MEMBRANE PROCESSING STRATEGIES FOR VALUE-ADDED UTILIZATION OF ACID WHEY FROM GREEK-STYLE YOGURT

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As we move into the Anthropocene, sustainability is achieving the status of a survival necessity. The food-water-energy nexus is as strained as ever, requiring thoughtful and innovative changes from all facets of human activity. In the center of this conundrum is the food supply chain, which is currently full of unsustainable practices. Food manufacturers represent part of this chain, and some solutions are already starting to show up on that front. They include the reuse and upcycling of byproducts and coproducts typically regarded as waste, turning them into new, sustainable, value-added products. Food processing is also going through a major change with the growing implementation of nonthermal technologies that could lead to the production of fresh, nutritious, safe foods while minimizing the consumption of energy and water.

The dairy industry is sometimes criticized for not always having the most sustainable practices. One example is the issue of Acid Whey, an abundant and challenging coproduct from the Greek-style yogurt manufacturing which can pose a significant environmental impact if improperly disposed of.

In **Chapter One** of this dissertation, sustainability is defined in the context of the food supply chain, ranging from production to processing to food waste and loss at

the consumer end. Some examples of sustainable and unsustainable practices are presented and discussed, and so is a quantitative tool for holistically assessing the sustainability of a food product.

Chapter Two delves into an extensive characterization of Acid Whey and Milk Permeate. In summary, they show low protein contents and pH, and high mineral amounts and Biochemical Oxygen Demands. This characterization can contribute to a database of properties that could help in finding a better destination for such streams.

Based on the composition of Acid Whey, two different nonthermal membrane strategies for the value-added utilization of this coproduct were studied and are presented in detail in this dissertation. In **Chapter Three**, the fractionation of some of the components in Acid Whey was investigated using a combination of cold Microfiltration and Ultrafiltration. This was shown to be feasible only when there was enough protein in the material, which is seldom the case.

Therefore, **Chapter Four** proposes the concentration of Acid Whey using a combination of Reverse Osmosis and Forward Osmosis. The process developed can produce concentrates comparable with those obtained by thermal evaporation, but without thermal damage to their components, and at a lower energy consumption.

Lastly, **Chapter Five** contains an empirical model to predict the flux during the Forward Osmosis of Acid Whey given the desired concentration and operating temperature. The information contained in this dissertation could help food manufacturers make more informed decisions about how to handle Acid Whey and other challenging byproducts, including using nonthermal alternatives such as Forward Osmosis for the concentration of challenging or sensitive liquid food products.

BIOGRAPHICAL SKETCH

Pedro Menchik was born and raised in São José dos Campos, SP, Brazil with his parents and an older sister. He first learned about Food Engineering as a junior in high school. After talking to a few people who were studying and working in the field, he decided that was the major he would pursue. He was admitted in the Food Engineering program at the State University of Campinas (Unicamp), Brazil, where he graduated in December 2011 on top of his class, earning a prize from the São Paulo State Engineering Council.

His first job in the food industry was as an intern in Coca-Cola FEMSA in Brazil, where he later stayed in a full-time position as an Operations Analyst for a while. After another temporary work experience in Belmar Dış Ticaret A.Ş. in Turkey, he returned to his hometown and got a Specialization in Integrated Management of Quality, Environment, Work Safety and Health, and Social Responsibility at Serviço Nacional de Aprendizagem Comercial (Senac). He then decided to pursue a Ph.D. degree in Food Science at Cornell University under the advice of Dr. Carmen Moraru, receiving a Science without Borders Fellowship from the Brazilian Government to do so.

During his time at Cornell University, Pedro was involved in many extracurricular activities, such as science outreach programs for high school students, the organization of symposiums and summits, product development competitions, and co-founding and serving as the first president for the Cornell Food Science Graduate Student Organization. He has also presented his research in both poster and oral formats during scientific conferences such as the annual meetings of both the Institute of Food Technologists (IFT) and the American Dairy Science Association (ADSA).

Pedro has received several awards during his five years as a Ph.D. student, including the Kosi Award in Food Science (2019), the Unilever United States Graduate Award (2018), the Food Market Institute Foundation Scholarship (2017), and the Western New York Institute of Food Technologists Award (2015-2016). He has also served twice as a Teaching Assistant and personally mentored six Undergraduate students in their research projects.

After completing his doctorate degree, Pedro will be returning to the food industry in a leadership position, with the hopes of bringing his scientific expertise to improve the everyday operations and manufacturing in a food processing facility.

This work is dedicated to my parents, Miriam and Wander, and to my sister, Catarina, for all the love and support provided throughout my life.

And to Biju and Molly, the best dogs anyone could ever have asked for.

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Finally, I am grateful for the support of all my friends, old and new, during this stage of my life. Special mention to my peers in the Department of Food Science, my roommates and neighbors, the patrons of Big Red Barn, my Brazilian community both back home and in Ithaca, and, of course, my family.

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LIST OF ABBREVIATIONS

ALA – alpha-lactalbumin
BLG – beta-lactoglobulin
BOD – Biochemical Oxygen Demand
CAW – Cottage cheese Acid Whey
CN – Casein Nitrogen
COD – Chemical Oxygen Demand
CV – Coefficient of Variance
DOA – Diluted Osmotic Agent
FO – Forward Osmosis
FSI - Food Sustainability Index
FU - Functional Unit
GAW – Greek-style yogurt Acid Whey
GHG – Greenhouse Gases
GSY – Greek-style Yogurt
LCA – Life Cycle Analysis
LCI - Life Cycle Inventory analysis
LCIA - Life Cycle Impact Assessment
MCC – Micellar Casein Concentrate
MF – Microfiltration
MP – Milk Permeate
NF – Nanofiltration
NMR - Nuclear Magnetic Resonance
NPN – Non-Protein Nitrogen
OA – Osmotic Agent
PEO - Pressure Enhanced Osmosis
PRO - Pressure Retarded Osmosis
RO – Reverse Osmosis
SDS – Sodium Dodecyl Sulfate
SP – Serum Proteins
SW – Sweet Whey
TMP – Transmembrane Pressure
UF - Ultrafiltration

JUSTIFICATION

The last decade (2010s) was defined by an increased interest in high protein foods, which resulted in the surge of dairy products such as Greek-style yogurt (GSY) or beverages fortified with proteins obtained by membrane fractionation of milk or cheese whey (O'Keefe, 2017). During the manufacturing of such products, a significant portion of the water and water-soluble components in milk such as lactose and minerals are removed as either whey or permeate. With growing volumes of the high protein products, high volumes of these streams are also produced. In the past, they were deemed as byproducts, and often times disposed of as waste (Ganju & Gogate, 2017). However, such streams can present a huge environmental concern due to their high content of organic matter, which can lead to algal bloom and depletion of oxygen in water streams (Arla Foods Ingredients, 2017; Erickson, 2017). For example, the average Biochemical Oxygen Demand (BOD_5) for some whey streams was reported to be around 40,000 mg/L (Jelen, 2011), which is about 30 times higher than the effluent limit prescribed for cultured dairy products and 130 times higher than the limit for cheese products (CFR, 2017). Therefore, pressure is mounting on the industry to fully utilize all milk components. To reflect the change of attitude towards these streams, in recent years the term coproducts started being used instead of byproducts.

Acid whey and permeate from membrane fractionation represent some of the most significant underutilized coproducts currently generated by the US dairy industry. Acid whey is generated from products such as cottage cheese or Greek-style yogurt, in which casein coagulation is driven by pH reduction by either lactic fermenta-

tion or direct acidification (Chandrapala et al., 2015a). Similar to the sweet whey obtained from cheese making, acid whey consists mostly of water with lactose as the main solid, but it has a much lower protein content and higher acidity and mineral content than sweet whey. This results in significant differences in sensory, nutritional and technological properties, as well as different strategies for the usage and processing of the two types of whey (Jelen, 2011). In particular, acid whey from Greek-style yogurt (GAW) is reported to have a lower protein content than even other types of acid whey, due to the depletion of whey proteins caused by the extended heat treatment used in yogurt making (Gyawali & Ibrahim, 2016).

Large volumes of Greek-style yogurt (GSY) are currently produced both in the US and globally (Statista, 2017). In 2004, GSY accounted for less than 2% of all yogurt types produced in the US, but in 2017 this number skyrocketed to almost 40%, amounting to almost 1 billion pints of Greek yogurt (Statista, 2017) (**Figure 0.1**). The straining or centrifugation associated with the manufacture of GSY results in high quantities of GAW since on average 2 kg of whey are produced for every 1 kg of Greek yogurt (Erickson, 2017). In New York State alone, which is currently the largest yogurt producing state in the US, almost 700 million pounds of GAW were produced in 2012 (DEC, 2012). To date, GAW utilization has been limited to low added-value applications, and most processors have yet to find an economically feasible way to incorporate it into higher-value products. A few solutions have been proposed, so far with mild success (Arla Foods Ingredients, 2017; Erickson, 2017). Current applications of GAW include irrigation, feed for livestock, and energy generation in wastewater bioreactors (DEC, 2012).

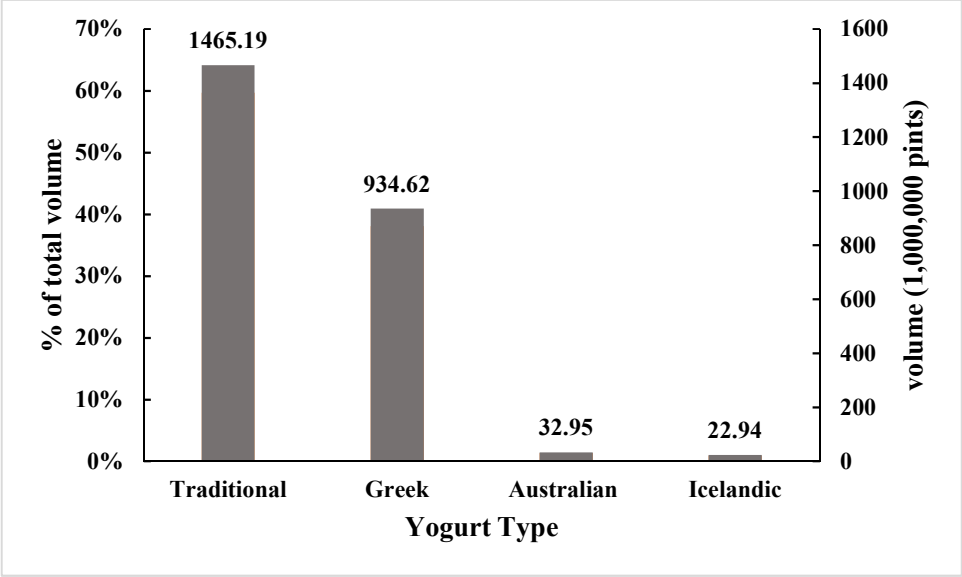

Figure 0.1 Total volume and market share of the main types of yogurt produced in the US in 2017 (Statista, 2017)

Solving the acid whey issue in an economically and environmentally sound manner requires the combined effort of farmers, dairy scientists, engineers, environmental researchers, nutritionists, product developers, government, and industry. This dairy coproducts still contain important components such as lactose, minerals, amino acids, and even small amounts of protein that could be used in products such as fermented goods, sports beverages, snacks, baby food formula, to name a few (Arla Foods Ingredients, 2017). Hence, stakeholders are actively seeking ways to find value-added utilization for dairy coproducts, which will help increase the value of milk and improve the sustainability of the dairy industry.

At the same time, the dairy industry is one of the most energy-intensive players within the food processing sector, and reducing energy usage is fundamental to improve the economic and environmental sustainability of dairy manufacturing (Briam,

Walker, & Masanet, 2015). Typical examples of energy-intensive thermal processes include pasteurization, sterilization, concentration, and drying. Thus, nonthermal alternatives such as membrane processing are currently drawing a lot of interest from the dairy industry lately. These technologies are reported to not only consume less energy and water than classic thermal processes (Chemat et al., 2017) but also promote minimal loss of sensory and nutritional quality of the products. Current membrane technologies used in the dairy industry include microfiltration (MF) for microbial removal from skim milk, which is used for the production of extended shelf-life milk, ultrafiltration (UF) to concentrate proteins in order to increase cheese yield, and reverse osmosis (RO) for the concentration of sweet whey (Cui & Muralidhara, 2010; Valentas, Rotstein, & Singh, 1997). Forward Osmosis (FO) is an emerging technique that is gaining increasing attention as an alternative to both RO and thermal evaporation, but it is still not as well-established in the food industry (Nicoll, 2013).

This dissertation focuses on both determining the composition of acid whey and UF permeate, and on evaluating the feasibility of membrane processing techniques such as MF, UF, RO, and FO for the fractionation and concentration of GAW.

It is anticipated that this research will have a significant impact on the dairy industry since it will provide processors with guidance on how to apply novel membrane technologies to obtain high added value products from GAW in a cost-effective, sustainable manner. Additionally, the predictive model developed and validated as part of this dissertation work could become a helpful framework for industry and researchers working on membrane processing of liquid foods and beverages in general and dairy products in particular.

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RESEARCH OBJECTIVES

The overall goal of this research was to assess the feasibility of a nonthermal processing strategy for the value-added utilization of acid whey from Greek-style yogurt. Specific research objectives include:

Objective 1. Discuss the Sustainability of the Food System

In the review presented in **Chapter One**, an extensive discussion of sustainability in the food system is presented, encompassing aspects ranging from agriculture to processing to food loss and waste at the customer level. Nonthermal processes, including membrane separation technologies, and the reutilization and repurposing of waste streams and byproducts are highlighted as part of the future approaches that could lead to a more sustainable food processing scenario.

Objective 2. Characterize the composition and variability of GAW and similar coproducts from the dairy industry

GAW streams and other related coproducts, namely UF permeate, were collected from a number of dairy processors in New York State and characterized in terms of composition (total solids, lactose, total protein and individual protein components, fat, ash, and minerals), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), pH, and titrable acidity. This objective is addressed in **Chapter Two** of this dissertation.

Objective 3. Investigate the feasibility of a membrane fractionation strategy for isolation of α-lactalbumin from GAW

Based on the composition of GAW, the feasibility of a combined microfiltration (MF) and ultrafiltration (UF) strategy for the fractionation of alpha-lactalbumin from GAW was tested.

This objective is addressed in **Chapter Three** of this dissertation.

Objective 4. Develop a membrane processing strategy for the nonthermal concentration of GAW using a combination of reverse osmosis and forward osmosis

A strategy that uses a combination treatment consisting of Reverse Osmosis (RO) followed by Forward Osmosis (FO) for the concentration of GAW was developed and assessed. A quantitative evaluation of the electrical and thermal energy used in the process was also performed. This objective is addressed in **Chapter Four** of this dissertation.

Objective 5. Develop a predictive model for the permeate flux of FO of GAW given the desired concentration factor and operating temperature

An empirical model relating flux to the initial concentration of the feed, concentration factor, and temperature was developed and validated. This objective is addressed in **Chapter Five** of this dissertation.

OVERALL EXPERIMENTAL APPROACH

The flow diagram below (**Figure 0.2**) summarizes the overall experimental approach for the objectives aforementioned. Specific methodologies will be described in detail in the following chapters.

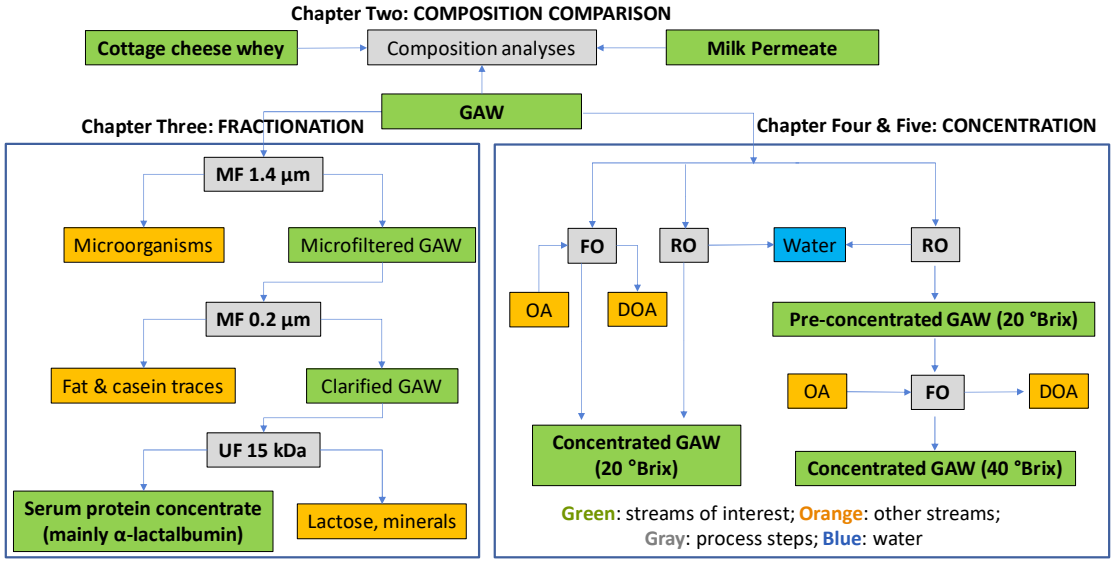

Figure 0.2 Overall experimental approach

CHAPTER ONE.

APPROACHES TO ACHIEVING SUSTAINABILITY IN THE FOOD SYSTEM: A REVIEW

1.1 ABSTRACT

Sustainability of the food industry has become a topic of increased interest in the past years. More than a buzzword, the term has evolved to encompass not only environmental concerns but also economic feasibility and social responsibility. In the context of food production, sustainability can entail an even larger spectrum, with the inclusion of matters such as food quality, safety, and security. In this chapter, a holistic review of sustainability issues concerning the food industry will be presented, illustrated with examples, and discussed. The current scenarios regarding food loss and waste and resource utilization in the primary agricultural production and food processing are defined, and some guidelines for sustainable practices are proposed. Finally, the Life Cycle Assessment (LCA) is discussed as a quantitative metric to assess the sustainability of a food product from farm to fork.

1.2 SUSTAINABILITY: DEFINITION AND CURRENT SCENARIO

Sustainability is a trendy, yet ambiguous concept that has gathered much attention recently. The term can assume different meanings to different people and industries. The Merriam-Webster learner's dictionary defines the adjective "sustainable" as "*involving methods that do not completely use up or destroy natural resources*" or

"*able to last or continue for a long time*" (Merriam-Webster, 2017). Most people interpret this solely from an environmental standpoint which, while an important aspect, is only one of the three pillars associated to sustainability in any field, the other two being economic feasibility and social responsibility (Murphy, McDonnell, & Fagan, 2014; Sellahewa & Martindale, 2010). All three aspects are intimately connected, which makes the discussions and policies towards sustainability rather complex.

In many industries, the management of issues such as product quality, productivity, and worker's health and safety can be partially or completely integrated with the pillars of sustainability. The food industry must also add the unique issues of food safety and security to this complex equation. To achieve sustainability, food processors have to answer the following question: "How, where, and when can we best source, process, and deliver enough nutritious, safe, high-quality foods without harming the environment, the community, our employees, or our finances?" While the answer is not straightforward, there are a few practices and guidelines that can help the food industry achieve this goal. These involve actions to be taken throughout the entire food supply chain, from agriculture and processing to retail and consumer end, and include changes regarding cultivation, harvesting, processing, packaging, transportation, and storage of raw materials or finished products, as well as the associated energy inputs and waste management.

The current model of food production, processing, and distribution is not highly sustainable. Agriculture accounts for 17% to 32% of all greenhouse gas (GHG) emissions worldwide; food processing is responsible for about 25% of the water consumption on the globe; food loss and waste are estimated to range between 30 and

50% of the total production (Boye & Arcand, 2013; Murphy et al., 2014), while malnutrition, undernutrition, and famine continue to occur in many developing countries. In parallel, an epidemic of obesity, diabetes, and coronary diseases affect developed nations, which are also responsible for a staggering 42% of the food waste in households and another 39% of the food losses during manufacturing (Van Der Goot et al., 2016). This alarming scenario is pushing the food industry towards adopting more sustainable practices.

Another significant driver for the adoption of sustainable practices by the food industry is the increasing number and stringency of local, federal, and transnational laws and regulations regarding the emission of pollutants into air, water, and soil (Murphy et al., 2014), or concerning the levels of sugar, sodium, and other nutrients in foods, associated with stricter oversight of labelling and label claims (Grunert & Wills, 2007). Working conditions and prevention of accidents for employees are also being considered as important components of sustainability.

In addition, we are currently witnessing increased awareness from customers, who are demanding evidence of sustainable practices from manufacturers and are often looking for specific certifications on food product labels (Sellahewa & Martindale, 2010). Furthermore, retailers, manufacturers, co-packers, and other intermediates often require their providers to meet or exceed the sustainability standards prescribed by legislation.

Besides the increase in business opportunities for companies that adopt sustainable practices, there are also other economic incentives, such as a) reduction of costs due to lower consumption of energy, water, and other materials; b) fewer expenses associated with waste handling and disposal; c) creation of high added-value

products from byproducts; and d) credits through the growing carbon market and energy exchange policies (Murphy et al., 2014). Finally, corporate performance assessment tools are giving increasing weight to sustainability-related indicators, including environmental, social, and economic factors.

Nevertheless, there are several reasons why the current food supply chain is currently not very sustainable. One of them is the focus on the purity of ingredients, which requires many unit operations to achieve, often consuming a large amount of water and energy, demanding extensive cleaning, and generating waste and low-value byproducts. These ingredients often come in a dry or concentrated form, requiring a high amount of energy to be produced, or must be refrigerated, increasing expenses with storage and transport. Furthermore, they need to be recombined or combined with water before further processing or use, which consumes yet more resources and generates even more waste. For example, soybeans are fractionated into carbohydrates (starch), proteins, and oil, some of which are later combined to produce meat analogs.

A system that focuses more on the functionality of products and ingredients, rather than on their purity, could minimize those intermediate steps and operations, and potentially be more sustainable (Van Der Goot et al., 2016). The focus on purity also has a negative impact on nutrition, since it usually generates foods high in simple components, namely sugars and fats, but low in minor important nutrients such as fibers, vitamins, and minerals (Van Der Goot et al., 2016).

1.3 SUSTAINABILITY AND AGRICULTURE

While the green revolution of the 1960s and the recent advances in genetic improvement of crops have had an invaluable contribution to increasing the food production and promoting food security globally (Pretty et al., 2010), agriculture still causes a strong negative environmental impact on the planet (Boye & Arcand, 2013; Murphy et al., 2014). One practice generally regarded as unsustainable in the current global environment is the preference for animal-based foods, especially in higher-income countries and households. Typically, animal products require a significant amount of feed and energy to produce and have a large impact on the environment. Paola, Rulli, & Santini (2017) estimated that animal proteins are 2.4 to 33 times more expensive than plant proteins in terms of land and water demand and could generate up to 240 more emissions of greenhouse gases (GHG). This is particularly significant since some emerging economies and regions that traditionally did not consume large amounts of animal-based foods, particularly meats, are changing their dietary preferences. For instance, by 2020 the consumption of meat in China is projected to be twice that of 2005, putting even more stress in the food supply chain (NSF, 2014).

A recent modeling study showed that replacing 50% of meat and dairy consumption in the UK by cereals, fruit, and vegetables would result in a 19% reduction in GHG emissions and could potentially avert or delay over 30,000 deaths per year (Scarborough, Allender, Clarke, Wickramasinghe, & Rayner, 2012). However, different metrics used to gauge sustainability can lead to different conclusions. Using a nutrient density to climate impact index, Smedman et al. (2010) compared milk to other commonly consumed beverages in Sweden and determined that milk had a far more favorable nutrient-to-GHG emissions ratio than soy drink, oat drink, or orange juice.

Similarly, another study pointed out that while removing animals from the agricultural system in the US would result in a significant reduction in GHG emissions, it would also result in nonviable diets for part of the population due to a lack of essential nutrients and excess of calories (White, Hall, & Turner, 2017). The discussion gets even more complicated when we factor in the social aspect of sustainability, since livestock production is fundamental for the economy of developing nations, currently supporting nearly a billion of low-income people (Pretty et al., 2010).

Animals or no animals, agriculture is currently responsible for 70% of all freshwater usage on the planet, which puts a big strain on the already limited water sources. To overcome this problem, there is a need for low-cost and energy-efficient processes to desalinate seawater and brackish water, as well as for reusing and recycling the wastewater generated during food production. Technical solutions include state-of-the-art high flux reverse osmosis, forward osmosis, membrane bioreactors, and artificial wetlands (NSF, 2014). Irrigation systems also need to be better designed to deliver the right amount of water where and when needed, minimize consumption, waste, and runoff. In a study conducted by Marino et al. (2018), for instance, traditional irrigation of a pistachio field was substituted by a technique called micro-irrigation, which used 85 to 90% less water and increased the yield by 30%.

The same concept of precision agriculture is valid for the application of fertilizers and pesticides (Bongiovanni & Lowenberg-Deboer, 2004). Nutrients such as Nitrogen and Phosphorous are costly to source and manufacture, so there is a need to recycle or recover them by composting when possible. This is particularly pressing in the US, one of the world's biggest importer of phosphate rock, with 1.7 Mtons/year on average (Khabarov & Obersteiner, 2017).

In addition, the excess of nutrients can lead to eutrophication when applied and disposed of improperly. Plant breeding and genetic engineering can also play a role in obtaining plants with better uptakes of nutrients and resistance to pests, minimizing the need for agricultural inputs. Bt maize, as an example, was shown to decrease insecticide usage and increase yields in five different countries (Qaim, 2009). Other ways to reduce classic pesticide usage are integrated pest management techniques (Roberts & Mattoo, 2018) and the application of low-toxicity biopesticides, usually materials derived from plants, fungi, bacteria, or even naturally-occurring mineral sources (NSF, 2014).

Therefore, to better control the efficiency of irrigation, pesticides, and fertilizers, there is a need to develop sensors and tools for monitoring the concentration of water, nutrients, and other chemicals in the field. These sensors should be reliable, but also low-cost and accessible, such as the ones proposed by Mohandas et al. (2017). Sensors should also be used to track parameters such as temperature and moisture changes during storage and transport of materials. Moreover, in order to improve food safety and quality, new tools should be developed for the rapid *in situ* detection of spoilage organisms, foodborne pathogens, and toxins in foods, since classical microbiological assessment is inconvenient and time-consuming. Viswanath et al. (2018) present a good review of some of those sensors and their applications.

1.4 FOOD LOSS AND WASTE MANAGEMENT

Another major reason for the current unsustainable food system is the culture of food waste. The terms “food loss” and “food waste” can have different definitions. Usually, food loss refers to a reduction in the mass or nutrition content of postharvest

edible parts of foods intended for human consumption (USDA, 2017). This includes moisture loss, degradation by pests and microorganisms, losses during transport, storage and processing, and end-user food waste. Some authors include non-edible parts of food, such as shells and bones, as part of food loss (Reich & Foley, 2014; USDA, 2017). Food waste, in turn, accounts for food discarded and/or spoiled in retail, households, and foodservice. Nevertheless, from the industrial standpoint, the term "waste" can assume a broader meaning and encompass various types of losses and emissions found throughout the food supply chain, including (but not limited to) food itself and other outputs such as ancillary materials, energy, and wastewater. In this chapter, the term "food waste" will be used to refer to any part of a food product, post-harvest, that has not been utilized to its full potential.

In developing countries, most food losses occur during production, manufacturing, and transport (NSF, 2014). Some of the contributing factors are ineffective agriculture and manufacturing practices, outdated industrial technology, and unreliable cold chains. On the other hand, in developed economies, the main culprits are actually the customers, including retailers, foodservice, and end consumers (NSF, 2014). Urbanization and affluence play a big role in this scenario since people who are living in smaller households and spending a relatively small fraction of their budget in food are not perceiving a significant penalty for wasting it. Consumer preference is also shifting towards more "fresh", hence perishable, foods, but at the same time, there is a rejection of perfectly safe and nutritious foods due to cosmetic or other minor quality reasons (NSF, 2014; Van Der Goot et al., 2016).

The concept of "zero waste", while somewhat utopian, is being used by some food producers and manufacturers as a goal to be met towards a more sustainable future (Lehmann, 2011). One of its main principles is the waste management hierarchy (Boye & Arcand, 2013; Kosseva, 2009; Van Der Goot et al., 2016), illustrated in **Figure 1.1**. Disposal (sometimes referred to as "**returning**") of waste in landfills or incineration is the least preferable option and many restrictions already apply in regulations throughout the world (Kazimierowicz, 2018). Treating (or "**remediating**") emissions to remove pollutants such as gases, heavy metals, or excessive organics comes next. This is followed by **recovering**, which refers to practices such as composting and digesting that can help recuperate part of the energy and/or the nutrients present in the waste, sometimes with positive economics. Examples of this include the studies carried by Hussain et al. (2018) about the vermicomposting of kitchen waste and paddy straw, and by Shukla et al. (2018), focusing on the economics of Nitrogen recovering from a vegetable farm drainage.

The next step up on the sustainability ladder is **reusing** materials, energy, and water. True reuse usually refers to re-introducing these inputs into the same process flow, with minimal or no transformation (e.g.: recirculating cooling water), while **recycling** involves some energy or water utilization to create new useful materials (such as recycled paper) and is less preferable. Depending on the added value of the new material in comparison with the original, the process can be either referred to as "upcycling" or "downcycling". In addition, a product that does not meet a specific quality or safety standard can be **reprocessed** (e.g.: re-pasteurizing undertreated milk using

temperature-activated diversion valves) or **repurposed** (e.g.: fruit pieces out of cosmetic or dimension specifications being used for jam production). Both of these examples can be included in the broader definition of reuse.

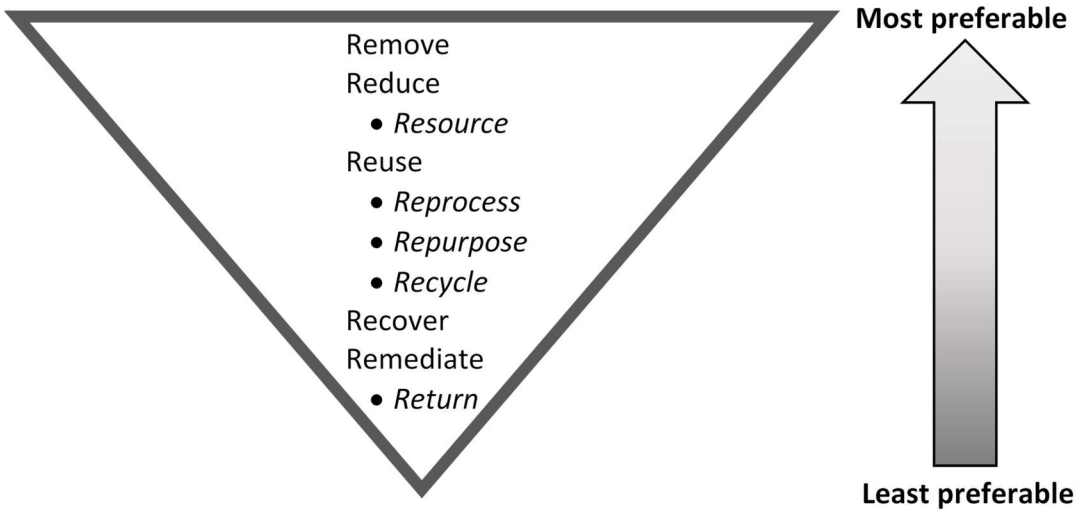

Figure 1.1 Food waste management hierarchy. Elements near the top should correspond to the bulk of the strategies adopted by organizations and individuals (adapted from Kosseva, 2009)

The second-best option in the sustainability pyramid is **reducing** the amount of waste produced by optimizing the number of resources needed, improving the processing techniques, productivity, and reliability, and/or improving the accuracy of consumption forecasts. The term **re-source** is sometimes related to this definition. It entails the usage of alternative sources of water, materials, or energy, (e.g.: renewable energy). The most preferred option – but also the hardest to achieve - is avoiding producing waste at all (also referred to as "**remove**"), which is basically the ultimate version of reducing waste to zero levels. Even though true zero waste is practically unachievable, many companies acquire and use the waste from others in their processes, aiming for an overall neutral waste balance.

Fruit and vegetable wastes can be used for numerous applications, including dietary fibers, vitamins, pigments, flavors, essential oils, gums, bioadsorbents, biofuels, antioxidants, and other phytochemicals. With the help of biotechnology, compounds of interest such as enzymes, organic acids, antimicrobials, and biopolymers can be synthesized from different plant wastes. For instance, apple pomace, a byproduct from the apple juice and cider industry, can be used for nutritional enrichment of feed and also as a substrate for the production of pectin and pectolytic enzymes, fruity aroma compounds, xanthan gum, citric acid, and ethanol (Kosseva, 2009). Bioadsorbents are a particularly interesting application of materials such as husks, piths, shells, and bagasse, in which "waste is used to treat waste". These materials can be used for the sorption and removal of metal ions and dyes from wastewaters that are hard to handle and usually require extensive pretreatment operations (Kosseva, 2009).

Animal-derived wastes have more specific applications than their plant counterparts. Common products include collagen from meat and fish waste and carotenoids and chitin from shrimp and other crustaceans. Cheese whey, a dairy processing byproduct, is usually converted into high added value whey protein powders. It can also be used in several other applications, such as feed enrichment and as a substrate for the production of other fermented goods, organic acids, gums, enzymes, bacteriocins, flavors, and yeast (Kosseva, 2009). Proteins, polysaccharides, and fats from both animal and plant-based wastes can be used to produce biodegradable (or even edible) films that can find many applications in food packaging, improving sustainability while protecting food safety and quality.

1.5 SUSTAINABILITY AND FOOD PROCESSING

On the food processing side, many of the classical unit operations involve extensive consumption of energy and water and generate a lot of waste. Typical examples of energy-intensive processes are heat treatments such as pasteurization, commercial sterilization, thermal concentration, and drying. The food industry already applies some practices aiming towards recuperating some of the energy used in those operations, including regeneration sections in pasteurizers, multiple-effect evaporators, and thermo-compression or mechanical compression during concentration, to name a few. However, a good amount of energy is wasted in these operations, which does not always find an adequate sink and ends up as thermal pollution to the environment. For instance, in the UK alone, almost 3 TWh of heat are estimated to be wasted by the food industry (Murphy et al., 2014). If recovered, this could be used to heat water or buildings, or even as a source of energy for other processing operations. This is another illustration of how the economic and the environmental pillars of sustainability can sometimes be positively coupled.

Recently, more disruptive innovations in food processing are targeting the partial or complete replacement of classical thermal operations by nonthermal alternatives. These include technologies such as ultrasound, supercritical fluids, high pressure processing, pulsed electric fields, irradiation, light-based treatments (UV, LED, pulsed light, etc.), cold plasma (Zhang, et al., 2011), and membrane filtration (microfiltration, utlrafiltration, reverse osmosis, etc.) (Cui & Muralidhara, 2010). Several non-conventional thermal techniques (i.e., not steam-based) are also receiving increased attention, namely microwave/radiofrequency, infrared, and ohmic heating.

Nonthermal technologies are also associated with minimal loss of sensory and nutritional quality in foods when compared to thermal methods, since there is less heat involved, and they could also help produce fresh-like and wholesome foods while reducing the number of chemical additives used. Hence, they meet both the sustainability drivers and the general food trends among consumers. While these technologies have their own particularities and applications, they are generally considered to consume less time, water, and energy than classic thermal processes (Chemat et al., 2017).

In this context, membrane technologies such as ultrafiltration and reverse osmosis have been increasingly applied in the dairy and beverage industries and for wastewater treatment and desalination. They are key operations in the transformation of sweet whey, a coproduct from the cheese industry, into high added value whey protein concentrates. State-of-the-art reverse osmosis, for instance, can have a specific energy consumption as low as 2 kWh/ m^3 of water removed (NSF, 2014). On the other hand, thermal concentration - even when utilizing multiple effects and mechanical vapor compression - has a specific energy consumption ranging between 7.7 and 11.4 kWh/ m^3 of water removed, as reported by Jamil & Zubair (2017). Similar estimates for energy usage during the desalination of saltwater via different thermal and nonthermal methods can be found elsewhere (El-Dessouky & Ettouney, 2002).

Another example of nonthermal technology is High Pressure Processing (HPP), one of the most mature of the nonthermal technologies, used mainly for the post-packaging treatment of liquid or paste-like foods, such as fruit juices, sauces, and guacamole. Chang et al. (2017) compared HPP-treated white grape juice with the classical thermally pasteurized version. At a similar level of bacterial inactivation, the HPP-treated products had better color, antioxidant activity, and overall sensory scores

compared to the thermally treated ones. Other emerging technologies are on earlier stages of development with more specific applications, such as UV for surface treatment, irradiation in spices, pulsed electric fields for potato processing, and supercritical CO_2 for extraction of oils and volatile compounds (Zhang et al., 2011).

Furthermore, there is a current move towards renewable, fossil fuel-free energy sources with the goal of improving overall sustainability. These include solar, wind, geothermal, and bioenergy sources. The first three have the disadvantage of being weather and/or region-dependent and considerable capital costs; however, the technologies are maturing and prices are dropping yearly, which means they are becoming more and more on par with traditional carbon-based sources (NSF, 2014). For bioenergy, a big concern is that biomass will compete with food production for resources such as land, water, and nutrients, which are already scarce. To avoid this extra strain on the food supply chain, it is important that biofuels focus on non-edible parts of crops or low-value byproducts, such as straws, husks, stems, and bagasse. Such materials are also a good source of feed for livestock as opposed to commodity grains, which should be targeted towards human nutrition. Moreover, some food waste and wastewaters can also be used as either animal feed or to generate biofuels using processes such as anaerobic digestion and gasification (Murphy et al., 2014). Recently, a process has been developed to convert acid whey – a byproduct from Greek yogurt production – into mid-chain carboxylic acids via chain-elongation in bioreactors, which could be used for both applications aforementioned (J. Xu et al., 2018).

1.6 PUTTING IT ALL TOGETHER: THE LCA METHODOLOGY

The most extensively studied and applied pillar of sustainability is the environmental component, which can be assessed by methods such as carbon and ecological footprints. On a country level, the Food Sustainability Index (FSI) is a new metric developed by The Economist Intelligence Unit with the Barilla Center for Food & Nutrition. The 2017 edition ranked 34 nations around the globe using 35 indicators related to the following pillars of sustainability: food loss and waste, sustainable agriculture, and nutritional challenges. Overall, France was the top performer, followed by Japan and Germany (Barilla Center for Food and Nutrition, 2017).

Recently, a tool that has gained popularity among food processors and researchers is the Life Cycle Assessment (LCA), which entails a comprehensive analysis of the impact of a product (or process, service, activity) on ecosystems, natural resources, and human health, which are referred to as endpoint impacts. Those are unraveled in more specific factors, such as ozone depletion, human toxicity, eutrophication, land occupation, and fossil depletion, among others - referred to as midpoint impacts. This method provides qualitative and quantitative evidence for the comparison of different competing products or different life cycle possibilities for the same product, helping processors make informed decisions with sustainability in mind. It can also be used to identify and prioritize specific steps of the life cycle that require improvement. While social and economic aspects are typically outside of the scope of the LCA (Finkbeiner, Inaba, Tan, Christiansen, & Klüppel, 2006), it provides a great starting point and can be combined with other tools (e.g.: hazard analysis and risk assessment) to address the other pillars of sustainability.

The LCA methodology is regulated by the international standards ISO 14040:2006 and ISO 14044:2006 and should be conducted following a specific framework and a set of requirements and guidelines. **Figure 1.2** depicts the overall stages involved in an LCA. The first step is defining the goal and the scope of the study, which entails its purpose and expected outcomes, the system boundaries, and the functional unit (FU) (Roy et al., 2008). The system boundaries can be represented by a flow diagram including the inputs and outputs of the processes in the life cycle of the product and may be as small or as big as necessary. In most cases involving food products, a "farm to fork" approach is preferred to encompass the entire supply chain when possible. The FU is a normalized reference unit of the inventory to be assessed and can be based on mass, energy, land area, or economic and nutritional values of the product (for instance: calories or protein content). This is important since one of the main purposes of the LCA is to provide insight to compare alternative products in terms of their impacts (ISO, 2006a, 2006b), and different FUs can lead to different results and conclusions.

The second step of the LCA is the life cycle inventory analysis (LCI), which involves the collection of data regarding all inputs and outputs pertaining to the process. Inputs include raw materials, water, energy, packaging materials, cleaning solutions, etc.; outputs include main products, coproducts, byproducts, solid and liquid waste, waste energy, and emissions to air. Some of the general data (for instance, on transport or electricity) can be found at LCA databases, while others are product-specific or even site-specific, and require extensive collection. This makes this step one of the most time-consuming and labor-intensive phases of LCA. The next stage is the impact assessment (LCIA) (**Figure 1.3**), in which the environmental impacts pertaining

to the previous inventory analysis will be classified, characterized, normalized, and valuated. This is when the impacts are going to be assigned into the endpoint and mid-point categories, quantified, and aggregated. The final stage is the interpretation of the results, in which the identified issues will be used as an input for decisions regarding potential changes in process and/or product design, resources and materials sourcing, and waste management (ISO, 2006b, 2006a).

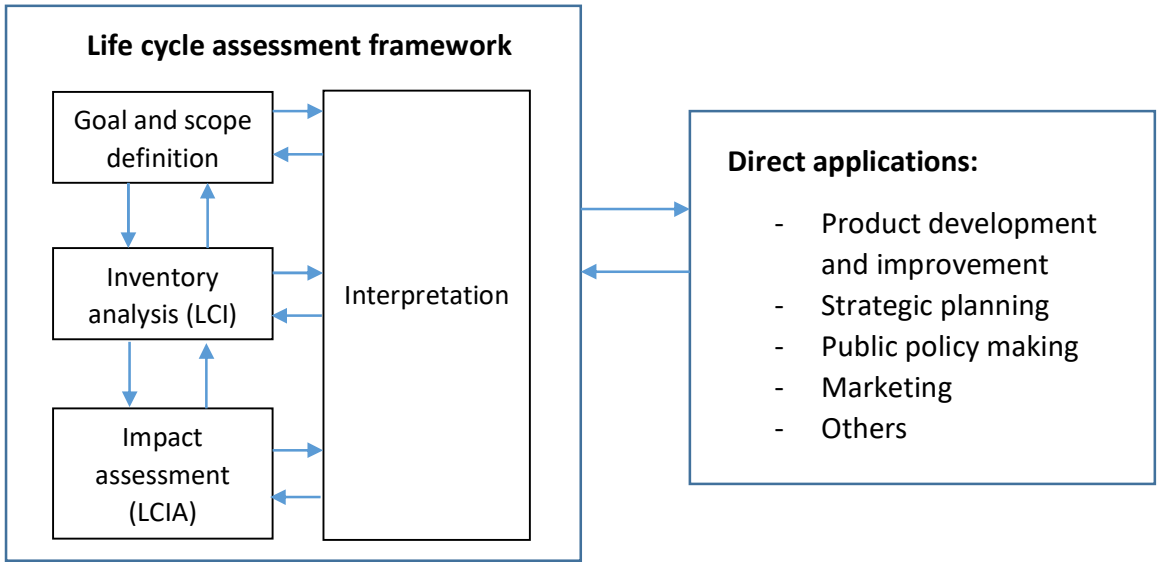

Figure 1.2 Stages in an LCA (Adapted from ISO, 2006a)

Smetana, Mathys, Knoch, & Heinz (2015) presented a comprehensive LCA for meat substitutes, comparing chicken, dairy-based, lab-grown, insect-based, gluten-based, soy-based, and mycoprotein-based analogs. The authors initially used ready-to-eat product mass as the functional unit and then did a sensitivity analysis using both calorie and protein contents as alternative FUs. For the mass and protein units, they determined that lab-grown meat had the highest impacts, followed by mycoprotein-

based. The lowest impacts were found in insect-based and soy-based alternatives. This result changed slightly when using the calorie content as FU, in which chicken, dairy-based, and gluten-based substitutes had the best performance, while the highest impacts were still found in lab-grown meat. Reviews of other examples of LCA for different food products can be found in Roy et al. (2008) and Murphy, McDonnell, & Fagan (2014).

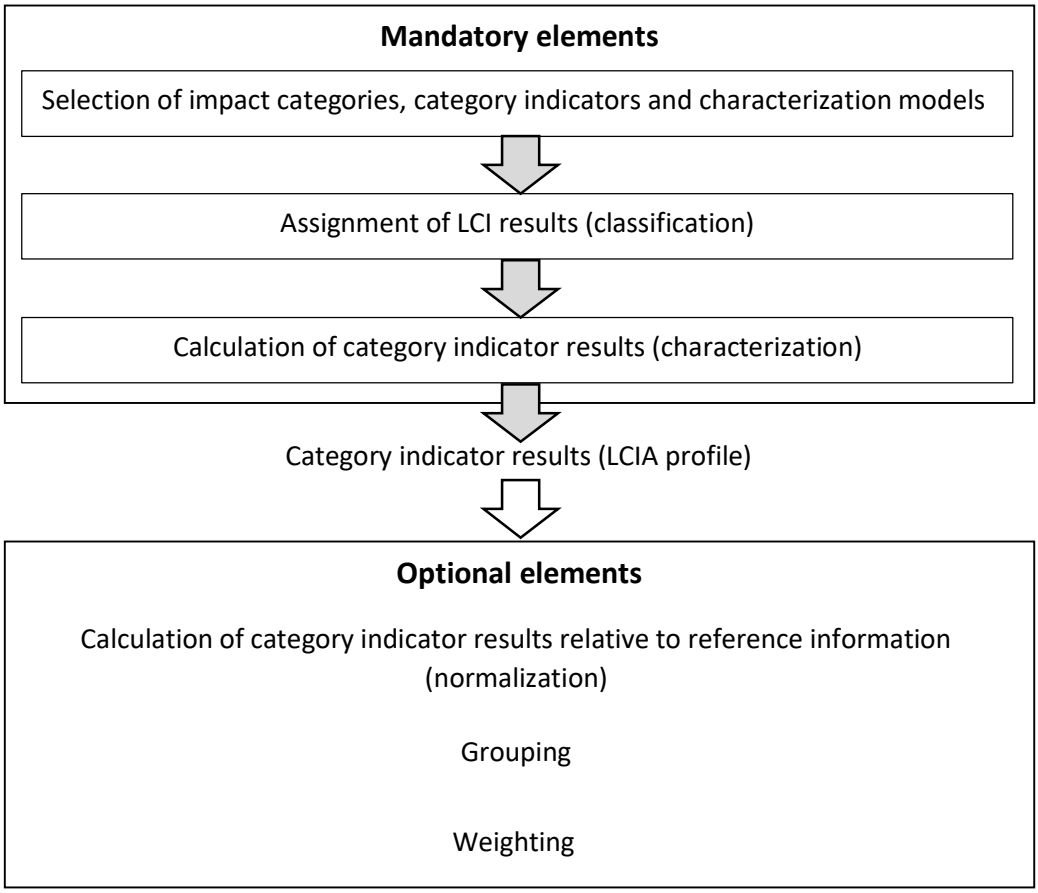

Figure 1.3 Steps in the LCIA stage (Adapted from ISO, 2006a).

1.7 CONCLUSIONS

In this review, sustainability was defined in the context of the food industry and analyzed in the context of food loss and waste in agriculture and food processing. Specific guidelines, examples, and comparisons were used to illustrate practices and changes that could lead towards a more sustainable food supply chain. In addition, the LCA methodology for assessing the environmental pillar of sustainability was discussed with specific examples. In the future, we hope that this tool will be increasingly implemented in the industry and that new universal metrics could also be developed for the economic and social pillars of sustainability, with the goal of stimulating a better decision-making process for food producers, manufacturers, and retailers.

1.8 ACKNOWLEDGEMENTS

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CHAPTER TWO.

COMPOSITION OF COPRODUCT STREAMS FROM DAIRY PROCESSING: ACID WHEY AND MILK PERMEATE

2.1 ABSTRACT

This chapter provides composition information for three abundantly available but little characterized dairy coproduct streams: acid whey from Greek yogurt (GAW), acid whey from cottage cheese (CAW), and milk permeate (MP). Three replicate samples obtained on different dates from several dairy processors were analyzed. The main component in all streams was lactose, with up to 3.5%, 2.1%, and 11.9% in GAW, CAW, and MP, respectively. Crude protein content ranged from 1.71 to 3.71 mg/g in GAW, 1.65 to 5.05 mg/g in CAW, and 3.2 to 4.35 mg/g in MP, and pH ranged from 4.21 to 4.48, 4.35 to 4.51, and 5.4 to 6.37, respectively. Chemical Oxygen Demand varied from 52,400 to 62,400 mg/L for GAW, 31,900 to 40,000 mg/L for CAW, and 127,000 to 142,000 mg/L for MP; Biochemical Oxygen Demand ranged from 45,800 to 50,500 mg/L (GAW), 32,700 to 40,000 mg/L (CAW), and 110,000 to 182,000 mg/L (MP), respectively. GAW had the lowest pH (4.21 - 4.48) and the highest mineral content of all streams. This data will assist processors and researchers in developing value-added utilizations for these dairy coproducts.

2.2 INTRODUCTION

Whey is the general name given to the liquid fraction that separates after the precipitation of casein from milk. There are two main types of whey: sweet and acid. ***Sweet whey*** is the most common type of whey and results from the manufacturing of cheeses via enzymatic (rennet) coagulation, such as cheddar, Swiss, parmesan and mozzarella (Chandrapala et al., 2015a; Jelen, 2011). ***Acid whey*** is generated from products such as cottage cheese or Greek-style yogurt, in which casein coagulation is driven by pH reduction due to lactic fermentation or addition of acids (Lievore et al., 2013; Nishanthi, Chandrapala, & Vasiljevic, 2017). Both types of whey consist mostly of water, with lactose as their main solid; however, they differ in acidity, protein, and mineral contents. This results in significant differences in sensory, nutritional, and technological properties, requiring different strategies for their usage and processing (Jelen, 2011). A third type, referred to as "salty whey", is generated during the dry-salting stage of cheeses such as cheddar, but it only accounts for 5% or less of the total whey removed (Blaschek, Wendorff, & Rankin, 2007). Another related coproduct sometimes called "native whey" is ***milk permeate***, obtained from ultrafiltration of skim milk, a process used by cheesemakers to increase yield. This permeate has a comparable composition to acid whey (minus the acidity) and consequently can find similar applications in the dairy industry (Jelen, 2011).

Acid whey from Greek-style yogurt (GAW) is substantially depleted of whey proteins - particularly beta-lactoglobulin (BLG) (**Figure 2.1**) - because of the extended heat treatment used in yogurt making followed by straining or centrifugation (Gyawali & Ibrahim, 2016). This, combined with its relatively high mineral content, especially

Ca and P, renders the processing and utilization of this byproduct a challenge (Menchik, Zuber, Zuber, & Moraru, 2018). Another caveat is the high variability in the composition and properties of the material, including its protein amount.

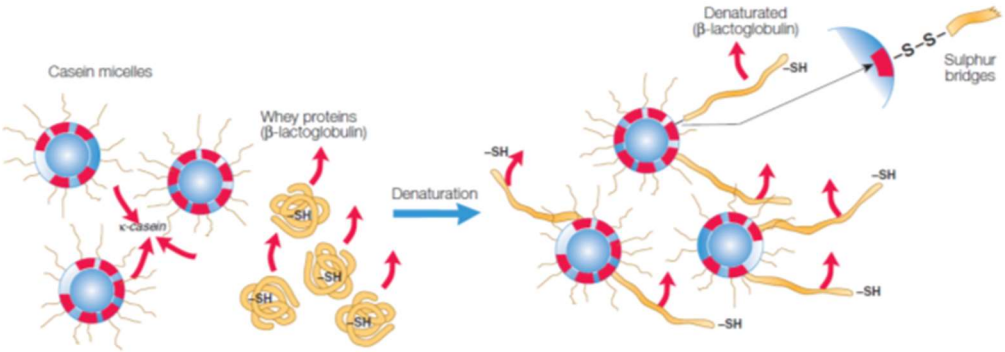

Figure 2.1 Denaturation and attachment of beta-lactoglobulin to casein micelles during yogurt making (Tetra Pak, 2015)

Whey and whey-like products were historically considered as waste; however, they can present a huge environmental concern due to their high organic pollutants content, which can lead to algal bloom and depletion of oxygen in water streams (Erickson, 2017). The average BOD_5 for most whey products is around 40,000 mg/L (Jelen, 2011), which is about 30 times the effluent limitation guidelines for cultured dairy products and 130 times those for cheese products (CFR, 2017).

Sweet whey is considered more valuable than its acid counterpart due to its higher protein content and pH, especially since it can be turned into protein powders targeted mainly towards the sports nutrition niche, in a process that is already well-stablished. Acid whey, on the other hand, is still constrained to low added-value applications and most producers have yet to find an economically feasible way to incorpo-

rate it into higher-end products. A few companies and researchers are already developing solutions with that goal in mind, with mild success (Arla Foods Ingredients, 2017; Erickson, 2017).

New York State is a big player in the national dairy industry, leading the manufacturing of cream cheese and Greek-style yogurt. In 2012, the state produced about 300 thousand tons of the latter (DEC, 2012). Companies with production sites in NY include the likes of Chobani, Dannon, Kraft, Fage, HP Hood, Byrne Dairy, O-AT-KA, Upstate Farms, Alpina Foods, and many more. In a survey conducted with 11 New York dairy producers by Cornell Dairy Extension (refer to APPENDIX A), 93% of them stated that acid whey is a byproduct with actual or potential value. However, there seems to be a lack of information regarding the composition and other physicochemical parameters of acid whey: 36% of them stated they do not conduct any kind of analyses in the material, and most of the others only do a few simple assays such as total solids or crude protein content.

Therefore, the main objective of this section is to provide a detailed composition panel of acid whey from both Greek-style yogurt and cottage cheese, as well of milk permeate, and to make comparisons between these coproducts. We expect that this database would help both producers and researchers make a more informed decision regarding the potential applications and processing of acid whey in the future.

2.3 MATERIALS AND METHODS

Sample Collection and Analytical Strategy

The following dairy byproducts were collected and analyzed in triplicate:

- Acid Whey from Greek-style Yogurt (***GAW***): Byrne Dairy – Cortland, NY. Dates of collection: 09/10/15, 02/16/16, and 02/17/16;

- Acid Whey from Greek-style Yogurt (***GAW***): Upstate Farms – West Seneca, NY. Dates of collection: 09/10/16, 02/15/16, and 02/17/16;

- Acid Whey from Cottage Cheese (***CAW***): Upstate Farms – West Seneca, NY. Dates of collection: 09/10/16, 02/15/16, and 02/16/16;

- Milk UF Permeate (***MP***): OATKA – Batavia, NY. Dates of collection: 09/10/16, 02/14/16, and 02/15/16.

Table 2.1 describes the assays performed for the first batch of products collected, the laboratories responsible, and the methodologies employed (AOAC, 1995; ASTM, 1995; EPA, 1978). Some of the methodologies will be detailed later in this section. After the results of the first batch were obtained, the number of analyses was reduced to just the most critical ones for the second batch, indicated in bold in **Table 2.1**. Another change was the laboratory responsible for BOD and COD assays, which switched from Certified Environmental Services to Community Science Institute (Ithaca, NY) for logistics reasons. Finally, the metal screening was reduced to just Ca, Na, P, K, and Mg.

Table 2.1 Methodology employed and laboratories responsible for each analysis

Test Analysis	Handling	Lab	Test Method
Acidity, Titratable (Lactic)	Samples	Medal-	AOAC 942.15, 962.12; 984.24
Amino Acid Profile (Acid hydrolyzed: Alanine, Arginine, Aspartic Acid, Glutamic	were kept	lion	AOAC 994.12 HPLC - UV
Acid, Glycine, Histidine, Isoleucine, Leucine, Lysine, Phenylalanine, Proline, Ser-	in vials	Labs	
ine, Threonine, Tyrosine, and Valine)	under re-	Minne-	
Amino Acid Profile (Cysteine & Methionine)	frigeration	apolis,	AOAC 994.12 HPLC - UV
Amino Acid Profile (Free Amino Acids)	and then	MN	AOAC 994.12 HPLC - UV
Amino Acids (Tryptophan)	shipped		AOAC 994.12 HPLC - UV
Ash	overnight		AOAC 923.03 Ash of flour Overnight (16h)
Chloride	to the		AOAC 915.01 - chloride in plants
Fat Analysis	lab's loca-		Roese-Gottlieb Method
Fat Analysis by GC with FA Profile (C40C24)	tion on		AOAC 996.06
Metals Screen II (**Ca**, Fe, **Na**, Cu, **K, Mg**, Mn, **P** Zn)	wet ice		AOAC 2011.14
Moisture (Vacuum oven @70°C/16 hours)			AOAC 925.09 with modifications
Nitrogen, Non-Protein			AOAC 991.21
Nitrogen, Total (no factor)			AACC 46-30; AOAC 992.15
Organic Acids (citric, acetic, glutaric, lactic, malic, oxalic, quinic, succinic, tartaric)			AOAC 986.13 via HPLC
pH (direct)			AACC 02-52; AOAC 943.02
Resistant Oligosaccharides with total soluble, and insoluble dietary fiber			AOAC 2001.03 & AOAC 991.43
Sugars by HPLC (Fructose, Glucose, Lactose, Galactose, Maltose & Sucrose)			AOAC 977.20 - HPLC - RI Detection
Vitamin A (Retinol, Concentrate)			AOAC 2005.07- HPLC-UV/VIS
Vitamin B9 (Folic Acid - Folate); Total IU			AOAC 2011.06 - UH Performance LC
Vitamin B3 (Niacin)			AOAC 944.13; AOAC 960.46
Vitamin B1 (Thiamine)			AOAC 942.23; AOAC 970.65; AOAC 981.15
Vitamin B2 (Riboflavin)			AOAC 942.23; AOAC 970.65; AOAC 981.15
Vitamin B12 (Cyanocobalamin)			AOAC 952.20; AOAC 986.23
Vitamin B6 (Pyridoxine)			AOAC 961.15; AOAC 985.32; AOAC 960.46
Vitamin B5 (Pantothenic Acid)			AOAC 945.74; AOAC 960.46; AOAC 992.07
Vitamin C			AOAC 967.22 with modifications and 984.26
Vitamin D			AOAC 2002.05 with modifications

Table 2.1 (continued)

Test Analysis	Handling	Lab	Test Method
Total Nitrogen - Ammonia, Nitrate, Urea, Organic	Samples were kept in vials and delivered the same date on wet ice	Dairy One Ithaca, NY	*Methodology described below*
COD*	Samples were kept in vials and delivered the same date on wet ice	Certified Environmental Services Syracuse, NY	ASTM 5220C
BOD*			ASTM 5210B
Ortho-Phosphorus			EPA 365.3 - Need to sample within 24 h
Alpha-lactalbumin, Beta-lactoglobulin, Alpha-S1-casein, Alpha-S2-casein, Beta-casein, Gamma-casein, Kappa-casein, Total Casein, Other Peptides, Total Low Molecular Weight	Samples were pasteurized (63°C for 30min) and frozen overnight, then shipped on dry ice	South Dakota State University (SDSU) – Metzger Lab Brookings, SD	*Methodology described below*

* BOD: Biochemical Oxygen Demand; COD: Chemical Oxygen Demand

Since some of the protein assays conducted by Medallion Labs were below the limit of detection, total crude protein in mg/g was later calculated by (total nitrogen/1000) x 6.38. The contents of individual protein fractions, such as alpha-lactalbumin and beta-lactoglobulin, were then calculated by multiplying the percentages present in these result tables by the total crude protein previously obtained. The other methodologies used are briefly described below:

Nitrates %NO3 or ppm NO3-N (conducted at Dairy One, Ithaca, NY)

RQflex® Reflectometer Method: 1g of dried, ground sample or 10g of wet sample is extracted in 50 mL deionized water for 20 minutes by shaking at 280 oscillations/minute. Samples are filtered through Whatman 934-AH (1.5 μm) filter paper, then analyzed by RQflex® Reflectometer using Reflectoquant® Nitrate test strips. When the Nitrate test strip is immersed in the aqueous sample, a reducing agent reduces nitrate ions to nitrite ions. In the presence of an acidic buffer, the nitrite ions react with an aromatic amine to form a diazonium salt. The salt reacts with N-(1-naphthyl)-ethylene-diamine to form a red-violet azo dye that is measured reflectometrically. Nitrate concentration is proportional to the color reaction. Each strip contains two reaction zones generating dual replicate analyses per sample. The RQflex® Reflectometer's double optic system measures the analyte concentration based on the light reflected from the dual reaction zones. Barcode-controlled software calculates the mean of those two measurements (EMD Chemicals Inc., Philadelphia, PA).

Ammonia Crude Protein Equivalent (CPE) or Ammonium-N (Dairy One, Ithaca, NY)

Timberline TL-2800 Analyzer (Timberline Instruments, Boulder, CO). Extraction: Samples are extracted in deionized water using a single speed blender at 20,000 rpm for 2 minutes (50 g / 750 mL) or a reciprocal shaker for 30 minutes at 280 rpm (Forage – 5 g / 100 mL wet or 1 g / 100 mL dry). For urea, a prepared urease solution is added to a duplicate sample prior to shaking (5 g / 100 mL wet or 1 g / 100 mL dry). All extracts are then centrifuged at 4000 rpm for 5 minutes, decanted into tubes, then analyzed. Analysis: A peristaltic pump directs the sample, caustic, and absorbing solutions into a diffusion cell. Within the cell, the sample is mixed with the caustic solution, resulting in a pH of 11-13 which converts the ammonium ion present in the sample to dissolved ammonia gas. The sample/caustic solution flows past one side of a membrane that is permeable to gases but not to liquids nor ionic species. The dissolved ammonia gas in the sample/caustic mixture diffuses across the membrane. On the other side of the membrane, a buffered solution absorbs the diffused ammonia gas then flows through a low volume heat exchanger to establish thermal equilibrium then into the conductivity detector. The conductivity cell measures the change in electrical conductance of the absorbing solution. This change is proportional to the concentration of ammonium in the original sample (Carlson, 1978; Kalra & Soil and Plant Analysis Council., 1998).

Urea CPE (conducted at Dairy One, Ithaca, NY)

Analyzed as above in Ammonia CPE after addition of prepared urease enzyme solution, using the Timberline TL-2800 Analyzer.

Capillary Gel Electrophoresis (conducted by SDSU)

The individual protein fractions present in the filtrate were determined using Capillary gel electrophoresis (CGE). A 10 µL sample was mixed with 85 µL of sample buffer (Beckman- Coulter) and 5 µL of β-mercaptoethanol in a micro-vial. Each micro-vial was capped tightly, mixed thoroughly and then heated in a water bath at 90°C for 10 min and then cooled to room temperature prior to injection. The CGE was carried out using a Beckman P/ACE MDQ capillary electrophoresis system (Beckman-Coulter, Fullerton, CA, USA) equipped with a UV detector set at 214 nm. The separation was performed using a 50-µm bare fused silica capillary (20.2 cm effective length from the inlet to the detection window). All solutions and reagents were obtained as a part of the ProteomeLab^{TM} SDS-MW Analysis Kit (Beckman-Coulter) that is designed for the separation of protein-SDS complexes using a replaceable gel matrix. The gel is formulated to provide an effective sieving range of approximately 10–225 kDa. A capillary preconditioning method was run every three samples. It consisted of a basic rinse (0.1 N NaOH, 5 min, 345 kPa), followed by an acidic rinse (0.1 N HCl, 2 min, 345 kPa), a water rinse (HPLC grade water, 2 min, 345 kPa) and finally an SDS Gel rinse (SDS gel fill, 10 min, 275 kPa). After the preconditioning steps, the sample was electrokinetically introduced at 5 kV for 20 s. The separation was performed at a constant voltage of 15 kV (25 °C temperature and 20 bar pressure) with reverse polarity in the SDS-molecular weight gel buffer. Actual current values were recorded to determine the efficiency of each electrophoretic run. Molecular weight standards (ProteomeLab and Beckman-Coulter) and available pure milk protein fractions (Sigma, USA) were also separated using the method as described above to determine migration

times. The peaks in the capillary electropherogram were identified by comparing the migration time of molecular weight standards and pure standard samples as well as by comparison to results reported by other researchers (Anema, 2009; Creamer & Richardson, 1984; Miralles, Ramos, & Amigo, 2000). The area of each identified peak was calculated from the electropherogram using a valley-to valley approach as described by Miralles et al. (2000). The area of the each identified individual casein (CN) fraction (αS1-CN, αS2-CN, β-CN, κ-CN and γ-CN), serum protein (SP) fraction (ALA, BLG, and peptides (peaks between 10 kDa and 20 kDa)), and non-protein nitrogen (NPN) fraction (all positive peaks below 10 kDa) was calculated as a percentage of total area (positive peaks).

Replication and Statistical Analyses

All analyses were completed in triplicate. Data were analyzed using R Studio (2015). Statistical differences among observed means were determined using an unpaired t-test with a significance level $\alpha = 0.05$.

2.4 RESULTS AND DISCUSSION

The complete results for the analyses done are presented in **Table B.1** and **Table B.2** in APPENDIX B. **Table 2.2** shows a summarized version, as well as a comparison with sweet whey for some selected parameters. Some highlights will be pointed out and discussed in the following paragraph.

Crude protein ranged from 1.71 to 3.71 mg/g for GAW (both producers), 1.65 to 5.05 mg/g for CAW, and 3.2 to 4.35 mg/g for MP. For pH, the corresponding ranges were 4.21 to 4.48, 4.35 to 4.41, and 5.4 to 6.37. COD varied from 52,400 to

64,400 mg/L for GAW, 31,900 to 40,000 mg/L for CAW, and 127,000 to 142,000 mg/L for MP; whereas for BOD the ranges were 45,800 to 50,500 mg/L, 32,700 to 40,000 mg/L, and 110,000 to 182,000 mg/L, respectively. The BOD ranges for GAW are over 30 times the effluent limitations for cultured dairy products (CFR, 2017).

Table 2.2 Average composition and physicochemical properties of milk permeate (MP) and acid whey from GSY (GAW) and from cottage cheese (CAW). Selected Sweet Whey (SW) components are provided for comparison purposes. Samples were collected from three companies in NY state, from October 2015 to February 2016

Component	GAW	CAW	MP	SW
Moisture	94.23%	93.08%	86%	91.81%[1]
Crude Protein	0.17% - 0.37%[a]	0.17% - 0.51%[a]	0.32% - 0.43%[a]	0.6% - 1.0%[2]
ALA*	170 - 770 mg/kg[a]	220 - 710 mg/kg[a]	520 - 1620 mg/kg[a]	
BLG*	0 - 200 mg/kg[a]	830 - 2110 mg/kg[b]	0 - 1180 mg/kg[a,b]	
Lactose	3.33% - 3.5%[a]	1.99% - 2.13%[b]	10.6% - 11.9%[c]	4.97%[1]
Ash	0.64% - 0.75%[a]	0.33% - 0.42%[b]	1.13% - 1.25%[c]	0.51%[1]
Ca	1200 - 1280 mg/kg[a]	680 - 710 mg/kg[b]	960 - 1060 mg/kg[c]	400 - 600 mg/kg[2]
P	670 - 690 mg/kg[a]	460 - 490 mg/kg[b]	1000 - 1130 mg/kg[c]	100 - 300 mg/kg[2]
pH	4.21 - 4.48[a]	4.35 - 4.41[a]	5.4 - 6.37[b]	6.29[1]
BOD*	45.8 - 50.5 mg/g[a]	32.7 - 40 mg/g[b]	110 - 182 mg/g[c]	32 mg/g[1]
COD*	52.4 - 64.4 mg/g[a]	31.9 - 40 mg/g[b]	127 - 142 mg/g[c]	

*ALA: α-lactalbumin; BLG: β-lactoglobulin; BOD: Biochemical Oxygen Demand; COD: Chemical Oxygen Demand

[a-c]Different superscripts in the same row indicate difference is statistically significant

[1](Huma, Pasha, Sarwar, Ahmad, & Shah, 2015); [2](Jelen, 2011)

The concentration of alpha-lactalbumin was found to be very low in GAW, ranging from 0.17 to 0.77 mg/g. Interestingly, this concentration was not found to be statistically different from those of CAW and MP, and neither were the crude protein contents ($p > 0.05$). Besides being very small, the concentration of alpha-lactalbumin in the coproduct was shown to present a lot of variability (CV = 99%), which is also true for the total amount of crude protein (CV = 29%). This low concentration and high variability, associated with low pH and high mineral content (Ca ranged from 120 mg/100g to 128 mg/100g, for instance), renders the product processing and usage very challenging. (Cui & Muralidhara, 2010)

2.5 CONCLUSIONS

Acid whey from Greek-style yogurt (GAW) is a coproduct of interest in the dairy industry due to the recent boom in the production of GSY and the potential negative environmental impact of its whey disposal. While GAW is generally considered a less valuable stream compared to other dairy coproducts such as sweet whey, it contains highly valuable components that could potentially be extracted and purified.

This study provides a detailed composition panel of GAW that could help dairy producers make more informed decisions about how to handle this challenging coproduct. Based on these results, the next chapters of this dissertation will focus on nonthermal methods for processing acid whey, namely membrane fractionation and concentration.

2.6 ACKNOWLEDGEMENTS

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CHAPTER THREE.

FEASIBILITY OF A MEMBRANE FRACTIONATION STRATEGY FOR ISOLATION OF ALPHA-LACTALBUMIN FROM GREEK-STYLE YOGURT ACID WHEY

3.1 ABSTRACT

Acid whey from Greek-style yogurt (GAW) is a coproduct of interest in the dairy industry. In this chapter, the feasibility of a membrane separation strategy for the fractionation of alpha-lactalbumin (ALA) from GAW was tested. This included two preliminary sequential pre-filtration steps using 1.4μm and 0.2μm pore microfiltration (MF) membranes, followed by ultrafiltration (UF) of the MF filtrate (permeate) using a 15 kDa UF membrane. Flux and composition of the UF concentrate were evaluated.

Fractionation of ALA was shown to be possible, but only feasible when there is enough protein in GAW. Several samples studied had minimal protein content, and therefore their fractionation by membrane filtration was deemed unfeasible. The permeate fluxes decreased from 43.2 L/m^{2}h to 17.7 L/m^{2}h after 8h of UF.

3.2 INTRODUCTION

Membrane technologies have a wide array of applications in the food industry, including cold sterilization, clarification, desalting, concentration, fractionation, product recovery, and dealcoholization (Cui & Muralidhara, 2010). For the dairy industry in particular, pressure-driven processes such as reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF), and microfiltration (MF) are well-established practices. **Figure 3.1** below depicts the relationship between these processes and the main milk components in terms of their particle sizes and average molecular weight.

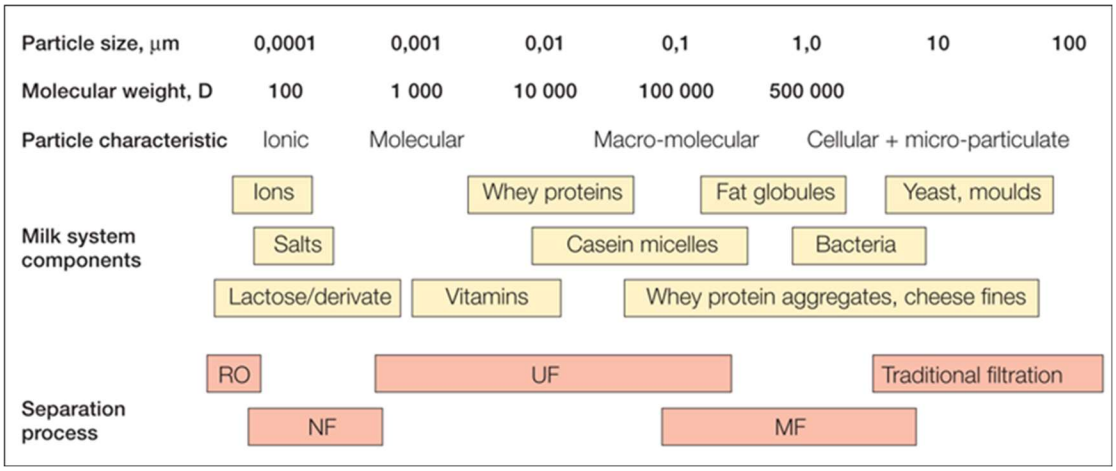

Figure 3.1 Applications of pressure-driven membrane processes in the dairy industry (Tetra Pak, 2003)

Microfiltration (MF) has two major applications in dairy processing: a preliminary step in the production of extended shelf life (ESL) milk through the removal of somatic cells and bacteria and the separation of micellar casein concentrate (MCC) from serum proteins (SP). Other emerging usages include separation of fat from whole milk and buttermilk as an alternative to centrifugation and the fractionation of milk bioactive compounds (Hu, Dickson, & Kentish, 2015; Pruksasri, 2015; Tomasula &

Bonnaillie, 2015). ***Ultrafiltration (UF)*** is also widely used, mostly for concentration and/or fractionation of components such as lactose and serum proteins (Etzel & Arunkumar, 2015; Pruksasri, 2015). UF can also be combined with other processes, such as diafiltration, for the production of low lactose skim milk (Solanki & Gupta, 2014). Regarding whey, previous studies have used ultrafiltration, dialysis, and egg membranes to concentrate the serum proteins from sweet and acid whey, with UF yielding the best results (Huma et al., 2015). Combinations of ultrafiltration and ***nano-filtration (NF)*** have also been used for the concentration of SP and lactose from cheese whey and milk, yielding high fluxes and retentions (Atra, Vatai, Bekassy-Molnar, & Balint, 2005).

Membranes for MF and UF come in a plethora of sizes, configurations, and materials. Compared to polymeric membranes, ceramic membranes present higher mechanical, thermal and chemical tolerances, as well as a longer lifetime, even though they are more expensive. Regarding configurations, multitubular modules have the advantages of being able to handle relatively large particles and easy cleanability by physical and chemical methods (Cui & Muralidhara, 2010).

One of the main challenges in MF and UF is ***membrane fouling***, caused by the deposition and adsorption of particles at the membrane surface or within its pores, leading to a decrease in permeate flux. Factors that affect the degree and the type of fouling include membrane material, feed composition, and operating conditions. When the particle size of the feed components is comparable with that of the membrane pores, complete (**Figure 3.2a**) or partial (**Figure 3.2b**) pore blockage may occur; when they are significantly larger, they can form a cake layer onto the membrane surface

(**Figure 3.2c**). Smaller solutes might get adsorbed onto the internal membrane channels causing pore constriction (**Figure 3.2d**), thus reducing the effective diameter of the pores (De Barros, Andrade, Mendes, & Peres, 2003). In membrane filtration of dairy streams, the main foulants are microorganisms, fats, proteins (casein and SP), lactose, and minerals such as calcium, magnesium, and phosphate (Cui & Muralidhara, 2010).

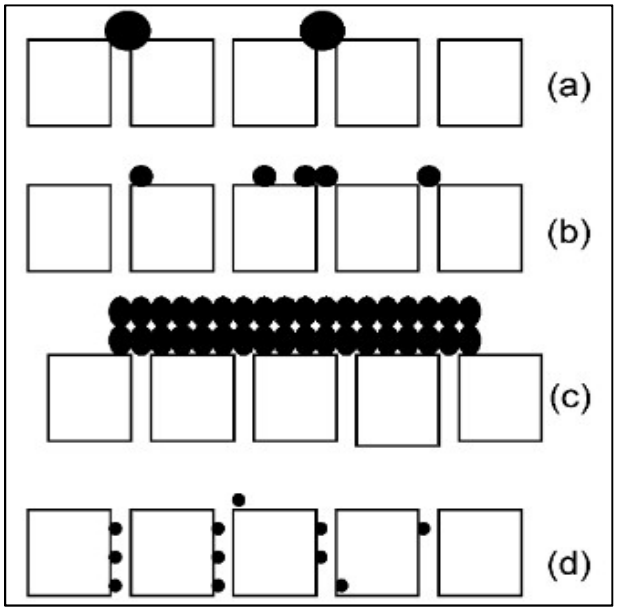

Figure 3.2 Membrane fouling mechanisms: a) complete pore blockage; b) partial pore blockage; c) cake formation; d) pore constriction (De Barros et al., 2003)

The two major serum proteins found in bovine milk are beta-lactoglobulin (BLG) and alpha-lactalbumin (ALA), which amount to 50% and 20% of all whey proteins (or 12 and 3.5% of all milk proteins), respectively (McSweeney & Fox, 2013). In human milk, however, there is no BLG, and this protein is a major allergen for infants (Bonnaillie & Tomasula, 2012). This is why ALA is preferred for applications such as baby foods or clinical nutrition. Besides having great nutritional value, ALA has been

reported to play a role in lactose synthesis and the apoptosis of tumor cells (McSweeney & Fox, 2013).

During the processing of Greek-style yogurt, the extended heat-treatment step causes BLG to denature, exposing reactive sites that can bind to the casein micelles in the curd, which is later strained (Gyawali & Ibrahim, 2016). Therefore, acid whey is depleted from BLG, having ALA as its major protein, and this presents an interesting fractionation opportunity. ALA has a nominal molecular weight of 14 kDa, but it is believed to occur as a dimer in the conditions present in GAW, which means that it could be retained by UF membranes with a molecular weight cut-off smaller than 25 kDa (Huma et al., 2015).

Based on the GAW protein composition presented in Chapter Two, the fractionation of ALA from GAW using a combination of MF and UF is investigated in this chapter.

3.3 MATERIALS AND METHODS

MF and UF Processing

Skim GAW was obtained from Byrne Dairy (Cortland, NY) and stored under refrigeration conditions (5 ± 1 °C) until usage for a maximum of two weeks. 180 L of the sample were pre-filtered using Whatman paper filters #41 to remove coarse particles such as pieces of curd and dirt that could cause fouling and damage to the filtration equipment. Then, the sample went through a series of MF and UF steps, as depicted in **Figure 0.2:** initially, a 1.4 μm MF membrane was used to retain microorganisms, followed by a 0.2 μm MF to remove traces of fat and/or casein that might still be

present; finally, the clarified acid whey went through a 15 kDa UF step, yielding a serum protein retentate and a permeate containing mostly lactose and minerals. For comparison purposes, UF of sweet whey obtained from Cornell Dairy (Ithaca, NY) was performed under similar conditions (minus pretreatment) as for acid whey.

All membranes used were monolithic multitubular ceramic membranes of Tami design (GEA Filtration; Hudson, WI) with an outside diameter of 25 mm and a length of 1,200 mm. The MF membranes were Isoflux® type with 23 internal channels of 3.5 mm hydraulic diameter each and a filtration area of 0.35 m^2. For the UF, an InsideCéram® membrane with 19 internal channels of 3.5 mm hydraulic diameter each and a filtration area of 0.25 m^2 was used. **Figure 3.3** illustrates the type of membrane used in these experiments. A picture of the batch pilot-scale filtration unit used and a schematic of the process are shown in **Figure 3.4**.

Figure 3.3 Monolithic multitubular ceramic membranes (Tami Industries, 2017)

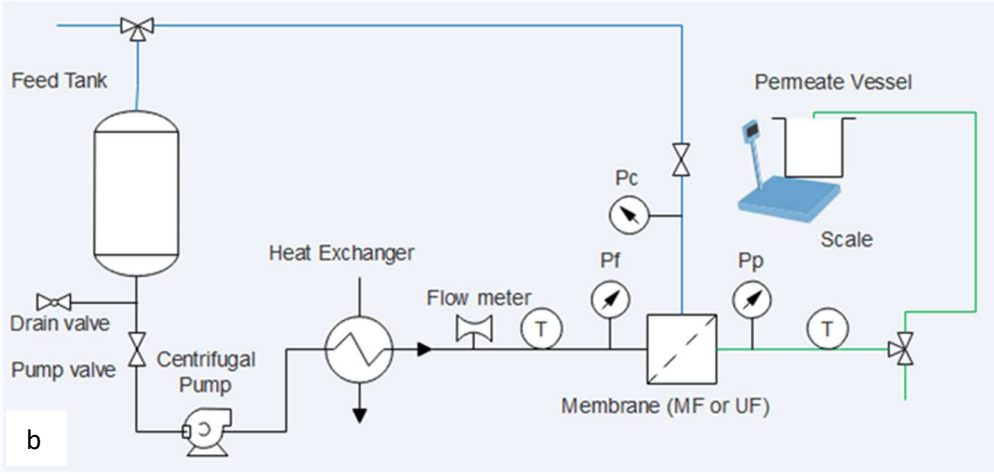

Figure 3.4 a) Picture and b) schematic of the membrane filtration rig

Using a centrifugal pump (Fristam Model FPR 1742 type 316L; Middleton, WI), GAW was pumped from a 200 L feed tank through a countercurrent shell-and-tube heat exchanger in order for the temperature to be kept at 10 ± 1 °C. The product then reached the membrane, from which the permeate started to be collected after the initial 2 min in a vessel on top of a scale that automatically logged mass readings

every 5 s. The concentrate was constantly recirculated into the feed tank in a closed loop. A concentrate valve on the outlet of the membrane was used to adjust transmembrane pressure. There were also drain valves for the feed, the concentrate, and the permeate. The setup also included instruments for measuring the process parameters: thermocouples in the membrane inlet and outlet, pressure gauges for the feed, concentrate, and permeate flows, and a flow meter placed after the heat exchanger.

The processing conditions used for the 1.4 μm MF, 0.2 μm MF, and 15 kDa UF were, respectively: transmembrane pressures (TMP) of 158, 165, and 255 kPa and cross-flow velocities of 4.3, 4.6, and 5.2 m/s. The duration of each experiment was 1.5 h. Permeate flux, TMP, and cross-flow velocities were calculated according to equations (3-1), (3-2), and (3-3), respectively:

$$J = \frac{m}{A \times t \times \rho} \tag{3-1}$$

Where: J is the permeate flux in L/m^{2}h, m is the cumulative mass collected in kg, A is the membrane filtration area in m^2, t is the time elapsed in h, and ρ is the density of the permeate in kg/L. Since the permeate is mostly water, density was taken as 1 kg/L, and this was later verified experimentally.

$$TMP = \frac{(P_1 + P_2)}{2} - P_p \tag{3-2}$$

Where: TMP is the transmembrane pressure, P_1 is the feed pressure, P_2 is the concentrate pressure, and P_p is the permeate pressure (all values in kPa).

$$v = \frac{4Q}{\pi D^2 n} \tag{3-3}$$

Where: v is the cross-flow velocity in m/s, Q is the flow rate in m^3/s, D is the channel diameter in m, and n is the number of channels.

Membrane Cleaning Procedure

After each experimental run, the filtration rig went through the following cleaning cycle to regenerate the permeability of the membrane: an RO water rinse for 10 min, followed by a caustic wash with Ultrasil-25 (Ecolab, Saint Paul, MN) 20g/L at 80 °C, another 10-minute RO rinse, then an acid wash with HNO_3 5g/L at 50 °C, and a final RO water rinse for 10 min. In order to verify if the membrane had been properly regenerated, water fluxes were determined before and after the experiments, and cleaning was considered effective if the latter was 95% or more of the former.

Composition Analyses

The raw material, the pre-filtered whey, and the permeates and concentrates of each filtration step were analyzed for moisture, total solids, fat, ash, protein and protein profile, minerals, BOD, COD, titrable acidity, and pH, as described in Menchik et al. (2019) and **Section 2.3**.

Particle Size Analysis

Particle size distribution was determined by dynamic light scattering, using a Brookhaven 90Plus Particle Size Analyzer equipped with a Peltier temperature control system (Brookhaven Instruments Corporation, Holtsville, NY). Measurements were performed at 20 °C, a fixed angle of 90°, and a wavelength of 658 nm. Data collection and analysis were performed using the BIC software (Brookhaven Instruments Corp., Holtsville, NY). The dust filter cut-off was set at 30 to reject random contaminating particles such as air bubbles or dust. No dilutions were made to the samples. Each

measurement consisted of 8 individual runs with a duration of 30 s each. The relative particle size distribution and the intensity weighted effective diameter were determined for each sample.

Statistical Analyses

All experiments were conducted in triplicate. Data were analyzed using R Studio (2015). Statistical differences among observed means were determined using an unpaired t-test with a significance level $\alpha = 0.05$.

3.4 RESULTS AND DISCUSSION

Flux Analysis

Figure 3.5 contains a plot of the UF permeate fluxes vs time. For GAW (black), the initial flux was 43.2 L/m^{2}h but dropped to 17.7 L/m^{2}h after 1.6 h due to fouling. These flux values are considerably smaller as compared to the flux for the UF of sweet whey (blue) under similar conditions (70.9 L/m^{2}h to 48 L/m^{2}h after 1h of processing). This indicates that GAW is more prone to membrane fouling than sweet whey, which means that anti-fouling strategies are required. Minerals are believed to play a critical role in membrane fouling, acting as a bridge that binds membrane pores to whey proteins (Kulozik & Kessler, 1988a; Meyer & Kulozik, 2016), which could explain the difference observed.

Composition of the Membrane Filtration Products

The full composition data for fresh and pre-filtered GAW, as well as for the permeates and the concentrates of the 1.4 µm MF, the 0.2 µm MF, and the 15 kDa UF

are presented in **Table C.1** in APPENDIX C. A summarized version with selected components is shown in **Table 3.1**, and some highlights will be discussed.

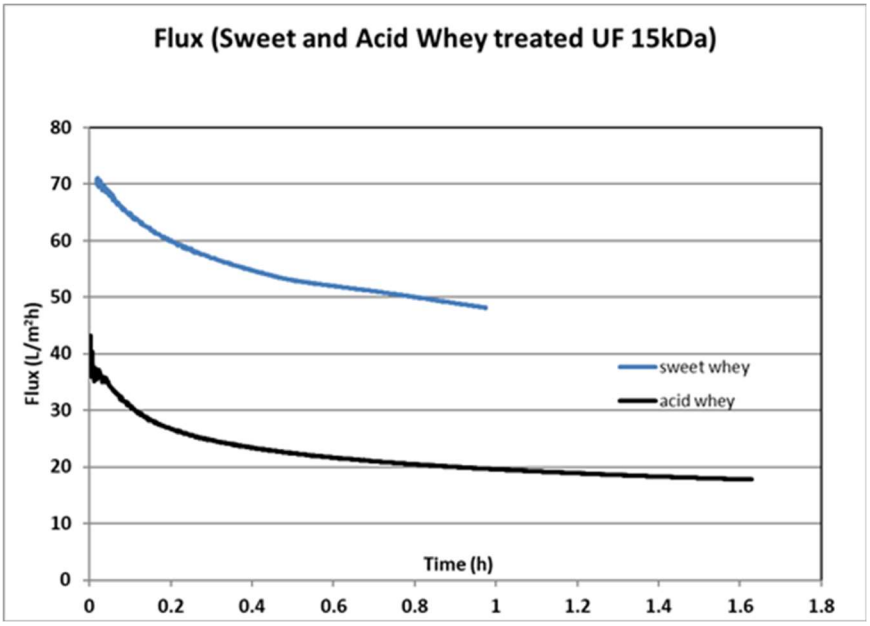

Figure 3.5 Permeate flux behavior during UF of GAW and sweet whey

Table 3.1 Composition and physicochemical properties of acid whey from GSY (GAW), the permeates after each inter-mediate microfiltration (MF) step, and both streams following the ultrafiltration (UF) step. All values are given in mg/g.

Component	Fresh GAW	1.4 µm MF Permeate	0.2 µm MF Permeate	15 kDa UF Permeate	15 kDa UF Concentrate
Total solids	57.00	59.00	57.00	50.00	58.00
total sugars	33.60	36.00	35.50	30.50	36.50
lactose	30.00	31.60	31.10	26.10	31.90
total protein	4.40	4.10	3.38	2.36	3.28
alpha-lactalbumin	0.86	0.70	0.42	0.00	0.50
beta-lactoglobulin	0.15	0.08	0.00	0.00	0.00
ash	6.84	6.56	6.80	5.88	6.54
Ca	1.20	1.20	1.16	1.12	1.18
Titrable acidity (lactic acid)	5.42	4.58	4.50	4.25	4.52
pH	4.47	4.58	4.57	4.57	4.58
COD	64.8	58	71.6	48	60
BOD	30.8	34.2	33.5	31.7	33.7

There were 4.4 mg/g of crude protein and 0.86 mg/g of alpha-lactalbumin in the feed (fresh GAW), which are slightly above than the ranges reported previously in Chapter Two. The concentration of the other components was very consistent with previous findings. Fractionation can be considered successful when looking at the UF streams: all the ALA was found in the concentrate (0.5 mg/g) and none in the permeate after the final step. Also, there was no BLG in any of the UF streams. The other components analyzed did not show much variation among streams since the experimental runs were not long enough to produce significant concentration differences.

Particle Size Data

Figure 3.6 depicts the results of the particle size analyses for all fractionation streams. In the 1.4 μm MF concentrate, the two peaks roughly correspond to larger casein micelles and fat globules (~500 nm) and bacteria (~2000 nm). For the 1.4 μm MF permeate, the peaks can be assigned to two different populations of casein micelles (~70 nm and ~300 nm), similar to the classes of particles observed in the 0.2 μm MF concentrate. The peaks shown in the 0.2 μm MF permeate likely represent whey proteins (~30 nm) and some smaller casein micelles (~100 nm), analogous to the classes of particles found in the 15 kDa UF concentrate. Finally, the main 15 kDa UF permeate peak (<1 nm) likely corresponds to lactose (**Figure 3.1**).

3.5 CONCLUSIONS

The fractionation of alpha-lactalbumin from GAW using a series of microfiltration and ultrafiltration steps is feasible, but only possible when there is enough whey protein in the raw material. As described in **Section 2.4**, this is not always the case since

the concentration of alpha-lactalbumin in GAW is often very low, and also shows much variability. Another caveat is the low permeate flux during the UF of acid whey when compared with sweet whey, which demonstrates the need for anti-fouling pretreatment strategies.

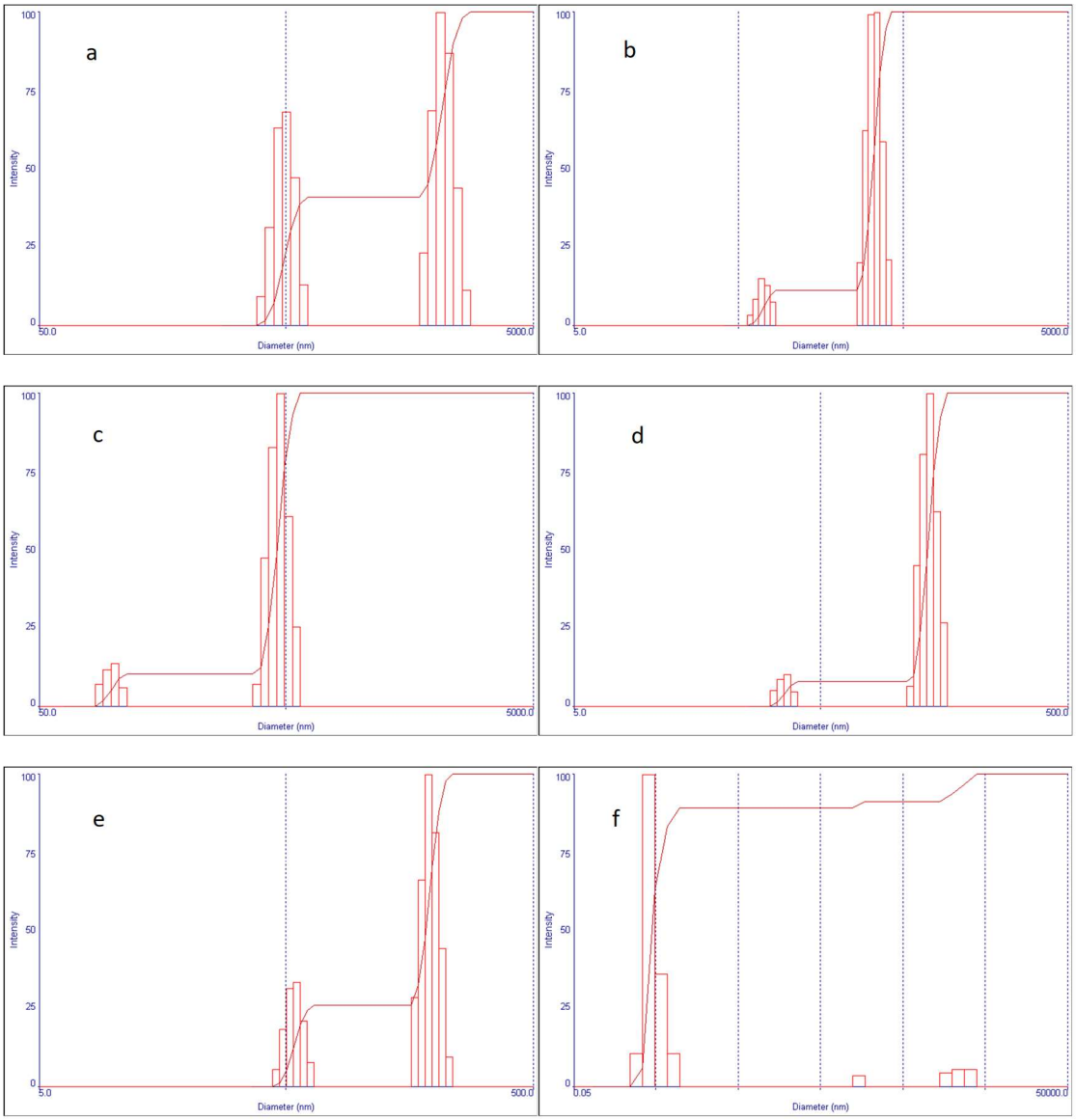

Figure 3.6 Particle size distribution for: a) 1.4 µm MF concentrate, b) 1.4 µm MF permeate, c) 0.2 µm MF concentrate, d) 0.2 µm MF permeate, e) 15 kDa UF concentrate, and f) 15 UF kDa permeate

3.6 ACKNOWLEDGMENTS

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CHAPTER FOUR.

NONTHERMAL CONCENTRATION OF LIQUID FOODS BY A COMBINATION OF REVERSE OSMOSIS AND FORWARD OSMOSIS. ACID WHEY: A CASE STUDY

4.1 ABSTRACT

Acid whey from Greek-style yogurt (GAW) has become abundantly available in recent years. Due to its low solids content, low pH, and high lactose and mineral content, GAW is challenging to transport and process. In this study, a combination of reverse osmosis (RO) and forward osmosis (FO) was developed for the nonthermal concentration of GAW. Permeate flux, retentate concentration, and specific energy were evaluated for the individual and combination processes. GAW (6.6°Brix) was concentrated to 19.6°Brix by RO (3×), then to 40.2°Brix (6×) by FO. During the concentration, permeate fluxes dropped from 33.2 L/m^{2}h to 2.6 L/m^{2}h for RO and from 3.6 L/m^{2}h to 1.6 L/m^{2}h for FO. The specific energy consumption was 0.29 kWh/kg water for RO and 0.65 kWh/kg water for FO, from which half pertains to the osmotic agent regeneration. This combination process could become an efficient nonthermal method for concentrating challenging or sensitive liquid foods and beverages.

4.2 INTRODUCTION

The dairy industry has experienced a boom in the production of Greek-Style Yogurt (**GSY**) in recent years. While in 2004 GSY accounted for less than 2% of all yogurt produced in the US, this number skyrocketed to almost 40% by 2015

(Erickson, 2017). GSY is very appreciated by consumers for its high protein content, which provides appealing nutritional and sensory properties with minimal or no additives. However, the straining step in its processing generates high quantities of Greek yogurt Acid Whey (**GAW**): 2 to 3 kg of GAW are produced for every 1 kg of GSY (Erickson, 2017). If directly disposed of, GAW can have a negative impact on the environment due to its high organic material and mineral content (Huma et al., 2015).

Other coproducts from the dairy industry have found successful utilizations over the years. For example, sweet whey obtained from the manufacturing of rennet cheeses is a valuable source of whey proteins and it is routinely used to make protein powders using well-established processes. GAW, however, has a much lower protein content and it is also more difficult to process than sweet whey due to its low pH, high lactose, and mineral content (Chandrapala et al., 2015b). Current utilizations of GAW are mainly limited to low added-value applications such as irrigation, feed, and energy generation (DEC, 2012). Nonetheless, GAW still contains some milk solids (proteins, lactose, and minerals) that could be used as ingredients in value-added products such as beverages, sauces, snacks, or baked goods (Arla Foods Ingredients, 2017). Due to the low solids level in GAW (around 6%), concentration can facilitate its usage as a food ingredient, further processing, storage, and transportation, by reducing both volume and water activity.

The concentration of liquid foods is typically achieved by thermal evaporation under vacuum, which is an energy-intensive operation that also causes undesirable sensory and nutrition changes in the concentrated products (Chemat et al., 2017). For this reason, nonthermal concentration processes, such as osmotic processes using semi-permeable membranes, have been gaining increasing interest in recent years.

Figure 4.1 illustrates the four main types of osmotic processes: forward osmosis (FO), pressure enhanced osmosis (PEO), pressure retarded osmosis (PRO), and reverse osmosis (RO).

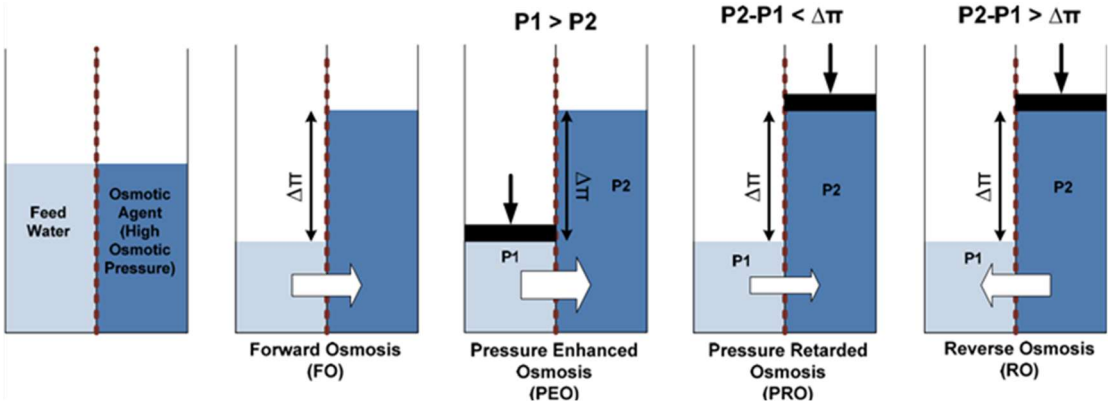

Figure 4.1 The four main types of osmotic processes (Nicoll, 2013)

RO consists of the application of high pressure to a liquid feed, which forces water out of it through a selective membrane. The technology is currently used mostly for desalination, but there are also some applications in the concentration of beverages such as juices, tea, and coffee (Cui & Muralidhara, 2010; Valentas et al., 1997). In the dairy industry, RO is used for the concentration of milk upstream from evaporation, drying, or cheese making, and to concentrate whey to reduce transport and storage costs. It is also employed in the treatment of wastewater from various dairy products processing (Zargar, Jin, & Dai, 2015).

RO is considered less expensive and less energy-intensive than thermal concentration (Valentas et al., 1997; El-Dessouky and Ettouney, 2002). Using desalination as an example, state-of-the-art RO was reported to have a specific energy consumption as low as 2 kWh/m^3 of water removed (Mathematical and Physical Sciences

Advisory Committee & NSF, 2014), while modern efficient thermal concentration has a specific energy consumption ranging between 7.7 and 11.4 kWh/m^3 of water removed (Jamil & Zubair, 2017). Unfortunately, RO itself has several shortcomings, including limited attainable concentration due to concentration polarization and membrane fouling caused by the high transmembrane pressures applied (Rastogi & Nayak, 2011). In RO filtration of dairy streams, the main membrane foulants are microorganisms, fats, proteins (casein and serum proteins), lactose, and minerals such as calcium, magnesium, and phosphate (Cui & Muralidhara, 2010).

FO is a more novel technique than RO and uses a highly-concentrated osmotic agent (OA, also called draw solution) to draw water from the feed through a membrane, based exclusively on the difference in osmotic pressure between the two streams. In FO, little or no applied pressure is involved (Nicoll, 2013). Even though it is not as well-established in the food industry as RO, recent studies report the use of FO for desalination, wastewater treatment, sugar processing, and the concentration of liquid foods such as fruit juices, tomato sauce, and coffee (Cath, Childress, & Elimelech, 2006; Nicoll, 2013; Sant'Anna et al., 2012).

FO is gaining increasing attention as an alternative to both RO and thermal evaporation, since it allows the concentration of sensitive liquid foods to high solid levels, without pre-filtration or significant membrane fouling (Babu, Rastogi, & Raghavarao, 2006; Beaudry & Lampi, 1990; K. Raghavarao, Nagaraj, Patil, Babu, & Niranjan, 2005; Ravindra Babu, Rastogi, & Raghavarao, 2006; Zhao & Zou, 2011; Zhao, Zou, Tang, & Mulcahy, 2012). The downside of FO is that it is a fairly slow process and has significantly lower initial permeate fluxes than RO, which can be an issue particularly for concentrating large volumes of dilute streams (Chun, Mulcahy,

Zou, & Kim, 2017). FO was also reported to require less energy than RO for product pumping, but additional energy is needed to pump and regenerate the osmotic agent utilized in the process (Lee, Boo, Elimelech, & Hong, 2010).

The most common membranes for RO applications are spiral-wound membranes made of either cellulose acetate or polyamide (Zargar et al., 2015) (**Figure 4.2a**). Traditional spiral-wound membranes cannot be used for FO because of spatial constraints; however, modified versions have been adapted specifically for this application (Cath et al., 2006) (**Figure 4.2b**). Cellulose triacetate is a common material choice for FO membranes.

Both RO and FO are also subject to concentration polarization, a reversible phenomenon that arises mostly during the start-up of the concentration process but lingers throughout the entire run. It occurs when the permeate flow causes a build-up of the non-permeable solids at the membrane surface, promoting a concentration gradient, increasing the pressure drop, and hindering water permeation. In RO, it can lead to cake formation and other forms of fouling. However, this is a concern particularly in FO, since it reduces the effective osmotic driving force.

Considering the advantages and disadvantages of both RO and FO, there is a complementarity between them that can be explored. Therefore, the present study focuses on combining RO and FO for achieving a high concentration factor of GAW as a case study of a dilute, challenging fluid. This combination process has numerous potential applications for the nonthermal concentration of a variety of other challenging or thermally sensitive liquid foods and beverages.

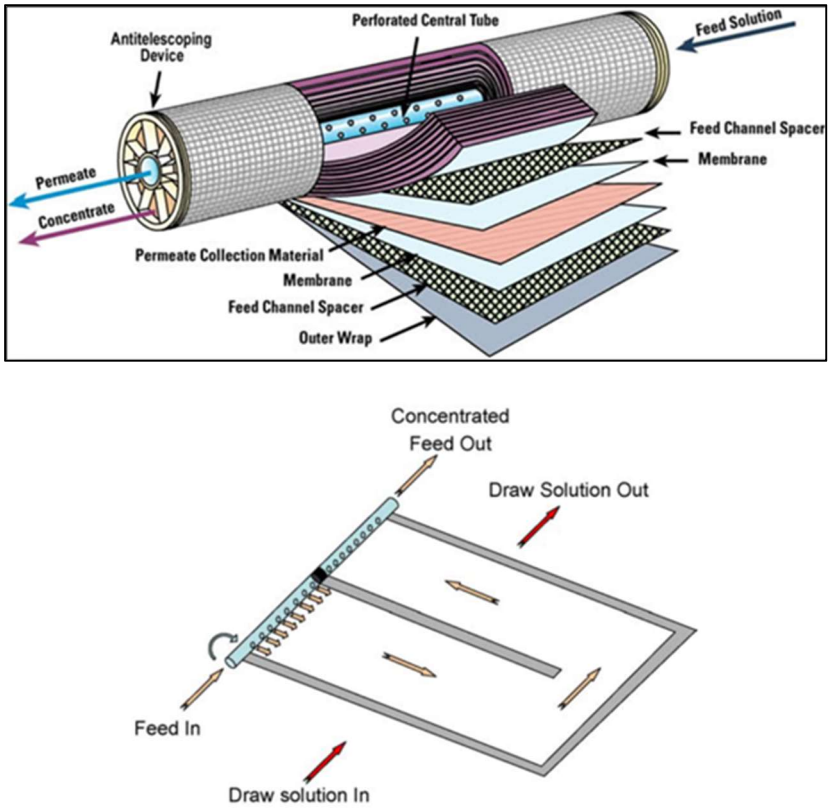

Figure 4.2 **a) Schematic of the spiral-wound membrane used for RO (Lixus Separation Technology, 2013). b) Open-sheet view of a modified spiral-wound module for FO membranes (Cath et al., 2006)**

4.3 MATERIALS AND METHODS

RO Processing

Fat-free GAW, in batches of 150 L, was obtained from Byrne Dairy (Cortland, NY) and stored under refrigeration (5 ± 1 °C) until use, for a maximum of two weeks. RO concentration was conducted using a pilot plant rig (Osmonics, WI), shown in **Figure 4.3.** The system was equipped with a Filmtec spiral-wound aromatic polyamide thin-film composite membrane (model XLE2540, The Dow Chemical Company, Webster, NY), with an outside diameter of 61 mm, a length of 1,016 mm, a spacer thickness of 0.71 mm, and a filtration area of 2.6 m^2 (**Figure 4.4**). The membrane consisted of two separate leaves, each of 850 mm length.

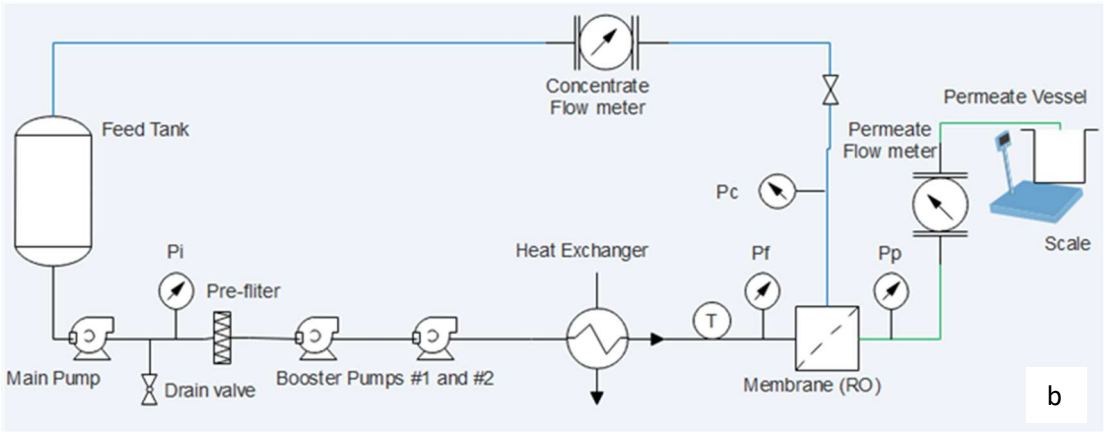

Figure 4.3 a) Picture and b) schematic of the RO rig

Figure 4.4 Spiral wound polymeric membrane used for RO

A centrifugal pump (Tonkaflo SS 1804 - GE, Trevose, PA) was used to pump GAW from the feed tank through an embedded pre-filter cartridge (5 μm cut-off) to remove coarse particles such as pieces of curd. Two booster pumps (Tonkaflo SS 526X 3290 - GE, Trevose, PA) were used to achieve the desired transmembrane pressure (***TMP***) and feed velocity. Next, the product was passed through a countercurrent shell-and-tube heat exchanger to maintain a temperature close to refrigeration during processing (product temperature varied between 5 and 13 °C in all runs), in order to minimize microbial proliferation. After 2 min from the beginning of processing, the permeate (water) was collected in a vessel placed on top of a scale that automatically logged its mass. The retentate was constantly recirculated into the feed tank in a closed loop. A concentrate valve close to the membrane outlet was used to adjust the pressure in the system when needed. The feed tank also had a drain valve. The instruments used to measure the process parameters included a thermocouple at the membrane inlet, pressure gauges on both the feed and concentrate sides, and flow meters for the concentrate and the permeate. The RO process was stopped when the instant fluxes became too small for the permeate scale detection limit.

Initially, 30-min RO concentration runs were performed at 5 different TMPs: 1900, 2450, 2850, 3425, and 3925 kPa, in order to determine the optimal TMP for processing. A chart of initial, final and average fluxes vs time was plotted. The lower TMP value (1900 kPa) corresponds to the minimal TMP that yields a measurable permeate flux, and the higher is slightly below the membrane's upper limit of operation (4100 kPa).

RO Processing Parameters

The RO permeate flux was calculated as in Equation (3-1), the transmembrane pressure *(TMP)* was calculated as in Equation (3-2), and the cross-flow velocity was determined as in Equation (3-3). The concentrations of both the concentrate and permeate, in °Brix, were measured every 15 min with a digital refractometer (Sper Scientific 300053 – Scottsdale, AZ). The concentration factor was then determined as:

$$c_f = \frac{Brix_i}{Brix_0} \tag{4-1}$$

Where: c_f is the concentration factor, $Brix_i$ is the concentration of the retentate at a given point, and $Brix_0$ is the initial concentration of the feed.

RO Membrane Cleaning & Storage

After each experimental run, a cleaning cycle to restore the RO membrane permeability was performed as follows: a rinse with RO water for 10 min, followed by a caustic wash with NaOH 0.1% and sodium dodecyl sulfate 0.025% at 35 °C for 10 min, a 10-min rinse with RO water, followed by an enzymatic wash with Hydrazyme 399 4% (Hydrite Chemical Co., Brookfield, WI) at pH 10 and 35°C for 35 min, another rinse with RO water for 10 min, a second caustic wash with the same parameters as the first caustic wash but with a duration of 15 min, and a final 10-min rinse with

RO water. Whenever available, permeates produced during the runs were used for the rinsing steps to reuse water and reduce waste.

To verify if the membrane was properly regenerated, pure water fluxes were determined before and after the RO experiments. Cleaning was considered effective if the water flux after cleaning was higher than 95% of the initial water flux. In cases when the membrane was not used for 48 h or longer, a sodium metabisulfite 1% solution was circulated for 10 min, then the membrane was left to soak in it until the next use to prevent microbial growth. This cleaning procedure was developed by consultation with the supplier of the cleaning solution (Hydrite Chemical Co.) and based on pilot tests that assessed membrane permeability recovery after processing and cleaning.

FO Processing

The FO concentration runs were conducted using batches of 12 L of fresh GAW, obtained and stored as described above. The FO rig was equipped with one spiral-wound cellulose triacetate membrane (Ederna, Toulouse, France) with an outside diameter of 63 mm, a length of 530 mm, and a filtration area of 0.5 m^2 (**Figure 4.5**).

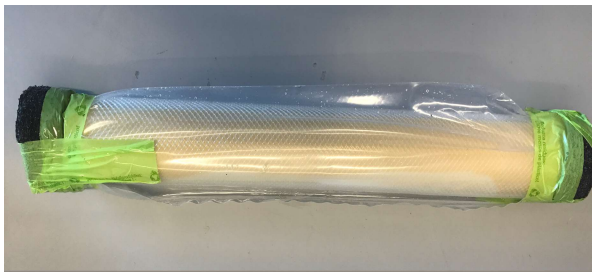

Figure 4.5 Membrane used for the FO runs

The schematic of the batch bench-scale EVAPEOS filtration unit used (Ederna, Toulouse, France) is shown in **Figure 4.6**. A centrifugal pump was used to pump the pre-concentrated GAW, at a flow rate of 3.5 L/min, from a feed tank through a countercurrent plate heat exchanger which maintained the product at low temperature (4 to 10 °C) to avoid microbial growth, and then into the membrane. The retentate was constantly recirculated into the feed tank in a closed loop. The concentrate valve on the outlet of the membrane was used to adjust pressure as needed. In parallel, the OA (potassium lactate of 60 °Brix from Ederna, Toulouse, France) was pumped from a feed vessel to a spent (dilute) OA vessel, passing along the other side of the membrane and drawing the permeate (water) out of the feed. Potassium lactate is the osmotic agent recommended by the manufacturer of the FO unit (Ederna); it is food grade, hygroscopic, has high osmotic pressure at relatively low viscosities, can be easily regenerated by evaporation, and has antimicrobial properties (Ederna, 2015). It is also compatible with GAW, which already contains both lactate and potassium.

The mass loss in the feed OA vessel and the gained mass in the spent OA vessel were monitored gravimetrically, in a continuous manner, by placing each OA vessel on top of a scale. The temperature of the product was monitored using a thermocouple installed in the inlet of the membrane, and the pressure was measured using pressure gauges installed in the feed, concentrate, and osmotic agent sides, respectively. The concentration of the retentate, expressed as °Brix, was measured every 15 min as described previously. The FO process was run until the pressure in the system reached the operational limits of the membrane or the pump.

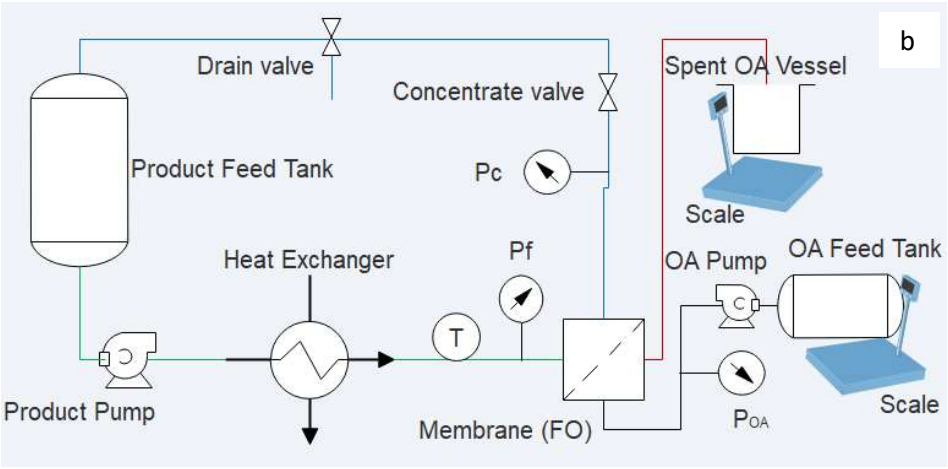

Figure 4.6 a) Picture and b) schematic of the FO rig

FO Processing Parameters

For the FO process, the permeate flux was calculated based on the amount of water that diluted the OA and was collected in the spent OA vessel, according to the following equation:

$$J = \frac{m_{s,i} - (m_{c,0} - m_{c,i})}{A \times t \times \rho} \tag{4-2}$$

Where: J is the permeate flux, in L/m^{2}h; $m_{s,i}$ is the mass of spent osmotic agent collected every 15 min, in kg; $m_{c,0}$ and $m_{c,i}$ are the initial and final masses of concentrated OA left in the feed tank for each 15 min, respectively, in kg; A is the membrane filtration area, in m^2; t is the time elapsed, in h; and ρ is the density of the permeate (water), in kg/L. The concentration factor was determined using equation (4-1).

FO Membrane Cleaning & Storage

After each experimental run, the FO rig was subjected to the following cleaning cycle to regenerate the membrane permeability: three rounds of DI water rinsing for 2 min each, followed by a caustic wash with 40 drops of Ultrasil-110 (Ecolab, Saint Paul, MN) in 2L of water for 11 min at 2 L/min and 4 min at 3.5 L/min, followed by another DI water rinse as previously described, then an enzymatic wash with 4 mL of Prolyve 1000 protease (Soufflet Biotechnologies, Colombelles, France) in 2L of water at pH 7 for 1h at 2 L/min, another DI water rinse, then an acid wash with citric acid 0.4% for 11 min at 2 L/min and 4 min at 3.5 L/min, another DI water rinse, a disinfection step with hydrogen peroxide 1% for 20 min at 2 L/min and 10 min at 3.5 L/min, and a final DI water rinse. The osmotic agent side was rinsed in parallel with DI water until Brix of the outlet stream reached zero, and then disinfected similarly to the product side (but only for 10 min), followed by a final DI water rinse.

This cleaning procedure was developed in consultation with the manufacturer of the FO equipment (Ederna). To verify if the membrane was properly regenerated, water fluxes were determined before and after the FO runs. Cleaning was considered effective if the water flux after cleaning was higher than 95% of the initial water flux.

A sample of the water remaining in the feed tank after the water flux runs was also analyzed by conductivity and nuclear magnetic resonance (NMR) to check for the presence of potassium lactate, as an indication of reverse solute flux.

When no experiments were planned for 48 h or longer, a sodium metabisulfite 0.5% solution was circulated on the product side for 2 min, then the membrane was left in this storage solution until the next use, in order to prevent microbial growth.

Osmotic Agent Regeneration

The spent OA (40 to 50 °Brix) was regenerated using an Evaled evaporator (model R 150V3 AA2 V, Veolia, Zoppola, Italy), at -60kPa, for 6 h. When the regenerated OA had more than 60 °Brix, it was diluted with DI water to exactly 60 °Brix before the next use for consistency among the different experimental runs. The evaporator was cleaned by conducting two 5-minute runs with RO water.

RO & FO Combination Process

The flux behavior and achievable concentration factor for the individual processes were used to develop an efficient nonthermal RO&FO combination process. The RO component of the combination process was performed as described previously. The FO stage was conducted with 5 L of RO pre-concentrated GAW (17 to 20 °Brix), using the procedure detailed above.

Physicochemical Analyses

The feed, concentrate, and permeate from the RO experiments, and the concentrate from the FO experiments were analyzed for moisture content (Fisher Scientific Isotemp oven, Waltham, MA), water activity (AquaLab series 3 Meter, Pullman, WA), pH, and titrable acidity (Easyplus titrator AP0002, Mettler Toledo, Columbus, OH).

The composition of fresh GAW was determined following the official analyses described in **Table 4.1** (AOAC, 1995), and the results are included in **Table 4.2**. Total crude protein, in mg/g, was calculated as (total nitrogen/1000) x 6.38. The methodology used is described in the Compendium of analytical procedures by Dairy One (Dairy One, 2015, p. 5-6). The composition of the concentrated streams was calculated by multiplying each the concentration of each individual component by the concentration factor achieved during RO and FO, since only pure water is considered to leave the feed during both operations, and no reverse solute flux was considered to take place during FO.

Table 4.1 Methodology employed and laboratories responsible for each analysis of fresh GAW

Analysis	Handling	Analytical Lab	Test Method
Moisture (Vacuum oven at 70 °C/16 h)	Samples were kept in vials under refrigeration and then shipped overnight on wet ice	Medallion Labs Minneapolis, MN	AOAC 925.09
Sugars by HPLC (Fructose, Glucose, Lactose, Galactose, Maltose & Sucrose)			AOAC 977.20 - HPLC - RI Detection
Ash			AOAC 923.03
Chloride			AOAC 915.01
Minerals (Ca, Na, K, Mg, P)			AOAC 2011.14
Nitrogen, Non-Protein			AOAC 991.21
Total Nitrogen – Crude Protein		Dairy One Ithaca, NY	*Methodology described elsewhere* (Dairy One, 2015)

Energy Consumption Calculation

Electrical Energy

For RO, the voltage and electrical current used during pumping (for both processing and cleaning) were measured using a multimeter (Fluke 324, Everett, WA)

and the measured values were used to calculate the true electrical power used, according to equation (4-3). This value was converted to specific energy using equation (4-4), following procedures described by Tremblay-Marchand et al. (2016).

$$W_{pump} = \sqrt{3} \times U \times I \times \eta \tag{4-3}$$

Where: W_{pump} is the electrical power in W, U is the voltage in V, I is the current in A, and η is the efficiency, taken as an average value of 0.65 (Tremblay-Marchand et al., 2016).

$$\widehat{E_{pump}} = \frac{W_{pump} \times t}{m_p \times 1000} \tag{4-4}$$

Where: $\hat{E}_{pump}$ is the cumulative specific electrical energy, in kWh / kg permeate, W_{pump} is the electrical power, in W, t is the elapsed time, in h, and m_p is the cumulative mass of permeate, in kg. Since the cleaning operation did not take place at the same time as processing, the total energy required for cleaning was calculated and allocated to the process in proportional installments divided throughout the entire duration of the RO runs.

The same procedure was used for FO, using the voltage and current information provided by the manufacturer of the equipment. In this case, the electrical energy also included the energy used by the chillers integrated into the unit. In addition, the electrical energy required for regenerating the osmotic agent ($\hat{E}_{reg}$) was calculated similarly to that of pumping, using equations (4-3) and (4-4), based on the current and voltage specifications from the manufacturer of the evaporator.

Thermal Energy

For the RO process, the cooling of the product was carried out with chilled water at ~ 4°C, in a process equivalent to bringing the product from 40°C to 8°C. In reality, the product never reached 40°C, but this is the temperature the product would reach due to friction and heat losses if no cooling was applied, based on previous experimental data (not shown). The theoretical maximum coefficient of performance for this process is given by the Carnot cycle efficiency, and it represents the ratio between the energy used by the compressor and the amount of energy removed at the evaporator (Borgnakke & Sonntag, 2013):

$$COP^{max} = \frac{T_{cold}}{(T_{hot} - T_{cold})} \tag{4-5}$$

Where: COP^{max} is the maximum coefficient of performance, in J/J; T_{cold} is the temperature of the chilled water, in K; and T_{hot} is the temperature of GAW, in K.

The heat transferred from the product to the chilled water was calculated as:

$$q_{GAW} = (m \times Cp \times \Delta T)_{GAW} \tag{4-6}$$

Where: q_{GAW} is the heat transferred from GAW, in J; m is the mass of the GAW batch, in kg, Cp is the specific heat of GAW at the experimental conditions, in kJ/kgK; and ΔT is the change in product temperature, in K. For simplification purposes, since GAW is mostly water (**Table 4.2**), a Cp value of 4.2 kJ/kgK was used (The Engineering Toolbox, 2017). In reality, lactose concentration would increase with time, which would cause Cp to decrease (Kawaizumi, Nishio, Nomura, & Miyahara, 1981). Therefore, q_{GAW} was overestimated in a conservative fashion. This

should also compensate for the fact the real *COP* is actually smaller than the theoretical maximum calculated. Therefore, the specific energy required for cooling was:

$$\widehat{E_{cool}} = \frac{q_{GAW}}{COP^{max} \times m_p} \tag{4-7}$$

Where: $\hat{E}_{cool}$ is the specific thermal energy, in J / kg permeate; q_{GAW} is the heat transferred from acid whey, in J; and m_p is the permeate mass, in kg.

Total Specific Energy Consumption

For RO, the total specific energy was calculated as:

$$\widehat{E_{RO}} = \sum (\widehat{E_{pump,p}} + \widehat{E_{pump,c}} + \widehat{E_{cool}}) \tag{4-8}$$

Where: $\hat{E}_{pump,p}$ and $\hat{E}_{pump,c}$ are the cumulative specific electrical energies, in kWh / kg permeate, consumed during processing and cleaning, respectively; and $\hat{E}_{cool}$ is the specific thermal energy required for cooling the system, in kWh / kg permeate.

For FO, the total specific energy was calculated as:

$$\widehat{E_{FO}} = \sum (\widehat{E_{pump,p+cool}} + \widehat{E_{pump,c}} + \widehat{E_{reg}}) \tag{4-9}$$

Where: $\hat{E}_{pump,p+cool}$ and $\hat{E}_{pump,c}$, represent the cumulative specific electrical energy consumed during processing (including cooling) and cleaning, in kWh/kg permeate. The term $\hat{E}_{reg}$, which is the electrical energy required for regenerating OA in the evaporator, was calculated using equations (4-3) and (4-4), but it will be discussed separately from the other energy components since it can differ greatly depending on the OA regeneration method used by different processors.

Statistical Analyses

FO concentration of single strength GAW was only conducted once, due to the excessively long duration of the run. Both the RO and the FO components of the combination process were conducted in triplicate, using different batches of GAW, and the data were analyzed statistically using R Studio (2018). For the physicochemical analyses, statistical differences among means were determined using one-way Analysis of Variance (ANOVA). Significant differences among samples were determined by the Tukey honestly significant difference (HSD) test at $p \leq 0.05$.

4.4 RESULTS AND DISCUSSION

Concentration of GAW by RO and FO Individually

Firstly, the processing conditions conducive of the highest RO permeate fluxes were identified by carrying out RO concentration experiments in a range of TMP. Cross-flow velocity varied simultaneously as TMP. **Figure 4.7** shows the initial flux and the final flux (flux at 3.5 h) for the RO of GAW as a function of TMP. At low TMP values, flux increased with increasing pressure until a plateau was reached, following a behavior typical of pressure-driven membrane processing (Brans, Schroën, Van Der Sman, & Boom, 2004; Fritsch & Moraru, 2008). Based on this data, a TMP value of 3375 kPa and a feed velocity of 0.37 m/s were used for all the subsequent RO runs. The FO process could not be optimized, due to the physical constraints of the system used. However, with the proper equipment, FO processes can also be optimized by varying the flow rate of the two fluids to improve diffusion and transport of water across the membrane (Babu et al., 2006).

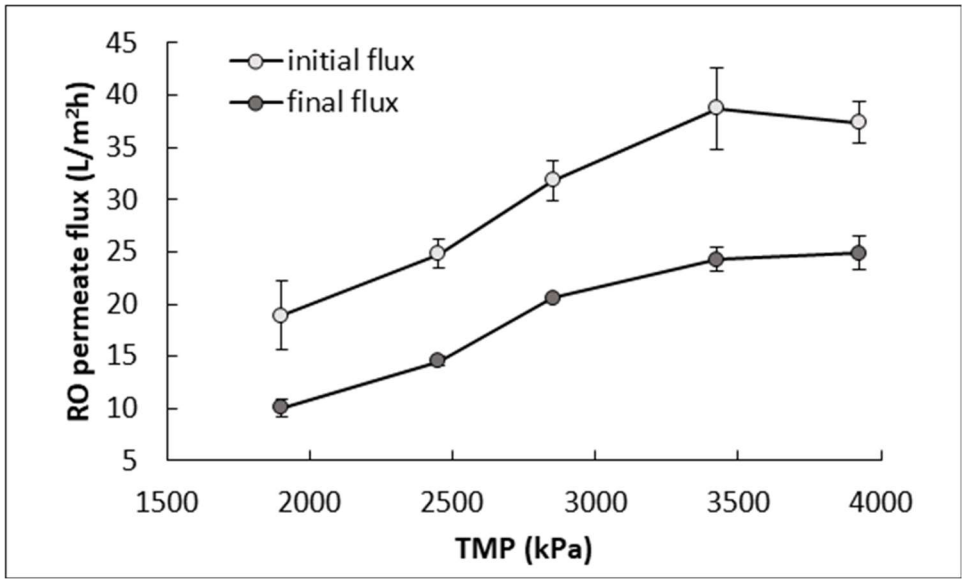

Figure 4.7 Permeate flux vs. transmembrane pressure (TMP) during Reverse Osmosis (RO) of Greek yogurt acid whey (GAW). Error bars represent standard deviations (n=3)

Fresh GAW, with an initial concentration of 6.6 °Brix, was concentrated by RO to a final concentration of 20.3 °Brix in about 3.5 h (**Figure 4.8**). A plateau in concentration was virtually reached at this point, and the RO process progressed very slowly in its last minutes, probably due to an increase in the osmotic pressure of the concentrated feed and significant membrane fouling (Rastogi & Nayak, 2011). A picture of the permeate, feed, and concentrate for one selected RO experiment is shown in **Figure 4.9**.

On the other hand, fresh GAW with an initial concentration of 5.9 °Brix was concentrated by FO to 26.4 °Brix, in about 7 h (**Figure 4.10**). Concentration by FO took considerably more time to reach the same concentration factor as RO (3x) (5.5 h for FO compared to 3.5 h for RO), which was due to both the lower fluxes in FO compared to RO and to the much lower surface area of the FO membrane (0.5 m^2 for FO compared to 2.6 m^2 for RO). Additionally, the volume of the feed in the RO and FO systems was different. Therefore, although total processing times for the two processes

will be reported here for completeness of information, direct comparisons between RO and FO in terms of total processing times should be avoided, since they are specific for the size of the processing units used in this study.

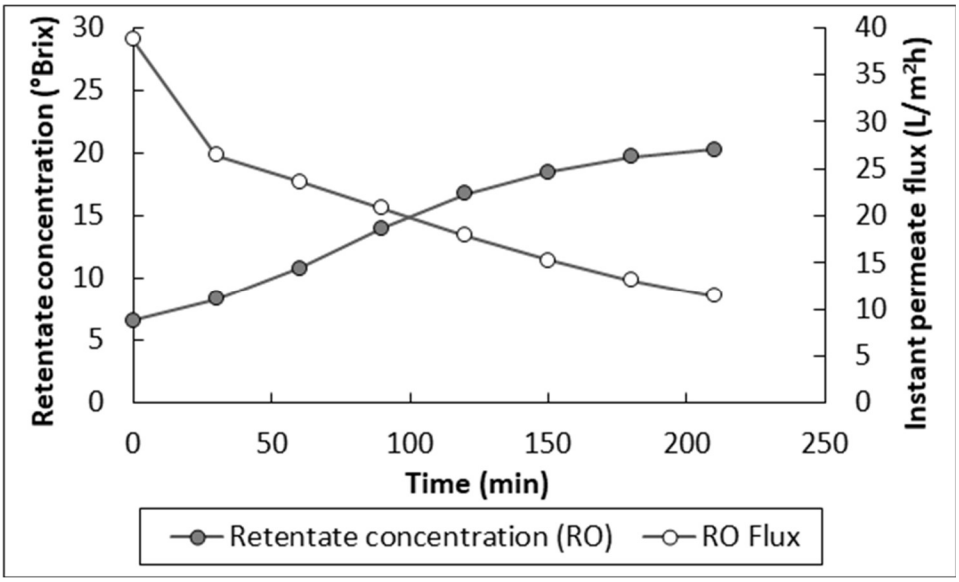

Figure 4.8 Example of retentate concentration and permeate flux as a function of time during Reverse Osmosis (RO) of Greek yogurt acid whey (GAW)

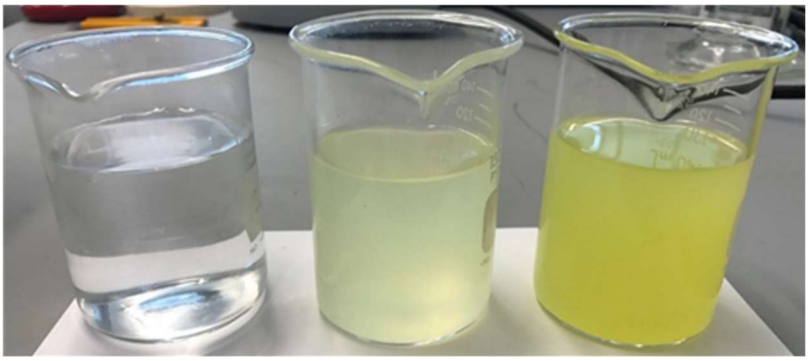

Figure 4.9 RO permeate (left), feed (center), and concentrate (right)

Most remarkably, though, the concentration during FO increased continuously and a plateau was not reached for the duration of the FO run in the current study. At

the same time, water fluxes in FO were much lower but more stable than in RO, consistent with previous reports in the literature (Babu et al., 2006; Sant'Anna et al., 2012). As discussed before, this can be explained by the lower propensity to fouling in FO, because of the very low pressure during processing (M. S. Raghavarao, Nagaraj, Patil, Ravindra Babu, & Niranjan, 2005). The main limitation of achievable concentration in FO is the concentration of the OA, since the driving force is the difference in concentration (and osmotic pressure) between the feed and the OA.

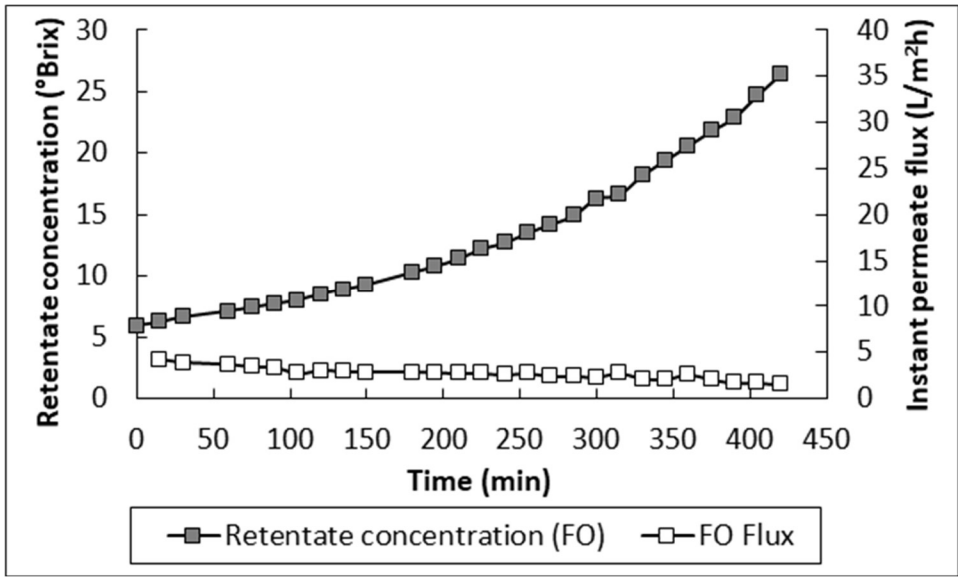

Figure 4.10 Example of retentate concentration and permeate flux as a function of time during Forward Osmosis (FO) of Greek yogurt acid whey (GAW)

A comparative analysis of RO and FO shows that RO performs better at low feed concentration, due to the high fluxes and the reduced propensity for membrane fouling by the dilute feed, while FO performs better at high feed concentration, where small volumes of water need to be removed from the feed to significantly increase its concentration factor. This behavior prompted the study of a combination process, where the dilute feed is first pre-concentrated by RO, then followed by FO.

RO + FO Combination Process: Concentration and Flux Behavior

Fresh GAW, with an initial concentration of 6.6 ± 0.4 °Brix, was pre-concentrated by RO to 19.6 ± 1.1 °Brix (**Table 4.3**). As seen in **Figure 4.11**, a plateau in concentration was almost reached at this point, similarly to the RO experiment described previously. At this point, the RO process was stopped and the concentrate was fed into the FO unit. In the FO step, the product reached a final concentration of 40.2 ± 1.9 °Brix (**Figure 4.12**), at which point the process had to be stopped due to reaching the maximum pressure drop between feed and concentrate allowed by the FO unit. In a larger unit, the FO process could continue until a final product concentration is close to the OA concentration (60° Brix for the OA used in this study). An even higher final concentration of the feed is theoretically possible if an OA with a higher osmotic pressure were available. Overall, RO achieved a concentration factor of about 3×; FO accounted for an additional 2×, bringing the total concentration factor to about 6×.

The evolution of the permeate (water) flux and flux drop during the RO and subsequent FO of GAW is shown in **Figure 4.13**. The permeate flux for RO decreased from 33.2 ± 2.8 L/m^{2}h to 2.6 ± 1.9 L/m^{2}h, which represents a drop to about 8% of the initial flux at the end of the 2.5 h run. The initial flux values are comparable to those reported before (Pepper & Orchard, 1982) for the RO of sweet whey under similar processing conditions. The permeate flux at the beginning of FO was of the same order of magnitude as the flux at the end of the RO run, with a value of 3.6 ± 0.7 L/m^{2}h. At the end of the FO run, the permeate flux dropped to 1.6 ± 0.3 L/m^{2}h, or about 46% of the initial flux. Although the initial RO fluxes were an order of magnitude higher than those of FO, the rate of fouling for the latter was much slower, even though the feed was much more concentrated than in RO. The application of high pressures in RO

(> 3000 kPa, compared to ~ 100 kPa in FO) likely leads to compacting of both the membrane and the fouling layer and consequently results in a drastic decrease in permeate flux and a plateau in the retentate concentration. On the other hand, the flux in FO was much steadier and the process could handle much higher concentrations of the retentate than RO, which is consistent with previous observations (Lee et al., 2010).

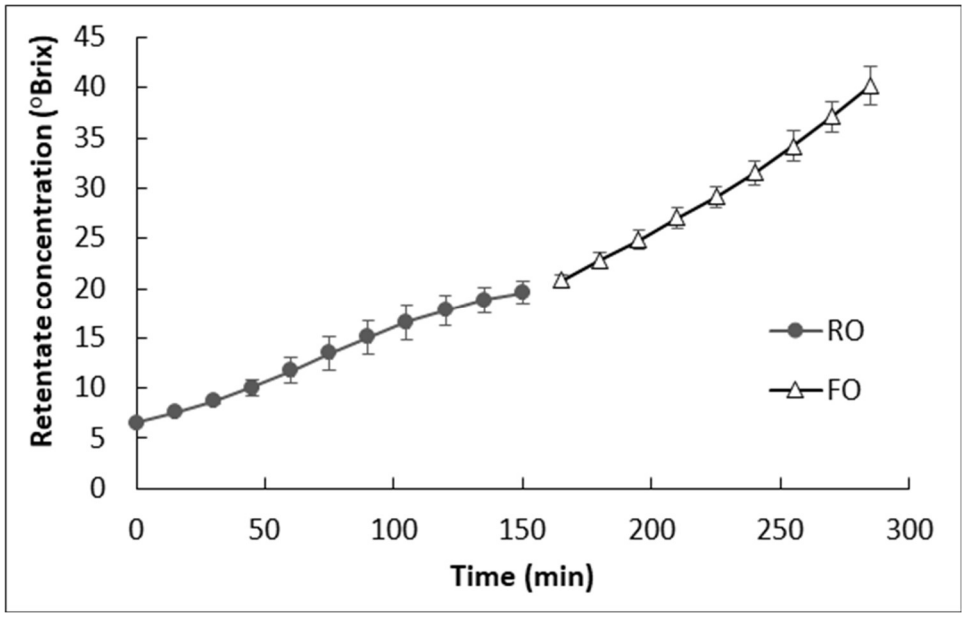

Figure 4.11 Concentration increase vs. time for the processing of Greek-style yogurt whey (GAW) by a combination of Reverse Osmosis (RO) and Forward Osmosis (FO). Error bars represent standard deviations (n=3)

Figure 4.12 Final Concentrate obtained using a combined RO + FO process

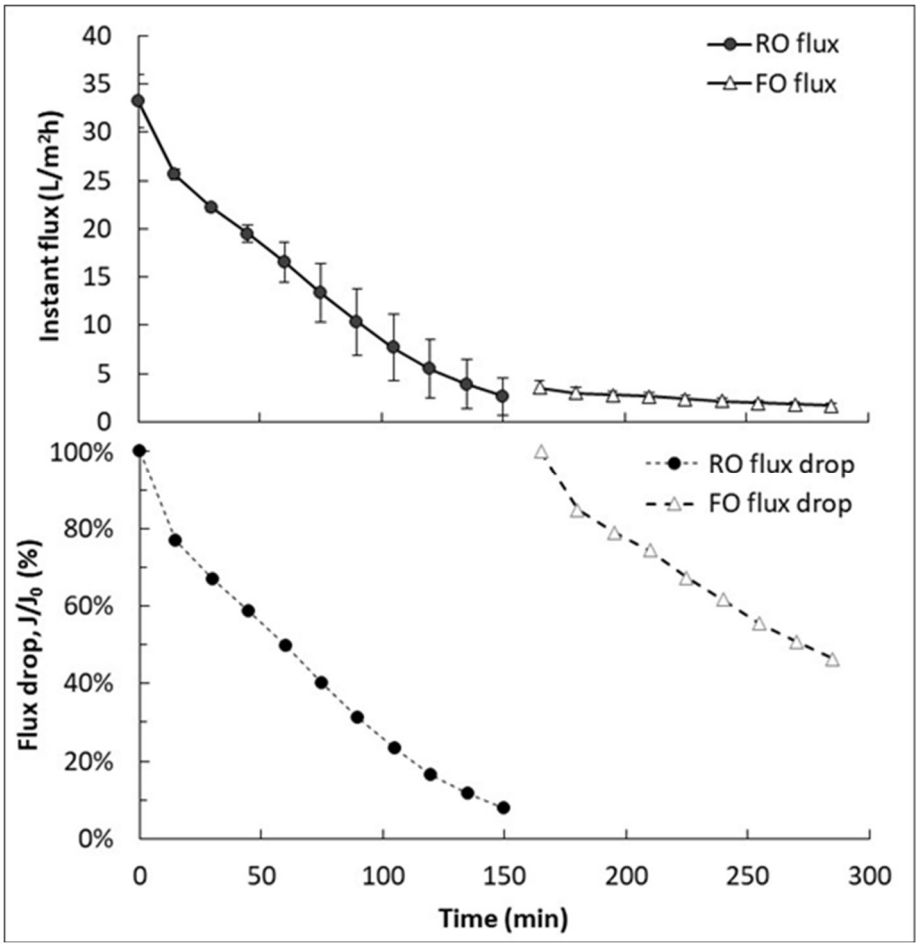

Figure 4.13 Permeate flux and flux drop (J/J_0, i.e., the flux as a percentage of the initial flux) as a function of time for the concentration of Greek-style acid whey (GAW) using a combination of Reverse Osmosis (RO) and Forward Osmosis (FO). Error bars represent standard deviations (n=3)

The measured composition of the fresh GAW and the calculated compositions of the RO and FO concentrates are shown in **Table 4.2**. GAW had a low protein content, but a high concentration of lactose and minerals. The lactose levels in the final FO concentrate actually reaches the solubility limit of lactose at 20 °C, which is ~20 g of anhydrous lactose per 100 g of water (Huppertz and Gazi, 2016). This may lead to lactose crystallization during storage, and thus needs to be considered when handling the final concentrates. On the other hand, the presence of high levels of Ca and P is relevant for membrane processing, as these minerals are known to promote membrane

fouling, particularly under the high pressures applied in RO. Polyvalent ions have been reported to form bridges between the membrane and the carboxyl groups in proteins, organic acids, and amino acids in whey (Kulozik & Kessler, 1988b; Madaeni & Mansourpanah, 2004; Meneses & Flores, 2016).

Table 4.2. Composition of GAW (1×) (measured), and its RO concentrate (3×) and FO concentrate (6×) (calculated)

Component	GAW* Feed	GAW RO* concentrate	GAW FO* concentrate
Total solids (%)	6.00 ± 0.22	18.00	36.00
Total sugars (%)	3.84 ± 0.33	11.53	23.05
Lactose (%)	3.29 ± 0.21	9.87	19.74
Crude protein (%)	0.30 ± 0.12	0.91	1.83
Ash (%)	0.67 ± 0.03	2.03	4.52
K (mg/g)	1.61 ± 0.03	4.83	9.66
Ca (mg/g)	1.23 ± 0.04	3.68	7.37
P (mg/g)	0.68 ± 0.01	2.03	4.07
Na (mg/g)	0.39 ± 0.02	1.17	2.34
Mg (mg/g)	0.10 ± 0.01	0.31	0.63
Cl (mg/g)	0.86 ± 0.09	2.57	5.15

*GAW: Greek yogurt Acid Whey; RO: Reverse Osmosis; FO: Forward Osmosis

Some select physicochemical properties of the fresh GAW, the RO concentrates, and the FO concentrates are included in **Table 4.3**. As expected, the concentrate streams had higher titratable acidity than the fresh GAW, due to the high concentration of organic acids, particularly lactic acid, and lower water activity, due to the higher concentration of soluble components. However, the difference in water activity was statistically significant compared to the initial GAW only for the FO concentrate. The pH did not change after concentration, probably because of the buffering capacity of some of the GAW components (Salau, Mietton, & Gaucheron, 2005).

Table 4.3 Physicochemical properties of GAW (1×), RO concentrate (3×) and FO concentrate (6×)

	GAW* Feed	GAW RO* concentrate	GAW FO* concentrate
°Brix	6.57 ± 0.40^{a}	19.57 ± 1.14^{b}	40.23 ± 1.93^{c}
Water activity	0.99 ± 0.01^{a}	0.98 ± 0.01^{a}	0.93 ± 0.01^{b}
pH	4.33 ± 0.25^{a}	4.31 ± 0.10^{a}	4.17 ± 0.12^{a}
Titrable acidity - lactic acid (g/L)	5.98 ± 0.91^{a}	15.94 ± 2.83^{b}	53.55 ± 10.40^{c}

*GAW: Greek yogurt Acid Whey; RO: Reverse Osmosis; FO: Forward Osmosis

[a-c]Values on the same row followed by different superscript letters are significantly different ($p < 0.05$)

Energy Consumption during the RO and FO Concentration

Besides superior product quality, one of the premises of nonthermal methods for concentration is a reduced energy consumption compared to thermal concentration. To assess this, the total cumulative specific energy required during the RO+FO combination process was calculated. It is important to state from the beginning that the values obtained here are highly dependent on the design and the scale of the units used in this study, as well as the feed. These values are expected to vary from system to system, and the calculations presented here should be used as an example of how such a process can be assessed in each particular situation.

The changes in specific energy with time during each step of the concentration process are shown in **Figure 4.14.** As described in **Section 4.3**, the calculated values accounted for pumping, cooling, and cleaning for both the RO and the FO stages of the process. It should be specified that certain components of the energy consumption, in this case cleaning costs, are fixed and do not depend on the yield of the membrane separation processes or their duration. Thus, the energy used for cleaning was distributed proportionally throughout the entire duration of the process, both for RO and FO. Total specific energy values for RO and FO (without the OA regeneration step) were

0.29 ± 0.04 kWh/kg and 0.32 ± 0.07 kWh/kg, respectively. The inclusion of the regeneration step brings the FO energy to about 0.63 kWh/kg. This is still below the latent heat of water at 45 °C and 0.1 bar (typical for thermal concentration), which is 0.66 kWh/kg (The Engineering ToolBox, 2003).

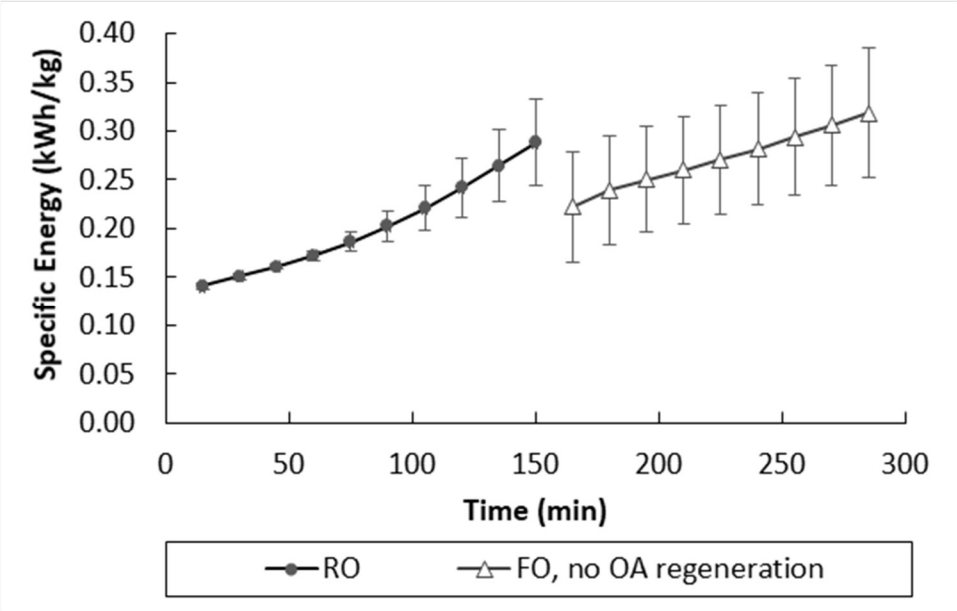

Figure 4.14 Specific cumulative energy consumption (kWh/kg water removed) over time for the combination Reverse Osmosis (RO) & Forward Osmosis (FO) of Greek-style acid whey (GAW). Error bars represent standard deviations (n=3)

By comparison, Xu & Rizvi (2016) estimated the specific energy for the thermal concentration of GAW up to a concentration factor of 8×, using a calandria vacuum evaporator, as 3.98 kWh/kg of water removed, which is an order of magnitude higher than the values obtained here for RO and FO. As discussed before, the RO and FO units had different membrane surface areas. If the total specific energy values shown above considered the membrane surface area, the energy consumption would

become lower in FO compared to RO. We chose however not to make these calculations and to focus on the total specific energy, which allowed an assessment of the other components of the energy consumption.

As shown in **Figure 4.15a**, in RO about half of the specific energy was due to membrane processing, with values similar to those reported in the literature (Tremblay-Marchand et al., 2016), while the other half was spent mostly for cleaning, with cooling having a very small contribution. In the case of the FO process, however, about half of the total required energy pertained to the OA regeneration operation. The contribution of the OA regeneration component to the total energy decreased with processing time, as shown in **Figure 4.15b**, as more permeate (water) was removed from the feed. The specific energy values for OA regeneration shown here are specific to the vacuum evaporation system used in this study, and these values will depend on the regeneration system used in each particular case.

This data shows that finding an economical solution for OA regeneration is key to reducing the energy consumption in FO. Another alternative would be to use osmotic agents that can be easily regenerated without much energy expenditure. Volatiles, thermolytic salts, flocculants, and magnetic particles have been proposed before as such alternatives (Zhao et al., 2012). Nonetheless, in any food application, the choice of OA will also depend on its food-grade status and compatibility with the feed material to avoid any issues of product contamination in case a membrane breach or reverse solid flux occur at any point during the process.

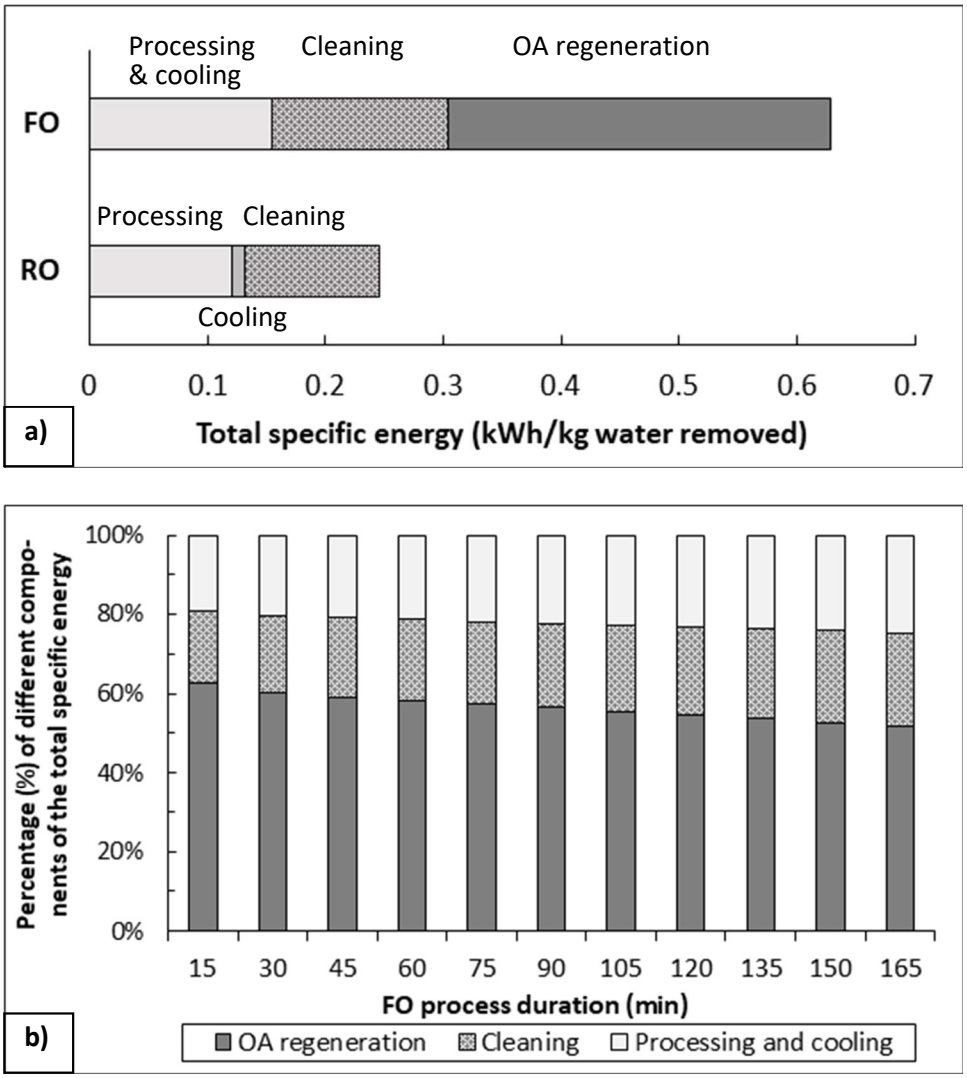

Figure 4.15 Calculated specific energy for the Reverse Osmosis (RO) and Forward Osmosis (FO) stages of the combined concentration process. a) Components of total specific energy for RO and FO; b) Percentage of the specific energy components of total specific energy for FO, as a function of process duration. OA refers to the osmotic agent for the FO process

4.5 CONCLUSIONS

This work demonstrates how a combination of RO and FO can be used to non-thermally concentrate a challenging liquid stream from the food industry, using acid whey from Greek yogurt processing as an example. The proposed combination process couples Reverse Osmosis, a step able to achieve high flux at a low solids content

of the feed, with Forward Osmosis, a step able to achieve a high concentration factor at a high solids content of the feed. Overall, the RO+FO combination can produce concentrates of concentration levels comparable to or higher than thermal evaporation, without thermal damage of their components, and potentially at lower energy consumption. The energy calculations provided here serve as an initial estimate for the RO+FO combination process. Scaling up the process can bring these values down due to large volumes of product processed, continuous operation, and overall more efficient processing. Such energy considerations, as well as the flux and concentration behaviors, can help processors decide about the moment when it is most advantageous to switch from RO to FO for each type of feed.

As progress is made in developing better performing FO membranes, better osmotic agents, and solutions for their regeneration, this combination process can become a very interesting nonthermal alternative for the concentration of challenging or sensitive liquid food products (i.e. protein concentrates, baby formula) and beverages (i.e. juices, cold brew coffee).

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CHAPTER FIVE.

DEVELOPMENT AND VALIDATION OF AN EMPIRICAL PREDICTIVE MODEL FOR THE PERMEATE FLUX DURING THE FORWARD OSMOSIS OF ACID WHEY

5.1 ABSTRACT

Forward Osmosis (FO) is receiving increasing interest as a nonthermal concentration process for liquid foods and beverages. In order to optimize the process, there is a need for simple models that can predict the permeate flux based on product composition and operating conditions. In this study, preconcentrated acid whey from Greek-style yogurt (GAW) was further concentrated by FO at temperatures ranging from 10 to 30 °C. The instant flux data was used to create a linear model correlating the permeate flux at a given time with the operating temperature, initial concentration of the feed, and concentration of the feed at a given time, with the form: $J(t) = \frac{m}{(1.04C_0+0.29)^{n-1}(1.04\ \ (t)+0.29)}\sqrt{T}$, where *J(t)* is flux at time *t, T* is the operating temperature, C_0 *is* the initial concentration of the feed, *C(t)* is the feed concentration at time *t*, and *m* and *n* are numerical constants specific to the system. The model was then validated using data from independent FO processing runs conducted under different conditions than those used for generating the model. The validation was successful since 95% of the measured flux values were within the prediction intervals. The model developed and validated in this study can become a helpful framework for predicting and optimizing the FO concentration of liquid foods and beverages.

5.2 INTRODUCTION

Forward Osmosis (FO) is a novel concentration technique that is emerging as an alternative to both reverse osmosis (RO) and thermal evaporation. The process consists of removing water from a product through a semipermeable membrane, based on the concentration gradient between the feed and a more concentrated solution (osmotic agent) on the other side of the membrane (Nicoll, 2013). Since FO uses little applied pressure, it is less prone to fouling than RO, which makes it a promising process for concentrating challenging streams to high solid levels without pretreatment or extensive membrane fouling (Babu et al., 2006; Beaudry and Lampi, 1990; Raghavarao et al., 2005; Ravindra Babu et al., 2006; Zhao et al., 2012; Zhao and Zou, 2011).

Nevertheless, FO is subject to concentration polarization, a reversible phenomenon that arises mostly during the start-up of the concentration process but affects the process throughout the entire run. Concentration polarization occurs when the permeate flow causes a build-up of the non-permeable solids at the membrane surface, which reduces the concentration gradient between the feed side and the osmotic agent side, increases the pressure drop across the membrane, and hinders water permeation (Cath et al., 2006; Gray et al., 2006; Xu et al., 2010). Specific to FO is the fact that the water flow through the membrane also dilutes the osmotic agent (OA) adjacent to the membrane, as shown in **Figure 5.1**. This process, called dilutive concentration polarization, is another concern since it reduces the effective osmotic driving force. In addition to the concentrative and dilutive concentration polarization described above, while not as severe as in RO, fouling caused by the deposition and adsorption of particles at the membrane surface and/or around the spacers of the spiral-wound membrane

may cause a decrease in permeate flux after several hours of processing, especially at high concentrations (Lee et al., 2010).

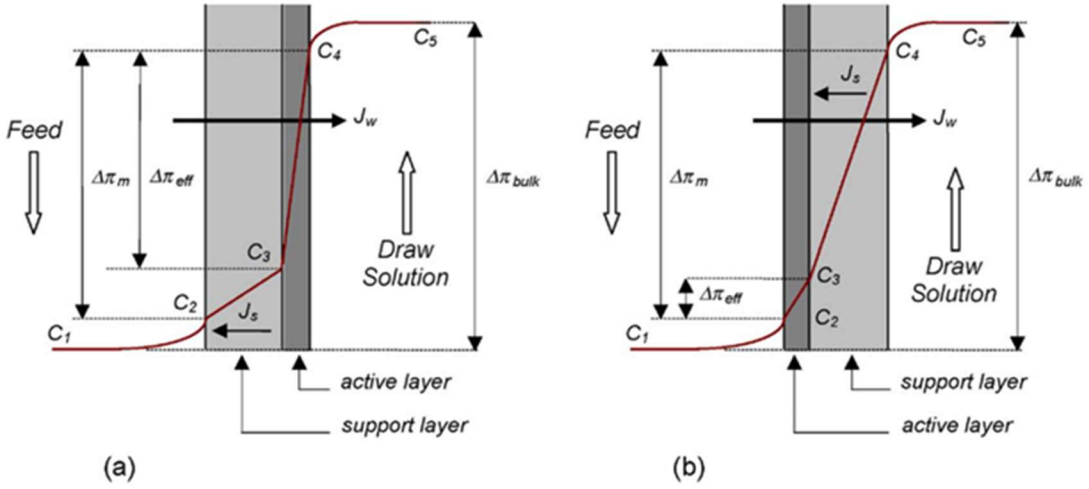

Figure 5.1. Concentration Polarization in FO when the feed is facing: a) the support layer; b) the active layer (Cath et al., 2006)

Recent studies report the use of FO for desalination, wastewater treatment, sugar processing, and the concentration of liquid foods such as fruit juices, tomato sauce, and coffee (Cath et al., 2006; Nicoll, 2013; Sant'Anna et al., 2012). Menchik and Moraru (2019) have proposed a combined reverse osmosis – forward osmosis process to concentrate acid whey from Greek-style yogurt, a challenging coproduct from the dairy industry that is currently an environmental issue in the US. As FO is increasingly explored for the concentration of challenging or heat-sensitive fluids in the food industry, there is a need for developing mathematical models relating the permeate flux with operating conditions and product characteristics. Such models could be used to quantitatively predict the flux and optimize the FO process by selecting those conditions capable

of achieving higher fluxes and concentration factors while minimizing energy consumption.

For dilute solutions, and ignoring the concentration polarization phenomena, the water flux of the FO process for a non-fouled membrane is given by (Cath et al., 2006):

$$J_W = a\sigma\Delta\pi \tag{5-1}$$

Where J_W is the water flux in L/(m^{2}h), a is the pure water membrane permeability in L/bar.m^{2}h, σ is the reflection coefficient, and $\Delta\pi$ is the osmotic pressure differential between the bulk of the feed and the bulk of the OA, in bar. The osmotic pressure of each solution can be determined using the Van't Hoff equation presented by Phuntsho et al. (2014):

$$\pi = \sum iCRT \tag{5-2}$$

Where π is the osmotic pressure in bar, i is the Van't Hoff coefficient of each component (related to the number of dissociated chemical species in the solution), C is the concentration of the component that gets concentrated in mol/L, R is the universal gas constant (0.08314 L.bar/mol.K), and T is the temperature in K.

Since concentration polarization is a prevalent issue in FO, it diminishes the driving force of the process to a point in which equation (5-1) needs to be revised. In order to solve this, Phuntsho et al. (2014) proposed the following modification based on boundary layer mass transfer theory:

$$J_w = \frac{1}{K_D} ln\left[\frac{a\pi_{D,b} + b}{a\pi_{F,b}e^{J_w/k_F} + J_w + b}\right] \tag{5-3}$$

Where K_D is the resistivity of diffusion of draw solutes within the porous support layer of the membrane, $\pi_{D,b}$ is the osmotic pressure of the draw solution in the bulk, b is the salt permeability coefficient of the membrane active layer, $\pi_{F,b}$ is the osmotic pressure of the feed solution in the bulk, and k_F is the mass transfer coefficient of the feed side boundary layer.

While comprehensive, this equation does not have a trivial solution and needs to be solved numerically. Moreover, it includes several constants and properties that need to be determined experimentally or calculated. Additional caveats of this equation include: i) both a and b changing with time as the membrane experiences fouling; and ii) the poor fit for Equation (5-2) to determine osmotic pressures at high feed concentrations (Valentas et al., 1997).

Based on the limitations of existing models for FO concentration, the present study focused on developing a more user-friendly, readily applicable empirical equation for permeate flux in FO that incorporates three simple key product and process parameters: initial Brix of the feed, concentration factor, and temperature. The first two parameters are intimately connected to the osmotic pressure (Equation (5-2)) and the viscosity of the feed. In addition, temperature affects both the osmotic pressure (Equation (5-2)), the viscosity of the feed (Fritsch, 2006), and the diffusivity of its solids (Tew, 2015).

5.3 MATERIALS AND METHODS

Concentration Calibration Curve

GAW was concentrated to 35 °Brix following the procedures described in Menchik and Moraru (2019) and **Section 4.3**, and later diluted with deionized water to

the following Brix levels: 1, 5, 10, 15, 20, 25, and 30 °Brix. Moisture content for each Brix level, in addition to the undiluted sample, was determined according to method AOAC 925.09 (AOAC, 1995) using the sand pan technique, as described in Penner (2010). Total solids were calculated from the moisture content and a calibration curve was obtained.

Osmotic Pressure Determination and Theoretical Flux Estimations

Fat-free GAW, in batches of 150 L, was obtained from Byrne Dairy (Cortland, NY) and stored under refrigeration (5 ± 1 °C) until use, for a maximum of two weeks. It was then pre-concentrated by RO to 15 °Brix following the procedures described in **Section 4.3**. FO of the pre-concentrated GAW was conducted at 20 °C using the same equipment and parameters detailed in Menchik and Moraru (2019). Permeate fluxes and concentration factor were calculated for the initial and final 15 minutes of the process, using equations (4-2) and (4-1), respectively.

The osmotic pressures of the fresh, RO-concentrated, and FO-concentrated GAW, as well as of the concentrated and diluted OA, were calculated based on their individual components using equation (5-2).

The osmolarities of the fresh and RO-concentrated GAW were later verified using a MicroOsmette^{TM} Automatic Osmometer Model 5004 (Precision Systems, INC, Binghamton, NY). The measurements were later converted to osmotic pressure at 20 °C using equation (5-2), in which the term *iC* was replaced by osmolarity values, in Osm/L. Since the osmolarities of the FO-concentrated GAW and the OA were above the maximum limit of the osmometer (3000 µOsm/L), they could not be verified using this method.

The membrane permeability (a) was determined using equation (5-1), with J_w being the pure water flux (osmotic pressure = 0 bar) and considering $\sigma = 0.95$, as specified by the membrane's manufacturer. After a was determined, equation (5-1) was used to calculate the theoretical initial and final fluxes for the FO concentration experiments, and those were compared to the actual values measured. Since the OA is diluted during the FO process, both a maximum and a minimum theoretical flux were estimated, using the osmotic pressures of the fresh and diluted OA, respectively.

Empirical FO Flux Model Development

Fat-free GAW, in batches of 150 L, was obtained from Byrne Dairy (Cortland, NY) and stored under refrigeration (5 ± 1 °C) until use, for a maximum of two weeks. It was then pre-concentrated by RO to 15 °Brix following the procedures described in **Section 4.3**. FO of the pre-concentrated GAW was conducted at 10, 15, 20, 25, and 30 °C (a triplicate each) using the same equipment and parameters detailed in Menchik and Moraru (2019). The temperature extremes were considered based on the limitations of the membrane and the heat exchangers available. Permeate flux and concentration factor were determined every 15 minutes, using equations (4-2) and (4-1), respectively.

The FO water flux (J) was related to the concentration factor (c_f), the initial concentration of the feed ($Brix_0$), and the FO processing temperature (T). The empirical constants were determined using a linear model fit (R Studio 2018). Predictors were considered statistically relevant to the model when their individual p-values were lower than 0.05. Overall goodness-of-fit of the model was assessed by its adjusted R-squared value. Overall assumptions for the model, such as independence of the data

points, normality and homoskedasticity of the residuals, and the absence of influential points were also tested. The full code is provided in APPENDIX D.

Validation of the empirical FO model

In order to validate the model previously proposed, three new FO experimental points were proposed using the following combinations of temperature and initial Brix: 10 °C and 20 °Brix, 20 °C and 15 °Brix, and 30 °C and 10 °Brix. They would correspond, respectively, to the lowest, mid, and highest points for prediction based on the model. All points were run in triplicate, and the experimental values of instant flux measured were compared with the 95% prediction intervals (R Studio 2018) for the previous model fit using the predictors (initial Brix, concentration factor, and temperature) from the new data points.

5.4 RESULTS AND DISCUSSION

Concentration Calibration Curve

The relationship between °Brix and total solids for GAW is shown in **Figure 5.2**. The slope and the intercept are close to 1 and zero, respectively, and R-squared = 0.999, which means that °Brix is a good surrogate for concentration for this material.

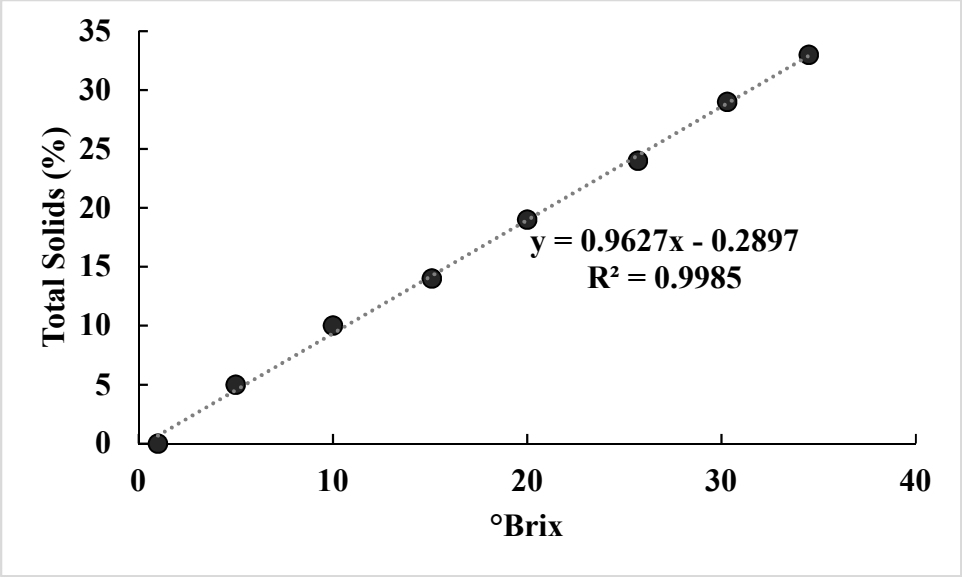

Figure 5.2 Calibration curve relating Total Solids (%) to °Brix

Osmotic Pressure Determination and Theoretical Flux Estimations

Table 5.1 shows the calculated osmotic pressures for the different streams involved in the FO concentration process, as well as the average measured osmolarities and their corresponding calculated osmotic pressures, when available. °Brix is also provided, for comparison.

Table 5.1 Osmolarity, measured, and calculated osmotic pressures for acid whey (GAW) and Osmotic Agent (OA) at different concentrations

	GAW F*	GAW ROC*	GAW FOC*	DOA	OA
°Brix	6	15	45	45	60
Osmolarity (mOsm/L)	369	915	-	-	-
Π measured (bar)	8.97	22.25	-	-	-
Π calculated (bar)	8.80	22.02	66.05	170.95	227.93

*F: fresh; ROC: Reverse Osmosis Concentrate; FOC: Forward Osmosis Concentrate; D: Diluted.

It is noticeable that the component-based osmotic pressure calculations for the feed and the RO-concentrated GAW are very close to the values determined based on

the measured osmolarity, confirming the previously reported accuracy of equation (5-2) for dilute solutions (Phuntsho et al., 2014). Unfortunately, the osmolarities for the other components could not be determined, since they were beyond the maximum threshold of the equipment used. Another important observation is that even though GAW FOC and DOA have similar concentrations, the calculated osmotic pressure of the latter is higher than that of the former due to the different nature of their solutes.

The pure water flux measured was, on average, 10.56 L/m^{2}h, and the calculated osmotic pressure of the OA was 227.9 bar (**Table 5.1**), which resulted in a membrane permeability of 0.049 L/bar.m^{2}h. **Table 5.2** shows the theoretical maximum and minimum fluxes calculated for the beginning and end of the FO concentration of GAW, as well as the actual measured values.

Table 5.2 Theoretical maximum, minimum, and actual permeate fluxes for the beginning (initial) and the end (final) of the FO concentration process

	Initial Flux (L/m^{2}h)	Final Flux (L/m^{2}h)
Theoretical Maximum	9.54	7.50
Theoretical Minimum	6.90	4.86
Actual measured	5.80	2.52

The actual measured fluxes were considerably lower than the theoretical minimum calculated values, which means that equation (5-1) could not provide good estimates for the FO process. As previously discussed, this is due to the concentration polarization phenomenon, which makes the concentrations of GAW and OA near the active layer of the membrane a lot closer to each other than one would expect based on the concentrations of their bulk solutions, thus reducing the effective driving force of the process (Cath et al., 2006; Phuntsho et al., 2014). The equation could perhaps still

be used if there were a simple, non-destructive way of measuring the true concentrations of the streams right at the active layer of the FO membrane, which was not the case for the equipment used in this study, and would not be practical for most industrial-scale operations. Therefore, there is a need for developing a simple yet accurate empirical model for the permeate flux of the Forward Osmosis process.

Empirical FO Flux Model Development

Based on the considerations discussed in the introduction, which were confirmed by correlation diagrams between flux and independent process variables (initial concentration, concentration factor, temperature), the following model was obtained:

$$ln(J) = a_1 + a_2 \times ln(Brix_0) + a_3 \times ln(c_f) + a_4 \times ln(T) \quad (5\text{-}4)$$

Where: J is the permeate flux, in L/m^{2}h; c_f is the concentration factor; $Brix_0$ is the initial concentration of the feed (pre-concentrated GAW); T is the FO processing temperature, in °C; and a_1, a_2, a_3, and a_4 are empirical constants.

By transformation, the following exponential form of Equation **Error! Reference source not found.** was obtained:

$$J = e^{a_1} \times {Brix_0}^{a_2} \times {c_f}^{a_3} \times T^{a_4} \quad (5\text{-}5)$$

The empirical constants were determined using a linear model fit of Equation **Error! Reference source not found.** of the data obtained from the FO runs.

Figure 5.3 shows the linear dependence of ln(J) with ln(cf) at different temperatures. Besides the demonstrated good linear fit (R-squared > 0.98 for all temperature groups), it is also noticeable the similar slopes of the curves at different temperatures, showcasing the independence between these two predictors. However, in order to account for the fact that the material could have been preconcentrated to different initial

Brix values, which would cause a change on its FO permeate flux, this parameter was also added as an extra predictor, and found to be statistically significant ($p < 0.05$). The summary for the full linear model proposed is provided in **Table 5.3.** All predictors used in the model are individually statistically significant ($p < 0.05$), and so is the model as a whole. The adjusted R-squared value is 0.8688, considered a very good fit ($n = 96$) (Agresti, 2007). The exponential form of the equation proposed is:

$$J = 1436.55 \times {Brix_0}^{-2.47} \times {c_f}^{-0.95} \times T^{0.44} \tag{5-6}$$

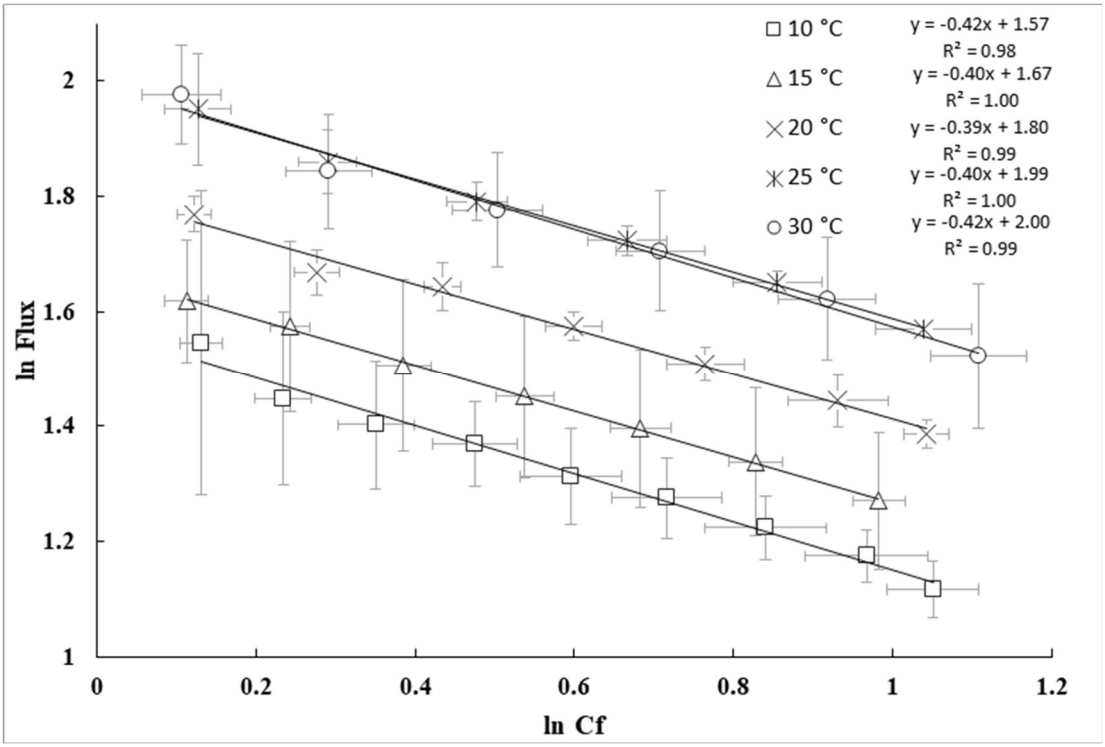

Figure 5.3 Log-log plot of Flux vs concentration factor (Cf) grouped by processing temperature. Error bars correspond to standard deviations (n=3, except for 15°C, where n=2)

Both initial Brix and concentration factor have negative exponents, as expected, since both are directly related with an increase in viscosity, concentration po-

larization, osmotic equilibration, and fouling propensity, all of which lead to a decrease in permeate flux. Interestingly, the exponent for concentration factor was very close to -1, indicating an inverse proportionality between flux and concentration.

Table 5.3 Summary output of the linear model proposed for FO instant flux

```
lm(formula = log(FluxInst) ~ log(BrixFeed) + log(Cf) + log(Temperature),
   data = TempProj)

Residuals:
    Min      1Q  Median      3Q     Max
-0.65816 -0.05773  0.01635  0.08006  0.21909

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)      7.27371    2.24669   3.238  0.00168 **
log(BrixFeed)   -2.46621    0.83951  -2.938  0.00418 **
log(Cf)         -0.95305    0.04358 -21.867  < 2e-16 ***
log(Temperature)  0.44203    0.03668  12.051  < 2e-16 ***
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 0.1342 on 92 degrees of freedom
Multiple R-squared:  0.873,    Adjusted R-squared:  0.8688
F-statistic: 210.8 on 3 and 92 DF,  p-value: < 2.2e-16
```

On the other hand, the positive exponent for temperature is likely explained by the decrease in viscosity and increase in diffusivity caused by higher temperatures, leading to an increase in flux. The value is ~ 0.5, which is reminiscent of the average velocity of particles being proportional to the square root of temperature (Bird et al., 2002). In fact, the 95% confidence intervals for the exponents of cf and T include, respectively, the values -1 and 0.5. Therefore, Equation (5-6) can be reasonably approximated with these rounded exponents and re-written as:

$$J = \frac{m}{{Brix_0}^n c_f}\sqrt{T} \tag{5-7}$$

Where *m* and *n* are empirical parameters that are material dependent. For the case of GAW, *m* = 4638 and *n* = 2.95, the change from Equation (5-6) being due to the new linear fit after fixing the exponents of *cf* and *T*.

The only two terms in Equation (5-7) that are time-dependent are *J* and *cf*. Therefore, by combining Equation (5-7) with Equation (4-1), we get the following:

$$J(t) = \frac{m}{{Brix_0}^{n-1} Brix_i(t)} \sqrt{T} \tag{5-8}$$

Replacing *Brix* with concentration C using the calibration curve presented in **Figure 5.2** yields the following equation for flux:

$$J(t) = \frac{m}{(1.04C_0 + 0.29)^{n-1}(1.04C(t) + 0.29)} \sqrt{T} \tag{5-99}$$

Empirical FO Flux Model Validation

In order to validate the model previously proposed, data from three independent FO runs conducted under temperature and initial Brix conditions different than those used for developing the model were used. The experimental values of instant flux were compared with the 95% prediction intervals for the developed model. The instant flux for the new set of data was within the prediction intervals for 95% of the points tested (using Equation (5-6)), as shown in **Figure 5.4**. This demonstrates that the empirical model developed is able to predict accurately the permeate flux during the FO of GAW in a range of feed concentrations and temperatures. It is important to notice, however, that this equation is only valid within the limits tested in this study, and that it cannot be extrapolated for different types of feed, membranes, or draw solutions without previous validation.

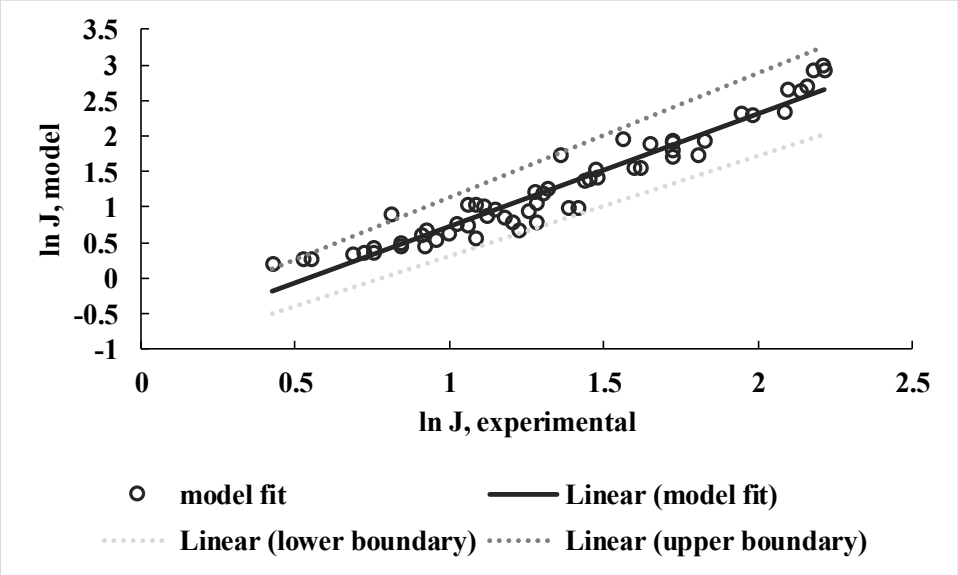

Figure 5.4 Comparison of the actual measured permeate fluxes with the values predicted by the model (fit), as well as the lower and upper boundaries of the 95% predicted interval

5.5 CONCLUSIONS AND FUTURE WORK

As Forward Osmosis becomes more prevalent in the food industry for the non-thermal concentration of a variety of liquid foods and beverages, models such as the one developed and validated in this study could help stakeholders make substantiated decisions about which parameters and conditions to use in order to increase permeate fluxes and optimize the manufacturing of various FO concentrates. Future work will test the same methodology to develop a similar model for the FO of other streams, such as milk, juices, or coffee. The study of how different draw solutions, membrane materials, and configurations affect the empirical model would also be very important. These results could be part of a framework for industry and researchers working on membrane processing of foodstuffs in general and dairy products in particular.

5.6 ACKNOWLEDGEMENTS

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APPENDIX A

SURVEY ABOUT GREEK-STYLE YOGURT ACID WHEY IN NEW YORK STATE

Initial Report

Last Modified: 03/05/2015

1. Site location:

Text Response
Alpina Foods, INC. at Batavia, NY
xd
Kraft- Lowville, NY
Kraft Foods- Walton, NY
Arkport, New York
HP Hood, LLC - LaFargeville, NY
West Seneca, NY
North Lawrence NY
Vernon NY
Vernon
Route 13, Cortland, NY
Friendship, NY
Chobani, New York

Statistic	Value
Total Responses	13

2. What was the volume of milk brought into your plant in 2014 for further processing?

Text Response
24,000,000 lbs
x
455,891,399 pounds
197,024,651 lbs
15 million gallons
140,197,318 pounds
178,690,000 lbs
33,000,000 pounds
238,000,000
25,000,000
720,000 gallons (partial year of operation)
1.5 mil gallons
500,000,000 lb
700 million lbs

Statistic	Value
Total Responses	14

3. The percent of milk that comes into your facility that is used to make products that may have acid whey as a byproduct (ie-cottage cheese, strained Greek yogurt, cream cheese)?

Text Response
98%
x
90-95% cream cheese
83%
60%
50 percent
52%
33%
93
100%
80%
90%
60
100%

Statistic	Value
Total Responses	14

4. What types of acid whey do you produce in your facility?

#	Answer	Bar	Response	%
1	Greek Yogurt		8	57%
2	Cottage Cheese		6	43%
3	Cream Cheese		3	21%
4	Ricotta		0	0%
5	Other		1	7%

Statistic	Value
Min Value	1
Max Value	5
Total Responses	14

5. If other, please indicate products:

Text Response
Quark

Statistic	Value
Total Responses	1

6. How does your company view acid whey?

#	Answer	Bar	Response	%
1	As a byproduct with no value		1	7%
2	As a byproduct with potential value		8	57%
3	As a byproduct with value		5	36%
	Total		14	

Statistic	Value
Min Value	1
Max Value	3
Mean	2.29
Variance	0.37
Standard Deviation	0.61
Total Responses	14

7. How much acid whey from Greek yogurt (in pounds of liquid weight) did your company produce in 2014?

Text Response
16,600,000 lbs
tt
0
0
0
0
30,552,028
14,285,000
100,000,000
About 6 million pounds
7 million lbs
0

Statistic	Value
Total Responses	12

8. How much acid whey from cottage cheese (in pounds of liquid weight) did your company produce in 2014?

Text Response
0
tt
0
300,151,954 lbs
900,000 pounds
2,604,303 pounds
76,649,040
0
104,000,000
None
0
45,000,000 lb

Statistic	Value
Total Responses	12

9. How many pounds of solids does this represent in total Greek Yogurt and/or cottage cheese?

Text Response
995,250 lbs
tt
0
58,232,064 lbs
?
156,258 in cottage cheese
4,680,020
837,145
12,240,000
About 2 million pounds
500,000
na

Statistic	Value
Total Responses	12

10. How much acid whey from other sources - i.e. Cream cheese, ricotta - (in pounds of liquid weight) did your company produce in 2014?

Text Response
0
tt
159,000,000 pounds
0
0
0
0
0
0
None
0
0

Statistic	Value
Total Responses	12

11. During what 4 months do you experience higher volumes of acid whey that your company must handle (Select 4 months for the products that you produce.)

#	Question	January	February	March	April	May	June	July	August	September	October	November	December	Total Responses
1	Greek Yogurt	2	3	3	3	4	3	3	1	1	1	0	0	24
2	Cottage Cheese	3	0	3	3	2	3	2	2	0	2	1	3	24
3	Other Sources (ie; cream cheese, ricotta)	0	0	0	1	1	1	1	2	2	2	2	0	12

Statistic	Greek Yogurt	Cottage Cheese	Other Sources (ie; cream cheese, ricotta)
Min Value	1	1	4
Max Value	10	12	11
Total Responses	6	6	3

12. Do you conduct compositional analysis an Greek yogurt or cottage cheese acid whey?

#	Answer	Bar	Response	%
1	Yes		7	64%
2	No		4	36%
	Total		11	

Statistic	Value
Min Value	1
Max Value	2
Mean	1.36
Variance	0.25
Standard Deviation	0.50
Total Responses	11

13. If yes, what do you test for?

Text Response
We check for minerals and protein content
We do not make these, only cream cheese
Portein, Solids, Ash, Lacteous, and Lactic Acid
Total Solids, Acid PH, butterfat, coliform, SPC, yeast, and mold
Total Solids, Fat, Protein, Lactose
Total solids, Protein, Fat, Lactose
Total solids, fats
Both Feed and Fertilizer value

Statistic	Value
Total Responses	8

14. On-site Handling: Are there any steps prior to storage or down the drain?
(Select all that apply.)

#	Answer	Bar	Response	%
1	No treatment-send down drain ordirectly to tanks		2	18%
2	Thermalize or pasteurize		2	18%
3	Cool immediately		1	9%
4	Reverse osmosis		4	36%
5	Neutralize		1	9%
6	Dispose it down the drain with no treatment		0	0%
7	Other		6	55%
8	Commingle with other waste		2	18%

Statistic	Value
Min Value	1
Max Value	8
Total Responses	11

15. If other, please indicate handling method:

Text Response
We send 100% of whey into tanks and ship to CH4 for digesting. It may include excess Yogurt base.
ff
Sent to anaerobic digester
RO condenses to 12-15% solids, then evaporator down to 38-40%.
Run through UF to remove protein
Ultra filtration of the acid whey to extract proteins. The permeate generated from this process we farm feed.
Whey is sent to a non-refrigerated silo and shipped to a biodigestor 3-4 times per week
None goes down the drain, all captured in tanks for disposal

Statistic	Value
Total Responses	8

16. How much did the handling of acid whey cost your organization in 2014?

Text Response
$0.075/gallon using 8.51 lbs/gal or $150,000
fff
Approx $5,000,000 between cost of digester, trucking and other costs.
~$500,000 (net loss)
$315,000.00
$ 280,000
$350,000
$50,000
840,000
Unknown
750,000

Statistic	Value
Total Responses	11

17. In pounds:

Default - On-site anaerobic digester
Volume
f444
47,000,000
6,000,000
Default - Off-site anaerobic digester
Volume
16,600,000
500,000
Default - City Discharge/sewer
Volume
107,201,000
Default - Off-site Land Application
Volume
112,000,000
200,000
16,680,000
28000000
Default - Animal Feed
Volume
8,024,717 lbs
700,000
41,782,040
3,500,000
28000000
Default - Drying for Animal Feed
Volume
-
Default - Drying for Human Feed
Volume
-
Default - On-site lagoon
Volume
14,285,700
Default - Other
Volume
11,646,933 lbs
180,000,000
400000

Statistic	On-site anaerobic digester	Off-site anaerobic digester	City Discharge/sewer	Off-site Land Application	Animal Feed	Drying for Animal Feed	Drying for Human Feed	On-site lagoon	Other
Min Value	-	-	-	-	-	-	-	-	-
Max Value	-	-	-	-	-	-	-	-	-
Total Responses	-	-	-	-	-	-	-	-	-

18. In pounds:

Default - On-site anaerobic digester
Solids
44f
360,000
Default - Off-site anaerobic digester
Solids
996,000
Default - City Discharge/sewer
Solids
4,680,020
Default - Off-site Land Application
Solids
1,000,800
1680000
Default - Animal Feed
Solids
3,121,615 lbs.
210,000
1680000
Default - Drying for Animal Feed
Solids
-
Default - Drying for Human Feed
Solids
-
Default - On-site lagoon
Solids
837,150
Default - Other
Solids
4,527,183 lbs
10800000
24000

Statistic	On-site anaerobic digester	Off-site anaerobic digester	City Discharge/sewer	Off-site Land Application	Animal Feed	Drying for Animal Feed	Drying for Human Feed	On-site lagoon	Other
Min Value	-	-	-	-	-	-	-	-	-
Max Value	-	-	-	-	-	-	-	-	-
Total Responses	-	-	-	-	-	-	-	-	-

19. Choose those that apply:

#	Question	Use	Total Responses	Mean
1	On-site anaerobic digester	2	2	1.00
2	Off-site anaerobic digester	3	3	1.00
3	City Discharge/sewer	1	1	1.00
4	Off-site Land Application	4	4	1.00
5	Animal Feed	5	5	1.00
6	Drying for Animal Feed	0	0	0.00
7	Drying for Human Feed	0	0	0.00
8	On-site lagoon	1	1	1.00
9	Other	3	3	1.00

Statistic	On-site anaerobic digester	Off-site anaerobic digester	City Discharge/sewer	Off-site Land Application	Animal Feed	Drying for Animal Feed	Drying for Human Feed	On-site lagoon	Other
Min Value	1	1	1	1	1	-	-	1	1
Max Value	1	1	1	1	1	-	-	1	1
Mean	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00
Variance	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Standard Deviation	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total Responses	2	3	1	4	5	0	0	1	3

20. Y/N

#	Question	Yes	No	Total Responses	Mean
1	Haul acid whey off-site	5	5	10	1.50
2	Use acid whey on-farm for feed or digestion	3	7	10	1.70
3	Use acid whey for further processing into ingredients	2	7	9	1.78
4	Dispose acid whey (ie; city discharge)	2	6	8	1.75

Statistic	Haul acid whey off-site	Use acid whey on-farm for feed or digestion	Use acid whey for further processing into ingredients	Dispose acid whey (ie; city discharge)
Min Value	1	1	1	1
Max Value	2	2	2	2
Mean	1.50	1.70	1.78	1.75
Variance	0.28	0.23	0.19	0.21
Standard Deviation	0.53	0.48	0.44	0.46
Total Responses	10	10	9	8

21. Cost

Default - Haul acid whey off-site
If yes, how much?
2,000,000
Farms accepting whey pay for the hauling, not Kraft Walton. Kraft Walton pays truck staging fees.
315,000.00
500000
Default - Use acid whey on-farm for feed or digestion
If yes, how much?
800,000
Default - Use acid whey for further processing into ingredients
If yes, how much?
300,000
Default - Dispose acid whey (ie; city discharge)
If yes, how much?
$350,000

Statistic	Haul acid whey off-site	Use acid whey on-farm for feed or digestion	Use acid whey for further processing into ingredients	Dispose acid whey (ie; city discharge)
Min Value	-	-	-	-
Max Value	-	-	-	-
Total Responses	-	-	-	-

22. What is the furthest acid whey is shipped off-site (in miles)?

Text Response
30 miles (Synergy Farm)
3r3
75
105 miles
100
30 miles
0
0
50
About 5 miles
30

Statistic	Value
Total Responses	11

23. Has your company explored other options for handling acid whey?

#	Answer	Bar	Response	%
1	Yes		9	82%
2	No		2	18%
	Total		11	

Statistic	Value
Min Value	1
Max Value	2
Mean	1.18
Variance	0.16
Standard Deviation	0.40
Total Responses	11

24. If yes, what sort of options? (Choose all that apply)

#	Answer	Bar	Response	%
1	On-site anaerobic digester		4	44%
2	Off-site anaerobic digester		3	33%
3	Land application		2	22%
4	Animal Feed		3	33%
5	Drying for human feed		0	0%
6	Drying for animal feed		0	0%
7	3rd party processing		5	56%
8	Other		1	11%

Statistic	Value
Min Value	1
Max Value	8
Total Responses	9

25. If "other", please indicate options:

Text Response
Animal Feed, 3rd Party Processing, as a Value added product, On-site anaerobic digesting
Favorings and chemicals

Statistic	Value
Total Responses	2

26. How do you plan on handling acid whey differently in the future?

#	Answer	Bar	Response	%
1	No changes		3	27%
2	Handling internally through a digester		2	18%
3	Handing externally through a digester		3	27%
4	Using a 3rd party		5	45%
5	Other		4	36%

Statistic	Value
Min Value	1
Max Value	5
Total Responses	11

27. If "other", please indicate how you plan to handle whey:

Text Response
We are developing value added products...
4f4f4
Looking into sending for ethanol production
As an ingredient

Statistic	Value
Total Responses	4

28. What are the limiting factors in creating new outlets for acid whey in your company?

Text Response
The product may be developed, but the challenge may be consumption by consumers. Also, any unique process and packaging of the product. Potential labeling issues: How do you/can you label the product: what do call it.
4f4f4
The distance to get it to a processor is too costly. Also, we remove all the good components and all that is left is lactose which has little value
Cost and availability.
funds
Costs
Inability to concentrate waste stream
Inability to concentrate waste stream
creating a viable product / saleable
No other known outlets
Capital costs, and Experienced Personnel to run the program

Statistic	Value
Total Responses	11

29. What types of acid whey research projects do you think would benefit all organizations that produce or process acid whey in New York State?

Text Response
I think there is enough understanding in regards to drying and concentrating and removing the Lactose as well as converting the product into a methane by-product to generate electricity. The challenge is how to convert it into a value added product. What would be some of the labeling challenges when you use it as an ingredient? Can it still be Grade-A? What processing/sanitation challenges could be associated with extended handling of the acid whey?
eefe
Ethanol production
I can't say I know for sure but we definitely need something. There is an overabundance of acid whey in New York. I'm positive that we've not utilized this byproduct to its full potential etiher.
use of the acid whey as an ingredient in other products.
Sending whey to Anaerobic digesters.
1. Value added ingredient 2. Value added substrate for third pary use 3. Animal feed
1. Value added ingredient 2. Value added substrate for third party 3. Animal feed
What can be done with a 20% TS product to be able to ship, use at a cost where there can be a profit/ break even opportunity.
Explore an economic method for extracting value added nutrients or content from the acid whey.
1) Identification of new uses for acid whey that can be done with equipment already owned by the mfg company. 2) Create a database/network platform for companies to share facilities, i.e. if a company installs a bioreactor sized for future capacity, can this excess capacity be used on another companies whey, so both organizations benefit. 3) Offer assistance for farmers wishing to navigate the regulatory web of permits, so that more demand for whey as a feed or fertilizer can be made - maybe additional assistance/research on the best way to feed whey.

Statistic	Value
Total Responses	11

30. Comments:

Text Response
I look forward to working with Cornell.
ere3f
We continue to look for any and all opportunities regarding this material
No active research in our organization at present.

Statistic	Value
Total Responses	4

31. Company Name (optional):

Text Response
Alpina Foods, INC
fr3f3rf
Kraft- Lowville, NY
Kraft Foods- Walton, NY
HP. Hood
Upstate Niagara Cooperative, Inc.
Upstate Niagara Cooperative, Inc.

Statistic	Value
Total Responses	7

APPENDIX B

COMPOSITION PANEL FOR GREEK-STYLE YOGURT ACID WHEY

Table B.1 Composition data for the first batch of products

Test	units	Byrne Dairy GAW**	Upstate Farms GAW	Upstate Farms CAW**	OATKA MP**
Moisture	% w/w	94.56	94.532	96.621	87.502
Dry Mass		6.0	6.0	3.7	13.4
Ash		0.641	0.746	0.416	1.134
ammonia – nitrogen	ppm	83	79	58	< 5
urea – nitrogen		N/D*	< 5	< 5	227
nitrate – nitrogen		N/D	N/D	N/D	N/D
total nitrogen (TN)		581	390	259	502
Non-Protein Nitrogen	% of TN	0.17	0.16	0.13	0.35
Total Protein (calculated)	mg/g	3.71	2.49	1.65	3.2
alpha-lactalbumin	mg/g	0.50	0.47	0.22	0.52
beta-lactoglobulin		0.11	0.13	0.83	1.18
alpha-S1-casein		0.00	0.00	0.00	0.00
alpha-S2-casein		0.00	0.00	0.00	0.00
beta-casein		0.00	0.00	0.00	0.00
gamma-casein		0.00	0.00	0.00	0.00
kappa-casein		0.00	0.00	0.00	0.00
total casein		0.00	0.02	0.07	0.00
other peptides		0.00	0.00	0.08	0.08
total low molecular weight		3.09	1.87	0.45	1.42
Calcium	mg/100g	121	120	69.9	96.3
Iron		< 1.00	< 1.00	< 1.00	< 1.00
Sodium		37.9	38.7	23.1	80.6
Phosphorus		66.8	66.5	46.3	99.9
Copper		< 1.00	< 1.00	< 1.00	< 1.00
Potassium		164	169	95.2	360
Magnesium		10.6	10.4	6.78	16.3
Manganese		< 1.00	< 1.00	< 1.00	< 1.00
Zinc		< 1.00	< 1.00	< 1.00	< 1.00
Total Chloride	%	0.078	0.094	< 0.06	0.207
Chemical Oxygen Demand	mg/L	62200	64400	40000	142000
Biochemical Oxygen Demand		> 22000	> 7300	> 7300	> 7300
Ortho-phosphorus		558	530	391	712
pH		4.4	4.4	4.41	6.37
Titrable Acidity (Lactic acid)	%	0.433	0.432	0.277	0.122
Oxalic Acid		< 0.01	< 0.01	< 0.01	< 0.01
Citric Acid		0.18	0.17	0.09	0.4
Tartaric Acid		< 0.01	< 0.01	< 0.01	< 0.01
Malic Acid		< 0.01	< 0.01	< 0.01	< 0.01
Quinic Acid		< 0.01	< 0.01	< 0.01	< 0.01
Succinic Acid		< 0.01	< 0.01	< 0.01	< 0.01
Lactic Acid		0.65	0.64	0.37	< 0.01
Glutaric Acid		0.06	0.06	0.04	0.14
Acetic Acid		< 0.01	< 0.01	< 0.01	< 0.01
Fumaric Acid		< 0.01	< 0.01	< 0.01	< 0.01

*N/D: not detected

**GAW: Greek-style yogurt Acid Whey; CAW: Cottage cheese Acid Whey; MP: Milk Permeate

Table B.1 (continued)

test	units	Byrne Dairy GAW**	Upstate Farms GAW	Upstate Farms CAW**	OATKA MP**
Insoluble Fiber	%	0	0	0	0
Soluble Fiber		0.4	0.3	0.2	0.2
Total Fiber		0.4	0.3	0.2	0.2
Resistant Oligosaccharides		0	0	0	0
Galactose		0.589	0.602	< 0.1	< 0.1
Fructose		< 0.1	< 0.1	< 0.1	< 0.1
Glucose		< 0.1	< 0.1	< 0.1	< 0.1
Sucrose		< 0.1	< 0.1	< 0.1	< 0.1
Maltose		< 0.1	< 0.1	< 0.1	< 0.1
Lactose		3.33	3.42	1.99	10.6
Total Sugar with Galactose		3.92	4.02	1.99	10.6
Total Fat, chromatography*		0	0.01	0.01	0
Saturated Fat		0	0.01	0	0
Monounsaturated Fat		0	0	0	0
cis-cis Polyunsaturated Fat		0	0	0	0
trans Fat		0	0	0	0
Total Fat, gravimetric		0	0	0	0.01
12:0 Lauric			0.001		
16:0 Palmitic			0.006		
18:0 Stearic			0.003	0.004	
18:1 Oleic			0.003	0.004	
18:2 Linoleic			0.001		
Folic Acid	µg/100g	< 5.00	< 5.00	< 5.00	8.12
Niacin	mg/100g	0.114	0.118	0.108	0.361
Vitamin B1 (Thiamine-HCl (US))		0.1	0.06	0.06	0.1
Vitamin B1 (Thiamine (EU))		0.0787	0.0742	0.0472	0.0787
Vitamin B2 (Riboflavin)		0.12	0.06	0.04	0.05
Vitamin B6		< 0.02	< 0.02	< 0.02	0.04
Vitamin B12	µg/100g	< 0.10	< 0.10	< 0.10	< 0.10
Pantothenic Acid	mg/100g	0.459	0.268	0.246	0.983
Vitamin A	IU/100g	< 50	< 50	< 50	< 50
Vitamin D Total		< 40	< 40	< 40	< 40
Vitamin C	mg/100g	< 0.5	< 0.6	< 0.5	< 0.5

* Fatty acids that were not found in the samples are not shown in the table.

**GAW: Greek-style yogurt Acid Whey; CAW: Cottage cheese Acid Whey; MP: Milk Permeate

Table B.1 (continued)

test	units	Byrne Dairy GAW**	Upstate Farms GAW	Upstate Farms CAW**	OATKA MP**
HydroxyProline	%	N/D*	N/D	N/D	N/D
Aspartic Acid		0.011	0.018	0.034	0.012
Threonine		0.006	0.008	0.015	0.004
Serine		0.006	0.008	0.013	0.004
Glutamic Acid		0.022	0.03	0.055	0.021
Proline		0.008	0.011	0.017	0.003
Glycine		0.002	0.004	0.005	0.005
Alanine		0.005	0.006	0.015	0.005
Valine		0.005	0.008	0.016	0.003
Isoleucine		0.006	0.009	0.017	0.004
Leucine		0.009	0.015	0.036	0.007
Tyrosine		0.002	0.005	0.011	0.002
Phenylalanine		0.004	0.007	0.012	0.002
Lysine		0.009	0.015	0.032	0.008
Histidine		0.004	0.005	0.007	0.002
Arginine		0.003	0.005	0.009	0.002
Total Hydrolyzed Amino Acids		0.102	0.154	0.294	0.084
Cysteine		0.002	0.003	0.008	0.003
Methionine		0.001	0.002	0.006	0.001
Taurine		M/I*	M/I	M/I	0.003
Asparagine		< LOQ*	< LOQ	< LOQ	< LOQ
Glutamine		< LOQ	N/D	< LOQ	N/D
Cysteine		N/D	N/D	< LOQ	N/D
Citrulline		N/D	N/D	0.001	< LOQ
GABA		0.003	0.004	0.007	< LOQ
Ethanolamine		0.001	0.001	0.001	0.007
Ornithine		< LOQ	N/D	0.001	0.001
Total Free Amino Acids		0.022	0.013	0.025	0.0035
Tryptophan		< 0.01	< 0.01	< 0.01	< 0.01

*N/D: not detected; M/I: matrix interference; LOQ: limit of quantification.

**GAW: Greek-style yogurt Acid Whey; CAW: Cottage cheese Acid Whey; MP: Milk Permeate

Table B.2 Composition data for the second batch of products (a duplicate each)

		Byrne Dairy		Upstate Farms				OATKA	
test	units	GAW**		GAW		CAW**		MP*	
Total protein (calculated)	mg/g	2.37	1.71	2.75	3.12	5.05	3.52	4.35	3.73
ALA*		0.25	0.17	0.77	0.63	0.71	0.39	1.62	1.57
BLG*		0.00	0.00	0.20	0.16	2.11	1.53	0.00	0.00
alpha-S1-casein		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
alpha-S2-casein		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
beta-casein		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
gamma-casein		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
kappa-casein		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
total casein		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
other peptides		0.00	0.00	0.25	0.22	1.02	0.57	0.00	0.00
total low molecular weight		2.11	1.54	1.53	2.10	1.20	1.03	2.73	2.15
COD*		56.1	53.7	52.4	54.9	31.9	38.7	127	133
BOD*		45.8	45.8	50.5	46.1	32.7	40	182	110
Dry Mass	% w/w	6.2	6.1	6.0	6.1	3.6	3.3	14.8	15.4
ammonia – N	ppm	79	79	87	64	55	57	< 5	< 5
urea - N		< 5	< 5	< 5	< 5	< 5	< 5	251	224
nitrate - N		N/D	N/D	N/D	N/D	N/D	N/D	N//D	N/D
total nitrogen		371	268	431	489	791	552	682	584
Titrable Acidity - Lactic Acid	%	0.484	0.529	0.45	0.424	0.299	0.307	0.205	0.312
Total Chloride		0.079	0.091	0.108	0.09	0.06	0.06	0.223	0.248
pH		4.21	4.22	4.35	4.48	4.37	4.35	5.88	5.4
Ash		0.666	0.71	0.674	0.688	0.406	0.326	1.196	1.248
Moisture		94.2	94.4	94.5	94.4	96.7	96.6	86.4	85.8
Total Fat, gravimetric		1.3	1.5	1.2	1.3	1.4	1.2	1.4	1.2
Calcium	mg/100g	122	128	122	122	68.3	70.7	102	106
Sodium		37.6	41.9	38.5	39.3	21.6	22.5	85.8	88.6
Phosphorus		68.2	69.2	69	68.5	48.1	48.9	108	113
Potassium		162	158	157	156	90.8	93.3	364	381
Magnesium		11	10.5	10.5	10.4	6.56	6.67	17.6	18.2
Galactose	%	0.62	0.65	0.59	0.56	0.14	0.15	0.13	0.17
Fructose		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Glucose		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.151	0.16
Sucrose		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Maltose		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Lactose		3.5	3.33	3.39	3.41	2.06	2.13	11.5	11.9
Total Sugar w/ Galactose		4.11	3.98	3.98	3.97	2.2	2.28	11.8	12.2

*ALA: α-lactalbumin; BLG: β-lactoglobulin; BOD: Biochemical Oxygen Demand; COD: Chemical Oxygen Demand

**GAW: Greek-style yogurt Acid Whey; CAW: Cottage cheese Acid Whey; MP: Milk Permeate

APPENDIX C

COMPOSITION PANEL FOR THE FRACTIONATION OF GREEK-STYLE YOGURT ACID WHEY

Table C.1 Composition data for all streams produced during the fractionation of GAW* (all values given in mg/g)

	Raw Material (GAW)		1.4µm MF**		0.2µm MF		15kDa UF**	
	Untreated	Pre-filtered	Permeate	Concentrate	Permeate	Concentrate	Permeate	Concentrate
total moisture	948.01	948.21	944.87	949.01	947.07	942.66	953.56	945.63
total solids	57.00	58.00	59.00	57.00	57.00	61.00	50.00	58.00
total sugars	33.60	32.80	36.00	31.60	35.50	37.20	30.50	36.50
lactose	30.00	29.50	31.60	28.50	31.10	32.70	26.10	31.90
total protein	4.40	4.50	4.10	4.37	3.38	4.57	2.36	3.28
alpha-lactalbumin	0.86	0.86	0.70	0.80	0.42	1.14	0.00	0.50
beta-lactoglobulin	0.15	0.12	0.08	0.07	0.00	0.11	0.00	0.00
beta-casein	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00
total casein	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
other peptides	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
total low molecular weight	3.38	3.52	3.31	3.44	2.96	3.33	2.36	2.76
total fat	1.00	1.00	1.00	2.00	1.00	1.00	1.00	0.00
ash	6.84	6.76	6.56	6.64	6.80	7.20	5.88	6.54
Ca	1.20	1.18	1.20	1.22	1.16	1.22	1.12	1.18
Na	0.38	0.38	0.38	0.39	0.37	0.39	0.38	0.38
P	0.67	0.65	0.66	0.67	0.63	0.67	0.61	0.64
K	1.60	1.56	1.60	1.62	1.56	1.61	1.57	1.57
Mg	0.10	0.09	0.10	0.10	0.09	0.10	0.09	0.10
Cl	0.95	0.87	0.84	0.90	0.30	0.97	0.99	0.89
titrable acidity (lactic acid)	5.42	5.38	4.58	5.61	4.50	4.95	4.25	4.52
pH	4.47	4.48	4.58	4.47	4.57	4.57	4.57	4.58
COD	64.8	54.8	58	60.4	71.6	64.8	48	60
BOD	30.8	33.6	34.2	36.7	33.5	35.8	31.7	33.7

Note: alpha-S1-casein, alpha-S2-casein, gamma-casein, and kappa-casein were not found in any of the samples.
*GAW: Greek-style yogurt Acid Whey
**MF: Microfiltration; UF: Ultrafiltration

APPENDIX D

FULL R CODE FOR THE EMPIRICAL MODEL DEVELOPED.

```
# Pedro Menchik
# temperature project - FO of GAW

# reading file into R, checking everything

library(readxl)
TempProj <- read_excel("C:/Users/pedro/Box Sync/PhD project 2019 apr/RO+FO experiments/temperature project/Temperature summary_forR.xlsx")
View(TempProj)

attach(TempProj)
names(TempProj)
summary(TempProj)
dim(TempProj)

# installing some packages

library(car)
library(MASS)
library(corrgram)
library(leaps)
library(outliers)

corrgram(TempProj,panel = "panel.pts", diag.panel = "panel.density")  # checking correlations between variables

grubbs.test(TempProj$FluxInst, type = 10, opposite = TRUE)  # checking for outliers
z = as.factor(TempProj$Tgroup)

# plotting the data

plot(TempProj$Cf, TempProj$FluxInst, col=c("red","blue","green", "yellow", "black")[z])
legend(x="topright", legend = levels(z), col=c("red","blue","green", "yellow", "black"), pch=1)

# building the model

TempProj$BrixFeed = BrixConc/Cf
```

```
choice_model = lm(log(FluxInst)~log(BrixFeed)+log(Cf)+log(Temperature), data=TempProj)
summary(choice_model)

#predictions

TempNew <- read_excel("C:/Users/pedro/Box Sync/PhD project 2019 apr/RO+FO experiments/temperature project/Temperature summary_forR_newdata.xlsx")
View(TempNew)

attach(TempNew)

TempNew$BrixFeedNew = BrixConcNew/CfNew

NewDat = data.frame(Temperature=TemperatureNew,Cf=CfNew, BrixFeed=TempNew$BrixFeedNew)
Pred.int = exp(predict(choice_model,NewDat,interval="prediction"))


TempNew$Lower<- Pred.int[,2]
TempNew$Fit<- Pred.int[,1]
TempNew$Upper<- Pred.int[,3]

TempNew$Check <- (FluxInstNew >= TempNew$Lower & FluxInstNew <= TempNew$Upper)
Percent = sum(TempNew$Check)/length(TempNew$Check)
Percent
TempNew$Delta <- (FluxInstNew - TempNew$Fit)

write.csv(TempNew, "modelcheck2.csv")

# checking assumptions
cooks.dist = cooks.distance(choice_model)  #outliers
plot(choice_model, which = 4)

stud.res = studres(choice_model)
qqPlot(stud.res)  #normality
plot(choice_model$fitted.values,stud.res, col=c("red","blue","green", "yellow", "black")[z])  #homoskedasticity
abline(0,0)
legend(x="bottomright", legend = levels(z), col=c("red","blue","green", "yellow", "black"), pch=1)
plot(1:nrow(TempProj), stud.res, col=c("red","blue","green", "yellow", "black")[z])
#independence
abline(0,0)
```

```
# fixed coefficient version

choice_model2 = lm(log(FluxInst)~log(BrixFeed)+offset(-1*log(Cf))+off-
set(0.5*log(Temperature)), data=TempProj)
summary(choice_model2)

anova(choice_model, choice_model2)  # the models are not statistically different!

# comparing goodness-of-fit

cp(choice_model7)
cp(choice_model5)
AIC(choice_model7)
AIC(choice_model5)
BIC(choice_model7)
BIC(choice_model5)

# assumptions

cooks.dist = cooks.distance(choice_model2) #outliers
plot(choice_model7, which = 4)

stud.res = studres(choice_model2)
qqPlot(stud.res)  #normality
plot(choice_model2$fitted.values,stud.res, col=c("red","blue","green", "yellow",
"black")[z])  #homoskedasticity
abline(0,0)
legend(x="bottomright", legend = levels(z), col=c("red","blue","green", "yellow",
"black"), pch=1)
```