PROJECT PROPOSALS
TO THE NYS MILK PROMOTION BOARD
FROM THE NORTHEAST DAIRY FOODS RESEARCH CENTER
August 19, 2019 (for August 26, 2019 meeting)

The Northeast Dairy Foods Research Center offers the following proposals for the NYS Milk Promotion Board to consider for funding for the period January 1, 2020 to December 31, 2020. The projects are:

**Continuing Projects: Projects 1 through 5.**

**Project 1 - Transfer of technology for the NEDFRC.** Project Leader - NEDFRC Director.

**DURATION:** (continuing core program activity)

The NEDFRC has been carrying out technology transfer activities in the past year. The NEDFRC requests that the NYS Promotion Board provide continued funding for this activity of the center in support of technology transfer, particularly to companies in NYS.

**SUMMARY OF PROPOSED PROJECT**

The NEDFRC has been conducting dairy product research since 1989. New knowledge and technologies have been developed that need to be transferred to the Northeast dairy foods industry.

**Objectives for 2020.**

To continue to work with dairy foods manufacturers in the Northeast to implement new dairy foods technologies developed in producer funded research programs.

1. To work on technology transfer of microfiltration (MF) and the milk refinery approach to converting milk to a family of higher value intermediate dairy ingredient products for use both in dairy and nondairy food applications. We continue to working with an equipment company and a milk processing companies within NYS and outside of NYS to scale up MF of skim milk. Recently, both individual companies and trade associations are working to provide information in support of a regulatory decision to all the use of MF of skim milk prior to cheese making to remove whey proteins from milk prior to the manufacture of cheeses with a standard of identity. Much work has been done on this technology at the NEDFRC. Technical information and data are being provided as support materials to justify a change in the regulations to allow MF retentates to be used for production of cheese with a standard of identity. There has been increased interest in use of MF retentate (i.e., micellar casein concentrate) in milk based beverages and the possibility that this technology could be the base for production of better tasting shelf stable milk based beverages.

2. Recently, research at Cornell has developed a number of new analytical methods for use of mid-IR to control the composition and quality of dairy products during the manufacturing process. A new method to analyzer cheese has been developed that should improve the accuracy of measuring the fat, protein, moisture, and salt content of natural cheeses. We
are working actively with dairy processing facilities in the NYS and Northeast to implement these new technologies.

3. We are currently working with milk testing laboratories in the Northeast to implement the new milk analysis tools to improve fat and protein content of milk and providing research information to dairy nutritionist and feed companies to help them more effectively utilize this new milk testing technology. As the use of the rapid analytical tools for milk fatty acid analysis (de novo, mixed origin, and preformed fatty acids) developed at Cornell become more common, we will start to explore the possibility that raw milk high in de novo milk fatty acid content may be used to produce cheeses with improve aged flavor characteristics. If we can collect data demonstrates added value in high de novo fatty acid milk, then it may be possible that quality premiums paid to farmers for high de novo fatty acid milk might be feasible.

Project 1: Request from NYS Milk Promotion Board for the period January 1, 2020 to December 31, 2020. $ 51,625.

Project 2: Nutritious Spreads and Fillings using Milk Ingredients
(Continuation project)

Project Title: Nutritious Spreads and Fillings using Milk Ingredients

New York State Milk Promotion Board Goal Addressed by this Project:
Conduct research to expand the demand for New York dairy products and dairy ingredients

Project Abstract:

Traditional spreads, fillings, and mayonnaise typically contain high fractions of oil (>70%) and are high in calories. As consumers’ demands for healthy and tasty products continue to grow, we plan to develop these products using a technology developed in our lab: water in oil (W/O) high internal phase emulsion (HIPE). This technology allows us to produce highly concentrated, and highly stable, W/O emulsions which can be used to create low-fat butter spreads (less than 20% oil), nutritious fillings, or mayonnaise with high quantities of milk proteins without sacrificing the creaminess of these products. We also intend to explore the possibility of delivering water-soluble vitamins and minerals using the HIPE approach.

Project PI: Alireza Abbaspourrad, Cornell University

Duration: January 1st 2020 – December 31st 2020 (year 2 of a 2-year project)

Background

Spreads, fillings and mayonnaise typically contain high fractions of oil. These products usually offer minimal nutritional benefits while remaining high in calories. For example, conventional spreads are
composed of 80% fat, along with synthetic emulsifiers, flavors and preservatives. To maintain the desired consistency in spreadable products while, at the same time, delivering higher nutritional value, we can utilize a technique which creates a high internal phase emulsion (HIPE). HIPE is a highly concentrated emulsion system with an internal phase volume fraction exceeding 0.74. The gel-like characteristic of HIPE allows the products to be self-standing and spreadable.\textsuperscript{1,2} However, current food products are limited to forming oil-in-water HIPE, while water-in-oil HIPE with high stability are difficult to fabricate and, thus, are rarely explored. By creating a water-in-oil version of HIPE, we can reduce the amount of fat used, and we can include nutritious milk proteins and vitamins in the water phase. In this research, we propose creating ultra-stable, water-in-oil HIPE made with milk-derived ingredients. We will emulsify 80\% water phase containing milk protein in 20\% milk fat phase using a high shear homogenizer. By applying a phase structuring approach within HIPE, we can modulate the rheological properties, stability, enable high loading of milk proteins, as well as provide functionality within a low-calorie, yet palatable, food matrix.

**Phase I. (first year summary)**

**(Summary of achievements in Phase I – milk ingredients)**

We first focused on the utilization of milk ingredients to create a low-calorie, nutritious, spreadable product. Using a high shear homogenizer as a tool, we incorporated a large portion of whey protein within fat to create a HIPE. The HIPE emulsification approach provides the ability to incorporate milk proteins into the water without any pretreatment. The concentration of protein (20 wt.\% in water phase), as well as fat proportion, could be easily varied to modulate the consistency and stability of the product. We evaluated the effect of a number of parameters on HIPE formation including highest volume ratio, droplet particle size, shelf life, and rheological properties. In addition, such HIPE enables us to encapsulate and protect other water-soluble bioactive ingredients in the water phase which results in low-cost, nutritious products with a creamy mouthfeel.

To summarize, we were able to fabricate multiple formulations using our pre-established HIPE technology.\textsuperscript{3} Our pre-established HIPE technology features the stabilization of highly-packed, water-in-oil emulsions by increasing the viscosity of both the water and oil phases (Figure 1). Using such platform, we evaluated the possibility of creating HIPE’s loaded with milk protein concentrate (MPC) and whey protein isolate (WPI). As a result, we were able to load 20\% of MPC or 20\% WPI into the water phase of the system with an improved stability, shown by increased viscoelasticity (Figure 2). Besides MPC and WPI, successful spread-like HIPE can be formed using milk fat, milk and chocolate milk (Figure 3). Since these dairy proteins are of high quality, they could contribute to increased nutritional value and become healthier alternatives of current conventional spreadable products. This platform can also be used to solve other dairy-related problems. Widely known, when WPI are added at high concentration (>3\%) and at acidic condition (pH 3.5), undesirable astringency can develop.\textsuperscript{4} Therefore, we also explored the possibility to mitigate the astringency of the acidified WPI (20\%) using the HIPE platform. The result from a sensory study clearly shows that our developed system can significantly reduce astringency perceptions from dairy proteins (Figure 4). Our system demonstrated great potential toward the production of a stable, protein-enriched, nutritious, palatable, and spreadable products, and is a promising, healthier choice for consumers.
Figure 1. Microstructure of HIPE under confocal laser scanning microscope (left three figures) and optical microscope (right figure). Green color represents aqueous phase, red represents oil phase.\(^3\)

Figure 2. HIPE containing MPC of different concentrations and its microstructures observed under optical microscope.

Figure 3. HIPE formed using milk fat, milk, and chocolate milk.
Figure 4. Astringency sensory ratings on HIPE containing 10% acidified WPI (pH 3.5), combined with different carrageenans (κ-/ι-/λ-car). 10% WPI solution at pH 3.5 was used as a control. The panelists (n=15) were asked to rate astringency level from 1-9 (9 being the most astringent).

Specific Objectives:

Objective 1: Create healthier spreads and nutritious fillings using milk ingredients by using the high internal phase emulsion (HIPE, W/O) strategy developed in our lab and simplify the production process so it can be applicable for large-scale production.

Objective 2: Determine the possibility of delivering water soluble vitamins and minerals contained in milk ingredients using HIPE approach.

Experimental Approach:

For Phase II of the project, we aim to utilize a variety of milk fat fractions, in combination with milk proteins, from processing companies in New York state, to formulate spreads and fillings with improved mouthfeel, stabilities, and physical properties. In preliminary experiments, utilizing milk fat will decrease the overall HIPE spreadability at refrigerated temperatures which will hinder applications as a refrigerated, spreadable product. Therefore, we would adjust the ratio of milk fat and internal volume and further tune the viscoelasticity, thus spreadability, of the resultant HIPE. We also aim to make HIPE utilizing solely dairy components, rather than adding emulsifiers. Current, conventional, spreadable products contain vast amounts of stabilizers and synthetic emulsifiers such as modified starch, gums, corn syrup, gelatin, monoglycerides, and lecithin that are undesirable to the consumers. In our effort to create healthier products for consumers, we will work toward minimizing the ingredient list while delivering products with high stability. This can be achieved by replacing all the stabilizers and emulsifiers with the crystals generated solely by milk fat upon cooling. Currently, this technique has not been explored to produce a W/O type of HIPE. Moreover, we will formulate HIPE utilizing milk fat and dairy protein, simplifying the production process so it can be applicable for a large-scale production. In addition, we will optimize parameters such as protein concentration, viscosity, pH, processing temperature, and we will evaluate the shelf-life, stability, and sensory attributes of the produced spreads and fillings. Specifically, sensory panels will be carried out to evaluate the creaminess of the sample and the overall mouthfeel and flavor as compared to current
market products (mayonnaise, dips, buttercream and spreadable butter). More importantly, we will perform studies to determine the possibility of delivering water soluble vitamins and minerals along with milk ingredients using HIPE approach.

**Deliverables:**

1. Healthier spreads and nutritious fillings with high proteins (spreads) or low fat (butter with <20% fat) and a simplified production process for a large-scale production.

2. Delivery of water-soluble vitamins and minerals along with milk ingredients using HIPE approach.
References


Project 3: Vacuum microwave drying of nonthermally concentrated milk and protein concentrates for the manufacture of dairy powders of superior quality and functionality (Continuation project)

New York State Milk Promotion Board Goal Addressed by this Project:
Conduct research to expand the demand for New York dairy products and dairy ingredients.

Project Abstract:
We will use Vacuum Microwave Drying (VMD) for the drying of nonthermally concentrated dairy products and ingredients (skim milk, whole milk and milk protein concentrates). The powders obtained by low temperature forward osmosis (FO) concentration followed by VMD will undergo minimal heat exposure, which will lead to superior physico-chemical, sensory, nutritional, functional, and microbiological quality of the obtained powders compared to powders obtained using traditional thermal concentration followed by spray drying. The energy consumption of the combination process will be determined and compared to traditional processing methods. The proposed combination process is novel, unique, and has the potential to lead to high quality powders, in an energy efficient way. This could bring a competitive advantage to US produced dairy powders on the global market, and increased consumer and customer satisfaction on the domestic market.

Project PI’s: Carmen I. Moraru

Duration: January 1st 2020 – December 31st 2020 (year 2 of a 2 year project)
1. **Background**

Market outlook for milk powders. Skim milk powder (SMP) is an important dairy product and ingredient. According to a recent report, the global SMP market was valued at $9,894 million in 2017, and is projected to reach $16,498 million by 2024, registering an annual growth of 7.3% during the time period 2018 to 2024 (*Allied Market Research, 2018*). While the EU-27 export most SMP, the US is the #1 single country exporter of SMP, followed by New Zealand and Australia ([https://www.indexmundi.com/](https://www.indexmundi.com/)). Asia Pacific region is an emerging market for SMP, which has the highest growth potential due to new consumer preferences and improvement in economic conditions in the region (*Allied Market Research, 2018*).

Over the last year, SMP prices increased (Figure 1). Additionally, the volume of U.S. milk powder exports was up 16% during the first three quarters of 2018 ([https://www.milkbusiness.com](https://www.milkbusiness.com)). Both of these trends represent a significant economic opportunity for US produced milk powder, both globally and domestically.

**Opportunity and need to manufacture powders of improved quality.** Milk powders are often times used for increasing the milk solids content for cheese, yogurt, ice cream or processed cheese manufacture, or for the manufacture of formulated foods (milk-based beverages, sauces, custards). The quality, functionality and stability of dairy powders are of critical importance for their targeted applications, consumer acceptability, and acceptance on export markets. One way for producers to differentiate themselves on the SMP market could be a significant increase in milk powder quality, which can open the way for new utilizations of this abundant dairy ingredient.

The sensory, nutritional and reconstitution properties of SMP are greatly affected by the heat treatment received during the manufacturing process, which involves pre-heating of the milk, concentration by evaporation, followed by spray drying of the concentrate. SMP is classified as low-, medium- or high-heat, depending on the heat treatment applied to skim milk prior to evaporation and drying (*Martin et al., 2007*). Typical heat treatments are 70–72 °C for 15 s for low-heat SMP, and 120 °C for 60–120 s, or 90 °C for 300 s high-heat SMP (*Kelly et al., 2003*).

The severity of the heat treatment affects the extent of serum (whey) protein denaturation, the complexation of denatured serum proteins with the casein micelle, and the partitioning of minerals, serum proteins and caseins between the serum and colloidal phases of milk (*Lin et al., 2018*). All of these affect the quality, functionality and stability of SMP, as well as the processing characteristics and the properties of the final products that use SMP as an ingredient. Low-heat powder, which is the product of the highest quality, is typically used as recombined milk for cheese manufacture, milk solids standardization for cheese milk or yogurt and other fermented milk products (*Patel et al, 2007*). High-heat SMP is used as an ingredient in bakery, sweetened condensed milk, and confectionery products (*Stewart et al., 2017*).

Before use, SMP has to be reconstituted to certain levels of total solids, depending on the application (*Lagrange et al., 2015*). The rehydration process consists of wetting, dispersing and...
solubilization of the powder. The feed solids content, as well as the drying conditions (dryer type, atomizer type, drying conditions) affect powder properties (e.g. particle density, particle size distribution, moisture content, air content). These in turn can have a significant impact on functional properties of the powder that are critical for reconstitution (bulk density, flowability, dispersibility, wettability, and solubility).

Recent years marked a renewed interest in milk powder quality and the factors that impact it (Stewart et al., 2017; Lin et al., 2018). There is however limited work available on new methods that can be used to produce milk powders of higher quality.

New technological developments relevant to milk powder manufacturing. Recent technological advances allowed the development of processing technologies that use moderate heat or no heat, some of which could be used as alternative methods for producing dairy powders of higher quality compared to the traditional thermal concentration followed by spray drying. For instance, with funding from the National Dairy Council (NDC), our group has investigated for the past two years the use of Forward Osmosis (FO) as a nonthermal method to concentrate milk. The use of FO allowed us to obtain similar concentration levels as thermal concentration, but in a completely non-thermal process. This process is also superior to Reverse Osmosis (RO), another membrane filtration process that is used by some dairy processors to replace thermal concentration, since it is less prone to fouling. Additionally, FO may be better suited for concentration of whole milk than RO, since it uses much smaller pressures and therefore it is much gentler and can better preserve the integrity of the milk fat globules.

The milk concentrates obtained by FO are of very high quality, since they are not subjected to heating. To take full advantage of this nonthermal process, it would be ideal to also use a gentle process for drying the FO concentrates.

One of the most remarkable recent advances in drying is Radiant Energy Vacuum dehydration (REV™), or Vacuum Microwave Drying (VMD), which uses microwave energy under vacuum to efficiently remove moisture at temperatures much lower than in traditional air drying methods such as spray drying.

In VMD, microwaves stimulate vibration and rotation of water molecules and the movement of ions inside the food, resulting in internal heat generation, which leads to water vapors, which are then removed through the application of vacuum (Kaensup et al., 2002). Since the process takes place under vacuum, oxidation is prevented, which can also be very beneficial for milk products, particularly those that contain fat. This technology is much faster than freeze drying, can be operated continuously, and leads to excellent retention of nutrition, color, flavor and functional properties of the dried products. Drying under vacuum also minimizes oxidation of the dried product, thus preventing unwanted changes in sensory and nutrient quality of the food (Cui et al., 2004; Böhm et al., 2002). This could be a significant advantage to dairy powders that contain fat, even in small amounts.

For the manufacture of milk powder, it is anticipated that the VMD process will allow lactose to be present in crystal form (non-hygroscopic) rather than in amorphous form (highly hygroscopic), which forms during spray drying. This could be a significant advantage for the storage of the final milk powders, since the powder will have a much lower tendency for caking, which happens when lactose is present in amorphous form.

Additionally, some earlier work pointed to the potential of VMD for microbial reduction in the dried samples (Bourdoux et al., 2017). Daglioglu et al (2002) reported the complete elimination of Staphylococcus aureus after microwave drying of a fermented yogurt–cereal mixture inoculated with S. aureus (10⁴ CFU/g) and concluded that microwave drying was a more efficient way to
decrease the microbial population than air drying. Yaghmaee and Durance (2007) showed that higher reductions in microbial population of freshly grated carrots and parsley leaves occurred in a shorter time and at a lower final temperature in VMD compared to traditional air drying, with a dramatic decrease of microbial population occurring during the first 5 min of the VMD process. Although we do not anticipate that full microbial inactivation will occur in products dehydrated by VMD, any level of microbial reduction can be beneficial for milk powders, as very few options for microbial inactivation exist for the final product.

2. Specific Objectives

We will use a combination of nonthermal concentration (Forward Osmosis) and Vacuum Microwave Drying for the manufacture of dairy powders of high quality, with a particular focus on skim milk powder. The powders obtained by this combination process will have minimal heat exposure and damage, which will lead to superior physico-chemical, sensory, nutritional, functional, and possibly microbiological properties of the obtained milk powders compared to powders obtained using traditional thermal concentration and spray drying. By starting with high °Brix milk concentrates, we will minimize the energy consumption for the drying process.

A. Research objectives for Year 1 of 2 (ongoing work)

Objective 1.1: Identify the optimal parameters and processing conditions (feed concentration, vacuum level, product temperature, drying time) for the Vacuum Microwave Drying of nonthermally concentrated skim milk.

Objective 1.2: Evaluate the quality (physico-chemical, microbiological), functionality and storage behavior of the powders.

Brief description of accomplishments to date

In the first months of the project, we began evaluating the use of VMD drying for the dehydration of skim milk concentrates of different initial concentration, at different vacuum levels and drying times (Obj. 1.1). We have successfully produced vacuum microwave dried skim milk powder (VMD-SMP), as shown in Fig. 2 below.

Figure 2: Images of VMD skim milk foam (left) and ground VMD-SMP (right)

Based on the initial observations, we are currently working on developing the most suitable drying setups for milk concentrates, primarily focusing on: a) controlling the crystallization of lactose; b) drying of the liquid in a thin layer, conducive of fast and uniform drying. The drying
conditions will be optimized in the coming months, and the quality (physico-chemical, microbiological), functionality and storage behavior of the powders will be evaluated (Obj. 1.2).

B. Research objectives for Year 2 of 2 (proposed work)

Objective 2.1: Evaluate the use of the combination process for other dairy products and ingredients: whole milk, milk protein concentrate and micellar casein concentrate.

Objective 2.2: Estimate the energy consumption for the combined nonthermal concentration and vacuum microwave drying process, and compare it with the energy consumption for traditional powder manufacturing (thermal concentration & spray drying).

3. Experimental Approach

Approach to Accomplish Objective 2.1.

Based on findings from the first year of the project, we will carry out VMD of whole milk, milk protein concentrate and micellar casein concentrate of different initial concentrations, under varying vacuum levels and drying times (Obj. 2.1). As part of Obj. 2.2, we will evaluate the quality (physico-chemical, microbiological), functionality and storage behavior of the powders. All processing runs and analyses will be conducted in triplicate and data analyzed statistically.

Materials. Whole, homogenized pasteurized milk will be obtained from Cornell Dairy. Milk protein concentrate and micellar casein concentrate will either be obtained in our Pilot Plant or procured commercially from a dairy company.

Nonthermal concentration. FO concentration will be conducted using a pilot scale FO unit (Ederna, France), using methodologies and processing conditions already established in our laboratory. The unit is equipped with pressure gauges and instrumentation to monitor flow rates and temperatures. The temperature during the run will be maintained constant using a plate heat exchanger. The flux data will be collected gravimetrically, and the concentration of the product during the process will be measured using a refractometer (°Brix) and will be determined for all runs.

Production of MPC and MCC concentrates will be conducted by ultrafiltration and microfiltration, respectively, using a ceramic membrane rig available to the PI. Alternatively, commercial protein concentrates – preferably produced in New York State, and with a known thermal history, may be used as a starting material.

Vacuum Microwave Drying (VMD) will be conducted using a 10 kW Medium-Scale REV unit (Enwave Corp.) in the Cornell Pilot Plant in Geneva, NY. Characteristics: microwave power: 10kW; frequency: 2450 MHz; vacuum: 25-300 Torr.

Spray drying: For comparison purposes, the concentrated dairy fluids will be spray dried using a Model 1 Niro Atomizer (Columbia, MD), at an inlet air temperature of 200°C and outlet air temperature of 95°C. The powders will be collected and packaged in opaque plastic containers with triple lead thread lids, which will be then stored at 21°C.

Characterization of concentrates: a) Composition: total solids, protein, lactose, minerals; b) Physical properties: color, refractive index (°Brix), water activity, viscosity

The concentration of the feed solution and feed temperature will be measured using a Sper Scientific Pocket Digital Refractometer (Scottsdale, AZ), and reported in °Brix. Conductivity (during FO) will also be determined, using a Fisher Scientific Traceable Conductivity, Resistivity, and TDS Meter (Waltham, MA).

The total solids content will be measured using the AOAC Method 925.23.
Water activity will be determined with an AquaLab Dew Point Water Activity Meter 4TE (Ramsey, NJ).

Instrumental color parameters will be determined using a Konica Minolta CR-400 Chroma Meter (Pullman, WA). Color will be recorded using the CIE-L* a* b* uniform color space, where L* indicates lightness, a* indicates hue on a green (−) to red (+) axis, and b* indicates hue on a blue (−) to yellow (+) axis.

Chemical composition (protein, lactose, minerals) will be determined using standard methods at the Dairy One Laboratory (Ithaca, NY), as well as in the PI’s laboratory, using a MilkoScan.

**Powder characterization:** a) Physical parameters: bulk density, color, water activity, moisture content; b) Functional properties: wettability, solubility; c) Heat damage: whey-protein nitrogen index; free fat (for whole milk powder); d) Lactose crystallinity (by differential scanning calorimetry and/or X-ray crystallography); e) microbiological characterization. Most methods are routinely used in the PI’s group.

**Bulk density** of the powders will be determined using the volumetric method (IDF, 1995; standard 134A).

The **solubility index** (SI) of the powders will be determined by centrifugation (IDF, 1988; method 129A) using a Sorvall RC-5B Refrigerated Superspeed Centrifuge (DuPont Instruments, Wilmington, DW).

Wettability of the powders will be determined using the IDF standard method 87:1979. A set quantity of powder (6 g) will be gently discharged into a 400 ml beaker containing 100 ml of distilled water at 20 °C and allowed to immerse spontaneously without agitation. Powder wetted in less than 60 s is usually considered easy to wet while powder which takes longer than 120 s is considered non-wettable.

The **structure of powder particles** will be evaluated using Scanning electron microscopy (SEM), using methodologies available in the PI’s laboratory.

**Microbiological quality** (total plate counts, thermophilic and mesophilic spore counts) will be determined using standard methodology used in the PI’s lab.

**Approach to Accomplish Objective 2.2.**

As part of Obj. 2.2, we will evaluate the energy consumption for the combination processes that lead to the highest quality powders.

**Energy calculations** will be conducted following a methodology currently used in PI Moraru’s laboratory, as described in the paper by Menchik and Moraru (2019). In short, the total specific energy for FO will be calculated as the sum of the specific (electrical) energy for pumping, the specific (electrical) energy used in FO for regenerating the osmotic agent in the vacuum evaporator, and the energy used for maintaining the temperature of the liquid in the FO unit (mostly cooling). For drying, the total electrical energy consumption will be determined, based on the measured voltage and electrical current.

4. **Deliverables**

After the completion of this project, we will obtain:

a) VMD dairy powders with well characterized properties and functionality

b) Optimized VMD conditions for each type of produced powder
c) Energy calculations for the combination of nonthermal FO followed by VMD, and comparisons with energy calculations for the manufacturing of powders by thermal concentration followed by spray drying.

5. **Advantages for the NY and US Dairy Industry**

The combination of nonthermal FO and VMD for the manufacture of dairy powders is novel, unique, and could offer a competitive advantage to US produced dairy powders on the global market, as well as increase consumer and customer satisfaction on the domestic market.

References


Project 3: Request from NYS Milk Promotion Board for the period January 1, 2020 to December 31, 2020. $99,238.

Project 4: Lactose Oxidase – The application of lactose oxidase to control Pseudomonas and improve UHT milk.

New York State Milk Promotion Board Goal Addressed by this Project:

Conduct research to ensure the safety and / or expand the demand for New York dairy products and dairy ingredients.

Project PI’s: Samuel Alcaine, Cornell University

Duration: January 1st, 2020 – December 31st, 2020 (year 2 of a 2 year project)

Summary of Proposed Project:

In our previous work support by the NYSMPB, we showed that lactose oxidase was an effective antimicrobial for the control of *Pseudomonas* spp. in pasteurized and UHT milk. One of the challenges for the successfully application of lactose oxidase (LO) in retail milk, is continued enzyme activate, which over extended times, significantly drops the pH of the milk. *Pseudomonas* spp, however, do not only represent a post-pasteurization spoilage challenge. Raw milk in the US is typically quickly cooled to below 45°F and stored in tanks for up 72 hours, transferred to insulated trucks, before finally being pasteurized at the processing plant. Typically, microbiological testing is done when the tank is originally filled, not after the storage time. We know from our previous study, that *Pseudomonas* spp. can grow to high levels at these storage temperatures and times. Some *Pseudomonas* spp also produce heat stable enzymes that can survive UHT pasteurization, and result in a product defects, including age gelation of these shelf-stable products. We believe that this challenge represents a unique application opportunity for lactose oxidase. Our previous research has shown that LO is potent against *Pseudomonas* spp. at these low temperatures. This application is also unique as there is a subsequent pasteurization step that would in inactive LO, thus preventing an impactful drop in pH. We thus propose investigating the use of LO to control heat stable-enzyme producing *Pseudomonas* spp. under raw milk storage conditions, and show that it can be used reduce age gelation in UHT milk with minimal impact on sensory properties.

Objectives:

1. Evaluate MQIPs library of proteolytic *Pseudomonas* spp. to identify 2-3 strains that produce heat-stable proteolytic enzymes.
2. Demonstrate the ability of lactose oxidase to control *Pseudomonas* spp in raw milk under typical storage conditions.
3. Demonstrate the subsequent shelf-stability of UHT milk made from proteolytic *Pseudomonas* spp. contaminated raw milk treated with and without lactose oxidase.
4. Demonstrate that there is minimal detrimentally sensorial impact on UHT milk made from lactose oxidase-treated raw milk.

**Background:**

Spoilage of finished and intermediate dairy products is a significant source of financial loss for the industry. Increasing product shelf life also reduces waste, lessens the environmental footprint, and potentially improves consumer preference for dairy. One industry survey indicated that 6% of US consumers would increase their dairy product consumption if the products stayed fresher longer (Ledenbach et al., 2009). A challenging headwind for improving dairy product shelf life is the concurrent consumer demand for clean labels, minimal processing, and removal of chemical preservatives. There is a clearly a need to investigate more “natural” methods for dairy preservation to satisfy these conflicting consumer preferences.

The lactoperoxidase system (LPS) is a well characterized, antimicrobial preservation system naturally present milk. The system is essentially composed of three components: Lactoperoxidase, thiocyanate, and hydrogen peroxide. Lactoperoxidase oxidizes thiocyanate using hydrogen peroxide resulting in an intermediate product with antimicrobial properties. The system has been shown to be effective at inhibiting a wide range of spoilage and pathogenic microorganisms common to dairy products (Seifu et al., 2005). Two of the three key components, the enzyme lactoperoxidase and thiocyanate, are endemic to the milk, but the third component, hydrogen peroxide, must be added for the system to work. Traditional sources of hydrogen peroxide for activation of the LPS are: i) endogenous generation via lactic acid bacteria; ii) the addition of glucose oxidase in combination with glucose; iii) and the addition of sodium percarbonate. These sources are not ideal due to the impact of lactic acid bacteria growth, flavor effect of residual glucose, and the negative consumer perception of chemical additives to foods, respectively.

We propose the investigation of a novel LPS activation mechanism utilizing a commercially available lactose oxidase called LactoYIELD produced by Novozyme. The lactose oxidase converts lactose to lactobionic acid and in the process generates hydrogen peroxide (Ahmad et al., 2004) thus providing a lactose driven activator for LPS. The lactose oxidase is GRAS (Ahmad et al., 2004), is currently used in certain US dairy applications for the removal of lactose, and represents a label friendly aid as enzymes are a common to dairy food production. There has been no published work on the use of lactose oxidase as an activator for LPS, subsequent levels of spoilage inhibition, or impact on the final product. Research into LPS use in cottage cheese has suggested no major impact on flavor for that product type (Earnshaw et al., 1989). We intend to use skim milk and cottage cheese as proof-of-principle models, but this scheme has potential applications in high pH/high moisture cheeses, raw milk cheeses, and may be leveraged to extend raw milk storage. This research represents an opportunity to evaluate a novel scheme for improving dairy product shelf life using components that would be readily available for dairy producers to implement.
Experimental Approach:

Identification of *Pseudomonas* strains producing heat-stable enzymes.

All known, proteolytic strains of *Pseudomonas* spp. will be selected from the MQIP strain library. These strains will be grown up to log phase in liquid media. A heat-treated and non-heat treated aliquots of these overnight cultures will be spotted onto D-BHI milk media and monitored for zone of hydrolysis. Strains showing hydrolysis under both conditions will be selected for use in the study. If no strains show heat stable enzyme production, we will reach out to colleagues at other institutions that have previously isolated such strains.

*Pseudomonas* spp. Inhibition in Raw Milk

Raw milk will be sourced from the Cornell Vet School Dairy. Aliquots will be inoculated with a cocktail of the proteolytic *Pseudomonas* strains and will be treated with or without lactose oxidase at the concentrations previously identified in our earlier study. Samples will be stored at 6°C for 96 hours. Samples will be taken every 12 hours to evaluate growth.

Prevention of age gelation in UHT

Raw milk will be sourced from the Cornell Vet School Dairy and taken to the Cornell FPDL. The milk will be inoculated the *Pseudomonas* cocktail, stored with and without lactose oxidase, for 72 hours at 6°C. Both treatments will then undergo UHT pasteurization using the microthermics unit in the FPDL, and placed into sterile containers. These containers will then be stored at room temperature. Samples will be evaluated monthly over 4 months for sedimentation, creaming, pH, and gelation.

Sensory analysis of lactose oxidase treated UHT Milk

Raw milk will be sourced from the Cornell Vet School Dairy and taken to the Cornell FPDL. The milk will be treated with and without lactose oxidase, for 72 hours at 6°C. Both treatments will then undergo UHT pasteurization using the microthermics unit in the FPDL, and placed into sterile containers. These containers will then be stored at room temperature. Samples will be evaluated monthly over 4 months for difference in taste and appearance but the trained milk sensory panel.

Progress

We have made significant progress since January 2019 against the objectives outlined.

1. Evaluate MQIPs library of proteolytic *Pseudomonas* spp. to identify 2-3 strains that produce heat-stable proteolytic enzymes.
Figure 1. Isolate screening for heat-stable proteolytic enzymes. Isolates circled in red were selected for challenge cocktail.

We evaluated 28 isolates, spanning three *Pseudomonas* spp. commonly associated with dairy spoilage, for both protease and heat-stable protease production (Figure 1). We identified 20 isolates that produced heat-stable proteases that could potentially impact UHT milk stability. We choose 2 heat-stable protease producing isolates from each of the three species to move forward with as our challenge cocktails trains.

2. Demonstrate the ability of lactose oxidase (LOX) to control *Pseudomonas* spp in raw milk under typical storage conditions.

Figure 2. Lactose Oxidase-based control of *Pseudomonas* outgrowth.
We choose a level for LOX incorporation based upon the research of a previously funded NY Dairy Board project. We then evaluated the ability of LOX to control the outgrowth of the heat-stable protease-producing *Pseudomonas* spp. cocktail under worst case conditions, where raw milk samples contain a bacterial load near the 300,000 CFU/mL PMO comingled limit. LOX was able to inhibit the outgrowth of the cocktail, with minimal impact on pH, whereas the untreated sample the cocktail was able to grow to > 100,000,000 CFU/mL within 72 hours at storage temperature that are compliant with the PMO (< 7 °C). When *Pseudomonas* grows to levels > 100,000,000 CFU/mL, they are reported to produce heat-stable proteolytic enzymes that impact UHT milk quality. Raw milk may be stored in silos for up to 72 hours, before the silos are required to be cleaned. When considering potential raw milk residency time at the farm bulk milk tank, transportation time to the processor, and time in the processor’s raw milk silo, our challenge conditions are within reason. We have thus demonstrated that LOX can control *Pseudomonas* outgrowth under these worst case conditions.

**Year 2 Work**

We will continue to work, as outlined above, against the remaining objectives: 3) demonstrating the shelf-stability of UHT milk made from proteolytic *Pseudomonas* spp. contaminated raw milk treated with and without lactose oxidase; and 4) Demonstrating that there is minimal detrimentally sensorial impact on UHT milk made from lactose oxidase-treated raw milk.

**References**


**Project 4: Year 2: Request from NYS Milk Promotion Board for the period January 1st, 2020 – December 31st, 2020 - $83,543.**

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**Project 5: – Development of a rapid method to determine raw milk protein and fat quality.**  
*(Continuation project)*
New York State Milk Promotion Board Goal Addressed by this Project:
Conduct research to expand the demand for New York dairy products and dairy ingredients.

Abstract: As high milk protein beverage and other high milk protein products increase in commercial importance, the impact of variation in protein degradation and fat degradation in raw milk may present challenges in the manufacture of new high quality high milk protein products and products using milk fat as an ingredient. Enzymatic degradation of milk protein (particularly casein) and milk fat (release of free fatty acids) alters flavor and (functionality) use value of milk in the manufacture of various dairy products. Factors such as cow age, health history, metabolic stress, and dairy cow genetics may influence degradation of the valuable components of milk. The objectives of our research are: 1) to develop a rapid mid infrared method to measure proteolytic breakdown of milk proteins in raw and pasteurized milks and 2) To develop a rapid mid infrared (MIR) method to measure lipolytic breakdown of milk fat in raw and pasteurized milks. This method would be incorporated into the analysis of milk infrared spectra that are produced every time a bulk tank milk is tested for milk payment analysis. During the first year of this project, the reference chemical testing methods to measure proteolysis and lipolysis are being optimized for routine operation so the results of these accurate but slow testing methods can be used to develop the rapid MIR milk analysis prediction methods.

Project PI’s: Dave Barbano, Cornell University, Ithaca, NY

Duration: January 1st, 2020 – December 31st, 2020 (year 2 of a 2 year project)

Summary of Proposed Project:

Enzymatic degradation of milk protein (particularly casein) and milk fat (release of free fatty acids) alters flavor and (functionality) use value of milk in the manufacture of various dairy products. Factors such as cow age, health history, metabolic stress, and dairy cow genetics may influence degradation of the valuable components of milk.

Proteolysis of casein causes lower cheese yield (Barbano et al., 1991; Klei et al., 1998) and off flavors (Santos et al., 2003 a, b) in all dairy products. The primary proteolytic enzyme that causes damage to milk protein is the native milk protease plasmin. Plasmin exists at high concentration as an inactive proenzyme normally in milk (Verdi and Barbano, 1991). When something causes the conversion of the inactive form of the enzyme to the active form, enzymatic degradation of milk protein (particularly casein) occurs. One common factor that is associated with this activation of plasmin and increased proteolysis of milk casein is high milk SCC. The proteolysis of casein caused by elevated milk SCC decreases cheese yield (Barbano et al., 1991; Klei et al., 1998) and as a result many milk quality programs include different payments for milk based on the benefit of keeping milk SCC low. SCC is a practical index for increased proteolysis in milk, but the limitation is that when milk SCC returns to low and normal level, the amount of proteolysis in the milk does not fully return to pre-infection levels. There may be cumulative effects of udder health history of infection on the overall background level of proteolysis and lipolysis in raw milk produced by some individual cows. Thus, there are cumulative effects of individual cow health
events on overall level of proteolytic damage to milk protein that are not reflected correctly by a milk SCC test.

Unfortunately, the enzyme (plasmin) is very heat resistant and can even partially survive UHT treatment. The action of this enzyme is often the cause for age gelation of UHT shelf-stable milk. There are classical chemical testing methods that can be measure enzymatic protein degradation. The Kjeldahl nitrogen fractionation method and by SDS Page electrophoresis are two classical methods, but these methods are slow, expensive, and not practical for routine quality milk testing. A rapid, cost effective method for measurement of proteolytic damage to milk protein is needed.

Lipolysis of milk fat can cause off flavors in many dairy products. Lipolysis is a degradation of milk fat triglycerides where an enzyme cuts off fatty acids from the milk triglyceride structure. When short chain fatty acids (C4, C6, C8) are broken off from triglycerides they produce a large sensory impact (called hydrolytic rancidity) at very low concentration because of the low molecular weight. Again, this is cause by an enzyme that is normally present in the milk and is blocked from breakdown milk fat by the native milk fat globule membrane that protects milk fat from this enzyme. When animal health, genetic, or a combination temperature and mechanical mishandling of milk occurs, then the milk fat globule membrane is damaged and an increased level of lipolysis occurs. Rancid off flavor is readily detected in bland tasting high fat dairy products (cream, butter, and mild cheeses).

There are three classical chemical methods for measurement of the free fatty acid content of milk. Two of the methods (Bureau of Dairy Industries method and the copper soap method) are slow and difficult to do but are possible for a dairy QC lab to run on a small number of milks (10 to 40 milks per day). However, both methods have the limitation that they do not recover the shortest chain free fatty acid (i.e., C4) and therefore are relatively insensitive to low levels of lipolysis that may be detected as a sensory defect. The third classical method is by a chemical sample preparation procedure to isolate free fatty acids using a ion exchange resin binding method, followed by gas chromatography to measure the quantity of individual free fatty acids (Melilli et. al., 2004). The sum of all the free fatty acids can be calculated to produce a reference value for total free fatty acid content of each milk. This method is really only practical in a research environment, is slow (less than 100 samples per week) and very expensive (more than $100 per test), but is highly accurate. A rapid, cost effective method for measurement of proteolytic damage to milk protein is needed.

In the last 5 years, there have been many innovations and methods developed in Fourier Transform Infrared Milk Analysis (FTIR). This milk technology is rapid and used for milk payment and DHIA individual cow milk testing in the dairy industry. A recent innovation has been the development of statistical models that analyzed the FTIR milk finger print of bulk tank and individual cow milk fatty acid composition and relate those changes to changes in dairy cow response to feed composition, farm management practices, and animal health (Woolpert et al., 2016). Recently, the same technique is has been used to estimate blood NEFA level in transition cows by testing milk instead of taking and testing blood samples from individual cows. The new milk testing technology uses the same equipment and samples that are being tested for milk payment or DHIA milk testing, it is just software prediction models that derive more information from the FTIR spectral figure print of a milk that is created when fat and protein are measured. The machines that do this can test up to 600 milk samples per hour and are commonly used in the dairy
The dairy herd management milk testing for milk fatty acid testing developed at Cornell is being implemented in many laboratories around the country and is currently done by St Albans Cooperative, AgriMark Cooperative, and Cayuga Marketing on individual farm bulk tank milks as part of the routine milk payment testing. The incremental cost of this new information is relatively low and is one of the reasons for its rapid implementation, but the value of the data in making farm management decisions to improve dairy farm sustainability is key.

The recent success in rapidly measuring fatty acids and milk estimated blood NEFA has demonstrated that this concept of method development and implementation is working. Thus, using the modern tools of FTIR partial least square statistical analysis of milk MID-IR spectra may be a feasible approach to develop rapid and cost effective methods for determination of proteolysis and lipolysis in milk. The research goal would be to improve the overall quality of raw milk and dairy products by providing cost effective milk testing tools to identify and correct the causes of proteolysis and lipolysis in milk. In accomplishing this goal it would provide two new cost effective milk quality testing methods that could applied to bulk tank milk as part of the milk payment testing sampling and milk analysis. Once farms are found with high levels of proteolysis or lipolysis at the bulk tank level, then individual cow milk samples from a farm could be tested using the milk testing technology to identify individual cows that have a higher impact the enzymatic degradation of milk protein and fat. This would provide information to be used in combination with other data to help guide culling decisions that would help improve bulk tank milk quality.

Objectives:

1) To develop a FT mid infrared method to measure proteolytic breakdown of milk proteins in raw and pasteurized milks.

2) To develop a FT mid infrared method to measure lipolytic breakdown of milk fat in raw and pasteurized milks.

Experimental Approach:

Objective 1. SDS PAGE (Verdi et al., 1987) will be used as the reference method for measurement of proteolytic breakdown of milk protein. Sets of 10 individual farm milks that vary in milk somatic cell count will be preserved and held at 4 to 6°C for 3 weeks to allow milk proteolytic enzymes to degrade milk protein. The milks will be sampled weekly and analyzed by SDS PAGE to measure extent of proteolysis and will be analyzed using a mid FTIR milk analyzer to collect spectra of the same milk samples as the protein is gradually broken down by native milk proteases. This will produce a population of samples that have the same overall composition but vary with time in the extent of proteolysis. If the increasing degree of proteolysis makes changes in the milk MIR spectra, then it will be possible to use the SDS PAGE reference chemistry values for degree of proteolysis in combination with their respective MIR spectra to produce a predictive milk analysis model for proteolysis caused by native milk proteases. The modeling will be done with Grams32 software and the PLS models will be run on a Delta FTA mid infrared milk analyzer. An external milk sample population will be used for the validation of the performance of the new predictive PLS model.
Objective 2. Resin binding for isolation of free fatty acids combined with GLC analysis of fatty acid methyl esters (Melilli et al., 2004) will be used as the reference method for measurement of lipolysis of milk fat. Sets of 10 individual farm milks that vary in milk somatic cell count will be preserved and held at 4 to 6°C for 3 weeks to allow native milk lipases to degrade milk fat. The milks will be sampled weekly and analyzed by GLC to measure extent of lipolysis and will be analyzed using a mid FTIR milk analyzer to collect spectra of the same milk samples as the milk fat is gradually broken down by native milk lipases. This will produce a population of samples that have the same overall composition but vary with time in the extent of lipolysis. If the increasing degree of lipolysis makes changes in the milk MIR spectra, then it will be possible to use the GLC reference chemistry values for degree of lipolysis in combination with their respective MIR spectra to produce a predictive milk analysis model for lipolysis caused by native milk lipases. The modeling will be done with Grams32 software and the PLS models will be run on a Delta FTA mid infrared milk analyzer. An external milk sample population will be used for the validation of the performance of the new predictive PLS model.

Deliverables. Two partial least squares prediction models for milk analysis that will measure the level of proteolysis of milk protein and level of lipolysis in a milk sample. The models will be developed and compiled so they will run on a commercially available infrared milk analyzer.

References


Project 5: Budget request from NYS Milk Promotion Board, $121,498. (Year 2 of 2)
New Projects:

Project 6: Encapsulating Cultures for HPP Dairy Products.

New York State Milk Promotion Board Goal Addressed by this Project:
Conduct research to ensure the safety and/or expand the demand for New York dairy products and dairy ingredients.

Project PI’s: Samuel Alcaine, Cornell University
Duration: January 1st, 2020 – December 31st, 2020 (year 1 of a 2-year project)

Summary of Proposed Project: High pressure pasteurization (HPP) technology is of interest to dairy processors due to its ability to eliminate vegetative pathogens, maintain the fresh flavor and textural characteristics of the food when compared to thermal processing; and reduce reliance on added preservative agents, thus allowing the production of foods with cleaner labels. The challenge for dairy processors is that this technology also inactivates the bacterial cultures we use to ferment dairy products, which makes leveraging HPP in fermented products challenging. Our goal is to investigate the use of encapsulation technologies to improve the compatibility of dairy cultures with HPP.

Objectives:
1. Evaluate the use milk fat and/or cocoa butter encapsulation of freeze-dried dairy cultures.
2. Evaluate the stability of the encapsulated cultures through HPP processing.
3. Evaluate the stability and subsequent activity of encapsulated cultures in milk pre and post HPP processing.

Background:
High pressure pasteurization (HPP) technology is being adopted by NY food producers as an alternative preservation method to traditional thermal pasteurization technologies because HPP is: i) effective at eliminating vegetative pathogens; ii) retains more of the fresh flavor and textural characteristics of the food when compared to thermal processing; and iii) reduces the reliance on added preservative agents, thus allowing the production of foods with cleaner labels. Dairy producers are interested in this technology, however, bacterial cultures – like starter cultures probiotics -are typically inactivated by HPP treatments, curtailing their use in these applications. Our goal is to investigate the impact of bacterial preservation technologies, like freeze-drying and encapsulation, on the survival of select lactic acid bacteria cultures through HPP.

Potential for Dairy Products
A key driver in consumer consumption of yogurt is the positive impact of probiotic cultures on health. The National Yogurt Association highlights the importance of these bacterial constituents by promoting the “Live and Active Cultures” seal on yogurt products. These cultures represent a
challenge to US producers looking to export yogurt, as they will continue to acidify the yogurt, negatively impacting final flavor and texture. Shelf-stable yogurts are available, but due to processing, lack live cultures and thus lack the probiotic benefits consumers desire. High pressure pasteurization (HPP) is a relatively new processes technology which can be leveraged to create shelf-stable acidic foods. While these treatments are effective at destroying vegetative cells in high-moisture foods like yogurt, they are not very effective at destroying vegetative cells in low-moisture foods. 

If freeze-dried bacteria survive HPP as we hypothesize in this project, we will have the foundation to pursue novel encapsulation process, that combines freeze-drying and hydrophobic materials to create microcapsules that would maintain the freeze-dried cultures in a desiccated, process-resistant state when added to yogurt. The yogurt could then be processed to destroy the non-encapsulated bacteria responsible for acidification, thus creating a shelf-stable product, while allowing the encapsulated, probiotic culture to survive to the consumer.

Another benefit of HPP, is that we typically process the food in the final package, thus eliminating the risk of contamination by spoilage organisms or pathogens from the environment. Some dairy producers have looked at using HPP as a replacement for thermal pasteurization. This may work for fluid milk, however for yogurts, which can also be produced within the final package (cup-set), this present a challenge because HPP would also kill the starter culture. In contrast to the probiotic cultures mentioned earlier, it may be possible to encapsulate the starter cultures in such way that when initially added to the milk, the culture is kept dry so that it survives HPP treatment, but after HPP treatment it is released from encapsulation, rehydrates in the milk become activing. The cultures then ferment the milk, dropping the pH, creating a yogurt with more of the distinct flavors and aromas of the non-thermally processed milk.

This work builds upon a small federal capacity fund grant to evaluate ways to improve the survival of protective bacterial cultures in high pH foods. Our current work has shown that freeze-dried cultures do have improved an HPP survival rate in comparison to hydrated cultures (Figure 1). The next challenge is to see how well encapsulation technologies can be used to maintain the stability of these cultures in high moisture products.
Experimental Approach:

Evaluate the use of cocoa butter and/or milk fat as encapsulating agents for freeze-dried cultures: We will explore three methods to encapsulate the freeze-dried cultures: 1) direct mixing of the freeze-dried culture with a lipid carrier (either cocoa butter or milk fat); 2) mixing of the freeze-dried culture with the lipid carrier followed by spray cooling; 3) mixing of the freeze-dried culture with the lipid carrier followed an oil in water emulsion, followed by spray cooling. The cultures will be evaluated for viability pre and post encapsulation. The processes providing the highest percent preservation will be selected for the next objective.

Evaluate the stability of the encapsulated cultures through HPP processing: Based on the results of the first objective, we will select at least 2 encapsulation methods for the freeze-dried bacterial cultures. We will then produce larger batches of the encapsulated cultures and process these encapsulated culture using the HPP unit at Cornell AgriTech in Geneva New York. The cultures will be evaluated for viability pre and post processing, stability of the encapsulation shell, and the ability to rehydrate in a liquid matrix.

Evaluate the stability and subsequent activity of encapsulated cultures in milk pre and post HPP processing: Based on the results of the second objective, we will select at least 1 encapsulation methods for the freeze-dried bacterial cultures. We produce a larger batches of the encapsulated cultures, add encapsulated and non-encapsulated cultures to milk, and process the inoculated milk samples using the HPP unit at Cornell AgriTech in Geneva New York. The samples will be evaluated for culture viability and activity (measured via pH drop).

Expected Results and Outcomes: The results of this research will expand the application of HPP to fermented dairy products through the development of new processes that will either allow the creation of higher quality, flavorful yogurts for the local market, or high quality, shelf-stable probiotic yogurts for the export and or local markets.

Project 6, Year 1 of 2: Request from NYS Milk Promotion Board for the period January 1st, 2020 - December 31st, 2020 - $68,203

Project 7: Fermenting value for dairy: Evaluating skim milk as a substrate for the biomanufacturing of value-added ingredients and products.

New York State Milk Promotion Board Goal Addressed by this Project:

Conduct research to ensure the safety and / or expand the demand for New York dairy products and dairy ingredients.

Project PI’s: Samuel Alcaine, Cornell University
Duration: January 1st, 2020 – December 31st, 2020 (year 1 of a 1-year project)

Summary of Proposed Project:
Decreasing demand for skim milk and skim milk powder, two major dairy products for the NY, represents a challenge and opportunity for the industry. One hurdle in skim milk utilization is its lactose content, to which many consumers have an intolerance to, thus limiting its consumption and use as an ingredient in other food products, despite its protein content. We propose building upon our current research and learnings from dairy by-product fermentation, to expand the utilization of fermentation to create novel, value-added ingredients from milk. Our previous research has shown that galactose-rich dairy-products can be potential be produced via fermentation, but we have not evaluated this process in protein containing dairy substrate like skim milk. The galactose-rich product could serve as inputs in to further value-added processes, like galactooligosaccharide production, tagatose production, and high protein/lactose-reduced consumer products. This research would lay the groundwork for launching a new category of dairy-based ingredients that would expand the utilization of milk, and position New York State Dairy as a leading innovator in the space.

Objectives:

1. Evaluate the fermentation of skim milk and milk concentrates by *Brettanomyces* and related yeast species to produce a galactose-rich fermented milk.
2. Optimize fermentation parameters for galactose production from skim milk and milk concentrates by *Brettanomyces*.

Background:

Lactose is a disaccharide composed of glucose and galactose. It is the key carbohydrate component in milk for young humans and other mammals. In humans, lactose is hydrolyzed by lactase produced in the small intestine. The resulting glucose is absorbed and used for energy, and galactose is taken to the liver and utilized to form parts of glycoproteins and glycolipids. As humans mature, many lose the ability to produce lactase and become lactose intolerant. The inability to digest lactose creates increased osmotic stress in the intestines, resulting in fluid loss. Bacterial fermentation of the lactose in the large intestine produces both gas and acids. These factors manifests themselves in gastrointestinal symptoms, such as diarrhea, abdominal cramping, and bloating, resulting in poor nutrient absorption. Estimates suggest that nearly 70% of the global population becomes lactose intolerant, including 30-50 million Americans, with intolerance rates above 75% among African American, Asian American, and Native American minorities. This inability to digest lactose is thought to be a major factor in decreasing US milk consumption, and makes successful utilization of excess lactose in foods and food ingredients problematic.

*Brettanomyces claussenii* is a yeast that has recently become popular in that craft brewing industry for its distinct flavor contributions to beer. *B. claussenii* is unique in its genus for its ability to ferment lactose into ethanol under anaerobic conditions. Under aerobic conditions, *B. claussenii* can produce high levels of acetic acid from sugars. Surprisingly, there has been little research exploring lactose utilization by *B. claussenii* under either condition. Current research in Dr. Alcaine’s lab has shown the successfully conversion of acid whey into alcoholic beverages, and whey permeate into acetic acid. Furthermore, current trials on sugar utilization by *B. claussenii* found that in the presence of free glucose, the yeast does not utilize galactose. This suggests that in
combination with lactase, B. clausenii could be used to selectively ferment glucose, leaving galactose, a low glycemic sugar that is twice as sweet a lactose.

Galactose is a monosaccharide, primarily derived from lactose, though it can also be found in cell wall components of vegetables and legumes. Galactose, unlike glucose, is a low-glycemic sugar (8) and unlike lactose, does not cause gastrointestinal discomfort (49). Because of these properties, there is interest in the potential health benefits of galactose (16, 36) and its utilization in sports beverages (8, 12, 44). Galactose is also a precursor for other value-added sweeteners, like tagatose, galactooligosaccharides (GOS), and pharmaceuticals compounds (20, 26, 28). Galactose production from lactose requires either a chemical or enzymatic hydrolysis step, followed by separation of the sugars. This separation may be achieved by a capital intensive chromatography step (34), secondary enzymatic treatment with glucose oxidase (16), or through a selective fermentation step with yeast (16) or bacteria (9). Current research in Dr. Alcaine’s lab has shown that B. clausenii could also be used to selectively, anaerobically ferment glucose post-hydrolysis, thus producing galactose and ethanol, the later which could be captured and valorized via distillation.

**Production of galactose via selective fermentation by B. clausenii.**

In order to better understand anaerobic fermentation, Dr. Alcaine’s research has been looking into sugar utilization in yeast nutrient broth by B. clausenii in comparison to K. marxianus, and S. cerevisiae (Figure 1). K. marxianus is effective at utilizing all the sugars (Figure 1B), glucose, galactose, and lactose. S. cerevisiae cannot utilize lactose (Figure 1C). B. clausenii, like K. marxianus, can also utilize all three sugars as well, but when both glucose and galactose are present it appears to ferment the glucose (Figure 1A), but then does not switch to utilizing galactose as noted by the plateau in density. This plateauing was also observed in a separate experiment when acid whey was supplement with glucose for B. clausenii (data not shown). The metabolic regulation of sugar utilization in B. clausenii has not been well studied, but our data suggests that B. clausenii could be used to selective ferment glucose and leave galactose. Further research is necessary to see if these observations hold when fermenting hydrolyzed skim milk, the impact of increasing starting solids & sugar concentrations, impact of the fermentation on skim milk protein stability, and post-fermentation levels of residual sugar and ethanol.

![Figure 1. Sugar Utilization (A) B. clausenii (B) K. marxianus (C) S. cerevisiae](image-url)
Experimental Approach:

Our goal is to optimize the conditions for the biomanufacture of galactose from skim milk and concentrated skim milk (2-3X) utilizing the yeast *B. claussenii*. This will be achieved through two steps: ii) screening of parameters that influence *B. claussenii* ethanol and residual galactose levels; and ii) use of response surface methodology to optimize the key fermentation parameters for either ethanol or galactose production for each dairy by-product.

**Screening of fermentation variables:** Screening will be performed using the in-house *B. claussenii, B. bruxellensis, K. marxianus,* and *S. cerevisiae* strain. We will use a full factorial design, with the appropriate center points, to screen the impact of temperature (25 °C and 35 °C), skim milk concentration (1X and 3X), cell concentration (5 million and 15 million CFU/mL), and lactase (presence at manufacturers recommend dose and absence). The following response outputs will be measured at regular intervals throughout the experiment: density, pH, and cell counts. The following response outputs will be measured at the start and end of each experiment: sugars (lactose, glucose, galactose) via HPLC, organic acids (acetic, lactic) via HPLC, ethanol via HPLC, and a visual assessment of protein coagulation. These experiments will be performed in triplicate. Experiments will be carried out in batch flask fermentations, with air locks, until the density measurements remains unchanged for three consecutive measurements or 14 days have been reached.

**Optimization:** Based on the results of the full factorial experiment, the most significant factors will be selected, and optimized using response surface methodology via a central composite design. Experiments will be performed with the in-house *B. claussenii* strain on hydrolyzed skim milk at three concentrations: 1X, 2X, 3X. The following response outputs will be measured at regular intervals throughout the experiment: density, pH, and cell counts. The following response outputs will be measured at the start and end of each experiment: sugars (lactose, glucose, galactose) via HPLC, organic acids (acetic, lactic) via HPLC, and ethanol via HPLC. Experiments will be carried out in batch flask fermentations, with air locks, until the density measurements remains unchanged for three consecutive measurements or 14 days have been reached.

**Expected Results and Outcomes:** This objective will provide foundational knowledge on alcohol and galactose production by *B. claussenii* in a skim milk system. All our previous work has been in protein-free dairy substrates. The screening and optimization work will provide key conditions for commercial biomanufacturing of ethanol and galactose. This final product can then be utilized as an input to multiple value-added manufacturing schemes. The galactose-rich, fermented skim milk will be used as input (Figure 2) to other inter-departmental projects that look to optimize the production of galactooligosaccharides from dairy substrates and as an input to extrusion process to produced dairy protein-rich, lactose-reduced, products. Lastly, the fermented skim milk itself will be preliminary evaluated to see if it would be suitable as the base of a stand-alone consumer facing product.
Figure 2. Value-added products from skim milk

References


Project 7, Year 1: Request from NYS Milk Promotion Board for the period January 1, 2020 - December 31, 2020 - $94,163.

Project 8 Title: Conversion of Sugar in Skim Milk to Galacto-oligosaccharides

New York State Milk Promotion Board Goal Addressed by this Project:
Conduct research to expand the demand for New York dairy products and dairy ingredients.

Project Abstract:
Skim milk contains 4.8% of lactose, a starting material to produce a prebiotic called galacto-oligosaccharides (GOS). Due to the increasing popularity and health benefits of GOS, the goal of this
The proposal is to increase the value of skim milk and expand the demand for New York originated skim milk by producing GOS-rich skim milk. We plan to use a continuous bioreactor we have previously built for the production of GOS. Such platform can convert 39% of lactose into GOS, a yield much higher compared to other reported methods. By collaborating with other faculty in the Cornell Food Science Department, we have organized an integrative plan to first produce GOS-rich skim milk, and then use GOS-rich milk as a key ingredient for novel extruded dairy products. In the first year of the study, we will use concentrated skim milk (2-3X) as starting material and adapt/modify our bioreactors to the physical properties of the concentrated skim milk to produce the highest amount of GOS in the skim milk by optimizing the processing parameters. In the second year of the study, we will use a fermented skim milk (galactose rich) as the starting material to explore a different reaction dynamic of GOS-synthesis in skim milk.

**Project PI: Alireza Abbaspourrad**

**Duration:** January 1st 2020 – December 31st 2020 (year 1 of a 2-year project)

**Background:**

An estimated ~65% of people worldwide suffer from lactose intolerance. The lactose (4.8%) found in skim milk can be hydrolyzed by lactase to produce lactose-free skim milk or low-lactose skim milk. Such skim milk, with glucose, galactose, and small amount of lactose, can be concentrated for fermentation and extrusion to create new value-added dairy products. The concentrated skim milk can also be directly subjected to enzymatic reactions to produce galactooligosaccharide (GOS) (Figure 1). In addition, the fermented skim milk can be utilized to produce a type of GOS-rich skim milk and then use as a starting material for extrusion.
Figure 1. Schematic illustration of use of the skim milk to produce value-added product.

The production of prebiotic GOS can be achieved by the enzymatic (β-galactosidase) conversion of lactose. Through the use of an enzyme immobilization technique, our group has built a cost effective, feasible platform to increase the efficiency of trans-galactosylation reaction and GOS production. In this recent work, we have also studied the bioconversion of whey permeates to GOS through the enzymatic action of β-galactosidase from Aspergillus oryzae in a continuous flow, packed-bed reactor. A novel enzyme immobilization, involving covalent immobilization of β-galactosidase on 3-aminopropyl triethoxysilane(3-APTES)-modified glass beads, was developed by our group using a cross-linking method.

Most studies use β-galactosidase in its free-state for the enzymatic conversion of lactose into GOS. Using the enzymes in their native form is often hindered by several limitations such as low operational stability, high costs, and difficulties in recovery and reuse. Our research on the β-galactosidase immobilization provides benefits such as enzyme reusability, increased enzyme stability and productivity, formation of products in continuous mode, reduced processing costs, improved product purity and quality, and eliminates the step needed to separate the enzyme from the reaction mixture (Figure 2). Our device also shows excellent reusability and can convert as high as 39% of lactose into GOS (Figure 3).
**Figure 2.** Schematic illustration of the continuous packed-bed reactor for the synthesis of galactooligosaccharide using β-galactosidase immobilized on porous glass spheres.

**Figure 3.** Repeated cycle reactions for GOS formation and pie chart showing the product composition (%) of lactose conversion in continuous packed-bed reactor after the 2nd cycle (39.3% GOS yield).
Specific Objectives:

Objective 1: adapt/modify our bioreactors to the physical properties of the concentrated skim milk
Objective 2: produce highest amount of GOS in the skim milk by optimizing the processing parameter

Experimental Approach:

Approach to Accomplish Objective 1 (year 1): The goal of this research is to convert the lactose, glucose, and galactose found in skim milk to a value-added product (GOS) using our continuous packed-bed reactor. We will use the concentrated skim milk as starting material. In the first year, we will perform experiments to convert sugars (lactose, glucose, galactose) in the concentrated skim milk (lactase hydrolyzed) to GOS using Aspergillus oryzae β-galactosidase covalently immobilized on porous glass spheres in a continuous flow, packed-bed reactor. Figure 2 displays the schematic of the continuous, packed-bed reactor for lactose conversion using β-galactosidase immobilized porous glass spheres. This device has shown promising results, i.e. 39% of yield, and great reusability when converting the lactose in the whey permeate to GOS. Furthermore, the continuous flow makes the conversion process very feasible and cost-effective to the dairy industry.

To be more specific, the β-galactosidase enzyme will be firstly immobilized on controlled porous glass (CPG) spheres using a cross-linking method. Activation will be achieved by the treatment of glass spheres with 3-aminopropyl triethoxysilane followed by the reaction with glutaraldehyde (GA). Then, the enzyme will be immobilized on the porous glass spheres and packed into the reactor equipped with thermal jacket. The size and packing density of the porous glass beads will be optimized to facilitate the flow of the concentrate milk. The loading and crosslinking of β-galactosidase on the glass beads will also be investigated and raised to increase the enzymatic reaction efficiency. The concentrated skim milk with adjusted pH, flow rates, and temperatures, will be pumped into the reactor to continuously convert sugars to GOS. Samples will be taken at different time intervals from the reactor and the concentrations of lactose, glucose, galactose, and GOS (tri-, tetra- and penta-saccharides, etc.) will be determined using a High-Performance Liquid Chromatography (HPLC) system. These evaluations will enable us to provide the most optimum conditions for the conversion of sugars (lactose, glucose, galactose) in the skim milk to GOS. Such treated skim milk can be further used for other applications to create novel dairy products rich in GOS.

Approach to Accomplish Objective 2 (year 2): In the second year of the project, we will use fermented skim milk, from our department collaborators, as starting material. In this phase, we will optimize the enzymatic conversion process for the fermented skim milk. The reactor performance will be evaluated using fermented skim milk with different lactose/galactose concentrations, pH’s,
temperatures, flow rates, reactor lengths, and reaction times. Since the fermented skim milk have a much more complex matrix than skim milk, we will also determine and optimize reusability and stability of the bio-reactor to ensure a high yield of GOS.

**Deliverables:**

1. An improved, continuous bioreactor design adapted to the physical properties of the concentrated skim milk for GOS production.
2. A proprietary process to produce GOS-rich concentrated skim milk which can be used as a starting ingredient to create novel dairy products.

**References**


**Project 8, Year 1: Request from NYS Milk Promotion Board for the period January 1, 2020 - December 31, 2020 - $107,380.**

**Project 9: Value-added products from lactose-hydrolyzed skim milk**

**New York State Milk Promotion Board Goal Addressed by this Project:**
Conduct research to ensure the safety and / or expand the demand for New York dairy products and dairy ingredients.

**Project Abstract:**
The proposed work will build a new route for direct conversion of lactose-hydrolyzed, preferably galacto-oligosaccharide-enriched, skim milk concentrates and powder into new food products using extrusion technology, test these products and demonstrate a path to prototype products of industrial utility. Work presented in this proposal is novel and unique in the following ways: First, use of skim milk for making extruded products for direct consumption using conventional cooking extrusion has not been successful despite several attempts. As practiced, the harsh processing conditions of cooking extrusion has precluded its utilization for the dairy products. Our proposed use of a low-temperature, and low-shear extrusion technology offers an attractive opportunity to create a new platform for utilization of skim milk via a new generation of products. Second, nonfat
Dry milk (NDM) contains over 50% lactose by weight, which is problematic for lactose intolerant consumers. Our proposal to enzymatically hydrolyze lactose in skim milk concentrate into glucose and galactose prior to extrusion would eliminate this issue and render products made with it highly consumer acceptable. **Third**, our preliminary data have shown that when lactose hydrolyzed whey is extruded, galactose preferentially polymerizes into galacto-oligosaccharides (GOS), a soluble dietary fiber. This is a very attractive proposition to convert a liability into an asset and make skim milk-based products more appealing to consumer. **Fourth**, using skim milk concentrate (20-30% solid) directly into the extruder will eliminate the drying step, resulting in big energy savings. **And finally**, experiments will be conducted with the objectives to i) determine the effects of adding lactose hydrolyzed skim milk concentrate and extrusion processing parameters on product quality and ii) compare the physico-chemical attributes of extruded prototypes (nutribar and baby puffs/melts) with their commercial counterparts.

**Project PI:** Sy Rizvi, Cornell University

**The full research project proposal is provided as a separate PDF file.**

**Duration:** January 1st 2020 – December 31st 2020 (year 1 of a 2-year project)

**Project 9, Year 1:** Request from NYS Milk Promotion Board for the period January 1, 2020 - December 31, 2020 - $99,120.

**Project 10:** High protein milk ingredients for value-added products

**New York State Milk Promotion Board Goal Addressed by this Project:**

Conduct research to ensure the safety and / or expand the demand for New York dairy products and dairy ingredients.

**Project Abstract:**

Milk proteins are well-recognized for their excellent nutritional qualities but their limited technological functionality poses constraints in their practical utilization in new, value-added products. Extension of low-shear, high-moisture extrusion to milk protein concentrates offers unique opportunities to achieve better functional properties in dairy proteins including viscosity building, gelling, emulsification, aeration, etc. in product formulations and to overcome some of the drawbacks of conventional products. The specific objectives of the proposed research are to i) investigate the effects of shear, temperature, moisture and carbon dioxide during high pressure extrusion processing to enhance the functionality of high milk protein concentrate (MPC85), ii) manufacture functionalized milk protein concentrate (f-MPC85) using the best operating conditions from objective 1 and evaluate its quality parameters for use an ingredient in food formulations. Development of high-protein, tailor-made ingredients based on these objectives will not only help produce many clean label food products but will also generate new commercial opportunities for dairy ingredients.

**Project PI:** Sy Rizvi, Cornell University
**Duration:** January 1, 2020 – December 31, 2020 (year 1 of a 2 year project)

The full research project proposal is provided as a separate PDF file.

**Project 10, Year 1:** Request from NYS Milk Promotion Board for the period January 1, 2020 - December 31, 2020 - $98,530.

**Project 11:** Making cool and nutritious milk based snacks for kids by 3D printing

**New York State Milk Promotion Board Goal Addressed by this Project:**
Conduct research to expand the demand for New York dairy products and dairy ingredients.

**Project Abstract:** We are proposing to use 3D printing to create novel, nutritious, tasty and attractive dairy snacks for children, from high quality milk concentrates. We will evaluate the effect of feed properties (concentration, pH) and temperature (preheating and post-deposition) on gelling and the structure and texture of 3D printed milk gels. The gel strength will be evaluated using rheological analyses, and the structure using scanning electron microscopy. We will also assess the effect of 3D printing parameters (rate of deposition, thickness of deposited layers, shape and size) on final product appearance, shape and texture. We will establish optimal feed composition, temperature treatment and 3D printing parameters for the creation of dairy snacks that are nutritious, attractive and with superior sensory properties for children. This work will lead to the creation of a new generation of dairy snacks, which will both satisfy the consumers and increase the utilization of milk solids. Due to its portability and low cost, this technology has the potential to be utilized in a range of food processing and food service facilities.

**Project PI’s:** Carmen I. Moraru (PI), Robin Dando (Collaborator)

**Duration:** January 1st 2020 – December 31st 2020 (year 1 of a 2 year project)

6. **Background**

   The opportunity for developing novel dairy snacks. Snacking between meals or even replacing whole meals by snacks in very popular in the US. In 2018, Americans spent an average of $195 on snack food which is approximately twice as compared to Canada, and more than twice as much compared to European countries. The snack food market in the U.S. will grow by 4.7% annually and will exceed 10% of the overall food market by 2023 (U.S. Snack Foods Industry - Statistics & Facts, Statista, 2019) leaving space for innovation. Savory snacks (chips, nachos, peanut puffs, cheese puffs and others) are the snacks purchased by US consumers in the highest amount, followed by candy and conventional cheese snacks. It should be noted that two types of dairy snacks can be found in the first six places in the billion-dollar US snack market. Dairy or dairy containing products continue to
be very popular, because they are perceived as clean label, low in sugar and high proteins. Additionally, they have a clean taste and numerous associated health benefits (Paddon-Jones et al., 2008).

**Dairy snacks for kids.** While very popular with adult consumers and parents, these traditional dairy snacks are not necessarily found very appealing by children, who are much more attracted by fun, colorful, “cool” snacks, often times high in sugar and calories, but no very healthy and nutritious. There is therefore a huge opportunity to develop dairy based snacks that are found attractive and fun by children, which could increase tremendously the consumption of dairy snacks – both at home, on the go, or as part of the school lunch program.

**Unique opportunities offered by 3D printing.** Edible 3D printing is becoming increasingly popular, both for professionals and for personal use. Most food 3D printers use extrusion 3D printing technology, in which a product with a paste consistency is printed layer after layer, generally through a syringe-like extruder (Fig. 1). Various shapes, including many that could be very attractive for children (see photos in Fig. 2) can be obtained this way.

Temperature control plays an important role both during the deposition by extrusion and in the post-deposition cooking, since it impacts the shape, texture and uniformity of the final products (Sun et al, 2018).

![Figure 1: Principle of 3D food printing by extrusion. Source: Sun et al, 2018](https://www.3dbyflow.com/designs-start)

The ingredients and products most commonly used for 3D food printing are chocolate, pancake batter and cream.

Here, we are proposing to use milk concentrates as a base for creating 3D printed dairy snacks that are nutritious and attractive, particularly for children. A desirable structure and texture of these products will be achieved by controlled gelling of the feed, by manipulating the feed (concentration and pH), and the 3D printing parameters (temperature, thickness of the deposited product, speed and total duration of deposition).

![Figure 2. Shapes of 3D printed food products. Source: https://www.3dbyflow.com/designs-start](https://www.3dbyflow.com/designs-start)

Gelation of milk / milk proteins and factors that affect it. The manufacture of dairy products such as yogurt or cheese are based on the gelling properties of milk proteins, achieved either by fermentation, enzymatic coagulation (i.e. renneting in cheese making).
or a combination of these two processes. The gelation process and the structure and texture of the obtained gels depend on a range of factors, most important being concentration and state of proteins, pH, calcium concentration and temperature.

Gelation in unheated milk occurs around pH 4.9; if acidification is performed at a very high temperature, a higher gelation pH can be seen (Vasbinder et al., 2001; Lucey, 2004). Typically, the strength of gels made from un-heated milk is low, due to the presence of dense clusters of aggregated casein particles, which prevent extensive particle rearrangement and cross linking during the gel formation.

Acidified milk gels made from heated milk form a gel at higher pH values because of the denaturation of whey proteins by high heat treatment. When heated milk is acidified, denatured whey proteins (β-lactoglobulin) become associated with the κ-caseins at the surface of casein micelles. β-lactoglobulin has an isoelectric pH of ~ 5.3, which is higher than the isoelectric pH of caseins, resulting in the high pH of gelation for heated milk. The gelation pH depends on the temperature of the preliminary heat treatment (see Fig. 3).

![Figure 3. Gelation pH (■) for the acidification of milk with 1.1% GDL at 32°C as function of the heat treatment temperature. Also shown: percentage of denatured whey protein; β-lg (•) and α-lac (○) in skim milk. Source: Vasbinder et al., 2001](image)

In terms of the effect of the temperature history of the milk, acid milk gels made from unheated milk are usually weak. The strength of gels made from heated milk is higher than those made from unheated milk, which is due to the fact that denatured whey proteins associated with casein micelles interact with each other and as a result increase the strength of the protein network.

Besides the temperature history of the milk, the structure and texture of acidified milk gels are influenced by several other factors, such as casein concentration / total solids content, pH, temperature, as well as the (Walstra, 1997; Lucey and Singh, 1998). The effect of these factors on the structure and strength of the 3D printed milk based products will need to be evaluated in the context of the current proposal.

7. **Specific Objectives**

The overall goal of this work is to use 3D printing of milk concentrates to create dairy snacks of desirable shape, structure and texture, which are nutritious, tasty and attractive for children. Specific objectives include:
Objective 1 (year 1): Evaluate the effect of feed properties (concentration, pH) and temperature (preheating and post-deposition) on gelling and the structure and texture of 3D printed milk gels.

Objective 2 (years 1 and 2): Evaluate the 3D printing parameters (rate of deposition, thickness of deposited layers, shape and size) that result in 3D printed dairy snacks that are attractive, uniform and stable.

Objective 3 (year 2): Optimize the 3D printing of dairy snacks that are attractive and palatable for children and other consumer categories.

8. **Experimental Approach**

**Approach to Accomplish Objective 1.**

We will use concentrated milks obtained by nonthermal concentration (FO) as a starting material, in order to preserve the quality of the product and to avoid as much as possible any heat damage to the product prior to the process. For preliminary studies, reconstituted milk concentrate of 30-50% solids will be obtained from low heat milk powders. Using a 3D printer with heated deposition plate, which we will acquire as part of this project, we will evaluate the effect of feed properties (concentration, pH) and temperature (preheating and post-deposition) on gelling and the structure and texture of 3D printed milk gels. The gel strength will be evaluated using rheological analyses, and the structure using scanning electron microscopy. Preliminary sensory studies will be conducted to inform the direction of the project in terms of size, shape, color and consistency of the created products.

**Approach to Accomplish Objective 2:** We will vary the 3D printing parameters (rate of deposition, thickness of deposited layers, shape and size) to obtain 3D printed dairy snacks that are attractive, and have a uniform and stable shape and texture.

**Approach to Accomplish Objective 3:** Based on the findings from the first two objectives, we will establish optimal feed composition, temperature treatment and 3D printing parameters for the creation of 3D printed dairy snacks that are attractive and palatable, primarily for children. This will be established by extensive product quality and sensory characterization of the most promising product prototypes.

**Experimental methods**

**Materials**

Milk concentrates – either obtained by reconstituting low heat milk powder, or obtained in house by nonthermal concentration (forward osmosis) of different levels of solids (30-50%) will be used in this study. For the reconstitution of powders, high shear mixing followed by a rest period at refrigeration temperatures will be used, to allow for fully hydration.

**Preliminary treatments**
Acidification. The pH will be lowered using chemical acidification with glucono delta lactone (GDL), at levels that will be established to obtain final pH values ranging between 6.7 (non-acidified controls) to 5.

Heat treatment prior to 3D printing. Pre-heating of samples up to 80°C will be achieved using water baths or incubators (Sauer and Moraru, 2012).

3D printing
For the purpose of the project, a byFlow Focus multi-material 3D printer (byFlow, The Netherlands) will be acquired and used. This 3D printer (see figure to the right) works with refillable cartridges containing paste-type ingredients, and is currently used for edible application, primarily bakery applications. The features of this 3D printer include different nozzle sizes (ranging from 0.3 mm to 1.60 mm) and a heated print bed made of glass, which can be heated to temperatures of up to 80°C. The printer is portable and has Wi-Fi connectivity.

Sample characterization
Textural analyses will be conducted using a TAXT2 texture analyzer to determine the samples’ texture.

Rheological analyses using a strain controlled rheometer will be conducted to monitor gelling behavior and gel strength.

Chemical evaluation of the samples. The samples will be subjected to proximate analyses (for fat, sugar, protein and minerals).

Microbiological evaluation of samples subjected to sensory evaluation will be carried out. Total aerobic counts, as well as the yeast and mold counts (Splittstoesser and Churey, 1989) will be determined.

Structure evaluation by Scanning electron microscopy (SEM) will be conducted to evaluate the structure of the various samples. A primary fixation will be done by treating the samples with 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer. After 1h the samples will be washed three times with cacodylate buffer. A secondary fixation will be done with 1% (w/v) osmium tetroxide in cacodylate buffer for 30 min, followed by 3 rinses with cacodylate buffer. Following dehydration with graded ethanol solutions, samples will be dried using supercritical CO₂, coated with evaporated carbon, and imaged using a Zeiss LEO 1550 SEM.

Sensory analyses will also be conducted using a non-trained sensory panel to evaluate the appearance, texture, taste and overall acceptability of the created products. In the initial stages of the study we will recruit panelists on the Cornell Campus, but in the second stage of the project we intend to recruit children as panelists.

We will use methodologies for consumer studies and sensory evaluation of samples that are established and routinely used by the Sensory facility at Cornell University, under the leadership of Collaborator Robin Dando.

Replication and statistical analysis
All experimental runs will be performed in triplicate. The data will be analyzed statistically, and significant differences between samples obtained under different experimental conditions will be determined (at p<0.05).

9. **Deliverables**
   After the completion of this project, we will obtain:
   
d) Quantitative data on the effect of feed and operating conditions on the structure and texture of 3D printed products obtained from concentrated milk
   
e) Data on sensory and quality attributes of 3D printed dairy snacks obtained by 3D printing
   
f) A novel, portable technology and several prototypes of dairy snacks of various shapes and sizes that are attractive for children and other consumers

10. **Advantages for the NY and US Dairy Industry**
    This work will lead to the creation of a new generation of dairy snacks, which will both satisfy the consumers and increase the utilization of milk solids. Due to its portability and low cost, this technology has the potential to be utilized in a range of food processing and food service facilities. To our knowledge, such snack products do not exist to date anywhere else. If successful, this technology will create a huge advantage to NYS and US Dairy Industry.

**References**
Piu, L. 2018. Dairy on the Go: Emerging Trends, Applications and Ingredients. Available at: https://www.naturalproductsinsider.com


**Project 11, Year 1: Request from NYS Milk Promotion Board for the period January 1, 2020 - December 31, 2020 - $95,342.**

**Project 12: Identification of sources of undesirable flavors in aseptic (UHT) milk.**

**New York State Milk Promotion Board Goal Addressed by this Project:**
Conduct research to ensure the safety and / or expand the demand for New York dairy products and dairy ingredients.

**Project PI’s:** D. M. Barbano (Cornell) and M. A. Drake (NC State)

**Duration:** January 1st 2020 – December 31st 2020 (year 1 of a 2 year project)

**Project Abstract:** *(for posting: one paragraph stating general approach and objectives)*

Numerous studies have demonstrated that US consumers (adults and children) prefer the flavor of HTST milk over ultrapasteurized (UP) milk, also called extended shelf life (ELS) milk (Chapman and Boor, 2001; Lee et al., 2017). This difference in liking is due to the distinct cooked/eggy flavor of UP milk (Lee et al., 2017), and recent work by the PIs has demonstrated the specific volatile compounds that cause these undesirable flavors and that these compounds are sourced from the serum protein fraction of fluid milk (Jo et al., 2018, 2019). Removal of serum protein from fluid milk followed by UP results in a milk beverage with no cooked/eggy flavor. More recent work in the PIs’ labs has demonstrated that aseptic or UHT milk is liked less by consumers than UP milk. Consumers noted both flavor and aroma as negative experiences in UHT milk, even following multiple exposure. Trained panel profiling of milks confirmed that the flavor profile of UHT milk was distinct from UP milk. ESL and aseptic processed milks have similar high temperature thermal treatment, but differ in the filling and packaging process and packaging materials, with aseptic milk having sterile filling and packaging. In commercial factories, the thermal processing is typically the same for aseptic and ESL milk from the same equipment and raw milk. The difference is that they are filled and packaged in different containers using different equipment and the time temperature conditions of product storage are different between ESL and aseptic milks. There have been no published scientific studies to fully characterize the sensory and chemical differences between ESL and aseptic milks. Our objectives are to determine the impact of time of storage (aseptic milk is stored longer than ESL), Temperature of storage (ESL is stored at refrigeration temperature and aseptic at room temperature) and differences in packaging (oxygen permeability of ESL and aseptic packaging material are different) on flavor of ESL and aseptic milks. Our research goal is to develop a technological approach to improve the flavor and consumer acceptability of aseptic shelf-stable milk and milk-based beverages.
Background: Previous and Preliminary work by the PI’s

The PI’s have extensive experience in the role of processing, flavor and consumer acceptance of fluid milk. PI Barbano has characterized the impact of raw milk somatic cell count on the enzymatic load and the impact of native milk enzyme action in the absence of microbial growth on degradation of milk protein during shelf-life of fluid milk (Santos, 2003 a, b). PI Barbano has conducted work on how milk fat influences the perception of milk flavor and mouthfeel (Phillips et al, 1995 a, b) and how microfiltration to remove spores influences the shelf life of fluid milk (Elwell and Barbano, et al. 2006; Caplan and Barbano, et al. 2013). PI Barbano has conducted research on protein standardization of fluid milk (Quinones et al, 1997, 1998) to improve consumer acceptance of beverage milk, which provided the technology foundation for the introduction of “ultra filtered” fluid milk products like Fairlife milk. PI Barbano and Drake have collaborated on the application of microfiltration to separate milk casein and milk derived whey protein (serum proteins) to determine the impact of individual protein groups on sensory properties of milk based beverages (Misawa et al, 2016) PI Drake has conducted work on the role of feed on milk flavor (Croissant et al., 2007), consumer perception of fluid milk (McCarthy et al., 2017; Harwood and Drake, 2018) as well as extensive work with chocolate milk, sugar reduction and child acceptance (Kim et al., 2013; Li et al., 2014; Li and Drake, 2015; Li et al., 2015). More recently, the PI’s have conducted joint work focused on fluid milk sensory quality including the role of raw milk cooling (Lee et al., 2016), milkfat (McCarthy et al., 2017), heat treatment (Lee et al., 2017) and vitamin premix (Yeh et al., 2017a, 2017b; Schiano et al., 2019). This body of work has demonstrated that consumers do not prefer the concept of UP(ESL) or the flavor of ESL milk and the PIs have recently documented the sources of these undesirable flavors and how to mitigate them with filtration technology (Jo et al., 2018, 2019). Ongoing fluid milk research that the PIs oversee includes work focused on child perception of school lunch milk packaging and flavor as well as the specific role of package on flavor transmission and leaching in HTST milk. This work has demonstrated that for school lunch milk the appearance of the package as well as the actual flavor of the milk are critical for child acceptance and that cardboard packaging of fluid milk can impart specific off flavors to milk that are noticed by children and adults. The sources of these off flavors include oxidation (due to high oxygen permeability) as well as transmission of off flavors from the package and the refrigeration environment.

The PIs also have conducted preliminary work with aseptic milk in tetrapak bricks and prisms. Although the heat treatment of aseptic milk is similar or identical to ESL milk, aseptic milks have distinct sensory profiles that are liked by consumers less than the flavor of ESL milk. Consumer dislike of aseptic milk was unchanged with age of the aseptic milk, multiple exposures to aseptic milk or by priming consumers with the advantages of aseptic milk. Consumer specific comments included aroma and flavor dislikes. There have been no published scientific studies to fully characterize the sensory and chemical differences between ESL and aseptic milks. There is a need to do so in order to pinpoint sources of consumer off flavors (package, storage temperature) and how to prevent or minimize them.
**Goal:** The goal of this study is to characterize the differences between aseptic and ESL milk and to identify the source(s) of these differences. This will provide insight into development of technological approaches to improve the sensory quality and consumer acceptance of aseptic milks.

**Specific Objectives:**

Determine differences between aseptic and ESL milk and understand the origin of those differences:

1) Determine the impact of differences in packaging (oxygen permeability of ESL and aseptic packaging material are different) on sensory quality and consumer acceptance of 1% milk.

2) Determine the impact of time of storage (aseptic milk is stored longer than ESL) and temperature of storage (ESL is stored at refrigeration temperature and aseptic at room temperature) on sensory quality and consumer acceptance of 1% milk.

**Experimental Approach:**

We will determine if differences in storage temperature or packaging of ESL and aseptic milks have an impact on milk flavor independent of the thermal process. An industry partner, Danone, has agreed to partner with the PI’s to process, package and provide the milks for the research study processed in one of their commercial facilities and cover all costs of that aspect of the project and shipping costs. The study will be replicated in two different months using different raw milks.

The proposed work will address the objectives.

Danone will process 1% fat milk on two different occasions at one of their commercial facilities. The same milk from each aseptic process run will be filled into ESL containers and into aseptic containers. This process will be achieved by processing of aseptic milk followed by overnight shipment to Broomfield Danone R&D where ESL treatments will be hand-filled into sterile puffs (i.e., premade and sealed ELS containers) under clean fill conditions. NCSU will provide at least one graduate student on site at Danone to assist with this process. Three different packaging treatments will be evaluated: 240 mL Tetra Bricks (aseptic) and two different types of ESL paperboard (TTS high oxygen barrier) and HP (low oxygen barrier) (objective 1). The package volume will be similar in all three treatments (about 240 mL / 8 oz). One group of aseptic tetra bricks will be stored at 4°C and another group at 21°C (room temperature) and the ESL paperboard containers will be stored at 4°C. This will make a total of 4 different treatments: aseptic product in one type of packaging material stored at two different temperatures and ELS product stored at one temperature but with two different packaging materials. The total length of the storage study for aseptic milks will be 24 weeks and the ESL milks will be 8 weeks (objective 2).

**Chemical and physical parameters to be measured during shelf life with indication of location where testing will be done and the number of containers needed for testing.**

**TIME 0 ASSESSMENTS - all treatments are the same at this point.**

1) Casein as a % of true protein (thermal treatment index) (T0) – Cornell 1
2) Furosine and furfuryl alcohol (thermal treatment index) (T0) - NC State 1
3) Denatured whey protein. (T0) (metric is Kjeldahl Casein as a % to TP) – Cornell. 1
4) Proteolysis during storage - Cornell 1
5) Volatile compound profile – NC State 6
6) Trained panel – NC State 6
7) Color – NC State 2
8) Viscosity at 4°C – NC State 3
9) Particle size (protein aggregates) – Cornell 1
10) Dissolved oxygen and headspace oxygen – Cornell 3

For the first timepoint – NCSU needs 18 240 ml containers and Cornell needs 7 240 ml containers

AFTER TIME 0, FOUR TREATMENTS ARE GENERATED – ASEPTIC 21°C, ASEPTIC 4°C AND ESL 4°C WITH TWO DIFFERENT PACKAGING MATERIALS

ITEMS TO BE MEASURED OVER TIME Volumes needed PER TIMEPOINT are estimated beside each location as the number of 240 mL containers needed

11) Changes in casein as % of true protein with time of storage (index of proteolysis) – Cornell 2
12) Change in Furosine and furfuryl alcohol – NC State 1
13) Particle size (protein aggregation and precipitation) - Cornell 2
14) Viscosity at 4°C. – NC State 3
15) Color (L, a, b-values) – NC State 2
16) Trained panel profiling – NC State 6
17) Volatile compound analysis – NC State 6
18) Dissolved oxygen and headspace oxygen – Cornell 3
19) Standard Plate Count – NC State 2
20) Consumer acceptance test at one time point (at 4 weeks of storage) – NC State 35

Timepoints of evaluation: 0, 1, 2, 4, 6, 8*, 10, 14, 18, 22 and 24 weeks.
*indicates end of ESL portion of study

There are 5 additional ESL timepoints and 10 additional aseptic timepoints

For each subsequent timepoint, NCSU will need 20 240 mL containers of each treatment + 35 240mL containers for one consumer test at 4 weeks. Thus for the ESL treatments this means (20 x 5) + 35 = 150* units of each of the 2 ESL treatments and for the aseptic treatments this means (20 x 10) + 35 = 250* units of each of the 2 aseptic treatments for a total of 800 containers of product.

For each subsequent timepoint, Cornell will need 7 240 mL containers of each treatment. Thus, for the ESL treatments this means (7 x 5) = 40* units of each of the 2 ESL treatments and for the aseptic treatments this means (7 x 10) = 85* units of each of the 2 aseptic treatments

*extra containers added just in case

Deliverable.
Data on the impact of different (packing materials) product oxygen levels on flavor changes during shelf storage at 4°C for all treatments and at 4 and 21°C for the aseptic product.
References


**Project 12, Year 1 of 2: Request from NYS Milk Promotion Board for the period January 1, 2020 - December 31, 2020 - $68,284.**

**Other Funding not requested from the NY SMPB:** DMI has indicated that the will fund the portion of this project to be carried out at NC State at a total level of $120,000 for 2 years. Danone will be a partner in the research and all results will be published. The processing, product and services contributed to the project by Danone has a value of about $60,000 and provides product for the processed in a commercial factory.
Previous Year Projects 2019

Total Budget from the NYS MPB for the Northeast Dairy Foods Research Center for the period Jan 1, 2019 to December 31, 2019 = $835,572

Continuing Projects in the year 2019

Project 1 - $87,557 (Abbaspourrad) - Conversion of lactose to GOS
Project 2 - $99,940 (Rizvi) – Functionalization of Whey protein
Project 3 - $63,648 (Alcaine) – Elasto-sense measurement of texture
Project 4 - $63,089 (Alcaine) – Upcycling of acid whey
Project 5 - $99,738 (Barbano/Drake/Grant) – Sources of autoxidized off flavor in milk
Project 6 - $50,548 (Barbano) continuing project (center technology transfer – on going)

Total Continuing Projects $464,520 for 2019 (Projects 1, 2, 3, 4, 5 and 6)

New Projects in the year 2019

Project 7 - $78,566 (Abbaspourrad) Nutritious Spreads and Fillings using Milk Ingredient
Project 8 - $101,210 (Moraru) Vacuum Microwave treatments.
Project 9 - $72,314 (Alcaine) Improving UHT shelf-life
Project 10 - $118,962 (Barbano) rapid method to determine raw milk protein and fat quality

Total New Projects $371,052 for 2019 (Projects 7 thru 10)
Proposed Projects for the Year 2020

Total Budget from the NYS MPB for the Northeast Dairy Foods Research Center requested for the period Jan 1, 2020 to December 31, 2020 = $1,094,499

Continuing Projects 2020

Project 1 - $50,548 (Barbano) continuing project (center technology transfer – on going)
Project 2 - $108,560 (Abbaspourrad) Nutritious Spreads and Fillings using Milk Ingredient
Project 3 - $99,238 (Moraru) Vacuum Microwave treatments.
Project 4 - $83,543 (Alcaine) Improving UHT shelf-life
Project 5 - $121,498 (Barbano) Rapid method for milk protein and fat quality

Total Continuing Projects $463,387 for 2020 (Projects 1 through 5)

Proposed New Projects 2020

Project 6 - $68,203 (Alcaine) Encapsulating Cultures for HPP Dairy Products
Project 7 - $94,163 (Alcaine) Fermenting Value for Dairy
Project 8 - $107,380 (Abbaspourrad) Conversion of Sugar in Skim Milk to Galacto-oligosaccharides
Project 9 - $99,210 (Rizvi) Value-added products from lactose-hydrolyzed skim milk
Project 10 $ 98,530 (Rizvi) High protein milk ingredients for value-added products
Project 11 $ 95,342 (Moraru/Dando) Milk based snacks for kids by 3D printing
Project 12 $ 68,284 (Barbano/Drake)

Total New Projects $631,112 for 2020 (Projects 6 through 12)
Project X: Value-added products from lactose-hydrolyzed skim milk

New York State Milk Promotion Board Goal Addressed by this Project:
Conduct research to ensure the safety and / or expand the demand for New York dairy products and dairy ingredients.

Project Abstract:
The proposed work will build a new route for direct conversion of lactose-hydrolyzed, preferably galacto-oligosaccharide-enriched, skim milk concentrates and powder into new food products using extrusion technology, test these products and demonstrate a path to prototype products of industrial utility. Work presented in this proposal is novel and unique in the following ways: First, use of skim milk for making extruded products for direct consumption using conventional cooking extrusion has not been successful despite several attempts. As practiced, the harsh processing conditions of cooking extrusion has precluded its utilization for the dairy products. Our proposed use of a low-temperature, and low-shear extrusion technology offers an attractive opportunity to create a new platform for utilization of skim milk via a new generation of products. Second, non-fat dry milk (NDM) contains over 50% lactose by weight, which is problematic for lactose intolerant consumers. Our proposal to enzymatically hydrolyze lactose in skim milk concentrate into glucose and galactose prior to extrusion would eliminate this issue and render products made with it highly consumer acceptable. Third, our preliminary data have shown that when lactose hydrolyzed whey is extruded, galactose preferentially polymerizes into galacto-oligosaccharides (GOS), a soluble dietary fiber. This is a very attractive proposition to convert a liability into an asset and make skim milk-based products more appealing to consumer. Fourth, using skim milk concentrate (20-30% solid) directly into the extruder will eliminate the drying step, resulting in big energy savings. And finally, experiments will be conducted with the objectives to i) determine the effects of adding lactose hydrolyzed skim milk concentrate and extrusion processing parameters on product quality and ii) compare the physico-chemical attributes of extruded prototypes (nutribar and baby puffs/melts) with their commercial counterparts.

Project PI: Sy Rizvi, Cornell University

Duration: January 1st 2020 – December 31st 2020 (year 1 of a 2-year project)

Background:

According to USDA, the United States is the world’s largest single-country producer and exporter of non-fat dry milk (NDM)/skim milk powder (SMP), producing in excess of 1 million metric tons per year and over 75% is exported without any value addition, fetching poor returns to dairy farmers and processors. Furthermore, the recent changes in trading policies of the European Union and Canada have exacerbated the issues and created hefty government stockpiles of NDM. It is a very highly underused and significantly cheaper than most dairy products. Its utilization is generally limited to either in the production of a number of other dairy products such as ice cream, yogurts, recombined milk products, etc. or as an ingredient in confectionery, bakery, soups and meat products. There is not a single value-added, non-dairy product in the market that contains NDM as the major ingredient. One of the main reasons for this is the fact that over 50% of skim milk and NFD milk solids is lactose and about 70% of the global population is lactose intolerant, including 30-50 million Americans (4). Thus consumption of skim milk-based product becomes
problematic for lactose intolerant consumers. Our proposal to enzymatically hydrolyze lactose in skim milk into glucose and galactose and then design and develop new value-added product would eliminate this issue and render products directly made with it highly consumer friendly.

Accounting for nearly $190 billion in U.S. sales across the store, protein is the most desired ingredient by consumers. And NDM powder contains 36-38% protein, with casein (80%) and whey protein (20%) in the same ratio as in milk. It also contains all the nutrients of milk, making it a very attractive ingredient. Yet, as shown in Fig.1, milk protein is at the lower end of per unit weight cost among different protein rich products, a real bargain for consumer but not for producers and processors. The identification of milk protein as a cost effective and readily available source of protein which lead to maximal health benefits is an important consideration in the development of nutritionally superior products. If properly exploited by converting NDM into value added products via judicious use of new approaches, there is no reason why it cannot be moved several notches higher in return on investment in dairy-based products.

![Fig.1. Average U.S. price (¢) per gram of protein (10)](image_url)

Key strategies to compete in this marketing space clearly requires that new approaches to converting skim milk into shelf-stable, organoleptically attractive, affordable and value-added products while retaining the maximum amount of nutrients are needed. This would not only capture latent demand gaps for dairy products in consumers’ changing preferences but would most ostensibly help increase demand for milk and milk ingredients.
Furthermore, in its quest to cater to a new generation of parents, the food industry launched a new line of infant/toddler first finger food – extruded puffs that melt in the mouth in less than 30 seconds. They are made of mostly white rice or other non-whole grains and a serving size of seven gram contains six grams of carbohydrate and no protein, Fig. 2. Designing such puffs with lactose-hydrolyzed and GOS enriched skim milk concentrate or powder that melts in the mouth offers a potential new market for such products. It is anticipated that hydrolyzed NDM will help in quick dissolution of extrudate while enriching it with milk protein and soluble dietary fiber (GOS), if the feed stream coming from other related, interdepartmental projects is utilized as planned.

Additionally, changing lifestyle, growing population of health-conscious consumers, and increasing disposable income are fueling the growth of the nutritious snack and cereal food markets globally. Sales of nutrition and energy bars in the U.S. increased by 71% and was valued at $1.7 billion in 2011(9). Packaged Facts reported the U.S. retail market for food bars, classified into two categories: cereal/granola bars and energy/nutrition bars at $5.7 billion in 2012 (14). New trends such as the blurring of meals and snacks, an emphasis on portion control, and on-the-go eating all favor the nutrition-based market. The category is positioned well for future growth, propelled by a strong demand to convert fun-for-you snacks into better-for-you snacks and cereals. This offers a unique opportunity to introduce hydrolyzed NFDM-based, nutritionally superior product into commerce using new and emerging technologies.

The conventional cooking extrusion is a high temperature short-time (HTST) process. It operates at temperatures in the range of 140-170°C and uses high mechanical shearing which induce excessive cross-linking, polymerization and Maillard browning reactions in products made with formulations containing significant quantities of dairy ingredients. Consequently, despite many efforts of the last decade, no expanded and texturally acceptable milk protein-rich, ready-to-eat bars or cereals are available in the marketplace today because the mechanics of structure formation during extrusion of these matrices without quality loss remain unattainable (3,5-8,11-13) and but offers an attractive segment for inroad by milk ingredient-rich counterparts. Key strategies to compete in this marketing space clearly requires that new approaches to processing and presenting milk ingredients into shelf-stable, organoleptically attractive, affordable and convenient products
while retaining the maximum amount of nutrients are needed. And extension of low-temperature and low-shear extrusion technology, which retains the nutritional and organoleptic qualities, is needed for delivery of dairy based extruded foods. We propose to use low-shear, low-temperature and temporarily-imposed acidity to hydrolyzed skim milk concentrate or powder containing formulations via high-pressure carbon dioxide-based extrusion to make puffed extrudates with minimum age-hardening and other quality degrading mechanisms commonly encountered in products made with high concentrations (>30 wt.%) of milk solids. The proposed process will be used to generate porous extrudates for use as baby puffs and nutribars. Preliminary studies on making expanded extrudates with skim milk powder and milk protein concentrate have been successfully conducted and prototypes have been tested with promising results in terms of color, flavor and texture (2,3). Critical work in the areas of hydrolysis of lactose, quantification of GOS formation and use of skim milk concentrate in extruder and their effect on product quality and process performance remain to be investigated and optimized to make the process robust and attractive.

**Preliminary Results:**

In order to understand the effect of lactose hydrolysis on skim milk quality, we converted 98% lactose-hydrolyzed milk to make 30% solid concentrates and milk powder. As shown in Fig. 3, the color of the powder was extremely white, compared to the slightly brownish color of comparable commercial sample. This is an attractive product indeed, waiting to be converted into new and novel products of commercial utility and we plan to undertake this activity under the proposed research.

Preliminary results have also been obtained to demonstrate that NDM mixed with a starch or milk protein matrix is amenable to expanded, porous extrudates of unique and architecturally different morphology with expansion characteristics similar to steam-based extrudate but at lower temperatures by using high pressure extrusion, which helps preserve the original color and heat sensitive ingredients. The interior of the expanded extrudate is porous with very smooth skin, ideal for surface coating. The color of the NDM and milk protein concentrate-containing product is well preserved through SCFX when compared with steam-expanded counterpart, Fig. 4.
Specific Objectives:

**Objective 1:** Determine the effects of adding lactose-hydrolyzed skim milk concentrate/powder and extrusion processing parameters on product quality

**Objective 2:** Compare the physico-chemical attributes of extruded prototypes (nutribar and baby puffs/melts) with their commercial counterparts.

**Approach to Accomplish Objective 1:**

The lactose-hydrolyzed skim milk produced directly by our group or obtained from related inter-departmental projects will be used as input (Figure 4) stream to develop extrusion process parameters to produce dairy protein-rich, lactose-reduced and possibly GOS enriched extruded crisps and puffs/melts. A factorial design will be used to set up experiments with various levels of lactose-hydrolyzed skim milk and rice starch in the formulations and the operating parameters of the extruder will be optimized.

A co-rotating twin-screw extruder with a length to diameter ratio (L/D) of 28.5 (Wenger Manufacturing, Sabetha, KS), available in Cornell’s Food Processing and Development Lab will be used for the proposed work. Formulations of 60-70 wt. percent milk protein concentrate or pregelatinized corn starch and 30 wt. percent lactose hydrolyzed skim milk concentrate will be extruded through a die with SC-CO2 injected at 0.4-0.7 wt. percent at 80°C. Process will be optimized through adjustment of specific mechanical energy, pressure, shear, and residence time. These response parameters will all be controlled through adjustment of operating parameters such as feed rate, screw speed, screw configuration, moisture content, and barrel temperature.

A factorial design will be used to set up experiments with various levels of Lactose-hydrolyzed skim milk concentrate and rice flour in the formulations. The extrusion parameter will be pressure, which will be varied between 1500, and 3000 psi. The temperature will be maintained between 60-80°C and the supercritical CO2 will be between 0.5% and 1.5%. All compositional analyses will be done use approved methods (1). Product ingredients and process parameters including shear, temperature, pressure, and dense phase carbon dioxide will be optimized for improved nutritional and textural qualities. We will study a detailed phytochemical composition and how
each step of the production process impact their retention. Five different tests will be used to characterize the product, as described below:

- **Expansion ratio**: The expansion ratio is an indicator of the degree of expansion of the extrudates and is defined as a ratio of the cross-sectional area of the extrudate to the cross-sectional area of the die.
- **Bulk density**: The bulk density is defined as the weight divided by total volume of the extrudate which will be determined by the sand displacement method.
- **Cellular density, size, and distribution using Scanning Electron Microscopy**: The cell density if defined as the number of cells per unit area and cell size is the diameter (microns) of the cell while the cell size distribution is the number of cells in a given range of cell sizes. These characteristics will be examined by scanning electron microscopy (SEM) for selected samples to observe the differences between samples obtained under different conditions. The cell densities and sizes will be determined from the micrographs using image processing software (Image-Pro Plus). Images will be viewed on the computer monitor and saved for later analysis of cell density, size, and distribution.
- **Melt test**: The dissolving/melting characteristics of the extrudate will be evaluated by dipping in water for 30 seconds followed by product integrity testing in a texture analyzer (TX-AT2).

**Approach to Accomplish Objective 2:**

Under this objective, we will make expanded, porous and shelf-stable milk protein-rich ready-to-eat nutri-bars and baby puff/melts as new products for children and adults who want more and better-quality, lean protein in their diets. Our preliminary results indicate the SCFX technology can indeed produce such products but additional research is needed to make viable products economically and establish the unique advantages of the new products and process combinations. Extrudate in the form of puffs and crisps will be manufactured and then formed into nutri-bars using simple formulations as shown in Fig. 5. Baby puff/melts will be based on hydrolyzed skim milk and rice flour. Physico-chemical attributes of selected commercial samples will be also determined using methodology outlined under objective 1 and compared with lactose-hydrolyzed skim milk-based samples for quality characteristics and acceptability. The following parameters of the final products will be quantified and results will be used to further optimize the extrusion process and product quality.

- **Differential Scanning Calorimetry (DSC)**: The DSC will be used to measure the gelatinization temperature of the products. Differences in the peaks of the heating curves for the different samples
will be analyzed. Glass transition and denaturation temperature of the products will be measured as a function of moisture content.

- **Breaking strength:** The breaking strength is used to characterize the texture of the dry extrudate. The breaking strength will be evaluated as the peak force required to shear the extrudate in the transverse or longitudinal directions using a texture analyzer (TX-AT2). A Warner-Bratzler shear cell will be used to measure the shear force along the transverse direction. A compression test will be performed to measure the shear along the longitudinal direction. These results will be compared with commercially available nutribars and puffs.

- **Bulk density:** The bulk density is defined as the weight divided by total volume of the product which will be determined by the sand displacement method and compared with commercial samples.

- **Sensory evaluation:** Consumer testing of samples made will be conducted in the Sensory Testing Lab maintained by our Department on a hedonic preference scale in duplicate and compared for preference and liking with commercial samples of approximately the same type. Approximately 100 panelists will be recruited for evaluation and data will be statistically analyzed using XLSTAT.

**Deliverables:**

Commercially available high-protein bars contain mostly imported caseinates, whey proteins and their hydrolysates, as well as others like soy proteins. Consumers’ cravings for milk protein-rich products is all time high. On the other hand, the recent surge in stockpiles of non-fat dry milk offers both an opportunity and a challenge for the dairy industry to make in-roads into new areas for continued growth. While relatively inexpensive source of milk solids, high lactose content (>50 wt. %) constraints its utilization as a major ingredient in may food formulations. Hydrolysis of lactose in skim milk concentrate, its direct use in extruded product without drying (and thus saving energy) and enhancing galacto-oligosaccharides (GOS) content to enrich the extruded product with a soluble dietary fiber would go a long way to open up new options for enhanced utilization of skim milk in high value products. By the end of the proposed work, we should have established a robust process for making skim milk-based products of high keeping and eating qualities. By understanding the process mechanics, we will define a basic set of requirements necessary for production of these novel products economically. In addition, it would help us understand the link between process and food product properties, in particular the preservation of micronutrients. Our ultimate goal is to provide an innovative technology and derived products for delivery of highly nutritious bars and melts via expanded, cellular foods that can be effectively marketed and thus help increase consumption of milk and milk ingredients.

**Requested Budget, Year 1:** $99,120
References:

Project 1: High protein milk ingredients for value-added products

New York State Milk Promotion Board Goal Addressed by this Project:
Conduct research to ensure the safety and / or expand the demand for New York dairy products and dairy ingredients.

Project Abstract:

Milk proteins are well-recognized for their excellent nutritional qualities but their limited technological functionality poses constraints in their practical utilization in new, value-added products. Extension of low-shear, high-moisture extrusion to milk protein concentrates offers unique opportunities to achieve better functional properties in dairy proteins including viscosity building, gelling, emulsification, aeration, etc. in product formulations and to overcome some of the drawbacks of conventional products. The specific objectives of the proposed research are to i) investigate the effects of shear, temperature, moisture and carbon dioxide during high pressure extrusion processing to enhance the functionality of high milk protein concentrate (MPC85), ii) manufacture functionalized milk protein concentrate (f-MPC85) using the best operating conditions from objective 1 and evaluate its quality parameters for use an ingredient in food formulations. Development of high-protein, tailor-made ingredients based on these objectives will not only help produce many clean label food products but will also generate new commercial opportunities for dairy ingredients.

Project PI:  
Sy Rizvi, Cornell University

Duration: January 1st 2020 – December 31st 2020 (year 1 of a 2 year project)

Background:

Changing lifestyle, growing population of health-conscious consumers, and increasing disposable income are fueling the growth of functional ingredient market globally and demand for high quality convenience foods with natural ingredients and without any synthetic additives has increased exponentially. Key strategies to compete in this marketing space clearly requires that new approaches to processing and presenting milk protein as a functionally superior, shelf-stable, organoleptically attractive, affordable and convenient products while retaining its nutritional superiority are needed. Several modification techniques such as enzyme hydrolysis (Banach, et al., 2013), pH adjustment (Chen et al., 2019), extrusion process (Manoi & Rizvi, 2009), etc. have been attempted to improve the emulsifying properties of whey proteins. No functionalized MPC is currently available in the market and non-dairy, chemically mediated sodium caseinate, introduced 50 years ago, is still considered as the gold standard of useful ingredients.

Extrusion processing is a way of improving the functional properties of proteins. During extrusion, the forces which stabilize the tertiary and quaternary structures of the proteins have been shown to be weakened by a combination of temperature and shear. Individual protein molecules unfold and align themselves with the flow of material towards the die. Exposure of proteins to shear during extrusion processing has also been shown to expose the reactive free SH groups, non-polar amino acids, and peptides that are normally concealed within the native proteins. A combination of fragmentation and aggregation, hydrophobic and electrostatic interactions, non-covalent
associations, and covalent cross-linking on extrudate structures and functionality has been reported (Harper, 1979, 1981, 1986; Kinsella, 1976, 1978). Flow-induced shear causes the aligning of the protein molecules in the axial direction via intermolecular bonds between protein molecules prior to leaving the die (Camire, 1991). The residence times and processing temperatures in the extruder provide the necessary conditions for cross-linking or matrix formation via disulfide bonds and non-covalent interactions (Hager 1984; Ledward & Mitchell 1988; Prudencio-Ferreira & Areas, 1993; Li & Lee 1996). It also known that acidic treatments increase protein solubility and produce non-oriented fiber arrangement of extruded proteins (Dahl & Villota, 1991; Onwulata et al., 2006). Queguiner et al. (1992) also reported that a microparticulated whey protein isolate (WPI) could be obtained by extrusion at acidic pH (~ 3.9). Ion additions to proteins’ diminished repulsive forces, protein-protein association occurs, forming a self-supporting gel. Electrostatic repulsive forces and interactions between charged species are particularly sensitive to the ionic strength of the intervening medium and are reduced with an increase in ionic strength (Mulvihill & Kinsella, 1988). Control of calcium concentration has been known to modify protein gel characteristics (Ju & Kilara, 1998). Controlled addition of salts to manipulate ionic strength is another avenue to explore to control protein functionality. Protein hydrophobicity has been increasingly recognized to be affected by thermal treatment during processing and that hydrophobicity plays a critical role in the determination of protein functionality (Mangino et al., 1987). For example, hydrophobicity has been shown to affect the function of proteins in foaming (Liao & Mangino, 1987; Townsend & Nakai, 1983), emulsification (Aoki et al., 1981; Kato & Nakai, 1980; Voutsinas et al., 1983), whipped topping overrun (Liao & Mangino, 1987; Mangino et al., 1984) and gelation (Voutsinas et al., 1983; Kohnhorst & Mangino, 1985; Mangino et al., 1987). This knowledge could profitably be also used to control certain end product functionalities during extrusion processing of proteins and offers attractive new avenues for exploration. The types of interactions that can occur at extrusion temperatures and high moisture levels (30–60% water) include hydrophobic interactions, ionic bonds and hydrogen bonds (Stanley, 1989).

And extension of low-temperature and low-shear extrusion technology, which retains the nutritional and organoleptic qualities, is needed for delivery of novel dairy-based ingredients. We propose to use low-shear, low-temperature and temporarily-imposed acidity to MPC80 via high-pressure, carbon dioxide-based high-moisture extrusion to impart new and improved functionality. The die design has also been shown to affect the functionality very profoundly and we plan to use three geometries (circular, annular and slit) to evaluate their role in enhancing desirable attributes. Preliminary, proof-of-concept studies with MPC80 have been successfully conducted and tested as discussed below.

**Preliminary Results:**

We have conducted some preliminary work with commercial milk protein concentrate containing 85% milk protein (MPC85). A feed formulation comprising (w/w) 99% MPC85 was extruded at 80 °C and 30% moisture (dry feed basis) in a pilot-scale Wenger TX-52 Magnum co-rotating twin screw extruder with a length to diameter ratio (L/D) of 28.5 It was configured to operate at a screw speed of 138 rpm and feed rate of 35 kg/h with 1% (dry feed basis) SC-CO2 injected at 10 MPa as a blowing agent. The resulting extrudate was dried, ground to less than 1mm particle size using a mill. This functionalized (f-MPC85) powder was then stored at room temperature in airtight containers until analyzed. The powder was reconstituted in deionized water and evaluated for its
rheological and physicochemical properties. Viscosity was evaluated instrumentally and the results indicated that the resulting functionally superior f-MPC85 had significantly higher viscosity at room temperature than the unextruded MPC85 sample (Fig. 1). Its dispersion (20% w/w) showed approximately 16 times higher apparent viscosity than the unextruded samples at 50°C. It also exhibited a highly viscous and creamy texture with particle sizes in the micron-range (mean diameter ~ 5 µm), which could serve as a thickening/gelling agent or as a fat substitute in food formulations. The sample showed a high stability of its rheological properties over a wide temperature range (25 to 80 °C).

Emulsification characteristics of the f-MPC85 were also determined to study its potential as an emulsifying/stabilizing agent. The extruded protein powder was incorporated as part of an aqueous phase and evaluated for its emulsifying activity and emulsion stability against droplet coalescence in oil-in-water (o/w) emulsions compared with those of commercial MPC-85 and sodium caseinate (Na-Cn), Fig. 2. The f-MPC85 also showed excellent emulsifying properties compared to its unextruded counterpart and sodium caseinate. Emulsions prepared with such small amounts of f-MPC showed an enhanced adsorption of proteins at the oil-water interface, which prevented flocculation and coalescence of the oil droplets, and an increase in the viscosity of the continuous phase prevented creaming by trapping the oil droplets within the gel matrix.

Fig. 1. MPC85: Viscosity of 20% dispersions with temperature

Fig. 2. Effect of different proteins on the viscosity of emulsions prepared using 2 wt.% protein and 40 wt.% oil
Additionally, we have used gel-like emulsifying properties of f-MPC85 to make spreads, using both clarified butter oil and vegetable oil that have no emulsifier or additives in their formulations, just oil and f-MPC85, Fig. 3. A unique feature of the butter spread is that it remains spreadable at room and after storage at refrigeration temperatures, a highly desirable property not hitherto available for butter containing products. With the ease and convenience of spreadability, two ingredients, and made with protein should downright ideal for today’s consumers. Some commercial interests have already emerged in making such products, including ghee spreads with unique flavor.

**Specific Objectives:**

**Objective 1:** Investigate the effects of shear, temperature, moisture and carbon dioxide during high pressure extrusion processing to enhance the functionality of high milk protein concentrate (MPC85).

**Objective 2:** Manufacture functionalized milk protein concentrate (f-MPC85) using the best operating conditions from objective 1 and evaluate its quality parameters for use as an ingredient in food formulations.

**Experimental Approach:**

**Approach to Accomplish Objective 1:**

Extrusion of MPC85 will be performed using a co-rotating twin-screw extruder (Wenger TX-52 Magnum) with a barrel diameter of 52 mm and an L/D ratio of 28.5. The schematic for producing functionally superior protein by the extrusion process is shown in Fig. 4 below. The die will be fitted with two circular inserts of 0.5 to 2.5 mm diameter each. SC-CO$_2$ will be injected into the protein melt through four valves located around the extruder.
barrel at L/D = 24. This increases the mixing effect and reduces residence time for complete mixing. A flow restrictor plate is installed on the exit end of the last barrel, before the die assembly to maintain and regulate pressure as described by Rizvi et al. (1995).

Commercially available MPC85 powder (99 wt.%), containing casein and whey protein in the same ratio as in milk, will be blended with Dimodan (Monoglyceride, extrusion processing aid)(1 wt.%). The feed formulation will be fed into the extruder through a constant-weight hopper and water will be delivered by a volumetric pump at the extruder barrel entrance. The extruder variables for the generation of f-MPC85 will be CO2 injection at various pressure, screw speed, final product temperature and added water. A central composite experimental design (Box & Draper, 1987) will be used for each experimental set, an example of the variables for specific experiment is shown in Table 1. These will be repeated for three die geometries (circular, annular and slit).

Table 1. The levels of independent variables (4-factors and 3-levels) used in the unblocked central composite rotatable design.

<table>
<thead>
<tr>
<th>Coded value</th>
<th>Extrusion variable</th>
<th>Added CO2 (wt.%)</th>
<th>Screw speed (rpm)</th>
<th>Product temp. (°C)</th>
<th>Added water (wt.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td></td>
<td>0</td>
<td>50</td>
<td>70</td>
<td>50</td>
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<tr>
<td>0</td>
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<td>1.0</td>
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<td>1</td>
<td></td>
<td>1.5</td>
<td>150</td>
<td>90</td>
<td>70</td>
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</table>

- **Surface hydrophobicity measurements**: Surface hydrophobicity of f-MPC85 will be determined using the fluorescence spectroscopy techniques described by Kato and Nakai (1980) and Moro et al. (2001). Fluorescence titration employing hydrophobic probes such as 1-anilinonaphthalene-8-sulfonic acid (ANS) and cis-parinaric acid (CPA) will be used to determine aromatic and aliphatic hydrophobicity, respectively. The relative fluorescence intensity of solutions will be measured at \( \lambda_{ex}=325 \) nm and \( \lambda_{em}=420 \) nm for the CPA-protein conjugates, and \( \lambda_{ex}=390 \) nm and \( \lambda_{em}=480 \) nm for the ANS-protein conjugates in a spectrofluorometer. Measurements will be made at ambient temperature. The hydrophobicity will be calculated from the slope of the relative fluorescence vs percent (w/v) protein concentration.

- **Process characterization**: The thermomechanical input history will be quantified as follows (Della Valle et al., 1994):

The specific mechanical energy (SME) input in the extruder will be calculated according to following equation, where \( n \) is the screw speed, \( P \) is the rated power, \( W \) is the mass flow rate, and \( %\tau \) is the load factor.

\[
\text{SME} = \frac{(n_{\text{actual}} \times P \times %\tau)}{(n_{\text{rated}} \times W \times 100)}
\]  

(i)
Approach to Accomplish Objective 2:

Rheological and functional properties measurements will be used as analytical tools and will in addition provide fundamental insights into the structural organization of these materials and to better understand the relationships between microstructure and functionality of f-MPC85. Characteristics of f-MPC85 in aqueous solution will be compared with those of un-extruded protein samples and commercially available sodium caseinate.

- **Liquid viscosity by shear rate ramp test**: The f-MPC85 powders will be reconstituted at different concentrations (3-20%, w/w) in deionized water at ambient temperature and gently stirred for 2 h or until dissolution is completed, and then stored at 4 °C prior to testing. This is done to ensure that dispersions are in the fully hydrated state. The viscosity of f-MPC85 solutions will be measured using the shear rate ramp test. The parallel plate geometry (diameter=50 mm, gap=1 mm) will be used for this test. All experiments will be conducted at 25 °C. Shear rate will be ramped from 0.1 to 100 s⁻¹. Shear stress (τ), shear rate (\(\dot{\gamma}\)), and apparent viscosity (\(\eta_a\)) will be recorded and the corrected flow curves will be fitted using the power law (ii) and the Herschel-Bulkley model (iii). The flow behavior index (n), consistency coefficient (k), and yield stress (\(\tau_{0HB}\)) will be computed.

\[
\tau = k\dot{\gamma}^n \quad -- \quad (ii)
\]

\[
\eta_a = \frac{\tau_{OHB}}{\dot{\gamma}} + k\dot{\gamma}^{n-1} \quad (iii)
\]

- **Gel properties by small-amplitude oscillatory (dynamic) tests**: Gels containing 15 to 30% + (w/w) will be prepared at ambient temperature by reconstituting f-MPC85 powder in deionized water and gently stirred for 2 h and then stored at 4 °C prior to testing. In this study, the dynamic mechanical testing approach is used to measure mechanical changes in the linear viscoelastic behavior of f-MPC85 gels. The small strain measurement is believed to leave the microstructure intact and thus it is possible to characterize the viscoelastic properties of the original structure (Gunasekaran & Ak, 2000). A viscoelastic network indicates the elastic and viscous behavior of the sample over a range of frequencies. A frequency sweep test will be conducted using the parallel plate geometry (diameter=25 mm, gap=1 mm) and the frequency will be oscillated from 0.1 to 100 rad/s at 25 °C. All measurements will be performed within the identified linear viscoelastic region and made at 1% strain. The storage modulus (G'), loss modulus (G") and loss angle tangent (\(\tan \delta\)) will be computed. G' represents a measure of the elastic response of the material whilst G" is a measure of the viscous response. The relative ‘strength’ of gels will be interpreted in terms of \(\tan \delta (G''/ G')\), measuring energy loss compared to energy stored during cyclic deformation. The temperature sweep test will be conducted to evaluate the rheological stability of F-MPC85 gels over a range of temperatures. Temperature will be ramped from 5 to 85 °C at 2 °C/min heating rate and at a constant frequency rate of 1 rad/s and 1% strain. The G’, G‘and \(\tan \delta\) will be then computed. To study the effect of pH conditions on the rheological stability of F-MPC85 gels, the dynamic moduli (G’, G"and \(\tan \delta\) ) will be measured at pH 4.0 to 8.0 and 25 °C as frequency is ramped from 0.1 to 100 rad/s.

- **Water holding capacity (WHC) of f-MPC85 powders**: f-MPC85 powders will be hydrated (15%, w/w) in deionized water for 3 h and centrifuged at 3,500 rpm for 30 min at 25 °C. After
centrifugation, the supernatant will be removed and the remaining pellet will be weighed. The amount of water held per gram of protein powder is calculated as the WHC.

- **Emulsifying activity index (EAI) and emulsion stability index (ESI):** The EAI and ESI are determined by the turbidometric technique described by Pearce and Kinsella (1978). The EAI is related to the surface area stabilized by a unit weight of proteins. It represents the ability of proteins to be adsorbed at the interface of fat globules and the aqueous phase. The ESI reflects the ability of proteins to impart strength to emulsion for resistance against coalescence upon storage (Patel & Kilara, 1990). The emulsions will be prepared from 10 mL corn oil and 40 mL of 3% (w/w) protein dispersions at pH 7. The emulsions (10 µL) are then diluted in 5 mL of 0.1 M phosphate buffer containing 0.1% (w/v) sodium dodecyl sulfate (SDS). The absorbance of the diluted emulsions is then determined at a wavelength of 500 nm in a spectrophotometer. The EAI is then calculated as

\[
EAI(m^2 g^{-1}) = \frac{2xTXD}{\Phi x C x 10,000}
\]  
(iv)

where \(T\) is the turbidity, \(D\) is the dilution factor, \(\Phi\) is the volumetric fraction of oil, \(C\) is the weight of protein per unit volume of aqueous phase before the emulsion was formed (g mL\(^{-1}\)) and 10,000 is the correction factor for square meters. The ESI is calculated as:

\[
ESI(h) = \frac{(TX\Delta t)}{\Delta T}
\]  
(v)

where \(T\) is the turbidity value at 0 h, \(\Delta T\) is the change in turbidity during the storage period, and \(\Delta t\) is the time interval.

- **Creaming index:** The creaming index is used to indicate the susceptibility of oil droplets to coalescence by such forces as gravitational, colloidal, hydrodynamic and mechanical, and the resistance of the droplet membrane to rupture during a certain period of time (McClements, 1999). Emulsions prepared with corn oil (10 mL) and protein dispersions (40 mL) containing various concentrations of protein samples (0.25, 0.5, 1, 2, 3, and 4%, w/w) will be measured for the creaming index as described by Firebaugh and Daubert (2005). The height of the serum (\(H_s\)) and the total height of the emulsions (\(H_t\)) will be recorded after storage at ambient temperature for 1, 7, and 14 days.

\[
Creaming\ Index\ (%) = \frac{H_s}{H_t} x 100
\]  
(vi)

- **Cold, gel-like emulsion preparation and characterization:** Emulsions containing oil levels of 20 to 80% (w/w) will be prepared at ambient temperature to study the effect of oil concentrations on gel-like emulsion properties. The emulsion of a given oil concentration will be prepared at ambient temperature by mixing the correct amount of corn oil with the appropriate quantity of aqueous protein dispersion, at 9,500 rpm for 3 min using a ULTRA-TURRAX high-speed dispersing and emulsifying unit. The resulting emulsions will be stored using three different conditions (refrigerated storage at 5 ºC; ambient storage at 25 ºC; and at 55 ºC in an oven) for up to 6 months and periodically measured for their stability in terms of rheological properties, oil droplet size, and microstructure. The rheological properties of cold, gel-like emulsions will be evaluated using the strain-controlled rheometer. A cone and plate geometry (diameter=25 mm,
nominal cone angle=0.1 radians) will be used for steady shear viscosity and small-amplitude oscillatory experiments as previously described. The droplet size distribution of cold, gel-like emulsions will be evaluated using the laser diffraction method of Mastersizer (2000). Emulsions will be diluted (0.5% w/w) with 1% SDS solution and stirred for 20 min prior to measurement. Drops of emulsion solution will be introduced into the sample presentation unit and dispersed in deionized water at 1200 rpm and 40 ºC until an obscuration rate of about 8% is obtained. Droplet size will be reported as the volume-weighted mean diameter: \[ d_{4,3} = \frac{\Sigma n_i d_i^4}{\Sigma n_i d_i^3}, \]

where \( n_i \) is the number of droplets of diameter \( d_i \). The emulsion samples will be stained with a mixture of Nile Red (0.01%) to visualize the oil phase and Fast Green FCF (0.001%) to visualize the protein phase. The microstructure of stained emulsions will be visualized using a confocal laser scanning microscopy (CLSM). The CLSM is performed on a Leica TCS-SP2 Confocal Laser Scanning head mounted on a Leica DMRE-7 (SDK) upright microscope equipped with a 20x HC PL APO/0.70NA oil immersion objective lens. Confocal illumination is given by an Argon laser with excitation at 488 nm and a HeNe laser with excitation at 633 nm.

- **Foaming properties:** Foam formation and stability of f-MPC85 solutions can be determined by conductimetric and optical measurements (Carrera & Rodriguez Patino, 2005). The foam is generated by blowing gas (nitrogen) at a flow of 45 mL/min through a porous glass filter (pore diameter 0.2 µm) at the bottom of a glass tube where 20 mL of the foaming agent solution under investigation is placed. In all experiments, the foam will be allowed to reach a volume of 120 mL. The bubbling is then stopped and the evolution of the foam is analysed at 20 ºC. The overall foaming capacity (OFC, mL/s) is determined from the slope of the foam volume curve up to the end of the bubbling. The foam capacity (FC is determined by Eq. (vii). The relative foam conductivity (\( C_f \), %) is a measure of the liquid retention in the foam and the foam density and is determined by Eq. (viii).

\[
\text{FC} = \frac{V_{\text{foam}}(f)}{V_{\text{gas}}(f)} \quad \text{(vii)}
\]

\[
C_f = \left( \frac{C_{\text{foam}}(f)}{C_{\text{liq}}(f)} \right) x 100 \quad \text{(viii)}
\]

where \( V_{\text{foam}}(f) \) is the final foam volume, \( V_{\text{gas}}(f) \) is the final gas volume injected, and \( C_{\text{foam}}(f) \) and \( C_{\text{liq}}(f) \) are the final foam and liquid conductivity values, respectively. The static foam stability is determined from the volume of liquid drained from the foam over time. The half-life time (\( \theta_{1/2} \), referring to the time needed to drain half of the volume of the liquid in the foam, will be used as a measure of foam stability.

- **Differential scanning calorimetry (DSC):** The degree of protein denaturation in f-MPC85 will be evaluated using DSC. A 20 µL f-MPC85 dispersion (10% w/w) will be scanned in a DSC from 5 to 110 ºC at a rate of 5 ºC/min to assess the thermal denaturation of proteins. Temperature at peak height and peak area will be recorded. Then the percent native protein remaining in the sample will be calculated as

\[
\text{Peak height of F-MPC85 sample x 100}
\]

\[
\text{Peak height of unextruded sample}
\]

\[
\text{Peak height of unextruded sample (ix)}
\]
Statistical Analysis:

The whole experiment will be replicated twice, with all sample treatments being done in triplicates, resulting in \( n=6 \). Statistical analysis will be done using MINITAB\textsuperscript{®} release 15 statistical software (State College, PA, USA). Significant differences \((p<0.05)\) will be determined by analysis of variance using the general linear models and least square means procedure.

For the central composite rotatable design five parameters will be studied at three levels. The levels of the factors have been determined based on the preliminary studies. The JMP statistics software (Version 7; SAS Institute, Cary, NC, USA) will be used to analyze the optimization data to define a regression model and to produce ANOVA tables and surface profile plots for the dependent variables.

Deliverables:

The once-predictable milk consumption in the U.S. has become dynamically fluid. To maintain and grow market share, the dairy industry needs to capture latent demand gaps for dairy ingredients in consumers’ changing preferences, most ostensibly noted in consumption of clean label, nutritionally superior products. New strategy must be devised to create and sell milk protein-based ingredients that address and deliver both these attributes. Development and delivery of such novel and value-added dairy protein would undeniably reverse the falling consumption of milk in the U.S. and help farmers realize better returns on their dairy farming investments.

By the end of the proposed work we should have established a process for making milk protein-based novel ingredient as the requirements for delivering a product of high functional and eating qualities. By understanding the process mechanics via objective 1, we will define a basic set of requirements necessary for production of these novel ingredient economically. Objective 2 would help us understand the link between process and properties, in particular the preservation of micronutrients while adding functionalities, to produce unique dairy ingredients of commercial utility not hitherto available. Through the series of tests described above that measures each functionality, a numerical ratings would be developed for each for use by product developers in making ingredient choices. Collectively, our ultimate goal is to provide an innovative technology and derived products for delivery of dairy protein-based ingredient that can be effectively marketed as a solution to many of the food industry’s unmet needs and thus help increase profitability of dairy farmers and processors.

Requested Budget, Year 1: $98,530
References:


Northeast Dairy Foods Research Center Overview

Dave Barbano

September 16, 2019
National Dairy Promotion and Research Board was established in 1983 by and act of congress.

First Draft of a Dairy Center Proposal was written by Dave Barbano and John Kinsella in 1985

August 26, 2019
### Areas of Major Accomplishments

<table>
<thead>
<tr>
<th>Technology Development</th>
<th>Product Quality &amp; Shelf Life</th>
<th>Broader Industry Impacts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane filtration technology for dairy</td>
<td>Significant improvements in fluid milk shelf-life</td>
<td>Fluid milk and dairy based beverage innovation</td>
</tr>
<tr>
<td>Milk chemical analytics for milk payment and dairy product manufacture</td>
<td>Raw milk quality and relationship to cheese yield and product quality</td>
<td>Maintain competitiveness of the NYS cheese and cultured product industry</td>
</tr>
<tr>
<td>On-farm technologies and their connection to dairy product yield and quality</td>
<td>Improved cultured product shelf-life and quality by using CO₂ technology</td>
<td>Adding value to cultured dairy product co-products</td>
</tr>
</tbody>
</table>
1. Maintaining competitiveness of the NYS cheese and cultured product industry

2. Raw milk quality and relationship to cheese yield and product quality

3. Membrane Filtration Technology

4. Fluid milk shelf-life

5. Fluid milk and dairy based beverage innovation

6. Cultured product shelf-life and quality: Use of CO₂ technology

7. Milk chemical analytics for milk payment and dairy product manufacture

8. On-farm technologies and their connection to dairy product yield and quality

9. Adding value to cultured dairy product co-products
## Areas of Major Accomplishments

1. Maintaining competitiveness of the NYS cheese and cultured product industry.

### Table 9.

<table>
<thead>
<tr>
<th>Year</th>
<th>Fluid Sales (2)</th>
<th>Used for Mfg. in NY Plants</th>
<th>Sold for Mfg. Out-of-State</th>
<th>Frozen Desserts Food Proc. and Other (3)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>3,502,732</td>
<td>8,419,698</td>
<td>135,422</td>
<td>1,28{2007}0,710</td>
<td>13,338,562</td>
</tr>
<tr>
<td>2008</td>
<td>3,395,894</td>
<td>8,437,043</td>
<td>139,368</td>
<td>1,242,007</td>
<td>13,214,312</td>
</tr>
<tr>
<td>2009</td>
<td>3,380,809</td>
<td>8,698,406</td>
<td>134,271</td>
<td>1,383,787</td>
<td>13,597,273</td>
</tr>
<tr>
<td>2010</td>
<td>3,385,736</td>
<td>9,265,779</td>
<td>139,741</td>
<td>1,577,324</td>
<td>14,368,580</td>
</tr>
<tr>
<td>2011</td>
<td>3,389,348</td>
<td>9,863,846</td>
<td>166,897</td>
<td>1,904,961</td>
<td>15,325,052</td>
</tr>
<tr>
<td>2012</td>
<td>3,255,006</td>
<td>10,654,384</td>
<td>170,230</td>
<td>2,298,875</td>
<td>16,378,495</td>
</tr>
<tr>
<td>2013</td>
<td>3,153,530</td>
<td>10,869,353</td>
<td>217,785</td>
<td>2,673,940</td>
<td>16,914,608</td>
</tr>
<tr>
<td>2014</td>
<td>3,289,971</td>
<td>10,787,618</td>
<td>313,165</td>
<td>2,420,432</td>
<td>16,811,186</td>
</tr>
<tr>
<td>2015</td>
<td>3,241,910</td>
<td>11,364,075</td>
<td>345,210</td>
<td>2,289,382</td>
<td>17,240,577</td>
</tr>
<tr>
<td>2016</td>
<td>3,194,482</td>
<td>11,468,216</td>
<td>422,839</td>
<td>2,476,269</td>
<td>17,561,807</td>
</tr>
<tr>
<td>2017</td>
<td>3,056,718</td>
<td>11,396,670</td>
<td>355,900</td>
<td>2,570,088</td>
<td>17,379,377</td>
</tr>
</tbody>
</table>

(Thousand Pounds)
1. Maintaining competitiveness of the NYS cheese and cultured product industry.

| Table 28. DAIRY PRODUCTS MANUFACTURED IN NEW YORK STATE DAIRY PLANTS, 2007 - 2017 |
|-----------------------------------------------|-----------------|----------------|-----------------|
|                                              | Butter        | Sour Cream    | Yogurt          | Egg Nog         | Condensed Whole Milk | Condensed Skim Milk |
|                                              | 15,388         | 18,812         | 16,234         | 16,133         | 16,174         | 18,412         | 21,089         | 21,366         | 24,480         | 27,488         | 27,565         |
|                                              | 256,967        | 241,718        | 268,792        | 251,547        | 272,891        | 270,045        | 282,361        | 276,686        | 267,031        | 278,417        | 272,899        |
|                                              |                |                |                |                |                |                |                |                |                |                |                |
|                                              | 233,844        | 229,755        | 266,697        | 369,458        | 553,681        | 695,345        | 742,551        | 651,320        | 667,723        | 676,525        | 700,249        |
|                                              |                |                |                |                |                |                |                |                |                |                |                |
|                                              | 1,156          | 1,261          | 1,395          | 3,555          | 3,981          | 1,794          | 2,100          | 2,245          | 1,815          | 2,913          | 3,184          |
|                                              |                |                |                |                |                |                |                |                |                |                |                |
|                                              | 7,088          | 6,873          | 4,440          | 13,809         | 1,699          | 1,423          | 338            | 1,388          | 1,435          | 83             | 30             |
|                                              |                |                |                |                |                |                |                |                |                |                |                |
|                                              | 89,913         | 74,924         | 59,434         | 62,751         | 62,032         | 73,051         | 72,085         | 87,604         | 105,555        | 124,691        | 76,208         |
Areas of Major Accomplishments

1. Maintaining competitiveness of the NYS cheese and cultured product industry.

![Graph 1: Cheese Production in NYS Dairy Plants, 2002-2007](image1)

- Italian
- Cottage
- Cream Cheese
- American
- Other

![Graph 2: Cheese Production in NYS Dairy Plants, 2012-2017](image2)

- Italian
- Cottage
- Cream Cheese
- American
- Other

Cream cheese
American cheese
Areas of Major Accomplishments

1. Maintaining competitiveness of the NYS cheese and cultured product industry.
Milk Quality and milk quality payments (i.e., milk SCC)

A series of papers were published on research on Cheddar and cottage cheese and provide the justification for cheese makers to pay farmer quality premiums for low somatic cell count milk. When NYS Ag and Markets was collection statistics on this, dairy farmers in NYS were receiving about $1.5 million dollars per year in additional milk quality payments based on milk. In the year 2000, several USDA Federal Milk Markets added milk SCC to the official make payment system. Today, milk SCC is about half what is used to be in the milk supply.

Influence of Milk Somatic Cell Count and Milk Age on Cheese Yield

JDS 1991: 369-388

D. M. BARBANO, R. R. RASMUSSEN, and J. M. LYNCH
Department of Food Science
Cornell University
Ithaca, NY 14853
Proposed Projects for the Year 2020
Total Budget from the NYS MPB for the Northeast Dairy Foods Research Center requested for the period Jan 1, 2020 to December 31, 2020 = $ 1,095,486

Continuing Projects 2020

Project 1 - $51,625 (Barbano) continuing project (center technology transfer – ongoing)
Project 2 - $108,560 (Abbaspourrad) Nutritious Spreads and Fillings using Milk Ingredient
Project 3 - $99,238 (Moraru) Vacuum Microwave treatments.
Project 4 - $83,543 (Alcaine) Improving UHT shelf-life
Project 5 - $121,498 (Barbano) Rapid method for milk protein and fat quality

Total Continuing Projects $464,463 for 2020 (Projects 1 through 5)
Proposed New Projects 2020

Project 6 - $ 68,203 (Alcaine) Encapsulating Cultures for HPP Dairy Products
Project 7 - $ 94,163 (Alcaine) Fermenting Value for Dairy (GOS)
Project 8 - $ 107,380 (Abbaspourrad) Conversion of Sugar in Skim Milk to Galacto-oligosaccharides (GOS)
Project 9 - $ 99,120 (Rizvi) Value-added products from lactose-hydrolyzed skim milk (GOS)
Project 10 $ 98,530 (Rizvi) High protein milk ingredients for value-added products
Project 11 $ 95,342 (Moraru/Dando) Milk based snacks for kids by 3D printing
Project 12 $ 68,284 (Barbano/Drake) Identification of sources of undesirable flavors in aseptic (UHT) milk

Total New Projects $ 631,022 for 2020 (Projects 6 through 12)

Total Dairy Center Budget Request 2020 (Projects 1 thru 12) $1,095,485
Project 6: Encapsulating Cultures for HPP Dairy Products.

Project PI’s: Samuel Alcaine, Cornell University
Duration: January 1st, 2020 – December 31st, 2020 (year 1 of a 2-year project)

Summary of Proposed Project: High pressure pasteurization (HPP) technology is of interest to dairy processors due to its ability to eliminate vegetative pathogens, maintain the fresh flavor and textural characteristics of the food when compared to thermal processing; and reduce reliance on added preservative agents, thus allowing the production of foods with cleaner labels. The challenge for dairy processors is that this technology also inactivates the bacterial cultures we use to ferment dairy products, which makes leveraging HPP in fermented products challenging. Our goal is to investigate the use of encapsulation technologies to improve the compatibility of dairy cultures with HPP.

Objectives:

1. Evaluate the use milk fat and/or cocoa butter encapsulation of freeze-dried dairy cultures.
2. Evaluate the stability of the encapsulated cultures through HPP processing.
3. Evaluate the stability and subsequent activity of encapsulated cultures in milk pre and post HPP processing.
Value-added Product from Skim Milk

Skim Milk + Lactase

Concentration 1-3X

GOS Production

High Value Ingredients

Fermentation To Galactose

Extrusion

Beverages

Ethanol

Novel “yogurt”

Novel Functional Ingredients

Food Product

Snacks

Rizvi

Abbaspourrad

Alcaine
Project 7: Fermenting value for dairy: Evaluating skim milk as a substrate for the biomanufacturing of value-added ingredients and products.

Project PI’s: Samuel Alcaine, Cornell University

Duration: January 1st, 2020 – December 31st, 2020 (year 1 of a 1-year project)

Summary of Proposed Project:

Decreasing demand for skim milk and skim milk powder, two major dairy products for the NY, represents a challenge and opportunity for the industry. One hurdle in skim milk utilization is its lactose content, to which many consumers have an intolerance to, thus limiting its consumption and use as an ingredient in other food products, despite its protein content. We propose building upon our current research and learnings from dairy by-product fermentation, to expand the utilization of fermentation to create novel, value-added ingredients from milk. Our previous research has shown that galactose-rich dairy-products can be potential be produced via fermentation, but we have not evaluated this process in protein containing dairy substrate like skim milk. The galactose-rich product could serve as inputs in to further value-added processes, like galactooligosaccharide production, tagatose production, and high protein/lactose-reduced consumer products. This research would lay the groundwork for launching a new category of dairy-based ingredients that would expand the utilization of milk, and position New York State Dairy as a leading innovator in the space.

Objectives:

1. Evaluate the fermentation of skim milk and milk concentrates by *Brettanomyces* and related yeast species to produce a galactose-rich fermented milk.

2. Optimize fermentation parameters for galactose production from skim milk and milk concentrates by *Brettanomyces*. 
Project 8 Title: Conversion of Sugar in Skim Milk to Galacto-oligosaccharides

Project Abstract:
Skim milk contains 4.8% of lactose, a starting material to produce a prebiotic called galacto-oligosaccharides (GOS). Due to the increasing popularity and health benefits of GOS, the goal of this proposal is to increase the value of skim milk and expand the demand for New York originated skim milk by producing GOS-rich skim milk. We plan to use a continuous bioreactor we have previously built for the production of GOS. Such platform can convert 39% of lactose into GOS, a yield much higher compared to other reported methods. By collaborating with other faculty in the Cornell Food Science Department, we have organized an integrative plan to first produce GOS-rich skim milk, and then use GOS-rich milk as a key ingredient for novel extruded dairy products. In the first year of the study, we will use concentrated skim milk (2-3X) as starting material and adapt/modify our bioreactors to the physical properties of the concentrated skim milk to produce the highest amount of GOS in the skim milk by optimizing the processing parameters. In the second year of the study, we will use a fermented skim milk (galactose rich) as the starting material to explore a different reaction dynamic of GOS-synthesis in skim milk.

Project PI: Alireza Abbaspourrad
Duration: January 1st 2020 – December 31st 2020 (year 1 of a 2-year project)

Specific Objectives:

Objective 1: adapt/modify our bioreactors to the physical properties of the concentrated skim milk
Objective 2: produce highest amount of GOS in the skim milk by optimizing the processing parameter
**Project 9: Value-added products from lactose-hydrolyzed skim milk**

**Project Abstract:**
The proposed work will build a new route for direct conversion of lactose-hydrolyzed, preferably galacto-oligosaccharide-enriched, skim milk concentrates and powder into new food products using extrusion technology, test these products and demonstrate a path to prototype products of industrial utility.

**Project PI:** Sy Rizvi, Cornell University

**Specific Objectives:**

**Objective 1:** Determine the effects of adding lactose-hydrolyzed skim milk concentrate/powder and extrusion processing parameters on product quality

**Objective 2:** Compare the physico-chemical attributes of extruded prototypes (nutribar and baby puffs/melts) with their commercial counterparts.

**Duration:** January 1st 2020 – December 31st 2020 (year 1 of a 2-year project)
Project 10: High protein milk ingredients for value-added products

Project PI: Sy Rizvi, Cornell University

Project Abstract:
Milk proteins are well-recognized for their excellent nutritional qualities but their limited technological functionality poses constraints in their practical utilization in new, value-added products. Extension of low-shear, high-moisture extrusion to milk protein concentrates offers unique opportunities to achieve better functional properties in dairy proteins including viscosity building, gelling, emulsification, aeration, etc. in product formulations and to overcome some of the drawbacks of conventional products. The specific objectives of the proposed research are to i) investigate the effects of shear, temperature, moisture and carbon dioxide during high pressure extrusion processing to enhance the functionality of high milk protein concentrate (MPC85), ii) manufacture functionalized milk protein concentrate (f-MPC85) using the best operating conditions from objective 1 and evaluate its quality parameters for use as an ingredient in food formulations. Development of high-protein, tailor-made ingredients based on these objectives will not only help produce many clean label food products but will also generate new commercial opportunities for dairy ingredients.

Specific Objectives:
Objective 1: Investigate the effects of shear, temperature, moisture and carbon dioxide during high pressure extrusion processing to enhance the functionality of high milk protein concentrate (MPC85).
Objective 2: Manufacture functionalized milk protein concentrate (f-MPC85) using the best operating conditions from objective 1 and evaluate its quality parameters for use as an ingredient in food formulations.
Project 11: Making cool and nutritious milk based snacks for kids by 3D printing  
Project PI’s: Carmen I. Moraru (PI), Robin Dando (Collaborator) 

Project Abstract: We are proposing to use 3D printing to create novel, nutritious, tasty and attractive dairy snacks for children, from high quality milk concentrates. We will evaluate the effect of feed properties (concentration, pH) and temperature (preheating and post-deposition) on gelling and the structure and texture of 3D printed milk gels. The gel strength will be evaluated using rheological analyses, and the structure using scanning electron microscopy. We will also assess the effect of 3D printing parameters (rate of deposition, thickness of deposited layers, shape and size) on final product appearance, shape and texture. We will establish optimal feed composition, temperature treatment and 3D printing parameters for the creation of dairy snacks that are nutritious, attractive and with superior sensory properties for children. This work will lead to the creation of a new generation of dairy snacks, which will both satisfy the consumers and increase the utilization of milk solids. Due to its portability and low cost, this technology has the potential to be utilized in a range of food processing and food service facilities.

Specific Objectives
The overall goal of this work is to use 3D printing of milk concentrates to create dairy snacks of desirable shape, structure and texture, which are nutritious, tasty and attractive for children. Specific objectives include:

Objective 1 (year 1): Evaluate the effect of feed properties (concentration, pH) and temperature (preheating and post-deposition) on gelling and the structure and texture of 3D printed milk gels.

Objective 2 (years 1 and 2): Evaluate the 3D printing parameters (rate of deposition, thickness of deposited layers, shape and size) that result in 3D printed dairy snacks that are attractive, uniform and stable.

Objective 3 (year 2): Optimize the 3D printing of dairy snacks that are attractive and palatable for children and other consumer categories.
Project 12: Identification of sources of undesirable flavors in aseptic (UHT) milk.

Project PI’s: D. M. Barbano (Cornell) and M. A. Drake (NC State)

Project Abstract: (for posting: one paragraph stating general approach and objectives)
Numerous studies have demonstrated that US consumers (adults and children) prefer the flavor of HTST milk over ultrapasteurized (UP) milk, also called extended shelf life (ELS) milk (Chapman and Boor, 2001; Lee et al., 2017). This difference in liking is due to the distinct cooked/eggy flavor of UP milk (Lee et al., 2017), and recent work by the PIs has demonstrated the specific volatile compounds that cause these undesirable flavors and that these compounds are sourced from the serum protein fraction of fluid milk (Jo et al., 2018, 2019). Removal of serum protein from fluid milk followed by UP results in a milk beverage with no cooked/eggy flavor. More recent work in the PIs’ labs has demonstrated that aseptic or UHT milk is liked less by consumers than UP milk. Consumers noted both flavor and aroma as negative experiences in UHT milk, even following multiple exposure. In commercial factories, the thermal processing is typically the same for aseptic and ESL milk from the same equipment and raw milk. The difference is that they are filled and packaged in different containers using different equipment and the time temperature conditions of product storage are different between ESL and aseptic milks. There have been no published scientific studies to fully characterize the sensory and chemical differences between ESL and aseptic milks.

Objectives:
1) Determine differences between aseptic and ESL milk and understand the origin of those differences.
2) Determine the impact of differences in packaging (oxygen permeability of ESL and aseptic packaging material are different) on sensory quality and consumer acceptance of 1% milk.
3) Determine the impact of time of storage (aseptic milk is stored longer than ESL) and temperature of storage (ESL is stored at refrigeration temperature and aseptic at room temperature) on sensory quality and consumer acceptance of 1% milk.