular structure of SSPS in aqueous solution.

1.5.2. Functionality and applications.

SSPS is reported to have very high adhesive strength (Nakamura, 1989). With this property, SSPS can be employed as a binder not only in dried food applications like snacks and cereals but also in paper, wood, or glass applications. Also, its film forming
properties allow to make colorless, transparent, water soluble, and edible films. It is reported that SSPS prevents oxidation of oils and could be employed in the manufacturing of flavor oils (Masumura et al., 2003). Some more functionalities and applications are shown in Table 2.5.

2. Greek yogurt whey.

Greek yogurt is a fermented semi-solid high protein dairy product obtained by concentrating the yogurt by draining part of whey. It is commonly referred as strained yogurt in Europe and Labneh in Middle Eastern countries and Balkans (Nsabimana et al., 2005). Traditionally, strained yogurt is produced by straining yogurt in cloth bags until the desired level of total solids is achieved. But the modern commercial manufacturing methods include the use of technologies like centrifugation and ultrafiltration for concentration of yogurt. In the United States, the Greek yogurt sales grew tremendously from just 2% of overall yogurt market in 2004 to approximately 40% in 2015 (Erickson, 2017). On average, every 2 to 3 kg of whey is produced from every 1 kg of Greek Yogurt manufactured. Most of the Greek yogurt whey (GAW) is currently applied as fertilizer onto the fields. However, there is a limit for this use because, with too much application of GAW on the land, the mixture will run off into nearby waterways, leading to algal blooms, low levels of dissolved oxygen, and fish kills (Erickson, 2017).

2.1. Composition.

The GAW is characterized by low pH, high lactic acid, high mineral content, and very low protein like that of other acid whey types for example cream cheese whey and
cottage cheese. GAW compositionally very different from that of cheese (sweet) whey as shown in Table 2.6.

2.2. Challenges with GAW processing.

Because of high mineral content and acidity, GAW poses different processing challenges compared to sweet whey processing and drying. For example, high mineral content decreases the performance of vacuum evaporators due to the increase in mineral fouling and overall heat transfer resistance. On the other hand, because of high lactic acid and monosaccharides for example galactose, the kinetics of lactose crystallization in the GAW concentrates are slower than that of sweet whey concentrates, with an increase in the crystal size dispersion and decrease in the final crystallization rate (Modler and Lefkovitch, 1986). The presence of lactic acid and galactose in the concentrated GAW makes the product sticky during spray drying, resulting in fewer yields.

3. Nanofiltration.

Membrane processing is a selective permeability-based technology through a porous membrane filter. Based on the selectivity, the membrane used allows passage of certain components called permeate and concentrates certain components referred as retentate. The separation of milk components through different membrane processes is shown in Figure 2.13. Nanofiltration removes particles of molecular weight with less than 300-1000 Daltons and retains the rest. Based on the operating pressures (20-30 bar TMP), pores the size of the membrane (1 and 10 nm), it technically falls between ultrafiltration and reverse osmosis processes and often referred as loose reverse osmosis or ultra-tight ultrafiltration process.
The NF has been successfully used in industry for simultaneous concentration and demineralization of the whey. Using NF process whey can be concentrated to 20-22% TS and a demineralization of 25% and 60%, without and with diafiltration step (Gregory, 1987; Kelly et al., 1992; Kelly and Kelly, 1995; Suárez et al., 2006; Suárez et al., 2009).

It is also possible to partially deacidify the GAW using NF. The NF can also help in reducing the sticky monosaccharides present in GAW for example galactose.
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Shi, X., & Zhong, Q. (2015). Crystallinity and quality of spray-dried lactose powder improved by soluble soybean polysaccharide. LWT-Food Science and
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Dissertation


Van Kreveld, A., & Michaels, A. S. (1965). Measurement of crystal growth of α-


Dairy Journal, 21(11), 839-847.


Figure 2.1. The molecular structure of α- and β-lactose (Dincer et al., 1999)
Figure 2.2. Mutarotation process between α- and β-lactose forms (Walstra et al., 2006)
Figure 2.3. Solubility in water of fructose $\Delta$ (Silva, 2010), sucrose $O$ (Ouiazzane et al., 2008), glucose $\Diamond$ (Alves et al., 2007), lactose $\Box$ (Brito, 2007)
Figure 2.4. Temperature-dependent solubility of $\alpha$- and $\beta$-lactose (Fox, 2009)
Figure 2.5. Typical process flow diagram for the manufacture of edible-grade α-lactose monohydrate (Paterson, 2009).
Figure 2.6. Types of nucleation (Muthukumarappan et al., 2019)
Figure 2.7. Lactose solubility diagram (Wong et al., 2011).
Figure 2.8. Typical tomahawk-shaped $\alpha$-lactose monohydrate crystal with face indications (Kreveld and Michaels, 1965)
Figure 2.9. The crystalline habit of lactose α-hydrate crystals, sketched by Herrington (1934b). (A) Needle shaped crystal, formed at a very high growth speed. (B) Crystal formed more slowly than A form. (C) Diamond-shaped plate, transition between prism and pyramid. (D) Pyramid resulting from an increase in the thickness of the diamond. (E)-(G) Different forms of tomahawk shaped crystals, where the latter is usually described as fully developed. (H) A crystal with 13 faces. (I) A profile view of H with sharpened tomahawk blade.
Figure 2.10. Viscosity comparison of various polysaccharide solutions at 25°C (Maeda and Nakamura, 2009)
Figure 2.11. Viscosity change by heating of 10% SSPS aqueous solution at various pH ranges (Maeda and Nakamura, 2009)
Figure 2.12. Effect of various salts on viscosity of a 10% SSPS solution at 20°C (Maeda and Nakamura, 2009)
Figure 2.13. Milk components separation using different membrane processes (Cheryan, 19198; Brans et al., 2004)
<table>
<thead>
<tr>
<th>Forms of lactose</th>
<th>State of Lactose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crystalline</strong></td>
<td>α-lactose monohydrate</td>
</tr>
<tr>
<td></td>
<td>α-lactose anhydrous - stable</td>
</tr>
<tr>
<td></td>
<td>α-lactose anhydrous – unstable</td>
</tr>
<tr>
<td><strong>Amorphous</strong></td>
<td>a mix of α- and β-lactose</td>
</tr>
<tr>
<td></td>
<td>β-lactose</td>
</tr>
</tbody>
</table>
Table 2.2. Effect of different factors influencing nucleation

<table>
<thead>
<tr>
<th>Factor</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agitation or energy input</td>
<td>Increased molecular movement and</td>
<td>Hartel and Shastry, 1991; Wong et al., 2012;</td>
</tr>
<tr>
<td></td>
<td>nucleation rate</td>
<td>Mcleod et al., 2016</td>
</tr>
<tr>
<td>Seeding</td>
<td>Promotes secondary nucleation</td>
<td>Nickerson, 1956; Guu and Zall, 1991</td>
</tr>
<tr>
<td>High pH</td>
<td>Increases the induction time</td>
<td>Raghavan et al., 2001</td>
</tr>
<tr>
<td>Cooling-slow</td>
<td>Early nucleation, large crystals</td>
<td>Shi et al., 1989</td>
</tr>
<tr>
<td>Cooling-rapid</td>
<td>Spontaneous nucleation, small crystals</td>
<td>Wong et al., 2012</td>
</tr>
<tr>
<td>Large quantities of β-lactose</td>
<td>Inhibits nucleation</td>
<td>Raghavan et al., 2001</td>
</tr>
<tr>
<td>Minerals and vegetable gums</td>
<td>Inhibits nucleation</td>
<td>Gänzle et al., 2008</td>
</tr>
<tr>
<td>Alcohols</td>
<td>Promote spontaneous nucleation</td>
<td>Gänzle et al., 2008</td>
</tr>
<tr>
<td>Acetone</td>
<td>Metastable zone width increased</td>
<td>Brito and Giulietti, 2007</td>
</tr>
<tr>
<td>Glycerol</td>
<td>Inhibits primary nucleation</td>
<td>Vu et al., 2009</td>
</tr>
<tr>
<td>Power ultrasound</td>
<td>Promotes secondary nucleation, reduced</td>
<td>Bund and Pandit, 2007; De Castro and Priego-Capote, 2007</td>
</tr>
<tr>
<td></td>
<td>induction times</td>
<td></td>
</tr>
<tr>
<td>WPI, Gum Arabic</td>
<td>Delayed nucleation</td>
<td>Das et al., 2013</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>Accelerated nucleation</td>
<td>Sánchez-García et al., 2018</td>
</tr>
<tr>
<td>Lactates, Citrates</td>
<td>Accelerated nucleation</td>
<td>Gernigon et al., 2013</td>
</tr>
<tr>
<td>Ultrasound and antisolvent</td>
<td>Promotes primary nucleation</td>
<td>Guo et al., 2015</td>
</tr>
<tr>
<td>Galacto-oligosaccharides</td>
<td>Retards nucleation</td>
<td>Ihli and Paterson, 2015</td>
</tr>
<tr>
<td>Addition of CO$_2$ and N$_2$ along with sonication</td>
<td>Promotes nucleation</td>
<td>Adhikari et al., 2018</td>
</tr>
</tbody>
</table>
Table 2.3. Effect of different factors influencing crystal growth (adapted from Pisponen (2017) and edited)

<table>
<thead>
<tr>
<th>Additive/impurity</th>
<th>Impact</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whey protein</td>
<td>Significantly lowered the final crystal size</td>
<td>Mioumoni et al., 2005</td>
</tr>
<tr>
<td></td>
<td>Crystal size decreased with increased denatured protein content</td>
<td>Modler and Lefkovitch, 1986</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>Retards lactose crystallization</td>
<td>Jelen and Coulter, 1973b; Nickerson and Moore, 1974b</td>
</tr>
<tr>
<td>Milk fat</td>
<td>Limits the crystal growth</td>
<td>Kelly, 2009</td>
</tr>
<tr>
<td>β-lactose</td>
<td>Influences α-lactose crystal morphology</td>
<td>Dincer et al., 1999; Nickerson and Moore (1974a); Raghavan et al., 2000; Raghavan et al., 2001</td>
</tr>
<tr>
<td>Glucose</td>
<td>Accelerates lactose crystal growth</td>
<td>Nickerson and Moore, 1974b; Nickerson and Patel, 1972; Hunziker and Nissen, 1926</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Retards the growth and influences the tomahawk shape</td>
<td>Nickerson and Moore, 1974b; Nickerson and Patel, 1972; Hunziker and Nissen, 1926</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>Low levels promote growth, high levels inhibit the growth</td>
<td>Jelen and Coulter, 1973b; Guu and Zall, 1919; Bhargava and Jelen, 1996</td>
</tr>
<tr>
<td>NaH₂PO₄</td>
<td>Promotes the growth</td>
<td>Jelen and Coulter, 1973b; Guu and Zall, 1919</td>
</tr>
<tr>
<td>LiCl</td>
<td>Increases growth rate</td>
<td>Bhargava and Jelen, 1996</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>Increases growth rate</td>
<td>Bhargava and Jelen, 1996</td>
</tr>
<tr>
<td>Ca lactate</td>
<td>Inhibits growth rate</td>
<td>Bhargava and Jelen, 1996</td>
</tr>
<tr>
<td>KCl</td>
<td>Low levels promote growth, high levels inhibit the growth</td>
<td>Jelen and Coulter, 1973b</td>
</tr>
<tr>
<td>Compound</td>
<td>Effect</td>
<td>References</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>---------------------------------------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>K$_2$HPO$_4$</td>
<td>Decreases the growth rate</td>
<td>Bhargava and Jelen, 1996</td>
</tr>
<tr>
<td>Combination of K$^+$, Ca$^{2+}$, PO$_4^{3-}$</td>
<td>Decreases the yield</td>
<td>Guu and Zall, 1919</td>
</tr>
<tr>
<td>Interaction of K$^+$ and PO$_4^{3-}$</td>
<td>Decreases the yield</td>
<td>Guu and Zall, 1919</td>
</tr>
<tr>
<td>Lactose phosphates</td>
<td>Inhibits lactose crystal growth</td>
<td>Visser, 1984; Visser 1988; Lifran et al., 2007; Hartel and Shastry, 1991</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>Influences growth direction and effects the shape</td>
<td>Kreveld and Michaels, 1965; Lifran et al., 2007</td>
</tr>
<tr>
<td>Alcohols</td>
<td>Promote β-lactose generation and influence lactose crystalline habit</td>
<td>Vu et al., 2009</td>
</tr>
<tr>
<td>Acetone</td>
<td>Retards crystal growth</td>
<td>Brito and Giulietti, 2007</td>
</tr>
<tr>
<td>Gelatin</td>
<td>Reduced growth rate</td>
<td>Gänzle et al., 2008</td>
</tr>
<tr>
<td>Gels</td>
<td>Decreases crystallization rate and affect crystal shape</td>
<td>Zend et al., 2000</td>
</tr>
<tr>
<td>Galacto-oligosaccharides</td>
<td>Retards the crystal growth</td>
<td>Ihli and Paterson, 2015</td>
</tr>
<tr>
<td>Soluble soybean polysaccharides</td>
<td>At 0.1% rate – crystal growth increased</td>
<td>Shi and Zhong, 2015</td>
</tr>
<tr>
<td></td>
<td>&gt;0.1% - retarded crystal growth</td>
<td>Shi and Zhong, 2014</td>
</tr>
</tbody>
</table>
Table 2.4. Summary of functionality and food applications of lactose

<table>
<thead>
<tr>
<th>Utilization</th>
<th>Functionality</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant formulae</td>
<td>Mimic human milk, prebiotic, low glycemic index, promote mineral absorption</td>
<td>Ziegler and Fomon, 1983; Schaafsma, 2008</td>
</tr>
<tr>
<td>Chocolate and sugar confectionery</td>
<td>Color, flavor, and texture enhancing, reduced sweetness</td>
<td>Schaafsma, 2008; Lee and Szilagy, 2012</td>
</tr>
<tr>
<td>Caramels and fudges</td>
<td>Improved body, texture, chewiness, and shelf life</td>
<td>Witter and Webb, 1970</td>
</tr>
<tr>
<td>Protein standardization of retail and powdered milk</td>
<td>Composition adjustment</td>
<td>Rattray and Jelen, 19196</td>
</tr>
<tr>
<td>Spices and flavorings</td>
<td>Aroma enhancement and anticaking agent</td>
<td>Nickerson, 1979</td>
</tr>
<tr>
<td>Sweetened condensed milk</td>
<td>Improve body and texture</td>
<td>Call, 1958</td>
</tr>
<tr>
<td>Cake formulations</td>
<td>Reduce the fat content</td>
<td>Brack, 1999</td>
</tr>
</tbody>
</table>
Table 2.5. Functions and applications of SOYAFIBE-S (Maeda and Nakamura, 2009)

<table>
<thead>
<tr>
<th>Functions</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble dietary fiber</td>
<td>General dietary fiber-fortified foods</td>
</tr>
<tr>
<td>Stabilizing effect under acidic conditions</td>
<td>Drinkable yogurt, ice cream, acidic desserts, sour cream</td>
</tr>
<tr>
<td>Emulsifying and emulsion stabilizing</td>
<td>Flavor emulsion, powdered flavor coffee cream, dressing, cleaner</td>
</tr>
<tr>
<td>Dispersing</td>
<td>Various types of paint, agricultural chemicals, ceramics, cement</td>
</tr>
<tr>
<td>Foam stability</td>
<td>Meringue, surfactant</td>
</tr>
<tr>
<td>Anti-sticking effect</td>
<td>Various types of cooked rice and noodles</td>
</tr>
<tr>
<td>Softening effect</td>
<td>Bread, cake, ham, sausage, Kamaboko (boiled fish paste), cream sauce</td>
</tr>
</tbody>
</table>
### Table 2.6. Composition of Sweet whey, GAW and Cream cheese whey

<table>
<thead>
<tr>
<th>Component</th>
<th>Units</th>
<th>CW (Jelen, 2003)</th>
<th>GAW (Menchik et al., 2019)</th>
<th>CAW (Menchik et al., 2019)</th>
<th>CCAW (Chandrapala, et al., 2015)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS</td>
<td>% wt./wt.</td>
<td>6.3-7.0</td>
<td>6.0</td>
<td>6.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Ash</td>
<td>% wt./wt.</td>
<td>0.2</td>
<td>0.75</td>
<td>0.42</td>
<td>0.61</td>
</tr>
<tr>
<td>Lactose</td>
<td>% wt./wt.</td>
<td>4.6-5.2</td>
<td>3.42</td>
<td>1.99</td>
<td>4.41</td>
</tr>
<tr>
<td>Protein</td>
<td>% wt./wt.</td>
<td>0.6-1.0</td>
<td>0.25</td>
<td>0.17</td>
<td>0.52</td>
</tr>
<tr>
<td>Fat</td>
<td>%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.32</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>%</td>
<td>0.2</td>
<td>0.64</td>
<td>0.37</td>
<td>0.58</td>
</tr>
<tr>
<td>Calcium</td>
<td>mg/100 g</td>
<td>40-60</td>
<td>120</td>
<td>69.9</td>
<td>92</td>
</tr>
<tr>
<td>pH</td>
<td>-</td>
<td>5.8-6.6</td>
<td>4.40</td>
<td>4.41</td>
<td>4.54</td>
</tr>
</tbody>
</table>

CW = Sweet whey from cheese; GAW = Greek yogurt whey; CAW = acid whey from cottage cheese; CCAW = acid whey from cream cheese
CHAPTER III
Feasibility of soluble soybean polysaccharide for enhancing lactose crystallization during lactose manufacture

ABSTRACT

Previous research has established that soluble soybean polysaccharide (SSPS) can enhance lactose crystallization in pure lactose solutions. However, commercial lactose is typically manufactured by crystallization of concentrated permeate (CP). The objective of this study was to determine the feasibility of using SSPS to improve lactose recovery during manufacturing of lactose from CP. A laboratory scale crystallization set up with parallel crystallizers was utilized to conduct control and treatment (with SSPS) experiments simultaneously. CP (total solids from 58 to 60 % and 48 to 49 % Lactose) obtained from a lactose manufacturer was used in the experiments. CP was heated to 80°C to dissolve lactose before transferring to the crystallization tanks. The CP solution in the tanks was cooled from 80°C to 18°C (rate, -0.0479°C/min) using an automatic temperature-controlled water bath. Constant agitation of 120 rpm was applied during the cooling cycle. Both the control and treatment solutions were seeded with lactose crystals (0.027 g/100 g of solution) and 0.1 % SSPS was added to treatment solution. After completion of crystallization, chilled water (at 4°C, 15 g per 100 g of solution) was added to the crystallized solution and centrifuged at 10,000 × g for 20 min at 4°C. The supernatant was decanted, weighed and an equal quantity of deionized water (4°C) was added to wash the crystals. A total of four washing cycles were applied to purify the lactose crystals. The mass of the washed lactose crystals (corrected for total solids) was used to calculate lactose recovery. The collected supernatant from each washing was
freeze dried and analyzed for SSPS. Lactose recovery with 0.1% SSPS addition was significantly ($P < 0.05$) higher (76.1%) as compared to the control (71.5%). Out of the total SSPS added to the treatment solution, 79.7% was recovered into the wash water. The findings of this study suggest potential feasibility of SSPS for enhancing lactose crystallization during lactose manufacture from concentrated permeate.

**Key words:** permeate concentrate, Soluble Soybean Polysaccharide, lactose crystallization, lactose recovery
INTRODUCTION

Lactose is the principal component of milk and whey permeate and composed of approximately 76 to 88% of lactose (U.S Dairy Export Council, 2015) and forms the starting material in lactose manufacturing process. Typically, Lactose is manufactured from concentrated permeate (65-75% total solids) by cooling and separating α-lactose monohydrate crystals (Wong et al., 2012). The cooling stage of lactose production involves crystallization of lactose, which is an important step towards determining the recovery of lactose and hence the production economics. Crystallization is a two-step process involving nucleation and crystal growth (Hartel, 2002) and the process objective of lactose crystallization is to produce large crystals with less number of fines to avoid loss of later during washing steps and achieve optimum yields (Pandalaneni and Amamcharla, 2016, Paterson, 2017). Several studies were conducted to understand the factors effecting the lactose crystallization for example, presence of mineral contents (Jelen and Coulter, 1973, Bhargava and Jelen, 1996), different proteins and their degree of denaturation (Jelen et al., 1973, Modler and Lefkovitch, 1986, Mimouni et al., 2005), pH levels (Modler and Lefkovitch, 1986; Sánchez-García et al., 2019).

During lactose production, both nucleation and crystal growth stages are influenced by super saturated conditions. Additives that can affect lactose solubility would alter the superstation conditions, there by the crystallization rates (Mullin, 2001). The addition of alcohol (Majd and Nickerson, 1976; Nicekerson and Lim, 1974) and sucrose (Nickerson and Moore, 1972; Nickerson and Patel, 1972; Sormoli and Langrish, 2013; Kedward et al., 1998;) reduced lactose solubility and promoted lactose crystallization. Similarly, several researchers studied the presence of other impurities like proteins and minerals
such as potassium, chlorides, calcium, sodium, and phosphorus, individually and in combination (Jenness, 1988). Presence of whey proteins at neutral pH was reported to promote the crystallization of lactose because of increased solubility of whey proteins, they served as the nucleation sites, on the contrary at acidic pH, whey proteins retarded the rate of lactose crystallization (Sánchez-García et al., 2019). Modler and Lefkovitch, (1986) reported that with the increasing denaturation level of whey proteins the crystallization rates retarded. The salts like calcium chloride or magnesium sulfate was reported to speed up the growth of crystals, but other salts (e.g., K₂HPO₄) slows down the growth rate of crystallization (Bhargava and Jelen, 1996). The effect of different minerals was summarized in the Table 3.1. The lactic acid, one of the major organic acid in whey permeate, was reported to inhibit the crystallization of lactose (Nickerson and Moore, 1974). In some recent studies, the promotional effect of nucleation with ultrasonication was reported both alone and in combination with antisolvents (Guo and Li, 2006; De Castro and Priego-Capote, 2007; Zhou et al., 2007; Dhumal et al., 2008; Patel and Murthy, 2011; Zamanipoor and Mancera, 2014; Sánchez-García et al., 2018; Singh et al., 2019). The addition of gases like CO₂ and N₂ in combination with ultrasonic assisted crystallization, was reported to increase the yield of lactose (Adhikari et al., 2018). It is a common industry practice to add polysaccharides such as locust bean gum, guar gum, gelatin, and xanthan gum as stabilizers in ice cream (Bahramparvar and Mazaheri, 2011). These hydrocolloids increase the viscosity system there by inhibiting formation of ice and lactose crystals (Nickerson, 1962). In another study addition of 0.9% κ-carrageenan delayed the crystallization (Kouassi et al., 2002).
Recently the influence of different levels of soluble soybean polysaccharide (SSPS) on lactose crystal growth was conducted (Shi and Zhong, 2014) in aqueous lactose solution and reported significant improvement in lactose recovery. SSPS is a by-product of soybean protein manufacturing process and cell wall extract of soybean cotyledons (Furuta and Maeda, 1999), majorly consisting of acidic polysaccharide and small conjugated polypeptide fractions (Chivero et al., 2014). The ability of SSPS to form lower viscous solution compared to other polysaccharides is enabling its applications as a functional ingredient to stabilize proteins (Pan et al., 2014) and as a source of dietary fiber (Maeda and Nakamura, 2000).

However, there was no information available on the effect SSPS on lactose crystallization in concentrate permeates under industry relevant conditions. This study was focused on evaluating the influence of SSPS addition to concentrated permeates while manufacturing lactose using an industrially common cooling rate, and under seeded conditions.

**MATERIALS AND METHODS**

**Experimental Design**

The effect of SSPS on lactose crystallization was studied at 0.1% addition level. The control (without SSPS) and treatment (with 0.1% SSPS) concentrate permeates were crystallized using 0.048 °C/min. cooling rate with 0.027% seed crystal addition. Experiments were conducted in triplicates with control and treatments simultaneously.

**Concentrated Permeate (CP) and Soluble Soybean Polysaccharide (SSPS)**

Concentrated permeate solutions from three different batches were obtained from a nearby lactose manufacturer. The total solid content was adjusted approximately to 60%
using Hei-VAP Value laboratory vacuum evaporator (Heidolph Instruments GMBH &Co. KG) at 70±2°C before crystallization. Fuji Oil Corp. (Osaka, Japan) gifted the SSPS used in the experiments.

**Experimental Setup**

A scaled down version of industry crystallizer was developed to carry out the control and treatment experiments simultaneously, drawing shown in Figure 3.1. The components include two double jacketed tanks of 1000ml capacity each (Chemgalss Life Sciences LLC, 3800 N Mill Rd, Vineland, NJ 08360) connected to a recirculating chiller type (Thermoscientific Arctic, A25 model) programmable cooling tower, to run desired crystallization cooling rates. The tank closure lids and agitators with two sets of stirring arms and one set of bottom scarping arm were printed using 3D printing technology. Overhead stirrer (IKA RW 20 model) was connected to the agitator to stir concentrate permeate solutions in each tank. The final construction of the experimental set up during working conditions is shown in Figure 3.2.

**Lactose Crystallization**

After adjusting the total solids of concentrated permeate (CP) to 50-60%, it was heated to 80°C for few minutes to dissolve most of the lactose before transferring into the crystallizer tanks. Lactose crystallization was carried out by cooling the contents under constant agitation (120rpm) to a final temperature of 18°C. SSPS was added at 0.1% w/w of CP to the treatment tank and seed crystals (refined 40-60 mesh, supplied by Davisco Food International Inc., Eden Prairie, MN) were added at 0.027% (to both control and treatment crystallization tanks.

**Lactose crystal recovery**
Following crystallization, lactose slurry was washed by adding cold water (4 °C) at the rate of 15% w/w of tank contents and gentle mixing. The slurry was transferred into 250ml centrifuge bottles and centrifuged at 10,000 × g for 20 min at 4°C using Avanti J-E high capacity centrifuge (Beckman Coulter Inc.). After decanting the supernatant, the washing steps were repeated two more times by adding cold water at 1:1 ratio to the supernatant collected. The supernatant from all the washing steps was collected for SSPS analysis. Composite sample of washed crystals was dried at 87°C for 16h in forced draft oven (Isotemp Oven, Fisher Scientific, Pittsburg, PA) to determine moisture content. Lactose crystal recovery was calculated using the equation.

\[
\text{Lactose Crystal recovery (\%)} = \frac{\text{mass of lactose monohydrate recovered (g)}}{\% \text{ Lactose in CP} \times \text{mass of CP (g)}} \times 100 \quad [1]
\]

**Analysis**

Percentage lactose in concentrated permeates was determined using HPLC system (Beckman Coulter Inc., Fullerton, CA) equipped with a solvent delivery module (System Gold 125), a multichannel wavelength scanning detector (190–600 nm; System Gold 168 detector), and a 20-μL sample injection loop (Rheodyne, Rohnert Park, CA) as described by (Amamcharla and Metzger, 2011). Total solids percentage was determined using the oven drying method (AOAC International, 2002; method 990.20). Ash content was determined after ignition of sample at 550°C (AOAC International, 2002; method 954.46).

The supernatant collection was freeze dried using FreeZone 4.5L freeze dry system (Labconco Corporation, Kansas City, MO) to analyses the SSPS content. Supernatant can be preconcentrate using Hei-VAP Value laboratory vacuum evaporator (Heidolph Instruments GMbH &Co. KG) before freeze drying to reduce load and save time. The
dried supernatant was analyzed for SSPS using Total Dietary Fiber Assay Kit (K-TDFR) supplied by Megazyme International Ireland, based on AOAC Method 985.29.

**Lactose Crystal morphology**

Composite lactose crystal slurry samples were observed using a polarized light microscope (model BX-53, Olympus America Inc., Center Valley, PA) and the crystal images were captured with the camera (Olympus DP70) connected to the microscope. The length of crystals was measured using an image processor (Fuji ImageJ). The samples for capturing crystal images were prepared by placing the representative sample of crystal slurry at the center of the microscopic slide, followed by placing a chilled water drop and spreading the crystals uniformly on the slide. The crystal images on different spots of the slide were captured for image processing. The longest side of the crystal along the tomahawk shape measured (µm) was expressed as crystal size and used to calculate crystal size distribution (CSD).

**Differential scanning calorimetry (DSC)**

The DSC measurements were carried out using a model Q1000 (TA instrument, New Caste, DE). The calorimeter was calibrated with indium at an onset temperature of 150°C. The lactose crystal slurry was vacuum dried at 80°C and 25 mm Hg vacuum, and the dried lactose powder thus obtained was used for DSC measurements. The powder lactose samples (5±0.2 mg) were hermetically sealed in aluminum pans and analysis was carried out under a flow of dry nitrogen at a temperature scan rate of 5°C/min. The samples were scanned from temperatures 20 to 180°C. The scans for pure lactose (Davisco Foods International, Inc.), SSPS (Fuji Oil Co., Ltd., Oska, Japan), lactose
powders with and without SSPS were compared. The scans were processed and analyzed using Universal TA Instruments software V4.5A.

**Fourier Transform Infrared Spectroscopy (FTIR)**

The FTIR spectra of dried lactose crystals were obtained in the range of 400 to 4000 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\) using spectrometer (Cary 630, Agilent, Santa Clara, CA) with OMNIC software (OMNIC V9.2, Thermo Fischer Scientific Inc.). Absorbance mode was used for the analysis after subtracting the background spectra. Each spectrum was an average of 32 scans and all measurements were done in duplicate (Chandrapala et al., 2016). FTIR spectra of pure lactose (99.8% purity) obtained from Davisco Foods International, Inc, (Eden Prairie, MN), (Fuji Oil Co., Ltd., Oska, Japan), lactose powders with and without SSPS were also acquired and compared with the dried lactose crystals recovered with and without SSPS addition sources.

**Statistical Analysis**

Statistical analysis was performed using R program (version 3.4.1). Independent sample t-test to compare control and treatment group means differences at a significance level of 0.05.

**RESULTS AND DISCUSSION**

**SSPS effect on lactose crystal recovery and purity**

Lactose crystal slurry obtained at the end of the crystallization was corrected for the moisture content to enumerate the mass of \(\alpha\)-lactose monohydrate crystals recovered. The lactose recovery was calculated using Equation 1. The lactose recoveries without and with 0.1% SSPS were in the range of 70.52-72.42% and 75.04-77.19% respectively, as shown in Table 3.2. The crystallization promotion effect of SSPS at 0.1% level was
significant \((P < 0.05)\). Lactose exists into anomic forms referred as, \(\alpha\) - and \(\beta\)-lactose that are converted to each other by mutarotation. In unsaturated solution, lactose exists in soluble form surrounded by a hydration layer \((\text{Hartel, 2001})\). As the conditions of supersaturation such as concentration and cooling are manipulated, a series of transformations between \(\alpha\) - and \(\beta\)-lactose occur. The added SSPS consisting of hydroxyl groups may show a preferential hydration in CP and influence the degree of supersaturation conditions by reducing the solubility of lactose. The retention of part of the SSPS used in the treatment with the lactose crystals may explain the possible mechanism of SSPS servicing as the nucleation site and promoting the secondary nucleation.

The ash content in the washed crystal slurry was in the range of 1.26-1.38\% and 1.29-1.38\% for without and without 0.1\% SSPS addition respectively and presented in Table 3.2. There was no significant change \((P > 0.05)\) in the ash content observed with addition of SSPS. Higher ash content compared to that of commercial grade edible lactose was observed in both control and treatment lactose crystals, and this may be due to the washing protocol that was used during the experiment differ from industry washing procedures. Manual mixing of the crystals with wash water gently, may not be sufficient to remove enough minerals in supernatant during centrifugation process. Whereas aggressive mixing will result in broken lactose crystals and possible loss of fines with supernatant.

The differential dietary fiber content between control and treatment wash water solutions was enumerated to calculate the SSPS content that was left in lactose crystal
slurry. Out of the total SSPS added to the treatment solution, 79.82% was recovered into the wash water. This corresponds to 0.04% wt./wt. of SSPS in lactose crystals.

The DSC curves of α-lactose monohydrate, SSPS and the lactose crystals recovered with and without SSPS addition were shown in Figure 3.3. The amorphous form of lactose was identified by the presence of an exothermic peak at 167°C, which represents the transformation of amorphous to crystalline form (Gombás et al., 2002). The absence of this exothermic peak in the DSC curves of the lactose crystals recovered with and without SSPS suggests that there was no amorphous form of lactose present in these crystals. The endothermic peak in case of α-lactose monohydrate at around 145°C represents the loss of crystalline water. The corresponding endothermic peaks in case of recovered lactose crystals from permeate appeared at around 147-149°C. This shift could be due to the presence of impurities like minerals and proteins. The remained SSPS (0.04% wt./wt.) in the lactose crystals treated with SSPS did not significantly affect the temperature of loss of crystalline water. This further confirms that addition of 0.1% of SSPS did not significantly affect the purity of the lactose crystals recovered from the permeate.

The FTIR spectra of SSPS, lactose crystals with SSPS addition, pure lactose monohydrate, and lactose crystals without SSPS addition were presented in Figure 3.4. A specific peak at 1636 cm⁻¹ in the spectra of SSPS corresponds to the amide groups which was not present in the spectra of both control and treatment lactose crystals recovered. This further indicates that there was no significant SSPS present in the lactose crystals recovered with SSPS addition.

*Crystal size distribution (CSD)*
The mean crystal size was significantly (P < 0.05) higher in case of treatment (138.46 µm) compared to that of control (116.94 µm). The microscopic images of lactose crystals with and without SSPS were shown in Figure 3.5. As shown in the picture the relatively smaller crystals were observed in case of crystals recovered without SSPS compared to those of crystals recovered with the addition of SSPS. The preferential water binding capacity of added SSPS may have facilitated lactose-lactose interactions and promoted the growth of lactose crystals. Crystal size distribution of lactose, without and with SSPS was compared in Figure 3.6. The crystal size distribution of crystals with SSPS was observed to be narrower and more centered towards bigger crystal size compared to that of without SSPS, which followed a bimodal distribution. These observations further support the better recovery of the crystals with the addition of SSPS.

CONCLUSION

SSPS was observed to facilitate lactose crystal growth and promote recovery from concentrated permeate solution. Addition of SSSP was observed to have no significant difference in the purity of the lactose crystals recovered. From the findings of this study, we can conclude that it is feasible to use SSPS to influence lactose crystal growth and recovery during manufacture of lactose from concentrated permeated solutions.
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crystallization. Ultrasonics sonochemistry, 13(4), 359-363.


Singh, K., Gupta, S. P., Kumar, A., & Kumar, A. (2019). The effect of high intensity


**Figure 3.1.** Drawing of the scale down crystallizer
Figure 3.2. Lactose crystallization experimental set up
**Figure 3.3.** DSC curves of Alpha lactose monohydrate, SSPS, Lactose crystals with and without SSPS
**Figure 3.4.** FTIR spectra of SSPS, lactose crystals with SSPS addition, pure lactose monohydrate, and lactose crystals without SSPS addition
Figure 3.5. Typical microscopic images of lactose crystals without and with 0.1% SSPS from left to right.
Figure 3.6. Crystal size distribution of lactose recovered without and with 0.1% SSPS addition.
### TABLES

**Table 3.1.** The effect of presence of different minerals on the lactose crystallization kinetics

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Impact</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl₂</td>
<td>Low levels promote growth, high levels inhibit the growth</td>
<td>Jelen and Coulter, 1973; Guu and Zall, 1991; Bhargava and Jelen, 1996</td>
</tr>
<tr>
<td>CaCl₂ under seeded conditions</td>
<td>No effect</td>
<td>Smart et al., 19192</td>
</tr>
<tr>
<td>NaH₂PO₄</td>
<td>Promotes the growth</td>
<td>Jelen and Coulter, 1973b; Guu and Zall, 1991</td>
</tr>
<tr>
<td>LiCl</td>
<td>Increases growth rate</td>
<td>Bhargava and Jelen, 1996</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>Increases growth rate</td>
<td>Bhargava and Jelen, 1996</td>
</tr>
<tr>
<td>Ca lactate</td>
<td>Inhibits growth rate</td>
<td>Bhargava and Jelen, 1996</td>
</tr>
<tr>
<td>KCl</td>
<td>Low levels promote growth, high levels inhibit the growth</td>
<td>Jelen and Coulter, 1973</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>Decreases the growth rate</td>
<td>Bhargava and Jelen, 1996</td>
</tr>
<tr>
<td>K⁺, Na⁺, Ca²⁺, PO₄³⁻</td>
<td>Inhibits nucleation and crystallization</td>
<td>Guu and Zall, 1991</td>
</tr>
<tr>
<td>Combination of K⁺, Ca²⁺, PO₄³⁻</td>
<td>Decreases the yield</td>
<td>Guu and Zall, 1991</td>
</tr>
<tr>
<td>Interaction of K⁺ and PO₄³⁻</td>
<td>Decreases the yield</td>
<td>Guu and Zall, 1991</td>
</tr>
</tbody>
</table>
**Table 3.2.** Comparison of experimental recovery of lactose, mean (n=3) results with and without 0.1% SSPS

<table>
<thead>
<tr>
<th></th>
<th>Lactose Crystal Yield (%)</th>
<th>Ash (%)</th>
<th>SSPS retained in crystals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Range</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Without SSPS</td>
<td>71.33±0.98a</td>
<td>70.52-72.42</td>
<td>1.33±0.06a</td>
</tr>
<tr>
<td>With 0.1% SSPS</td>
<td>76.31±1.13b</td>
<td>75.04-77.19</td>
<td>1.35±0.05*</td>
</tr>
</tbody>
</table>

SD is standard deviation

Mean values with different superscript were significantly different (P < 0.05)
CHAPTER IV

Evaluate commercial feasibility of using SSPS to improve the drying ability and powder characteristics of Greek yogurt whey

The composition of Greek yogurt acid whey (GAW) is considerably different from that of cheese whey making it difficult to process into powder ingredients. The high lactic acid and mineral content in GAW delays lactose crystallization which causes stickiness during spray drying and caking during storage of the powder. Previous studies have shown that soluble soybean polysaccharide (SSPS) can enhance lactose crystallization in aqueous lactose solutions as well in concentrated permeate. However, the effect of SSPS on the crystallization of lactose in concentrated GAW has not been evaluated. The objective of this study was to determine the feasibility of using SSPS to improve lactose crystallinity and drying characteristics of GAW powder. GAW obtained from a Greek yogurt was vacuum concentrated to 56% total solids (30% Lactose) and crystallized using a laboratory scale crystallization tank. During crystallization, the concentrated GAW at 70°C was fast cooled to 30°C followed by slow cooling to 18°C (rate, -0.05°C /min) under constant stirring. Both the control and treatment solutions were seeded with lactose crystals (0.027g/100g of solution) and 0.1 % SSPS was added to the treatment solution. After crystallization, the concentrate was spray dried using a pilot scale NIRO dryer. GAW powder yield with 0.1% SSPS addition was observed to be less sticky on the dryer surface. The crystallized lactose content in treatment (77.46%) was also significantly higher than control (66.56%). The hygroscopicity at 75% relative humidity properties of treated GAW powder (10.21%) were significantly (P < 0.05) better than the control (15.87%). However, the glass transition temperature of the control (51.40±0.20°C) and
treatment (54.76±0.73°C) powders were not significantly different (P > 0.05). The findings of this study indicate that SSPS can enhance lactose crystallization in concentrated GAW during crystallization, reduce the sticking of the powder in the dryer and improve the drying characteristics such as hygroscopicity and caking of the GAW powder.

**Key words:** Greek yogurt acid whey, SSPS, lactose crystallization, spray drying
INTRODUCTION

In general, based on the production parameters whey is categorized into two basic groups - sweet whey, generated during manufacturing of rennet type of hard cheeses, and acid whey, obtained during production acid coagulated dairy products as soft cheese or strained yogurt (Schmidt et al., 1984). Increased consumer demand for high protein dairy products such as Greek yogurt whey lead to the generation of large quantities of acid whey (GAW) creating utilization challenges for dairy industry. In comparison to sweet whey, GAW is characterized by lower pH, lower protein, and lactose contents, but contains higher minerals, particularly soluble calcium, and higher lactic acid (LA). These compositional differences make it difficult to process GAW using the known sweet whey processing technologies (Chandrapala and Vasilijevic, 2018). Spry drying is one of the most convenient technology for processing whey powders. The challenges with using the existing sweet whey processing techniques for processing GAW are summarized below:

- High mineral content limits the performance of evaporators due to increased fouling (Bédas et al., 2017).
- The presence of high lactic acid and calcium influence the lactose crystallization in concentrated GAW during cooling step (Chandrapala et al., 2016). Lactic acid was reported as the inhibitor of the lactose crystallization (Jelen and Coulter, 1973a; Jelen and Coulter, 1973b; Saffari and Langrish, 2014). Because of insufficient lactose crystallization, most of the lactose remains in amorphous form leading to sticking and caking of powder during spray drying leading to minimal yields. The amorphous lactose in the powder continues to cause lumping and caking during storage of GAW powder.
• Additionally, the presence of higher monosaccharide content for example galactose interferes with evaporative concentration, lactose crystallization and spray drying.

During spray drying of sugar rich fruit and vegetable juices, due to the low glass transition temperatures of these sugars, these sugars get sticky leading to deposition onto the drying chamber resulting lower yields (Bhandari et al., 19197). It is common to add some carrier material while spray drying sugar rich fruit and vegetable juices (Barbosa et al., 2015). Generally, these carrier materials are used to increase the glass transition temperatures and reduce the stickiness of the sugars. The most used carrier materials are maltodextrin and gum Arabic. However, recent studies have shown proteins such as whey protein isolate, sodium caseinate, and soy protein isolate and low molecular weight surfactants are efficient to increase product yield even in lower concentration (Jayasundera et al., 2011).

Soluble soybean polysaccharide (SSPS) is a dietary fiber extracted from “Okara”, the byproduct of soya protein production. It is characterized as a branched chain polymer with a small amount of protein (Chivero et al., 2014). Shi and Zhong, 2014, studied the effect of addition of SSPS to lactose solution by evaluating the crystallinity and powder quality. They reported an improved degree of crystallinity and delayed the crystallization of amorphous lactose during storage of the powder. The aim of this study is to evaluate the feasibility of addition of SSPS for improving the drying characteristics of GAW.

**MATERIALS AND METHODS**

*Experimental Design*
The effect of SSPS on drying characteristics of GAW was studied at 0.1% addition level. The control (without SSPS) and treatment (with 0.1% SSPS) GAW concentrate were crystallized before spray drying and compared for drying and powder characteristics. Experiments were conducted in triplicates studies.

**Greek yogurt whey (GAW) and Soluble Soybean Polysaccharide (SSPS)**

GAW from three different batches were obtained from a Greek yogurt manufacturer. The GAW was obtained in a frozen and concentrated form (16.02 ± 0.17 % TS). The product was kept in the freezer in small lots until it was used for experiments. The required amount of frozen GAW was thawed and the total solids content was adjusted approximately to 55% using Hei-VAP Value laboratory vacuum evaporator (Heidolph Instruments GMbH &Co. KG) at 70±2°C before crystallization. Fuji Oil Corp. (Osaka, Japan) gifted the SSPS used in the experiments.

**Experimental Setup**

A scaled down version of industry crystallizer was developed to carry out the control and treatment experiments simultaneously, drawing shown in Figure 3.1. The components include two double jacketed tanks of 1000ml capacity each (Chemgalss Life Sciences LLC, 3800 N Mill Rd, Vineland, NJ 08360) connected to a recirculating chiller type (Thermoscientific Arctic, A25 model) programmable cooling tower, to run desired crystallization cooling rates. The tank closure lids and agitators with two sets of stirring arms and one set of bottom scrapping arm were printed using 3D printing technology. Overhead stirrer (IKA RW 20 model) was connected to the agitator to stir concentrate permeate solutions in each tank. The final construction of the experimental set up during working conditions is shown in Figure 3.2.
**GAW - Lactose Crystallization**

After adjusting the total solids of GAW to 55%, the pH of the solution was adjusted to 6.25 using edible grade NaOH, followed by heating to 80°C for few minutes to dissolve most of the lactose before transferring into the crystallizer tanks. Lactose crystallization was carried out in two step cooling process. First, the concentrated GAW was fast cooled to 30 °C, followed by a slow cooling (rate, -0.05°C /min) under constant agitation (120rpm) to a final temperature of 18°C. The SSPS was added at 0.1% wt./wt. of concentrated GAW to the treatment tank and seed crystals (refined 40-60 mesh, supplied by Davisco Food International Inc., Eden Prairie, MN) were added at 0.027% (to both control and treatment crystallization tanks.

**GAW spray drying**

Following crystallization, the concentrated GAW was spray dried using a pilot scale NIRO dryer with a two-fluid internal nozzle spray system. In this system, the feed fed at pressure mixed externally with compressed air to produce a completely automated spray. The drying operating conditions are described below.

- Feed rate and temperatures are 60 ml/min and 19±1°C, respectively
- Air pressure and feed pressures were 60 and 25±2psi, respectively
- The inlet and outlet temperatures of the spray dryer were 190±5°C and 90±5°C, respectively

GAW powder was collected from both the collection vessel and the cyclone, and the yield was calculated using the below formula.

\[
\text{GAW yield (\%)} = \frac{\text{mass of GAW concentrate (g)} \times \% \text{TS of GAW concentrate}}{\% \text{TS of GAW powder} \times \text{mass of GAW powder (g)}} \times 100
\]
Analysis

The lactose in concentrated GAW was determined using HPLC system (Beckman Coulter Inc., Fullerton, CA) equipped with a solvent delivery module (System Gold 125), a multichannel wavelength scanning detector (190–600 nm; System Gold 168 detector), and a 20-μL sample injection loop (Rheodyne, Rohnert Park, CA) as described by (Amamcharla and Metzger, 2011). Total solids percentage was determined using the oven drying method (AOAC International, 2002; method 990.20). Ash content was determined after ignition of sample at 550°C (AOAC International, 2002; method 954.46).

GAW powder characterization

Microstructure

The SE micrographs of the GAW powder samples were obtained using the Hitachi 3400N VP-SEM scanning electron microscope (Hitachi Science Systems Ltd., Tokyo, Japan). A small amount of powder sample was scattered onto a carbon tape, which was mounted on Al stub. Excess material was removed using air, and the sample was coated with a thin gold film for 3 min using a sputter coater. The imaging was conducted using a S-4800 (Hitachi Science Systems Ltd., Tokyo, Japan) and examined by a secondary electron detector operating at 10 kV.

Morphological analysis

Morphological characteristics of GAW powders were analyzed by Malvern Morphologi G3ID (Malvern Instruments, Worcestershire, UK). The circle equivalent diameter (CED), high sensitivity circularity (HSC), elongation, and convexity were calculated from the 2-dimensional images. Circularity describes the particle closeness to
a perfect circle and the values range from 0 to 1. Whereas convexity is a measure of the surface roughness of a particle (range 0 to 1). A convexity of 1 represents a smooth particle, and an irregularly shaped particle or very spiky has a convexity value closer to 0. Circle or square has an elongation value of 0, whereas shapes with large aspect ratios have an elongation closer to 1 (Li et al., 2016). The measures for each GAW sample were carried out in triplicate, and the mean values were calculated.

**Hygroscopicity properties of the powder**

The hygroscopicity of the GAW powder samples was determined by exposing the measured quantity of the sample to an environment of known relative humidity (RH) until equilibrium has been reached. Saturated salt solutions of NaCl and KCl were used to generate environments of 75 and 85% RH. The moisture gain by the unit sample was calculated and expressed as the % hygroscopicity. The classification of the powders as a function of hygroscopicity at 75% RH is shown in Table 4.2. Generally, the powder with a % hygroscopicity value ≤ 10.0 is considered as non-hygroscopic and those with ≥ 25.0 % hygroscopicity value are considered as extremely hygroscopic (Schuck et al., 2004).

**Differential scanning calorimetry (DSC)**

The DSC measurements were carried out using a model Q1000 (TA instrument, New Caste, DE). The calorimeter was calibrated with indium at an onset temperature of 150°C. The lactose crystal slurry was vacuum dried at 80°C and 25 mm Hg vacuum, and the dried lactose powder thus obtained was used for DSC measurements. The powder lactose samples (5±0.2 mg) were hermetically sealed in aluminum pans and analysis was carried out under a flow of dry nitrogen at a temperature scan rate of 5°C/min. The samples were scanned from temperatures 20 to 180°C. The scans for pure lactose
(Davisco Foods International, Inc.), SSPS (Fuji Oil Co., Ltd., Oska, Japan), lactose powders with and without SSPS were compared. The scans were processed and analyzed using Universal TA Instruments software V4.5A.

**Rate of lactose crystallization**

To determine the content of crystallized lactose ($\dot{C}$), first the total moisture content (TM) and free moisture (FM) of the GAW powders were determined and the bound moisture (BM) of the GAW powders were calculated using the following formula.

$$BM = TM - FM$$

FM of the GAW powders were measured by evaporating water from the test sample in the presence of sand after a period of 5 h in an oven at a temperature of $102 \pm 2^\circ C$ (Schuck et al., 2012). Whereas total moisture (TM) was determined by evaporation of total water from a sample in the presence of sand and in a vacuum after a period of 7 h in an oven at a temperature of $102 \pm 2^\circ C$ (Schuck and Dolivet, 2002).

The rate of lactose crystallization is the concentration of crystallize lactose expressed as a percentage of the overall lactose content of the powder. This is because lactose in concentrated milk products can crystallize with one molecule of water in the form of a $\alpha$-monohydrate. Measurement of bound water in GAW powders can give a good indication of the degree of lactose crystallization. The content of crystallized lactose ($\dot{C}$), expressed as % of lactose, is equal to:

$$\dot{C} = \frac{BM \times 19}{L} \times 100$$

Where $L$ is the % concentration of lactose in powder samples. The 19 factor represents the ratio between mass of lactose ($342 \text{ g. mol}^{-1}$) and water ($18 \text{ g. mol}^{-1}$). In complex
mixtures like GAW the bound water is not only bound to α-lactose monohydrate but also to soluble proteins and minerals. Hence, the formula here is used for relative comparison of the content of crystallized lactose in control and GAW powders.

**Statistical Analysis**

Statistical analysis was performed using Minitab® 19 Statistical Software (Minitab LLC, PA). The response means were compared using Tukey’s multiple comparison test at a significance $P$-value of 0.05.

**RESULTS AND DISCUSSION**

**Composition of preconcentrated GAW from factory**

The chemical composition of the pre concentrated GAW obtained from the factory is shown in Table 4.1. The analytical results of GAW from factory show that, GAW can be characterized with low pH, high titratable acidity, low lactose, high lactic acid, low protein compared to sweet whey and like other acid whey produced from cream cheese or cottage cheese (Jelen, 2003).

**Effect of SSPS on the yield of GAW powder**

The total, free, bound moisture contents, and the yields of the spray dried powders, with and without SSPS, were shown in Table 4.3. There was no significant difference ($P > 0.05$) in the total moisture and free moisture contents between GAW powders with and without SSPS addition. However, the bound moisture in GAW powder with SSPS is significantly ($P < 0.05$) higher than GAW powder without SSPS addition. This difference corresponds to an increased degree of lactose crystallization in case of GAW powder with SSPS, which is discussed in further section. The yield of GAW powder with SSPS (52%) was observed to be significantly higher compared to that of
GAW powder without SSPS (45%). The yield of GAW without SSPS were closer to the yield (44%) of another type of acid whey, cream cheese whey without any treatment (Chandrapala and Vasiligevic, 2017). As shown in Figure 4.1, the GAW powder without SSPS addition was sticky and lot of powder deposition was observed in the cyclone and was difficult to recover, whereas the power of GAW with SSPS addition was less sticky and easy to recover from the cyclone and the collection chamber.

The rate of crystallization described as the amount of lactose present in crystalline form out of total lactose in the concentrate before spray drying is an important parameter determining the yield and powder characteristics of lactose rich dairy powders. The rate of crystallization of GAW powders with SSPS addition (81.46±2.0) was observed to be significantly (P < 0.05) higher than GAW powders without SSPS (74.56±1.60). The rate of crystallization obtained in case of GAW powders without SSPS was observed slightly higher than that reported by Bedas et al., (2017). This could be due to the differences in the cooling rates and final crystallization temperatures.

**Effect of SSPS on GAW powder characteristics**

The SEM images of spray dried GAW powder samples were shown in Figure 4.2. The GAW powder particles had a greater presence of agglomerates than individual particles compared to that of GAW powder with SSPS addition. This suggests the presence of more amorphous lactose in GAW powders without SSPS, which is hygroscopic and affects the stickiness (Ross and Karel, 1992). The amorphous lactose present tend to crystallize during storage of the powders and decreases the shelf life of the powders by the formation of lumps (Aguilera et al., 1995). Also, the presence of
smooth surfaces indicates the presence of amorphous lactose (Cano-Chauca et al., 2005) which were noticeable with the GAW powders without SSPS.

The particle morphology analysis results indicated that there is no significant (P >0.05) difference between circulatory characteristics of the powder particles of GAW with and without SSPS addition. Same with the case of convexity characteristics of the powders. This suggests that addition of 0.1% SSPS does not have an impact on particle morphological characteristics.

The hygroscopicity values of GAW powders were shown in Table 4.4. The GAW powder with SSPS addition falls under slightly hygroscopic powders category whereas the powders without SSPS can be classified as hygroscopic powders (Schuck, 2004). The % hygroscopicity values of GAW with SSPS were significantly (P < 0.05) lower than those of GAW at both 75 and 85% RH conditions.

Differential scanning calorimetry (DSC) technique was used to observe the phase transitions such as crystallization. A clear exothermic peak around 150-170°C was associated with crystallization of lactose and the area under the peak was designated as the degree of amorphicity as described by Saffari and Langrish (2014). The DSC scans of GAW powders were presented in Figure 4.3. The temperature of glass transition (T_g) did not change significantly with the addition of SSPS. However, a wider exothermic peak in case of GAW powders without SSPS indicates presence of more amorphous lactose content in it compared to the GAW powders with SSPS.

CONCLUSION

The results from this study clearly showed that feasibility of SSPS addition to improve the drying characters of GAW. The addition of 0.1% SSPS significantly
improved the yield of GAW and the powder characteristics like degree of lactose

 crystallization, and hygroscopicity.
REFERENCES


Chandrapala, J., Wijayasinghe, R., & Vasiljevic, T. (2016). Lactose crystallization as affected by presence of lactic acid and calcium in model lactose systems. Journal of
Food Engineering, 178, 181-189.


Saffari, M., & Langrish, T. (2014). Effect of lactic acid in-process crystallization of


Figure 4.1. Top left to right: the GAW-SSPS powder deposition in the chamber and cyclone; Bottom left to right: GAW powder deposition in the chamber and cyclone.
Figure 4.2. SEM micrographs of spray dried GAW powder without and with SSPS, from left to right.
**Figure 4.3.** DSC scans of GAW powders

GAW = The powder obtained by spray drying of Greek acid whey

GAW-SSPS = The powder obtained by spray drying of Greek acid whey with SSPS addition
**TABLE 4.1.** Mean (n=3) composition of three different lots of preconcentrated Greek yogurt whey (GAW) from factory

<table>
<thead>
<tr>
<th>Item</th>
<th>Unit</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids</td>
<td>%</td>
<td>16.98 ± 0.28</td>
</tr>
<tr>
<td>Lactose</td>
<td>%</td>
<td>8.68 ± 0.06</td>
</tr>
<tr>
<td>Ash</td>
<td>%</td>
<td>1.96 ± 0.02</td>
</tr>
<tr>
<td>Protein</td>
<td>%</td>
<td>0.69 ± 0.02</td>
</tr>
<tr>
<td>Fat</td>
<td>%</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>%</td>
<td>1.82 ± 0.03</td>
</tr>
<tr>
<td>Titratable Acidity</td>
<td>%</td>
<td>1.38 ± 0.03</td>
</tr>
</tbody>
</table>
Table 4.2. Powder classification as a function of hygroscopicity at 75% RH (adapted from Schuck et al., 2004)

<table>
<thead>
<tr>
<th>Class</th>
<th>Hygroscopicity (%) at 75% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-hygroscopic powder</td>
<td>≤10.0</td>
</tr>
<tr>
<td>Slightly hygroscopic powder</td>
<td>10.1-15.0</td>
</tr>
<tr>
<td>Hygroscopic powder</td>
<td>15.1-20.0</td>
</tr>
<tr>
<td>Very hygroscopic powder</td>
<td>20.1-25.0</td>
</tr>
<tr>
<td>Extremely hygroscopic powder</td>
<td>≥25.0</td>
</tr>
</tbody>
</table>
Table 4.3. Mean responses (n=3) of total moisture, free moisture, bound moisture, and yield of GAW powders with and without SSPS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>(^1)GAW</th>
<th>(^2)GAW-SSPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total moisture</td>
<td>%</td>
<td>6.49 ± 0.16(^a)</td>
<td>6.69 ± 0.05(^a)</td>
</tr>
<tr>
<td>Free moisture</td>
<td>%</td>
<td>4.47 ± 0.11(^a)</td>
<td>4.40 ± 0.10(^a)</td>
</tr>
<tr>
<td>Bound moisture</td>
<td>%</td>
<td>2.02 ± 0.08(^a)</td>
<td>2.29 ± 0.08(^b)</td>
</tr>
<tr>
<td>Yield</td>
<td>%</td>
<td>44.51 ± 1.37(^a)</td>
<td>51.88 ± 1.64(^b)</td>
</tr>
</tbody>
</table>

\(^1\)GAW = spray dried powder obtained from Greek yogurt whey

\(^2\)GAW-SSPS = spray dried powder obtained from Greek yogurt whey with the addition of SSPS

\(^a\)-\(^b\) Mean responses in the same row with different superscripts were significantly different (P < 0.5)
Table 4.4. Mean responses (n=3) of % hygroscopicity of GAW powders with and without SSPS at 75% and 85 % RH

<table>
<thead>
<tr>
<th>Sample</th>
<th>Unit</th>
<th>Hygroscopicity at 75 % RH</th>
<th>Hygroscopicity at 85 % RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAW¹</td>
<td>%</td>
<td>15.87 ± 0.74ᵃ</td>
<td>18.15 ± 1.02ᵃ</td>
</tr>
<tr>
<td>GAW-SSPS²</td>
<td>%</td>
<td>10.21 ± 0.09ᵇ</td>
<td>12.09 ± 0.37ᵇ</td>
</tr>
</tbody>
</table>

¹GAW = spray dried powder obtained from Greek yogurt whey

²GAW-SSPS = spray dried powder obtained from Greek yogurt whey with the addition of SSPS

ᵃᵇMean responses in the same column with different superscripts were significantly different (P < 0.5)
CHAPTER V

Evaluate the feasibility of partial demineralization and deacidification of GAW by nanofiltration (NF) to improve the drying ability and powder characteristics

ABSTRACT

Greek yogurt acid whey (GAW) contains high concentrations of lactic acid (LA) and minerals as compared to cheese whey. LA and the minerals, particularly the calcium (Ca) in GAW cause stickiness during spray drying, thus limiting the processing capability and utilization of GAW. Nanofiltration (NF) has been in use for partial removal of minerals from cheese whey and milk to produce high value-added dairy ingredients. Similarly, NF can potentially be applied for partial demineralization and deacidification of GAW to improve spray drying and powder properties. The aim of this study was to evaluate the effectiveness of NF to remove minerals and LA from GAW for improving the spray drying ability of GAW and thus the drying characteristics of the powder. GAW (5.52±0.2% total solids) obtained from a Greek yogurt manufacturer was pasteurized at 72°C for 15 seconds followed by cooling to 4°C before concentrating to 22.35±0.8% using semi-industrial scale nanofiltration (Molecular Weight Cutoff – 300 to 500Da) plant. GAW feed, NF retentate and NF permeate compositions in terms of total protein nitrogen, non-protein nitrogen, total ash, minerals (sodium, potassium, and calcium) and Lactic acid were analyzed. The corresponding percent reduction of each component was calculated and expressed on a dry matter basis. The lactic acid and total ash concentrations were reduced significantly (P < 0.05) by 34.3±0.2 and 37.8±0.7, respectively. The reduction of monovalent ions, i.e., sodium and potassium, were observed to be higher (66% and 62%) than calcium (41%). There was no significant
difference (P > 0.05) in the total protein content and pH of GAW feed (4.13±0.2, 4.44) and NF GAW retentate (3.95±0.2, 4.38). The yield of GAW-NFR (56%) was observed to be significantly higher compared to that of GAW (45%). The results show that it is feasible to reduce the mineral content and lactic acid in GAW by approximately 38% and 34% respectively using NF.

**Key words:** Greek yogurt acid whey, nanofiltration, demineralization, deacidification, GAW spray drying
INTRODUCTION

Nanofiltration (NF) is a membrane filtration technology between ultrafiltration (UF) and reverse osmosis (RO). Hence, NF membrane process of whey and milk permeate, retains lactose and other high molecular weight components greater than around 200 Daltons. But it permeates low molecular weight components and ions. The permeated components of whey and milk permeate include organic components like, glucose, galactose, amino acids, and lactic acid and monovalent ions, particularly sodium, chloride. The multivalent salts, such as calcium, magnesium, and phosphate are partially removed based on the chemical nature, electric charge, and the hydrated state of these salts.

The current applications of nanofiltration in dairy processing are, removing water and salts from whole milk (Matsui et al., 2006), whey (Gernigon et al., 2011) and whey permeate (Cuartas-Uribe et al., 2009). Nanofiltration can achieve only 30% demineralization, with 70% reduction in monovalent ions (Greiter et al., 2002), whereas Electrodialysis can achieve up to 90% of desalination (Hoppe and Higgins, 19192; Kelly et al., 1991). But NF has the advantage of simultaneous concentration affect by a factor of 3 to 4 times (Kelly et al., 1991) which helps in upstream evaporation concentration process of milk and whey by reducing the scaling (Kentish and Rice, 2015). Another major advantage is that NF is more selective towards removal of the monovalent sodium ions, while the more nutritionally relevant divalent ions, calcium, zinc, and magnesium, can be retained (Rice et al., 2005; Van der Bruggen et al., 2004) which means calcium and magnesium fortified dairy ingredients can be prepared using this process. The dairy
minerals from the NF retentate can be recovered by precipitating the retentate either by heat or pH adjustment using chemicals or both (Vembu and Rathinam, 1997).

As discussed in chapter 4, acid whey, generated from cottage cheese or cream cheese production is very different in composition compared to that of sweet whey, shown in Table 5.1. Similarly, Greek yogurt whey (GAW) is another type of acid whey with composition like that of whey from cottage or cream cheese production. Due to the high concentration of lactic acid and the low pH, spray drying of GAW is not easy because the powder sticks to the dryer and cyclone walls (Bhandari, 2011; Salamesh and Taylor, 2006). In addition to the high lactic acidity, the high mineral content of GAW interferes with the industrial concentration due to the scaling of the evaporator. The high mineral and lactic acid content also affect the kinetics of lactose crystallization during the cooling stage of GAW powder manufacturing.

An earlier study reported that the reduction of lactic acid and calcium in lactose model systems helped in improving the crystallization properties of lactose, resulting in an enhanced yield of spray dried powders (Chandrapala and Vasilijevic, 2017). In another study nanofiltration was utilized to reduce the mineral content and lactic acid in acid whey by 37% and 32%, respectively, and reported improved drying characteristics of the powders thus obtained (Bedas et al., 2017). Thus, the aim of the current study was to utilize nanofiltration technology to partially demineralize and deacidify the GAW and evaluate the feasibility of this technology to improve the drying ability of GAW and the characteristics of the powder.

MATERIALS AND METHODS

**Experimental Design**
The effect of partial demineralization and deacidification of GAW on the drying characteristics and yields of GAW powder was studied. The control (no nanofiltration treatment) and treatment (nanofiltration) GAW concentrate were crystallized before spray drying and compared for drying and powder characteristics. Experiments were conducted in triplicates studies. The schematic diagram of the experimental steps is shown in Figure 5.1.

**Greek yogurt whey (GAW)**

GAW from three different batches were obtained from a Greek yogurt manufacturer. The GAW was obtained in a frozen and concentrated form (16.02 ± 0.17 % TS). The product was kept in the freezer in small lots until it was used for experiments. For control GAW drying experiments, the preconcentrated GAW from the factory is further concentrated to approximately to 60.35±1.36% using Hei-VAP Value laboratory vacuum evaporator (Heidolph Instruments GmbH &Co. KG) at 70±2°C before crystallization.

**Nanofiltration (NF)**

A semi commercial experiments were conducted using the pilot NF unit shown in Figure 5.2. Polymeric spiral wound membrane of 300-500 Dalton molecular weight cut-off (NFW-3B-3838, Synder filtration, Vacaville, CA 95688) was used for filtration. The element was 3.8mm in diameter and 380mm long, with a total surface area of 6.69 m². The base line pressure of 450 kPa maintained throughout the filtration process. The preconcentrated GAW obtained from the factory was diluted back to 5.52±0.15% total solids and fed to the filtration unit. The feed was concentrated to a total solid content of 22.35±0.78% in the nanofiltration retentate of Greek yogurt whey (GAW-NFR).

**Crystallization Setup**
A scaled down version of industry crystallizer was developed to carry out the control and treatment experiments simultaneously, drawing shown in Figure 3.1. The components include two double jacketed tanks of 1000ml capacity each (Chemgalss Life Sciences LLC, 3800 N Mill Rd, Vineland, NJ 08360) connected to a recirculating chiller type (Thermoscientific Arctic, A25 model) programmable cooling tower, to run desired crystallization cooling rates. The tank closure lids and agitators with two sets of stirring arms and one set of bottom scrapping arm were printed using 3D printing technology. Overhead stirrer (IKA RW 20 model) was connected to the agitator to stir concentrate permeate solutions in each tank. The final construction of the experimental set up during working conditions is shown in Figure 3.2.

**GAW - Lactose Crystallization**

For control experiment, after adjusting the total solids of GAW to 60.34±0.78% TS using vacuum concentration, the pH of the solution was adjusted to 6.25 using edible grade NaOH, followed by heating to 80ºC for few minutes to dissolve most of the lactose before transferring into the crystallizer tanks. Whereas in case of treatment the GAW-NFR was further vacuum concentrated to 60.63±0.97% TS using vacuum concentration, the GAW-NFR concentrate was heated to 80ºC for few minutes to dissolve most of the lactose before transferring into the crystallizer tanks. Lactose crystallization was carried out in two step cooling process. First, the concentrated GAW was fast cooled to 30ºC, followed by a slow cooling (rate, -0.05°C/min) under constant agitation (120rpm) to a final temperature of 18ºC. The crystallization of both GAW and GAW-NFR concentrates was carried out under seeded conditions by adding seed crystals (refined 40-60 mesh, supplied by Davisco Food International Inc., Eden Prairie, MN) at 0.027%.
**GAW spray drying**

Following crystallization, the concentrated GAW was spray dried using a pilot scale NIRO dryer with a two-fluid internal nozzle spray system. In this system, the feed fed at pressure mixed externally with compressed air to produce a completely automated spray. The drying operating conditions are described below.

- Feed rate and temperatures are 60 ml/min and 19±1°C, respectively
- Air pressure and feed pressures were 60 and 25±2 psi, respectively
- The inlet and outlet temperatures of the spray dryer were 190±5°C and 90±5°C, respectively

GAW powder was collected from both the collection vessel and the cyclone, and the yield was calculated using the below formula.

\[
GAW \text{ yield (\%)} = \frac{\text{mass of GAW concentrate (g) } \times \% \text{ TS of GAW concentrate}}{\% \text{ TS of GAW powder } \times \text{ mass of GAW powder (g)}} \times 100
\]

**Analysis**

The lactose in concentrated GAW was determined using HPLC system (Beckman Coulter Inc., Fullerton, CA) equipped with a solvent delivery module (System Gold 125), a multichannel wavelength scanning detector (190–600 nm; System Gold 168 detector), and a 20-μL sample injection loop (Rheodyne, Rohnert Park, CA) as described by (Amamcharla and Metzger, 2011). Total solids percentage was determined using the oven drying method (AOAC International, 2002; method 990.20). Ash content was determined after ignition of sample at 550°C (AOAC International, 2002; method 954.46).

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The SE micrographs of the GAW powder samples were obtained using the Hitachi 3400N VP-SEM scanning electron microscope (Hitachi Science Systems Ltd., Tokyo, Japan). A small amount of powder sample was scattered onto a carbon tape, which was mounted on aluminum stub. Excess material was removed using air, and the sample was coated with a thin gold film for 3 min using a sputter coater. The imaging was conducted using a S-4800 (Hitachi Science Systems Ltd., Tokyo, Japan) and examined by a secondary electron detector operating at 10 kV.

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The hygroscopicity of the GAW powder samples was determined by exposing the measured quantity of the sample to an environment of known relative humidity (RH) until equilibrium has been reached. Saturated salt solutions of NaCl and KCl were used to
generate environments of 75 and 85% RH. The moisture gain by the unit sample is calculated and expressed as the % hygroscopicity. The classification of the powders as a function of hygroscopicity at 75% RH is shown in Table 4.2. Generally, the powder with a % hygroscopicity value ≤ 10.0 is considered as non-hygroscopic and those with ≥ 25.0 % hygroscopicity value are considered as extremely hygroscopic (Schuck et al., 2004).

Differential scanning calorimetry (DSC)

The DSC measurements were carried out using a model Q1000 (TA instrument, New Caste, DE). The calorimeter was calibrated with indium at on onset temperature of 150°C. The lactose crystal slurry was vacuum dried at 80°C and 25 mm Hg vacuum and the dried lactose powder thus obtained was used for DSC measurements. The powder lactose samples (5±0.2 mg) were hermetically sealed in aluminum pans and analysis was carried out under a flow of dry nitrogen at a temperature scan rate of 5°C/min. The samples were scanned from temperatures 20 to 180°C. The scans for pure lactose (Davisco Foods International, Inc.), SSPS (Fuji Oil Co., Ltd., Oska, Japan), lactose powders with and without SSPS were compared. The scans were processed and analyzed using Universal TA Instruments software V4.5A.

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To determine the content of crystallized lactose (Ĉ), first the total moisture content (TM) and free moisture (FM) of the GAW powders were determined and the bound moisture (BM) of the GAW powders were calculated using the following formula.

\[ BM = TM - M \]

FM of the GAW powders were measured by evaporating water from the test sample in the presence of sand after a period of 5 h in an over at a temperature of 102 ± 2°C
(Schuck et al., 2012). Whereas total moisture (TM) was determined by evaporation of total water from a sample in the presence of sand and in a vacuum after a period of 7 h in an oven at a temperature of 102 ± 2°C (Schuck and Dolivet, 2002).

The rate of lactose crystallization is the concentration of crystallize lactose expressed as a percentage of the overall lactose content of the powder. This is because lactose in concentrated milk products can crystallize with one molecule of water in the form of a α-monohydrate. Measurement of bound water in GAW powders can give a good indication of the degree of lactose crystallization. The content of crystallized lactose ($\hat{C}$), expressed as % of lactose, is equal to:

$$\hat{C} = \frac{BM \times 19}{L} \times 100$$

Where $L$ is the % concentration of lactose in powder samples. The 19 factor represents the ratio between mass of lactose (342 g. mol$^{-1}$) and water (18 g. mol$^{-1}$). In complex mixtures like GAW the bound water is not only bound to α-lactose monohydrate but also to soluble proteins and minerals. Hence, the formula here is used for relative comparison of the content of crystallized lactose in control and GAW powders.

**Statistical Analysis**

Statistical analysis was performed using Minitab® 19 Statistical Software (Minitab LLC, PA). The response means were compared using Tukey’s multiple comparison test at a significance $P$-value of 0.05.

**RESULTS AND DISCUSSION**
The chemical composition of GAW and GAW-NFR is presented in Table 5.2. The nanofiltration of GAW enabled the volume concentration factor of 4 resulting GAW-NFR of 22.35±0.78% TS content.

**Effect of NF on the composition of GAW**

The composition results presented in Table 5.2. showed that the lactic acid, ash, potassium, sodium, magnesium, and calcium concentrations were reduced by 34, 38, 62, 66, 13 and 41% respectively by nanofiltration of the GAW. All the reductions were comparable to those reported by Bedas et al., (2017) except calcium and magnesium. There was no reduction of calcium and magnesium reported in that study. The differences could be due to the type of NF membrane used (MWCO is different) and the processing conditions like pH and transmembrane pressures. However, a reduction in calcium through nanofiltration of acid whey was reported by Chandrapala and Vasilijevid, (2018). The reduction in the protein and lactose was observed to be minimal, 4.31% and 4.01%.

**Effect of NF on the yield of GAW powder**

The total, free, bound moisture contents, and the yields of the spray dried powders, with and without SSPS, were shown in Table 5.3. There was a significant difference (P < 0.05) in the total moisture and free moisture contents between GAW powders with and without nanofiltration treatment. The lower free moisture corresponds to low water activity and better shelf life of the GAW-NFR powders (Kentish and Rice, 2015). Also, the bound moisture in GAW powder with SSPS was significantly (P < 0.05) higher than that of GAW powder without SSPS addition. This difference corresponds to an increased degree of lactose crystallization in case of GAW-NFR powder, which is discussed in further section. The yield of GAW-NFR powder (56.35 ± 0.65%) was
observed to be significantly higher compared to that of GAW powder (44.51 ± 0.65%) 5%\). The yield of GAW was closer to the yield (44\%) of another type of acid whey, cream cheese whey without any treatment (Chandrapala and Vasiligevic, 2017). The GAW powder was sticky and some powder deposition was observed in the cyclone and was difficult to recover, whereas the power of GAW-NFR was less sticky and easy to recover from the cyclone and the collection chamber.

The rate of crystallization described as the amount of lactose present in crystalline form out of total lactose in the concentrate before spray drying is an important parameter determining the yield and powder characteristics of lactose rich dairy powders. The rate of crystallization of GAW-NFR powders (82.58±1.27) was observed to be significantly (P < 0.05) higher that GAW powders (74.56±1.60). The rate of crystallization obtained in case of GAW powders was observed slightly higher than that reported by Bedas et al., (2017). This could be due to the differences in the cooling rates and final crystallization temperatures.

**Effect of NF on GAW powder characteristics**

The SEM images of spray dried GAW powder samples were shown in Figure 5.2. The GAW powder particles had a greater presence of agglomerates than individual particles compared to that of GAW powder with SSPS addition. This suggests the presence of more amorphous lactose in GAW powders without SSPS, which is hygroscopic and affects the stickiness (Ross and Karel, 1992). The amorphous lactose present tend to crystallize during storage of the powders and decreases the shelf life of the powders by the formation of lumps (Aguilera et al., 1995). Also, the presence of smooth surfaces indicates the presence of amorphous lactose (Cano-Chauca et al., 2005)
which were noticeable with the GAW powders compared to those in GAW-NFR powders.

The particle morphology analysis results indicated that there is no significant (P > 0.05) difference between circulatory characteristics of the powder particles of GAW and GAW-NFR. Same with the case of convexity characteristics of the powders. This suggests that Nanofiltration of GAW does not have an impact on particle morphological characteristics.

The hygroscopicity values of GAW powders were shown in Table 5.4. The GAW-NFR powders were falling under slightly hygroscopic powders category whereas the GAW powders can be classified as hygroscopic powders (Schuck, 2004). The % hygroscopicity values of GAW with SSPS were significantly (P < 0.05) lower than those of GAW at both 75 and 85% RH conditions.

Differential scanning calorimetry (DSC) was used to observe the phase transitions such as crystallization. A clear exothermic peak around 150-170°C was associated with crystallization of lactose and the area under the peak was designated as the degree of amorphicity as described by Saffari and Langrish (2014). The DSC scans of GAW powders were presented in Figure 5.4. A wider exothermic peak in case of GAW powders indicates presence of more amorphous lactose content in it compared to the GAW-NFR powders.

CONCLUSION

The results from this study clearly showed that it was feasible to partially demineralize and deacidify the GAW using nanofiltration technology. The nanofiltration retentate GAW powders showed better drying ability and powder characteristics.
REFERENCES


Technology (Japan).


B, 110(20), 10190-10196.


FIGURES

Figure 5.1. The schematic diagram of the experimental procedure
Figure 5.2. Schematic of pilot NF/RO skid used for nanofiltration of GAW.
Figure 5.3. SEM micrographs of spray dried GAW powders without and with Nanofiltration treatment, from left to right
Figure 5.4. DSC scans of GAW powders

GAW = The powder obtained by spray drying of Greek acid whey

GAW-NFR = The powder obtained by spray drying of nanofiltration retentate of Greek acid whey
### Table 5.1. The typical composition of sweet and acid whey

<table>
<thead>
<tr>
<th>Components</th>
<th>Sweet whey (g/l)</th>
<th>Acid whey (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids</td>
<td>63-70</td>
<td>63-70</td>
</tr>
<tr>
<td>Lactose</td>
<td>46-52</td>
<td>44-46</td>
</tr>
<tr>
<td>Protein</td>
<td>6-10</td>
<td>6-8</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.4-0.6</td>
<td>1.2-1.6</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1-3</td>
<td>2-4.5</td>
</tr>
<tr>
<td>Lactate</td>
<td>2</td>
<td>6.4</td>
</tr>
<tr>
<td>Chloride</td>
<td>1.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

(Source: Jelen, 2009; Panesar et al., 2007)
**Table 5.2.** Composition of GAW liquid and GAW-NFR (g/100 g, dry basis) (n=3)

<table>
<thead>
<tr>
<th></th>
<th>GAW liquid</th>
<th>GAW-NFR</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.55 ± 0.01</td>
<td>4.22 ± 0.01</td>
<td>-</td>
</tr>
<tr>
<td>Dry matter (g/100 g)</td>
<td>5.52 ± 0.15</td>
<td>22.35 ± 0.78</td>
<td>-</td>
</tr>
<tr>
<td>Titratable acidity</td>
<td>7.54 ± 0.37</td>
<td>6.35 ± 0.32</td>
<td>15.77</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>12.39 ± 0.32</td>
<td>8.15 ± 0.19</td>
<td>34.25</td>
</tr>
<tr>
<td>TN</td>
<td>4.13 ± 0.02</td>
<td>3.95 ± 0.21</td>
<td>4.31</td>
</tr>
<tr>
<td>NPN</td>
<td>2.25 ± 0.04</td>
<td>2.21 ± 0.04</td>
<td>13.18</td>
</tr>
<tr>
<td>Ash</td>
<td>11.44 ± 0.11</td>
<td>7.12 ± 0.07</td>
<td>37.75</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.74 ± 0.12</td>
<td>1.03 ± 0.07</td>
<td>40.64</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.15 ± 0.03</td>
<td>0.12 ± 0.03</td>
<td>13.39</td>
</tr>
<tr>
<td>Potassium</td>
<td>1.93 ± 0.08</td>
<td>0.74 ± 0.02</td>
<td>61.60</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.79 ± 0.08</td>
<td>0.27 ± 0.03</td>
<td>66.01</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1.12 ± 0.25</td>
<td>0.86 ± 0.28</td>
<td>30.36</td>
</tr>
</tbody>
</table>

GAW = Greek acid whey diluted to feed to nanofiltration

GAW-NFR = Nanofiltration retentate of GAW feed
Table 5.3. Mean responses (n=3) of total moisture, free moisture, bound moisture, and yield of GAW and GAW-NFR

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>¹GAW</th>
<th>²GAW-NFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total moisture</td>
<td>%</td>
<td>6.49 ± 0.16ᵃ</td>
<td>5.08 ± 0.20ᵇ</td>
</tr>
<tr>
<td>Free moisture</td>
<td>%</td>
<td>4.47 ± 0.11ᵃ</td>
<td>2.69 ± 0.2ᵇ</td>
</tr>
<tr>
<td>Bound moisture</td>
<td>%</td>
<td>2.02 ± 0.08ᵃ</td>
<td>2.39 ± 0.08ᵇ</td>
</tr>
<tr>
<td>Yield</td>
<td>%</td>
<td>44.51 ± 1.37ᵃ</td>
<td>56.35 ± 0.65ᵇ</td>
</tr>
</tbody>
</table>

¹GAW = spray dried powder obtained from Greek yogurt whey
²GAW-NFR = spray dried powder obtained from Greek yogurt whey nanofiltration retentate

ᵃᵇMean responses in the same row with different superscripts were significantly different (P < 0.5)
Table 5.4. Mean responses (n=3) of % hygroscopicity of GAW powders with and without SSPS at 75% and 85 % RH

<table>
<thead>
<tr>
<th>Sample</th>
<th>Unit</th>
<th>Hygroscopicity at 75 % RH</th>
<th>Hygroscopicity at 85 % RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAW(^1)</td>
<td>%</td>
<td>15.87 ± 0.74(^a)</td>
<td>18.15 ± 1.02(^a)</td>
</tr>
<tr>
<td>GAW-NFR(^2)</td>
<td>%</td>
<td>11.34 ± 0.11(^b)</td>
<td>14.59 ± 0.66(^b)</td>
</tr>
</tbody>
</table>

\(^1\)GAW = spray dried powder obtained from Greek yogurt whey

\(^2\)GAW-NFR = spray dried powder obtained from Greek yogurt whey nanofiltration retentate

\(^a\)\(^b\) Mean responses in the same row with different superscripts were significantly different (P < 0.5)
CHAPTER VI

Evaluate the effect of addition of SSPS to partially demineralized and deacidified GAW (GAW-NFR) on the drying characteristics of the GAW.

ABSTRACT

The objective of the final study was to evaluate the effect of addition of SSPS to NFR-GAW on the drying characteristics of the GAW. In this study, the yield, and drying characteristics of GAW with and without 0.1% SSPS were compared. There was no significant (P > 0.05) difference observed between the yields of GAW-NFR (56.35%) and GAW-NFR with SSPS addition (58.24%). However, the yield of NFR-GAW (56.35%) was significantly (P < 0.05) higher than that of GAW with SSPS addition (51.87%). From this study it can be concluded that there may not be any additional advantage in addition of SSPS to NFR-GAW in improving the spray drying characteristics of GAW.
INTRODUCTION

After conducting the GAW with SSPS addition and GAW nanofiltration experiments, it was worthwhile to evaluate the effect of the SSPS addition on the drying characteristics of the nanofiltration retentate of GAW, GAW-NFR.

MATERIALS AND METHODS

The all materials and methods used for this study were as previously explained in chapter V, except with the treatment conditions where 0.1% SSPS was added to GAW-NFR concentrate during crystallization step. All other experimental conditions remained same as mentioned in chapter V materials and methods section.

RESULTS AND DISCUSSION

In this section, apart from comparing the drying characteristics of GAW-NFR and GAW-NFR-SSPS, these results obtained were also compared with the results from the previous experiments i.e., GAW powder and GAW-SSPS powders.

Drying ability of GAW

The yields of Greek yogurt whey (GAW), Greek yogurt whey with the addition of SSPS (GAW-SSPS), nanofiltration retentate of Greek yogurt whey (GAW-NFR), and nanofiltration retentate of Greek yogurt whey with the addition of SSPS (GAW-NFR-SSPS) were presented in Figure 6.1. The yield of GAW (control) was significantly different from all the treatments (GAW-SSPS, GAW-NFR, and GAW-NFR-SSPS), however there was no significant different between the yields of GAW-NFR and GAW-NFR-SSPS powders. These observations indicate that both addition of SSPS to GAW and
nanofiltration of GAW had significant effect on the yields of powders compared to GAW powders. Whereas the addition of SSPS to GAW-NF did not have a significant impact on the yield of GAW-NFR-SSPS over GAW-NFR, suggesting that only nanofiltration treatment would be sufficient to improve the yields of GAW. However, the yield of GAW-NFR-SSPS powder was higher than GAW powder (control).

**GAW powder characteristics**

The rate of crystallization and powder hygroscopicity results of all the GAW powders were presented in Table 6.1. There was no significant difference between the rates of crystallization of lactose in the powders of GAW-SSPS, GAW-NFR, and GAW-NFR-SSPS. However, the rate of crystallization of these powders (81.76 to 83.31%) was significantly higher ($P < 0.05$) than that of only GAW powder without any treatment. In general, the better the rate of lactose crystallization the better the drying ability, because of the presence of less amorphous lactose. These outcomes were further supported by the hygroscopicity results of the GAW powders. Similar significance trend was observed with the hygroscopicity values of the powders. The GAW-SSPS, GAW-NFR, GAW-NFR-SSPS were falling in the category of slightly hygroscopic powders whereas GAW powder (control) was a hygroscopic powder.

The SEM monographs of all the GAW powders were presented in Figure 6.2. The presence of smooth surface particles is an indication of amorphous lactose, this observation was increasingly pronounced with GAW-NFR-SSPS, GAW-NFR, GAW-SSPS and GAW powders indicating that all the treatments, i.e., addition of SSPS and nanofiltration of GAW had helped in improving the lactose crystallization during the cooling step of the manufacturing process. The tomahawk shaped lactose crystals were
more pronounced with GAW-NFR and GAW-NFR-SSPS. This observation further supports the improved rate of crystallization with these treatments.

The DSC scans of all the GAW powders were presented in the Figure 6.3. The appearance of exothermic peaks in the differential scanning calorimetry scans at around 150 – 170°C indicates the presence of amorphous lactose. These exothermic peaks are formed due to the recrystallization of the amorphous lactose present in the powders. The initial endothermic peaks that appear at around 146-147°C represent the loss of water of crystallization from the α-lactose monohydrate. The reappearance of the endothermic peaks at around 180°C was due to the loss of water of hydration from the recrystallized lactose monohydrate. The peak observations indicate qualitatively, the increasing presence of amorphous lactose in GAW-NFR-SSPS, GAW-NFR, GAW-SSPS, and GAW (control) powder respectively in the order presented. The DSC scans clearly show that the treatments helped in reducing the amorphous lactose content in the GAW powders.

CONCLUSIONS

From the results of all the above studies, it can be concluded that the addition of SSPS and nanofiltration of GAW could be feasible for commercial scale spray drying of the GAW with improved drying ability and powder characteristics. However, there was no significant benefit in addition SSPS to the nanofiltration retentate of GAW.
**FIGURES**

**Figure 6.1.** The mean yield % (n=3) of control and different treated GAW powders

GAW = Greek yogurt whey powder, control

GAW-NFR = Powder obtained from nanofiltration retentate of Greek yogurt whey

GAW-SSPS = Powder obtained from Greek yogurt whey with the addition of SSPS

GAW-NFR-SSPS = Powder obtained from nanofiltration retentate of Greek yogurt whey with the addition of SSPS

a-cMean values (n=3) with different letters on the bars were significantly different (P < 0.05)
Figure 6.2. SEM micrographs of spray dried GAW powders. Top: left to right, GAW, GAW-SSPS. Bottom: left to right, GAW-NFR, GAW-NFR-SSPS

GAW = Greek yogurt whey powder, control

GAW-SSPS = Powder obtained from Greek yogurt whey with the addition of SSPS

GAW-NFR = Powder obtained from nanofiltration retentate of Greek yogurt whey

GAW-NFR-SSPS = Powder obtained from nanofiltration retentate of Greek yogurt whey with the addition of SSPS
GAW = The powder obtained by spray drying of Greek acid whey

GAW-SSPS = The powder obtained by spray drying of Greek acid whey with SSPS addition

GAW-NFR = The powder obtained by spray drying of nanofiltration retentate of Greek acid whey

GAW-NFR-SSPS = The powder obtained by spray drying of nanofiltration retentate of Greek acid whey with the addition of SSPS
TABLES

Table 6.1. The crystallization rates of lactose in different GAW powders with their hygroscopicity values at 75% RH (n=3)

<table>
<thead>
<tr>
<th>Powder</th>
<th>Crystallization rate</th>
<th>Hygroscopicity at 75% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAW</td>
<td>74.56 ± 1.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.87 ± 0.74&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GAW-SSPS</td>
<td>81.76 ± 1.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.21 ± 0.90&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GAW-NFR</td>
<td>82.58 ± 1.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.34 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GAW-NFR-SSPS</td>
<td>83.31 ± 1.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.86 ± 0.96&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

GAW = Greek yogurt whey power, control

GAW-NFR = Powder obtained from nanofiltration retentate of Greek yogurt whey

GAW-SSPS = Powder obtained from Greek yogurt whey with the addition of SSPS

GAW-NFR-SSPS = Powder obtained from nanofiltration retentate of Greek yogurt whey with the addition of SSPS

<sup>a-c</sup>Mean values (n=3) with different letters on the bars were significantly different (P < 0.05)