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, 2019 to December 31, 2019. There are 4 continuing projects and 5 new proposed projects. The projects are:

**Continuing Projects:**

**Project 1:** Enhanced Conversion of Lactose to Galacto-oligosaccharides (GOS)

**PI:** Alireza Abbaspourrad, Cornell University

**Duration:** January 1st, 2018 – December 31st, 2018 (year 2 of 2 years)

**1st Year Progress Summary**

Lactose is the major solid component in sweet whey produced as a byproduct of renneted cheesemaking. The estimated world whey production, as a byproduct, is several million tons. Whey residues like lactose can cause environmental problems due to their high chemical and biochemical oxygen demand [1]. Prebiotics such as oligosaccharides are of great interest because of their potential as ingredients to produce functional foods. Galacto-oligosaccharides (GOS), a type of non-digestible oligosaccharides typically made of linked galactose units, are a popular prebiotic ingredient in the food industry [2]. The production of GOS by enzyme-catalyzed conversion of lactose has become commercially important. However, there are still challenges to improving this process: namely, improving the efficiency of the trans-galactosylation reaction and GOS production to enhance the quality of converted GOS. Most studies use β-galactosidase in a free state for enzymatic conversion of lactose into GOS. However, the use of enzymes in a free state has several limitations; for example, high expenses, low operational stability, and challenges in recovery and reuse. The immobilization of enzymes onto suitable supports can overcome some of the drawbacks that are related to using free-state enzymes in conventional batch reactors [3]. The enzymatic production of GOS under continuous flow mode using different reactor setups has been reported. However, the true challenge for traditional immobilized systems is the lower conversion rate of whey permeate to GOS and the difficulty in recycling them for reuse in industrial applications.

In our recent work [4], we have studied the bioconversion of whey permeates to galacto-oligosaccharides (GOS) by the enzymatic action of β-galactosidase from Aspergillus oryzae in a continuous flow packed-bed reactor. A novel method of enzyme immobilization involving covalent immobilization of β-galactosidase on 3-aminopropyl triethoxysilane(3-APTES)-modified glass beads was developed by the cross-linking method (Eqs. 1 and 2).

\[
\text{(1)}
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\[
\text{(2)}
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The schematic for the configuration of the continuous flow packed-bed reactor for whey permeate bioconversion is demonstrated in Figure 1(a). A borosilicate glass column packed with ~80 g of β-
galactosidase immobilized glass beads was used as the reactor for the bioconversion of whey permeate to GOS. The temperature was kept at 60 °C by circulating tap-water through the supporting column. Using a peristaltic pump, whey permeate was continuously fed from the bottom of the reactor, with different flow rates (0.5-6 mL/h). To retain the glass beads, a glass wool filter was used inside the column. The final samples were collected from the outlet at the top and were analyzed by an HPLC system for determination of the concentrations of GOS (tri- and tetra-saccharides), glucose, galactose, and residual lactose.

Figure 1. (a) Schematic illustration of the configuration of the experimental setup for whey permeate bioconversion, with dimensions. (b) Pie chart showing the product composition (%) of whey permeate bioconversion in continuous packed-bed reactor after the 2nd cycle.

The pH and temperature dependence of the enzymatic efficiency of the glass bead-immobilized enzyme was compared with that of the free enzyme. Increased pH and thermal stabilities were observed for the immobilized enzyme over the free enzyme, with a maximum activity at pH 5.2 and temperature of 60 °C. The conversion of lactose to GOS as a function of feed flow rate was studied, and it was observed that the GOS formation rate increased with a decrease in feed flow rate due to the higher residence time of reactants. A maximum GOS yield of 25.2% was observed at feed flow rate of 0.5 mL/h with 43.9% whey permeate bioconversion. With the aim of improving the production process, the reactor was subjected to repeated cycle reactions to examine reusability. The results showed that GOS formation increased after the second cycle of passing, at which point a maximum GOS yield of 39.3% with 56.4% whey permeate bioconversion was obtained (Figure 1(b)). The immobilized system lost only about 4.6% of GOS yield after eight reuses, demonstrating its high efficiency and reusability.

Phase II. (second year)

As part of our continuing efforts to develop efficient systems for GOS production in the second phase of this research, we propose an efficient protocol for enhancing the conversion of whey permeate to GOS through parameter optimization. Magnetic monodisperse, dendritic, and functionalized mesoporous silica nanospheres (MDMSNs) with sub-200 nm size, large pores of dendritic funneling shape, and various inherent functionalities in a simple one-pot reaction in the presence of surfactant may provide a better alternative for β-galactosidase immobilization. As a support, MDMSNs possess features such as a high surface area and a dendritic mesoporous structure, which offer molecules an easy accessibility through its funneling shape (as opposed to the traditional use of pores) [5]. The dendritic structure can chemically attach to enzymes and the space between arborizations can improve mass transfer and enhance the adsorption–desorption rate of lactose to the reactive active sites of the enzyme but can also make the functional core directly respond to magnetism to realize synergistic effects. Considering its economic and environmentally friendly properties, high efficiency β-galactosidase-immobilized MDMSNs, can be designed and employed for conversion of lactose to GOS. The main procedures to prepare the large pore MDMSNs and following enzyme immobilization,
In the second year of the project we aim to utilize permeates obtained from different dairy processing companies in the state of New York as starting material as starting material. In this phase we will optimize the production process; the reactor performance will be evaluated using permeates (liquid and powder) with different lactose concentration, pH, temperature, flow rate, reactor length, and reaction time. More importantly, we will perform studies to determine and optimize reusability and stability of enzymatic packed-bed reactor to provide an economically feasible platform for conversion of permeate to high value added product GOS.

References


Project 1, Year 2 of 2: Request from NYS Milk Promotion Board for the period January 1st, 2018 - December 31st, 2019 - $89,444.

Project 2: Functionalization of whey protein by high-pressure, reactive extrusion

Project PI: Sy Rizvi, Cornell University

Duration: January 1st, 2019 – December 31st, 2019 (year 2 of 2)

Summary of Proposed Project:
Today’s consumers demand cleaner labeled, functional foods that provide health benefits. This has led food processors to constantly look for alternatives to negatively perceived ingredients like chemical emulsifiers, stabilizers and thickeners (xanthan gum, hydroxypropyl methyl cellulose or polyglycerol esters of fatty acids). Research carried out by our lab has shown that whey proteins could offer a potentially clean-label, nutritionally attractive alternative to the emulsifiers and thickeners currently used by the food industry.

A combination of shear, temperature and pressure during extrusion processing creates opportunities for both conformational changes and chemical reactions in whey proteins. It has been shown to expose the reactive free sulfhydryl groups, non-polar amino acids, and peptides that are normally concealed in the native proteins. It is indeed well-known that protein unfolding and aggregation are particularly sensitive to pH and ionic strength due to its dependence on electrostatic interactions, resulting in different gel structures.

Our prior research has shown that microparticulated whey protein could be obtained by precisely superimposed extrusion parameters at pH of 2-3 by adding acidic solution, which displays a semi-solid, smooth texture with a wide range of improved functional properties such as enhanced interfacial activity (emulsification) and cold-gelling and thickening characteristics. Although the process has received considerable attention, in situ modification of pH and ionic strength by introduction of high-
pressure, dense carbon dioxide in the extruder and restoration of the original pH upon depressurization offers an attractive approach for further functionalization of proteins but has not been fully explored. Key mechanical parameters required for extruder design and operation principally involve generation of very high pressures and the rheological response of the material to deformation via extensional and simple shear, while key product quality parameters are related to the microstructure of the final product. Linking these types of data is not straightforward; both for reasons of difficulty in quantifying and imparting appropriate microstructural and functional characteristics in the extrudates.

**Objectives:**
Based upon our promising preliminary data, we believe reactive high-pressure extrusion process in acidic environment given by dense carbon dioxide, combined with controlled shear and heat in the presence of mineral salts (CaCl$_2$ and NaCl) offers great potential to alter the gelling and emulsification properties of proteins. The three seminal and mutually supportive objectives of this proposal are to:

- Investigate the effects of superimposed process variables (shear, temperature, carbon dioxide pressure, and ionic strength) on the physicochemical properties of whey protein and milk protein concentrate (MPC-80) extruded at pH 4-7
- Determine the maximum pressure of CO$_2$ necessary to eliminate the need of added acid during extrusion
- Quantify the experimentally observed responses of whey protein and MPC-80 to functionalization by linking chemical and mechanical response of cellular extrudates with the desired properties.

**Background:**
Extrusion processing has 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). The proposed mechanism and reaction sequence of proteins during extrusion is shown in Fig. 1.

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 diminish repulsive forces, and protein-protein association occurs, forming a self-supporting gels. 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 used to control certain end product functionalities during extrusion processing of proteins and offers attractive new avenues for exploration.

Inclusion of a polysaccharide in a protein formulation aids in functionality enhancement through the formation of separate protein and carbohydrate phases and layering (Cheftel et al., 1992; Tolstoguzov, 1993). Pre-gelatinized corn starch has been used as an inactive filler and a binding agent to hold protein matrices due to its ability to form hydrogen bonds in the extruded products (Amaya-Llano et al., 2007). Depending on the amount used, it also determines the extent of protein/carbohydrate cross-linking. 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).

**Summary of Results To Date:**

Investigation was conducted with commercial whey protein concentrate (WPC-80). A feed formulation comprising (w/w) 98% WPC-80, 1% each of lecithin and diglyceride was extruded at 90 °C and 60% moisture (dry feed basis) at pH 3.0 and 5.4 with 1% (dry feed basis) SC-CO₂ injected at 10 MPa as a blowing agent. The average specific mechanical energy (SME) input for the process was 57 Wh/kg. The resulting protein extrudates were dried, ground into powder, reconstituted in deionized water and evaluated for their rheological and physicochemical properties. The functionality of whey protein concentrate containing 80 wt.% protein (WPC80) was evaluated at two (3.0 and 5.4) pH values. Different levels of oil (20, 40, 60 and 80 wt.%) and protein (1, 2 and 4 wt.%) were used to quantify the emulsion characteristics such as emulsion activity index (EAI), creaming index, droplet size and viscosity of the functionalized whey protein (f-WPC80) and compared with the control (c-WPC80) and commercial sodium caseinate (Na-CN). This study showed that EAI values of f-WPC80 (both pH 3.0 and 5.4) were similar to that of Na-CN but significantly higher as compared to c-WPC80, Figure 2. Additional results showed that gel-like emulsions, with uniform droplet size distribution, were formed by f-WPC80 (4%) with 80% oil which was stable over three months of storage at room temperature. The emulsions formed by f-WPC80 resulted in shear thinning behavior with higher
viscosity as compared to Newtonian emulsions formed by control WPC80. The minimum protein percentage required for stable emulsion formation was found to be 2% for f-WPC80 and Na-CN and 4% in the case of c-WPC80. The functionalized whey protein provides the unique emulsion with good stability at room temperature and may be used as a replacement of sodium caseinate and provide nutritionally superior products.

![Figure 2. Emulsifying activity index of protein emulsions](image)

Viscosity and gelation were evaluated instrumentally and the results indicated that the resulting functionally superior WPC had significantly higher viscosity at room temperature than the unextruded WPC sample. It showed approximately 258 and 275,000 times higher apparent viscosity (η25~2000 mPa·s) than the unextruded WPC (η~8 mPa·s). Its dispersion (20% w/w) 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 85 ºC).

Creaming index is a measure of emulsion stability, higher the value of creaming index, lesser is the emulsion stability. Creaming index was determined to evaluate and compare the stability of f-WPC80 products (pH 3.0 & 5.4) with Na-CN and c-WPC80 based emulsions. Results indicated that creaming stability increased with increasing levels of added oil (Figure 3a) and protein (Figure 3b) to the emulsions. Earlier researchers have also reported the dependency of creaming stability on the oil and protein fractions as it also increases the lag time of creaming, thus delaying the process and adding to the overall stability of the emulsions.

Creaming and coalescence oil droplets are a direct measure of the stability of an emulsion. Coalescence of droplets adds to the creaming rate of an emulsion. There is a number of forces involved in increasing the rate of coalescence in an emulsion like colloidal, gravitational and mechanical forces, and the strength of the viscoelastic layer of the emulsifier at the surface of oil droplets. In this study, emulsions with 60 wt.% oil and stabilized by SC-CO2 extruded f-WPC 80 (both pH3.0 and 5.4) were more stable against creaming as compared to both Na-CN and c-WPC80.
Emulsions made with f-WPC80, pH3.0 were very stable and showed no creaming at 60 and 80 wt.% oil (Fig 3a & b). Comparatively, c-WPC80 stabilized emulsions were very susceptible to creaming at all the studied concentrations (1, 2 and 4%) of protein and all the fractions of oil used.

Figure 3. Creaming index of emulsions after 7 days of storage as a function of (a) oil fractions at 4% protein concentration and (b) protein fractions at 60% oil content.
Surface hydrophobicity and emulsification characteristics of the functionalized WPC-80 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 surface hydrophobicity, emulsifying activity, and emulsion stability against droplet coalescence in oil-in-water (o/w) emulsions compared with those of control WPC-80. The surface hydrophobicity (binding sites) increased from 197 in unextruded WPC to 296 in f-WPC-80. The f-WPC-80 also showed excellent emulsifying properties compared to the c-WPC-80. Emulsions prepared with such small amounts of f-WPC 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.

Droplet size is one of the most important characteristic of an emulsion which determines its stability, texture, shelf life, and rheology. Smaller the droplet size, lesser is the creaming and phase separation. Droplet size for all the emulsions made using various different proteins were measured. The smallest droplet size (1511 nm) was observed in 80% oil emulsion stabilized with 4% f-WPC80, pH 3.0. At constant protein fraction, droplet sizes increased with an increase in oil concentration from 20 to 60 wt.% and then further increase in oil fraction resulted in a decrease in the droplet size. CLSM images of these emulsions also supported the above observation (Figure 4). On the other hand, emulsions with oil fractions of 20, 40 and 60 %, the droplets became progressively larger and polydisperse. However, in case of emulsion with 80 wt.% oil, the droplets became smaller and of uniform size with an average diameter of 1564 nm and showed no visible coalescence, adding to the stability of the emulsion.

![Figure 4. Confocal laser scanning microscopy (CLSM) images of emulsions using 4wt.% protein and 80wt.% oil.](image)

Thus very stable emulsions with finely dispersed fat droplets and homogeneous o/w gel-like emulsions could be produced. Only 2-4% (w/w) f-WPC and 16% (w/w) water was needed to emulsify 80% (w/w) oil, producing an emulsion with long-term storage stability.

The work is in progress to quantify the remaining parameters of functionalized whey protein concentrate at pH 2.9 and 5.7. Also, functionalization of milk protein concentrate (MPC-80) as a replacement of sodium caseinate is under way with promising results and the product has generated considerable industry interest.

**Experimental Details:**
Reactive supercritical fluid extrusion (RSCFX) system

RSCFX 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 (FSP) by the RSCFX process is shown below.

The die will be fitted with two circular inserts of 0.5 to 2.5 mm diameter each. SC-CO2 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). In-barrel moisture content will be maintained at 60% (dry feed basis). 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 injection pressure system will be modified, if needed, to enable extrusion with CO2 at pressures in the range of 3,000 to 4,000 psi.

Experimental design

A mixture of prehydrated (10% wet basis) protein powder (whey protein concentrate, WPC-80, 94% w/w) and pre-gelatinized corn starch (6% w/w) is the base formulation and will be used as the control. A 1:2 CaCl₂: NaCl mixture by molarity will be added to the dry mix. 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 additional variables for the generation of functionalized wpc will be CO2 injection rate (C) at various pressure, screw speed (S), final barrel temperature (Tb) and die diameter (Dd). Extrudates will be dried at 60 °C to a moisture content of 5 wt% on a wet basis and then ground in a hammer mill and sieved for functionality analysis.

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 the following equation, where n is the screw speed, P is the rated power, W is the mass flow rate, and %τ is the load factor.
SME = (n_{actual} \times P \times \%\tau) / (n_{rated} \times W \times 100)

- The temperature (T_p) and pH of the extrudates at the die exit
- The apparent shear rate at the die (SRD) defined by

\[ \text{SRD} = \frac{4 W}{\pi \rho R_d^3} \]

Where \( W \) is the mass flow rate of the feed, \( R_d \) is the die radius and \( \rho \) is the density of the feed.

**Extrudate Evaluation**

- **Expansion ratio, bulk density and void fraction:** Expansion ratio is calculated by dividing the cross-sectional area of the extrudate by the cross-sectional area of the die. Bulk density is measured using a sand displacement method (Park, 1976). Void fraction \( (V_f) \) is calculated using the following equation;

\[ V_f = 1 - \rho_b / \rho_s \]  

(i) where \( \rho_b \)=bulk density of the expanded sample (g/cm\(^3\)) and \( \rho_s \)=density of the unexpanded material (g/cm\(^3\)).

- **Color:** An average of 20 extrudate pieces will be grounded and measured for their color values including the lightness value ‘L’, color parameters ‘a’ and ‘b’, and color difference (ΔE) using a Macbeth Color-Eye Colorimeter.

**Functional Characterization**

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 FSP. Characteristics of FSP in aqueous solution will be compared with those of un-extruded protein samples.

- **Liquid viscosity by shear rate ramp test:** The FSP 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 FSP 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 (\( \tau \)), 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 (\( \tau_{0\text{HB}} \)) will be computed.

\[ \tau = k \dot{\gamma}^n \]  

(ii)  

\[ \eta_a = \frac{\tau_{0\text{HB}}}{\dot{\gamma}} + k \dot{\gamma}^{n-1} \]  

(iii)
• Gel properties by small-amplitude oscillatory (dynamic) tests: Gels containing 15 to 30% FSP (w/w) will be prepared at ambient temperature by reconstituting FSP 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 FSP 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 δ) 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 δ (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 FSP gels over a range of temperatures. Temperature will be ramped from 5 to 85 °C at 2 ºC/min heating rate and at a constant frequency rate of 1 rad/s and 1% strain. The G’, G'” and tan δ will be then computed. To study the effect of pH conditions on the rheological stability of FSP gels, the dynamic moduli (G’, G’” and tan δ ) will be measured at pH 4.0 to 8.0 and 25 ºC as frequency is ramped from 0.1 to 100 rad/s.

• Water holding capacity (WHC) of FSP powders: FSP powders will be hydrated (15%, w/w) in deionized water for 3 h and centrifuged at 3,500 rpm for 30 min at 25 ºC. 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.

• Surface hydrophobicity measurements: Surface hydrophobicity of FSP will be determined using the florescence 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.

• 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

\[ EAI \left( m^2 \cdot g^{-1} \right) = \frac{2xTxD}{\phixCx10,000} \]  

(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.

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

(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 \(^\circ\)C; ambient storage at 25 \(^\circ\)C; and at 55 \(^\circ\)C 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 \(^\circ\)C 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 FSP 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 \( \mu \)m) 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 \( ^\circ \)C. 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).

\[
FC = \frac{V_{foam}(f)}{V_{gas}(f)} \\
C_f = \frac{C_{foam}(f)}{C_{liq}(f)} \times 100
\]

where \( V_{foam}(f) \) is the final foam volume, \( V_{gas}(f) \) is the final gas volume injected, and \( C_{foam}(f) \) and \( C_{liq}(f) \) 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 FSP will be evaluated using DSC. A 20 \( \mu \)L FSP dispersion (10\% w/w) will be scanned in a DSC from 5 to 110 \( ^\circ \)C at a rate of 5 \( ^\circ \)C/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

\[
\text{Peak height of FSP sample} \times 100 \over \text{Peak height of unextruded sample}
\]

**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.

References


Project 2, year 2 of 2: Functionalization of whey protein by high-pressure, reactive extrusion
Request from NYS Milk Promotion Board for Jan 1st, 2019 – Dec. 31, 2019: $102,070


Project PI’s: Samuel Alcaine, Cornell University
Duration: January 1st, 2019 – December 31st, 2019 (year 2 of a 2-year project)
Summary of Proposed Project:

We have recently purchased the ElastoSens, an analytical benchtop technology that enables us to directly measure the viscoelastic properties of food gels, non-destructively, in real time. This of interest as other methods commonly used to infer gelation properties are either indirect (pH, optics, conductivity) or destructive (rheometer, penetrometers) resulting in either less precise info on gel strength or the inability to follow changes in a given sample overtime. There are many opportunities to apply this new technology to better understand how multiple factors influence gel formation in the production of dairy products like cheese and yogurt. Because of the speed and consistency of this tool, it could be implemented as a rapid method to evaluate incoming milk quality in regards to gelation, prior to use. The ElastoSens will also enable us to evaluate the impact of different bacterial cultures, temperature abuse, coagulants, functional ingredients, and contaminants (phage, antibiotics, etc) not on gel formation but on gel stability over the shelf life of products like yogurt.

Objectives:

1. Evaluate the use of the ElastoSens as a rapid method to screen milk quality for rennet-set dairy gel formation, in particular in regards to seasonal variability and temperature abuse.
2. Evaluate the use of the ElastoSens as a method to monitor and potentially predict syneresis in yogurt.

Background:

The ElastoSens is a patented benchtop technology that uses ultrasonic vibrations to measure the viscoelastic properties of a material overtime. A sample is placed in a detachable holder, the bottom of the holder is composed of a soft and flexible membrane. The sample and the membrane form a bilayer system capable of vibrating and resonating. Changes in the sample structure, for example gel formation, change vibration and resonance of the system, and these deformations are measured by laser and converted into storage and loss moduli. Because the measurements are contactless, changes in the sample can be measured overtime. The system allows the monitoring of three different samples in real time with temperature control from 4 °C to 70 °C. Sample holders can also be nondestructively disconnected, stored under various conditions, and placed back into the machine for measurement. This allows for monitoring of gel properties over many samples, under various conditions, formulations, over long periods of time. This novel method has been evaluated in biomedical applications for the characterization of hydrogels, and it performance has compared favorably to more common viscoelastic analytical devices like rheometers¹. For dairy gels, it allows us to not only measure gel strength and gelation rate in the first few hours of processing, but allows us to understand how these gels degrade over the shelf life of a product.

A production floor model of the ElastoSens, called the CoagusSens, has been targeted to the dairy industry. Its primary application has been vat side, as a consistent, automated method to monitor gel strength formation to optimize cutting time, and subsequently cheese yield. The manufacturer, Rheolution, along with researchers at the Agriculture and Agri-Food Canada Research & Development Center in Saint-Hyacinthe, Quebec have evaluated it use to successfully improve yields in cheddar cheese, though the data has not yet undergone peer review. Other industries have adopted this technology in a quality control role, where it used to measure the gelation of food products contain hydrocolloid gels and ensure that the properties are within parameters prior to shipment. Our aim is to see whether or not this technology has any potential utilization at the receiving as a rapid screen for incoming milk quality for fermented dairy producers prior to use, or if it can be used to evaluate gel degradation (ie syneresis) overtime.
Experimental Approach:

Evaluating Milk Quality
The goal of this portion of the study will be to build a data set that will allow us to ask deeper questions about the correlation of incoming milk attributes: fat, protein, lactose, total solids, solids no fat, total acidity, density, free fatty acids, calcium; and data collected using the ElastoSens on gelation strength, time, and speed. This will allow us to evaluate whether the ElastoSens provides any additional information about the incoming milk that would be valuable to cheese makers. We will work with the Voluntary Shelf Life program and Cornell Dairy to get access to milk samples from across New York State throughout the year. Each sample will be analyzed for the standard milk components mentioned above. A sample of the milk will then be adjusted to pH 6.4. A 7 mL sample of the pH adjusted milk will be added to the ElastoSens, and then rennet will be added per the manufacturer’s dosage and temperature recommendation. The sample will then be monitored in the ElastoSens for gelation speed, time, and strength. A subset of our milk samples will also be exposed to various time-temperature abuse conditions. The samples will then be adjusted to pH 6.4 and analyzed as previously described. By comparing these abused samples to the control, we will be able to evaluate whether or not the ElastoSens can capture additional milk quality information, like abuse, not caught by other analysis but that may have an impact on cheese making.

Evaluating Syneresis
In this set of experiments, we will evaluate the use of the ElastoSens to monitor yogurt gel formation and stability over shelf life. Three 1 L yogurts will be made from skim milk obtained from the Cornell dairy, and will be supplemented with either 0%, 2%, or 4% non-fat dry milk powder. A standard, commercially available yogurt starter culture will be added to each of the yogurts. Once the cultures are mixed in, 7 mL samples of each will be added to the ElastoSens for monitoring, and 200 mL will be added to a bottle and monitored for pH, temp, and redox using the iCinac. Samples will be held at the culture supplier’s recommended fermentation temperature until a pH of 4.5 is reached for all three samples as measured by the iCinac. Samples within the ElastoSens will be cooled to either 4 °C, 10 °C, or 20 °C and monitored until visual syneresis is observed. The data will then be reviewed to evaluate if there were detectable changes in gel strength that preceded visual detection of syneresis, thus indicating whether the ElastoSens could be used for shelf life prediction in yogurt. All NFDM fortification levels and incubation temperatures will be performed in triplicate.

Product Analysis and Statistical Validity
All products attributes in these studies will be analyzed using standards methods for the evaluation of dairy products. All experimental conditions will be performed in triplicate, and for the milk quality tests, each milk sample will be evaluated in triplicate. The yogurt data will be analyzed using standard methods for statistically significant (p <0.05) differences between conditions and University statisticians will be consulted to preform principle component analysis of the data collected for milk quality and gelation performance.

Progress Report:

Evaluate the use of the ElastoSens as a rapid method to screen milk quality for rennet-set dairy gel formation.
For the first half of the year the focus was on becoming familiar with ElastoSens, understanding how to appropriately calibrate the equipment, run samples, what errors were common during processing (ex. Drying out of sample), and how to download and analyze the data. We then started evaluating different levels of rennet and CaCl concentrations for a simple and rapid protocol for incoming raw milk coagulation evaluation. All samples are run in triplicate.

As can be seen from the graph, the coagulation attributes of our raw milk samples has been fairly consistent over the six month period we have evaluated. Our intention is to continue collecting this data to understand and potential trends throughout the year. Retain samples have also been saved for fat, protein, lactose, and mineral analysis.

_Evaluate the use of the ElastoSens as a method to yogurt (soft gels)._  

A question that arose in our early evaluation of the ElastoSens was what was the variability of this new technology to consistently evaluate products like yogurt, that are soft gels. To understand this potential variation, we are evaluating various factors that impact yogurt gel formation: i) protein fortification; ii) culture type; and iii) fermentation temperature. Each condition is being running six times to understand the variability range of the method. We are also using the iCinac to continuously monitor pH and its relation to gelation, since we know yogurt coagulation is driven by reaching a pH below the isoelectric point of casein.
We have currently completed running the protein fortified samples (data above) and are in the process of evaluating the impact of cultures. We will also use this data to look at the relationship between pH and the rate of gelation, the rate of acidification and the rate of gelation, and lastly the rate of acidification and gel strength. Finally, we also intend to validate the final yogurt gel strength values generated by the ElastoSens against those generated by a traditional rheometer to understand how comparable the methods are.

References


Project 3, Year 2: Request from NYS Milk Promotion Board for the period January 1st, 2018 - December 31st, 2019 - $65,005.

Project 4: Refermented: Upcycling dairy by-products into a new category of value-added consumer beverages.

**Project PI’s:** Samuel Alcaine, Cornell University  
**Duration:** January 1st, 2019 – December 31st, 2019 (year 2 of a 2-year project)

**Summary of Proposed Project:**

Food waste and new flavors are on the tongues of American consumers. The rise of kombucha, sour beers, and vinegar tonics creates a space where fermented dairy beverages could blossom. Furthermore, dairy by-products, like permeate and acid whey, with little current value to the industry make the ideal raw ingredients for upcycling due to their lactose and mineral content. We propose building upon our current research on acid whey utilization in brewing, to further characterize and develop fermentation processes to produce low to moderate alcoholic beverages from dairy by-products common to New York State. This research would lay the groundwork for launching a new dairy-based category with growth potential for the dairy industry and position New York State Dairy as the leading innovator in the space.

**Objectives:**

1. Evaluate the fermentation of concentrated acid whey using a strain of *Brettanomyces* to achieve yields of 2.5 to 10% ABV.
2. Evaluate the fermentation of milk permeate with lactic acid bacteria and Brettanomyces to achieve yields of 2.5 to 10% ABV and final pH between 3.2 to 4.8.
3. Develop prototypes for sensory analysis and consumer feedback in comparison to competitive products (i.e. kombucha, sour beers).

**Background:**
Over the last 20 years, the American palette has changed. It has not only become adventurous, seeking out novel flavors and experiences that challenge the taste buds, but it has gained a consciousness via new buds that sense and react to the environmental impact beyond the flavor of a food. These new sommeliers represent a risk to dairy, they are receptive to the creeping taste-tendrils of plant-based beverages and hear the accusatory tweets of waste and inefficiency. These new sommeliers also represent an opportunity for dairy to evolve, grow, and challenge preconceptions.

The Food and Agricultural Organization estimates that 1.3 billion tons of edible food is lost or wasted globally per year\(^1\). The FAO’s trumpet call has inspired the rise of startups that are upcycling food waste into virtuous ingredients for new products that resonate with both the environmental and flavor taste buds of consumers. From converting spent brewers grain into nutritious energy bars to transforming underutilized fish and produce into New England fish cakes, to face-lifting ugly fruits into delicious juices, these startups are gaining traction in the marketplace. In the world of dairy, one of our biggest challenges, acid whey from cottage cheese and Greek yogurt and permeate from ultrafiltered milk, represent our biggest opportunity. These by-products - currently destined to field application, animal feed, and anaerobic digesters - are rich with nutrients and minerals and have the potential to be converted into value-added products that resonate with consumers.

There is a well-documented history of whey based-beverages\(^2\), but they have received little renewed attention in light of the changing market. We believe a new opportunity exists because: i) upcycling of by-products has gained such resonance, ii) fermented\(^3\) and probiotic\(^4\) foods are also gaining share in the market, iii) and the growth in beverages like kombucha, sour beers, and vinegar tonics demonstrate a growing market in the flavor profiles that can be readily achieved with fermented dairy beverages. The Alcaine Research Group (ARG) is already working in this area, through a grant from NYS Department of Conservation and the Environment, where we are investigating acid whey utilization in “beer” like production. This work has resulted in developing a novel beer mash that activates enzymes that hydrolyze lactose. ARG has also been looking into mono and co-culture fermentations of lactic acid bacteria and yeast using non-concentrated acid whey. This work stream has resulted in a beverage containing nearly 2.5% ABV alcohol and that tastes like a Gose, a low alcohol sour beer style with growing popularity in the craft beer market. With more support we can further investigate these fermentations and understand the impact of pre-concentration on alcohol yields, use of UF milk permeate, look at non-alcoholic fermented beverages from these by-products, and perform larger scale pilot plant trials to gauge the acceptability of these products to consumers. This research represents the opportunity to develop a novel category of dairy-based products that engage consumers and grow the dairy market.

**Experimental Approach:**

_Evaluating Acid Whey Concentration and Fermentation_

All experiments will be performed on acid whey obtained from a local Greek yogurt producer. Acid whey will be transported to Cornell’s Food Processing Development Lab and concentrated using reverse osmosis to ~35% solids. This concentrated acid whey will then be diluted so that the diluted lactose content would theoretically yield 2.5%, 5%, 7.5%, and 10% ABV products after fermentation. These various wheys will then be fermented by two commercial strains of _Brettanomyces_ strains. Fermentations will be monitored for cell count, pH, organic acid profile, nitrogen, ethanol, and residual lactose content.

_Evaluating Milk Permeate and Fermentation_
All experiments will be performed on either permeate obtained from a local producer or if necessary, produced in Cornell’s Food Processing Development Lab using ultrafiltration of skim milk. The permeate will be diluted so that that the diluted lactose content would theoretically yield 2.5%, 5%, 7.5%, and 10% ABV. These various permeates will then be fermented by two commercial strains of *Brettanomyces* strains. We will also evaluate initial fermentation with yogurt strains, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, to drop permeate pH to 5.5 and 4.5, followed by fermentation by *Brettanomyces spp.* Fermentations will be monitored for cell count, pH, organic acid profile, nitrogen, ethanol, and residual lactose content.

**Evaluating non-alcoholic Fermentation**
In this series of experiments, we will evaluate the use of yogurt cultures (*S. thermophilus* and *L. bulgaricus*) with probiotics (*Bifidobacterium* and *Lactobacillus acidophilus*) to convert permeate into a non-alcoholic probiotic beverage. We will also investigate the use of in-house kefir cultures to ferment both permeate and acid whey into a non-alcoholic beverage. Fermentations will be monitored for cell count, pH, organic acid profile, nitrogen, ethanol, and residual lactose content.

**Prototype Development**
In year 2, based on the result of the fermentation work, we will develop prototypes, which may or may not contain added ingredients (fruits, spices, etc). With input from our sensory team we will develop a roundtable to evaluate these prototypes with consumers in the context of other fermented beverage with similar flavor profiles (kombucha, gose, etc). The attempt will be to gauge acceptability, interest, and overall flavor profile. We will also explore incorporating the value proposition, ie upcycling of dairy by-products to reduce food waste, and it is impact on interest, acceptability, and purchase intent.

**Product Analysis and Statistical Validity**
All products in the studies will be analyzed pre and post fermentation for cell count, pH, organic acid profile, nitrogen, ethanol, and residual lactose content. All fermentations will be performed in triplicate. Data will be analyzed using standard methods for statistically significant (p <0.05) to evaluate differences in finished product attributes and strains used.

**Progress**

*Evaluate the fermentation of concentrated acid whey using a strains of Brettanomyces*

In our initial work we evaluated two commercial strains of *Brettanomyces claussenii*, a yeast reported to have the ability to utilize lactose to produce ethanol. As can be seen in Figure 1, only one of these commercial strains, BC1, successfully produced detectable alcohol – at a final average concentration of 2.8% ABV, from acid whey. Sugar analysis revealed that strain BC1 had utilized all the lactose and galactose present in the acid whey, whereas strain BC2 had not utilized any lactose and just a little galactose. Both strains we sent off for genotyping, and BC1 matched *B. claussenii*, whereas strain BC2 came back as *B. bruxellensis*, a species unable to utilize lactose. This highlights the importance of quality control at commercial yeast supply companies.

We have proceeded to evaluate the fermentation of three known lactose utilizing yeast: i) *Kluyveromyces lactis* – a yeast used for bioethanol production from lactose; *Dekkera anomola* – an anamorph of *B. claussenii*; and iii) our original *B. claussenii*. As can be seen in figure 2, while *K. lactis* initially ferments fast than the other two yeasts, it does not completely ferment the sugar, thus producing a lower final alcohol.
We have done some preliminary work evaluating the fermentation of sweet whey, and have obtained milk permeate for future evaluation.

**Evaluating non-alcoholic Fermentation**

Kombucha has become a very popular fermented, non-alcoholic beverage in the market. It’s primary prebiotic component is acetic acid, which is thought to be responsible for many of the health benefits of the beverage. *B. clausenii* is an interesting yeast, in that it has been found kombucha, and is known to produce acetic acid from glucose under aerobic conditions. It has not evaluated for the production of acetic acid from lactose. We thus looked at *B. clausenii* and a group of other yeast that could potential produce acetic acid, and looked at their ability to do so using acid whey as a substrate (Figure 3). Of these yeast, two strains of *B. clausenii* and one of *D. anaomla*, were the best producers of acetic acid. We will continue experiments to evaluate the levels of acetic acid production and lactose utilization. Subsequently will evaluate beverages based on these fermentations.
Prototype Development

To gauge consumer acceptance of this novel class of alcoholic beverage, we recruited 100 participants to evaluate the acid whey fermented by *B. clausenii* and version of that plain base flavored with ginger and lime. Consumers preferred the flavored prototype (Figure 4), and nearly 50% claimed they would likely or would definitely buy this type of product (Figure 5). This makes the initial case for a potential market for these whey-based alcoholic beverage.

![Figure 3. Acetic Acid Production from Acid Whey by Yeast](image)

![Figure 4. Consumer Preference Testing of Whey Beer](image)

<table>
<thead>
<tr>
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<th>Plain</th>
<th>Ginger/Lime Flavored</th>
<th>Paired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Liking (9 pt scale)</td>
<td>3.99</td>
<td>6.45</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Purchase Intent (5 pt scale)</td>
<td>1.98</td>
<td>3.21</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Appearance (9 pt scale)</td>
<td>5.10</td>
<td>4.94</td>
<td>0.232</td>
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<tr>
<td>Aroma Liking (9 pt scale)</td>
<td>4.66</td>
<td>6.97</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Flavor Liking (9 pt scale)</td>
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<td>6.44</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Purchase Intent</td>
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<td>Ginger/Lime Flavored</td>
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<tr>
<td>5=Definitely would buy it</td>
<td>1</td>
<td>9</td>
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<tr>
<td>4=Probably would buy it</td>
<td>7</td>
<td>37</td>
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<tr>
<td>3=May or may not buy it</td>
<td>20</td>
<td>29</td>
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<tr>
<td>2= Probably would not buy it</td>
<td>33</td>
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</tr>
<tr>
<td>1= Definitely would not buy it</td>
<td>39</td>
<td>9</td>
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Figure 5. Consumer Purchase Intent - Whey Beer
Project 4, Year 2: Request from NYS Milk Promotion Board for the period January 1st, 2019 - December 31st, 2019 - $64,434.

Project 5: – Identification of farm/feeding sources of autoxidation based off flavors in fluid milk.

Project PI’s: Dave Barbano, Cornell University
MaryAnne Drake – North Carolina State University
Rick Grant and Heather Dann – Miner Institute, Chazy, NY

Duration: October 1st, 2017 – December 31th, 2018 (year 2 of a 2 year project)

Summary of Proposed Project:

Auto oxidation based off flavor in fluid milks (particularly skim milk) occurs more frequently in the spring of each year. This is a time when milk composition is changing (typically milk fat and protein content are decreasing) and ambient conditions that impact feed and forage quality are changing. Our recent work with more detailed analysis of the fat portion of milk by both reference chemistry (gas chromatography) and rapid infrared milk analysis has identified farm to farm and seasonal variation in milk fatty acid composition that relate to dairy cattle feeding and management and may be related to increased susceptibility of milk fat to develop oxidized off flavors in fluid milk products. The oxidation occurs in fluid milk at all fat levels, but the sensory thresholds for detection of oxidized off flavors are lower in skim milk. This has been reported as an issue in western NY and other regions of the US. Off flavors in fluid milk result in consumer complaints and decreased consumption of fluid milk. The susceptibility of milk fat to oxidation and off flavors may be related to the nutritional quality of the feed consumed by the cow and specifically the rumen unsaturated fatty acid load (RUFAL) placed on the rumen. Analysis of the double bonds per fatty acid in milk fat using mid-infrared spectroscopy may reflect the dietary RUFAL and therefore be used to effectively diagnose feeding issues that contribute to oxidation and off-flavors.

Objectives:
1) To produce milk by feeding cows different standard diets that vary in fat level, fat quality, and fat sources (particularly corn and soy) that are likely to relate to increased susceptibility of milk fat to oxidation and to provide these milks for chemical and sensory analysis.

2) To determine differences in milk fatty acid composition that relate to feeding and make that information available for correlation to the flavor characteristics of milk from different feeding treatments.

3) To determine difference in sensory levels of auto oxidation in the milks produced with different feeding treatments and determine the chemical characteristics of the oxidation product as they relate to sensory impact and dairy cattle feeding.

**Experimental Approach:**

**Cow Feeding, Management, and Milk Collection: (Miner Institute)**

We will conduct two feeding studies in which the diets are formulated to supply normal and elevated RUFAL (i.e., rumen unsaturated fatty acid load) content to mid-lactation Holstein cows. The design will be a 2-period crossover so that all cows are fed all diets, and milk from cows fed low and high RUFAL will be available at the same time for IR, oxidation, and sensory analyses. Experiment 1 will use ground, full-fat soybeans to raise the RUFAL. Often, when oxidized flavor is observed in the milk the problem can be traced back to a farm that is feeding soy products. In Experiment 2, we will use dried distillers grains with solubles (DDGS) to increase RUFAL because they are a commonly used byproduct and can easily lead to high RUFAL if diets are not formulated properly. In both experiments, we propose using corn silage as the predominant forage because it is commonly used in the northeast and, although its unsaturated FA content is relatively low, its high inclusion level in the diet makes it a significant (and too often overlooked) contributor to RUFAL.

**Proposed Procedures:**

We propose two feeding studies to test whether using IR to measure double bonds per fatty acid accurately reflects dietary RUFAL and in the context of the complete project determine if this is related to development of auto oxidation (not light induced) based off flavor development in fluid milk.

Experiment 1: Sixteen mid-lactation Holstein cows (balanced for parity) will be used in a crossover design with 21-d periods such that cows are exposed to the following diet sequences: control – high RUFAL or high RUFAL – control.

Cows will be housed in a tie stall facility equipped with individual feed boxes and fed once daily for ad libitum intake. Cows will be removed from the tie stall three times daily for milking.

Diets will be formulated using CNCPS version 6.55 with the AMTS platform and will contain approximately 55% forage (% of ration DM) with the majority being corn silage (~75% corn silage and 25% haycrop silage). The concentrate mix will be formulated to provide either a low (control) or high RUFAL by addition of roasted, ground full-fat soybeans.

The first 2 weeks will be used an adaptation period to the diet with milk sample collection occurring during week 3. Feed ingredients and TMR will be collected daily during each collection week, composited by period, and analyzed for nutrient composition. Milk will be measured at each milking during the collection week with milk sampling for compositional analysis occurring on two consecutive days (total of six milkings) during that week.
Specific measurements will include:
1. Chemical composition of the primary forage and grain ingredients. Key measures will be: DM, CP, protein degradability, NDF, NDF digestibility, starch, starch digestibility, fatty acid content, and fatty acid profile.
2. Dry matter intake and intake of specific nutrient fractions with a specific focus on RUFAL (DMI x dietary content of rumen unsaturated FA).
3. Milk yield (measured daily) and milk composition measured at each milking for two consecutive days.
4. Body weight and body condition score measured immediately after milking on day one of Period 1 and then at the end of each subsequent period. This information will be related to any observed changes in preformed milk fatty acids that may be reflective of body fat deposition or mobilization.

Experiment 2: Same design and measurements as Experiment 1, but the diets will be formulated using corn silage as the primary forage and DDGS as a source of unsaturated fatty acids.

For both experiments, thirty liters of raw milk will be collected per treatment in light shielding milk jugs and shipped on ice by overnight carrier to NC State. Samples of the same raw milks will be shipped to the laboratory at Cornell for chemical analysis.

**Chemical Analysis of Milk Fat and Feed: (Cornell)**

Both infrared milk analysis with the new metrics (de novo, mixed, preformed fatty acids, chain length and double bonds per fatty acid) for milk fat analysis [Woolpert et al. 2016, 2017; Wojciechowski and Barbano (2016)] along with GLC fatty acid profiles will be conducted on the raw milks from different treatments. Fatty acid composition of feed samples from Miner Institute will be determined at Cornell for different feeding treatments. These will be correlated with the differences in feeding and differences in flavor profiles of the pasteurized fluid milks. We will determine if any of the rapid infrared milk analysis metrics could be used to screen milks to indicate susceptibility of milk to developing auto oxidized (not light induced) off-flavors.

**Sensory and Instrumental Analysis of Oxidized Off-flavor in Milks: (NC State)**

**Milk Processing**

Upon receipt (30 liters of raw milk from Miner Institute per treatment) of milk by overnight shipping, temperature will be confirmed (< 4°C). Proximate analysis of raw milk will include coliforms, aerobic plate count, fat content and total solids content using standard methods (Yeh et al., 2017). Raw skim and raw cream will standardized to 3.25% fat. Fat content will be measured again by the mid-infrared milk analyzer (calibrated monthly) using a set of modified milk calibration samples (Kaylegian et al., 2006 a, b) with all lab mean reference chemistry (Wojciechowski et al., 2016) to confirm proper standardization of 3.25% fat milk. A Microthermics EHVH pasteurization unit (Microthermics, Raleigh, NC) with a two-stage homogenizer (GEA Niro Soavi, Parma, Italy) will be used to process the milks. Each batch will be preheated to 60°C, homogenized (first stage at 17.3 MPa and second stage at 3.4 MPa), and pasteurized (73°C for 15 s) before cooling to 10°C. Final products will be collected in sanitized containers and then placed at 4°C and tested for complete pasteurization by the alkaline phosphatase test. Fat content and microbial quality will be determined using the same methods described previously. Milk fat globule size distribution will be measured using a Malvern Mastersizer 3000 (Malvern Instruments, Westborough, MA) operated in the Mastersizer 2000 emulation mode for
the 3.25% fat milks. The focus of the study is on skim milk as this is the product where autoxidation is most obvious and is the primary industry problem. Standardized whole milk will be processed and included in the study for volatile compound analysis as a point of reference. The same volatile compound profile (sourced to autooxidation) should be present in whole milk.

**Descriptive Analysis**

Descriptive sensory analysis of milk flavor will be conducted at days 5, 10 and 14 post processing using a trained descriptive sensory panel and an established milk flavor language (McCarthy et al., 2017; Yeh et al., 2017). Multiple time points are needed as autoxidation may not be detected until 10 to 14 days post processing. Panelists (n=8, 6 females, 2 males, ages 22-49 y) each have more than 150 h of previous experience with the sensory analysis of food aromas and flavors using the Spectrum™ descriptive analysis method. Milks will be dispensed (60 mL) directly into three-digit-coded 120 mL lidded soufflé cups for evaluation. Preparations will be conducted with overhead lights off to avoid exposure to light. Samples will be evaluated by each panelist in duplicate. Sensory data will be collected using paper ballots. All sensory testing will be conducted in accordance with North Carolina State University Institutional Review Board for Human Subjects guidelines.

**Volatile Compound Analysis**

M MILks (skim and whole) will be sampled at each time point (5, 10 and 14 days post processing) for volatile compound analysis. Volatile flavor compounds will be extracted by sorptive stir bar extraction (SBSE) as described by Park and Drake (2016). Prior to analysis, stir bars (PDMS, 10 x 0.5 mm; Gerstel Inc., Linthicum, MD) will be cleaned by soaking 10 stir bars in 40 ml of a 1:1 mixture of methanol and methylene chloride for 4 h, air drying in a fume hood for 2 h, and conditioning at 280°C for 1 h with 75 ml/min nitrogen gas. Five ml of fluid milk will be placed in a 10 ml amber screw top vial with a Teflon lined lid (Gerstel Inc.) along with 10 µl of internal standard (0.81 mg/L 2-methyl-3-heptanone in water; Sigma Aldrich, St. Louis, MO). In order to more efficiently extract multiple classes of compounds, sequential stir bar extraction will be employed. The TDU tubes with stir bars will be injected using an autosampler (MPS Autosampler, Gerstel, Inc.). The tubes will be heated to 250°C for 10 min in a TDU (Gerstel Inc.) with cryogenic trapping of the compounds at -120°C (CIS 4, Gerstel Inc.). The volatile compounds will be injected onto an Agilent 7890B GC (Agilent Technologies, Santa Clara, CA) and separated using a non-polar column (ZB-5MS, 30 m x 0.25 mm x 0.25µm; Phenomenex, Torrence, CA). Oven conditions will be as described by Park and Drake (2016). Helium will be used as the carrier gas with a column flow rate of 1 ml/min and a purge time of 1.2 min. Compounds will be detected with an inert mass selective detector (model 5970A, Agilent) using selective ion monitoring mode. Compounds will be identified using the 2014 NIST mass spectral library (NIST, 2014), retention index, and retention time of authentic standards (Sigma Aldrich) injected under identical conditions. Relative abundance will be calculated for each compound based on recovery of the internal standard. Aroma active compounds in skim milks will be identified by gas chromatography olfactometry to pinpoint specific compounds responsible for autooxidized flavors. Volatile compounds will be extracted and injected as previously described. GC effluent will be split between the mass spectrophotometer and a sniffer port supplied with humidified air. Each sample will be evaluated by 2 highly experienced sniffers (each with > 50 h previous experience with GCO) who record retention time, aroma character and perceived intensity. GCO results will be cross-referenced with GCMS results and sensory results to identify key compounds responsible for auto-oxidized flavor. Once compounds are identified we may want to determine if some of these compounds are present directly in the dairy cattle feed.

**References**


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**Project 5, Year 2: Request from NYS Milk Promotion Board for the period January 1st, 2019 - December 31, 2019 - $101,864.**

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**Project 6 - 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 and those activities have been reported. *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 2019.

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 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 all MF retentates to be used for production of cheese with a standard of identity.

2. Many companies and cooperatives are interested in processing methods for reducing spore content of milk prior to dairy product manufacturing. The Northeast Center has conducted research on several approaches for spore removal. Depending on the dairy product being made, the spore reduction needed, and the scale of processing per day, different approaches may be appropriate depending on the site and milk supply specific details. We are working with companies to help evaluate options and help them decide on the best approach for their circumstances.

3. 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.

4. 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.

Project 6: Request from NYS Milk Promotion Board for the period January 1, 2019 to December 31, 2019. $ 51,625.

Proposed New Dairy Center Projects 2019:

Project 7: Nutritious Spreads and Fillings using Milk Ingredients
PI: Alireza Abbaspourrad, Cornell University

Duration: January 1st, 2019 – December 31st, 2019 (year 1 of 2 years)

Two Years Project Summary

Background
Spreads, fillings and mayonnaise contain high fractions of an oil phase. These products usually have high caloric values and lack nutritious ingredients. For example, in conventional spreads, 80% fat provides spreadable properties by selecting right combination of fat with different melting temperature. One approach to achieve the desired consistency in spread, fillings and mayonnaise products is to take advantage of high internal phase (HIPE) emulsion technique to reduce amount of fat used. Moreover, using HIPE approach enables us to include nutritious ingredients such as milk proteins, as well as flavors in both water and oil phases. High internal phase emulsion (HIPE) is a highly concentrated emulsion system with internal phase volume fraction exceeding 0.74, the gel-like characteristic of HIPE enables products such as low-fat spread and fillings. However, water-in-oil HIPE with high stability are difficult to fabricate thus are rarely explored. 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 high shear homogenizer. By applying phase structuring approach within HIPE, we can modulate rheological properties, stability and enable high loading of milk proteins, as well as flavors within a low-calorie yet palatable food matrix.

**Phase I. (first year)**

**Experimental Approach**

The aim of proposed research is to utilize milk ingredients including milk proteins, and milk fat to create low calorie, and nutritious fillings and spread like products. We will utilize high shear homogenizer to emulsify water phase within milk fat phase to create HIPE. The HIPE emulsification approach provides the ability to incorporate milk proteins without any pretreatment. In addition, this approach provides the platform to incorporate water soluble flavors within protein emulsion phase as well as fat phase. We will emulsify water phase enriched with milk protein within fat phase using high shear homogenizer. We will place the fat phase within water bath kept at temperature between 30 - 40 °C, water phase will be added to the fat gradually under high shear condition. The product will be cooled down to room temperature upon completion of homogenization. The concentration of protein (10-40 wt.% in water phase) as well as milk fat proportion could be easily varied to modulate the consistency and stability of the product. We will evaluate the effect of these parameters on HIPE formation, thermal stability, shelf life and rheological properties. In addition, using HIPE enables us to encapsulate and protect other water-soluble bioactive ingredients in water phase which will result in creating low cost nutritious products, with mimicking fat mouthfeel properties.

In our preliminary experiments, we evaluated the possibility of creating HIPE using gums as the internal phase. The HIPE stability increases with addition increased concentrations of protein and other water-soluble ingredients in water phase. For example, HIPE internal phase (water phase) made with 1% κ-carrageenan shows the most stable product, with no implication of droplets coalescence over time. Rheological results show that carrageenan-incorporated HIPE exhibits stable solid-like gel performance. Our preliminary data indicate that HIPE made with increased structured internal phase, i.e. loaded with high concentration of proteins could be used potentially in production of protein-enriched, palatable and nutritious spreads and fillings. Figure below demonstrates (preliminary data) microscope image of HIPE made with different consistency:

![Microscope image of HIPE](image-url)
Phase II. (second year)
In the second year of the project we aim to utilize variety of milk fat fractions and milk proteins from processing companies in the state of New York to formulate spreads and fillings with different mouthfeel and physical properties. In this phase we will optimize the production process such as shear rate, pH, processing temperature and evaluate the shelf-life, stability, as well as sensory attributes of produced spreads and fillings. More importantly, we will perform studies to determine the possibility of delivering water soluble vitamins and minerals along with milk ingredients using HIPE approach.

References


Project 7, Year 1: Request from NYS Milk Promotion Board for the period January 1st, 2019 - December 31st, 2019 - $80,240.

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Project 8: Vacuum microwave drying of nonthermally concentrated milk and protein concentrates for the manufacture of dairy powders of superior quality and functionality
PI: Carmen I. Moraru
Summary of Proposed Project

Skim milk powder (SMP) is an important dairy product and ingredient, which will be produced in increasing amounts in the coming years. The increase in production of SMP also coincides with a significant drop in its market price, which creates a very competitive environment for powder processors, both globally and domestically. 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 important and abundant dairy ingredient.

We propose to use a combination of nonthermal concentration and Vacuum Microwave Drying for the manufacture of milk 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. This project is building on work that was already completed on the nonthermal concentration of milk, and the proposed work will only focus on the Vacuum Microwave Drying process.

The proposed combination process for the manufacture of dairy powders 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, as well as increased consumer and customer satisfaction on the domestic market. The newly developed process can also become very attractive for drying of specialty powders, including protein concentrates obtained by membrane filtration, and infant formula.

Background

Market outlook

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).

North America has the major share of the SMP market, followed by Europe, while New Zealand is the major exporter of SMP. 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). This increase in production of SMP unfortunately coincides with a significant drop in prices (Fig. 1), which creates a very

![Skim Milk Powder Prices](https://www.globaldairytrade.info/en/product-results/skim-milk-powder/)

Figure 1: Skim milk powder prices between 2013-2018.
competitive environment, both globally and domestically.

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.

**Opportunity to create powders of improved quality**

SMP is 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 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). Low-heat powder is 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).

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 will 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.

Before use, skim milk powder has to be reconstituted to different 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

![Figure 2. Concentration of skim milk by Forward Osmosis (FO) (Beldie and Moraru, 2018, unpublished)](image)
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 (~ 50 °Brix), but in a completely non-thermal process (we conducted trials both at 4 °C and at 15 °C, as shown in Fig. 2). This process is also superior to Reverse Osmosis (RO), another membrane filtration process that is used by some dairy processors to replace thermal concentration, because RO cannot reach concentration levels higher than ~ 30 °Brix, due to membrane fouling.

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 from a variety of materials, including foods, at low temperatures.

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 is expected to happen because the process is slower than spray drying, which will allow lactose crystals to form. 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.
Proposed work

We propose to 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 – which is already less energy intensive than thermal dehydration.

This combination process 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.

This project is building on work that was already completed on the nonthermal concentration of milk, and the proposed objectives will only focus on the Vacuum Microwave Drying process. The following research objectives are proposed:

Objective 1 (year 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 2 (year 1): Evaluate the quality (physico-chemical, microbiological), functionality and storage behavior of the powders.

Objective 3 (year 2): Evaluate the use of the combination process for other dairy powders. Proposed additional dairy fluids to be tested: whole milk, milk protein concentrate of different protein concentrations.

Objective 4 (year 2): Estimate the energy consumption for the combination nonthermal concentration & drying process (under optimal conditions) and compare it with the energy consumption for traditional powder manufacturing (thermal concentration & spray drying).

Experimental methods

Approach. We will first evaluate the use of REV drying for the dehydration of skim milk of initial concentrations from 30°Brix to 50°Brix, at different initial product temperatures, vacuum levels and drying times (Obj. 1). As part of Obj. 2, we will evaluate the quality (physico-chemical, microbiological), functionality and storage behavior of the powders. All processing and analytical testing will be conducted in triplicate, and the data analyzed statistically. In Year 2 of the project, we will evaluate the use of the combination process for other dairy products (whole milk, milk protein concentrate) (Obj. 3), and then will evaluate the energy consumption and product quality for the combination processes that lead to the highest quality powders (Obj. 4).

All processing runs and analyses will be conducted in triplicate and data analyzed statistically.

Materials. Skim and whole pasteurized milk (Cornell Dairy) will be used as raw materials. Milk protein concentrates for Obj. 3 will be obtained by UF in our Pilot Plant.

Nonthermal concentration. FO concentration will be conducted with 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 concentrates** will be conducted by ultrafiltration, using a rig with a ceramic membrane available to the PI.

**Vacuum Microwave Drying (VMD)** will be conducted using a 10 kW Medium-Scale REV unit (Enwave Corp.) in the Pilot Pkant 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).

The Lab 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) 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.

Energy calculations will be conducted following a methodology currently used in PI Moraru’s laboratory. 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 specific (thermal) 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.

References cited


Project 8: Request from NYS Milk Promotion Board for Jan. 1, 2019 – Dec. 31, 2019: $103,367
Project 9: A novel approach for the manufacture of cheese-like products from nonthermally concentrated milk, without whey generation

PI: Carmen I. Moraru

DURATION: January 1, 2019 – December 30, 2019 (year 1 of 1)

Summary of Proposed Project

The consumers’ demand for food products rich in protein is at an all-time high both in the US and worldwide. Milk proteins and in general dairy products are very popular, since they have a very good image, a clean taste, and known health benefits. Consumers are also more open than ever to new types of foods, ideally products that are minimally processed, have high nutritional value, flavor, and texture, and have a minimal environmental impact, which could complement traditional sources of dairy proteins such as yogurt and cheese. Some of these characteristics can be obtained using nonthermal technologies, such as membrane filtration and high pressure processing (HPP), which are increasingly replacing traditional processing methods.

We are proposing to process skim milk solids using a combination of forward osmosis, as a nonthermal concentration method, and high pressure processing, as a structure formation method, to create high quality cheese-like foods, in a feasible and sustainable way. The pressure treatment used will also serve as a cold pasteurization step of the final product, with food safety and shelf life extension benefits. We will evaluate the effect of processing conditions (pressure and holding time) and product composition (skim milk concentration, pH) on the structure and texture of the nonthermally processed novel cheese-type product. We will also evaluate the effect of rennet addition on the properties (texture, appearance) and stability of the final products (texture, proteolysis). The microbiological composition of the samples will be evaluated prior and after the HPP treatment, in all cases.

The advantages of the proposed approach compared to “traditional” processing methods include: 1) the creation of new textures in a quick, reproducible manner; 2) high quality and nutritional value of the final products, due to limited use of heating; 3) final products with “built-in safety” and long shelf life, due to microbial inactivation by the HPP step and zero risk of post-process product contamination; 4) full utilization of the skim milk concentrate, with no whey removal. The proposed approach is realistic for today’s dairy industry, because the accessibility of nonthermal processing, particularly HPP, has increased, primarily due to the advent of toll processing.

Background

Consumers are still “hungry for proteins”

The consumers’ demand for food products rich in protein is still on the rise, and it is at an all-time high both in the US and worldwide (Mintel, 2013). This trend is a result of the proteins’ healthy image, and the incidence of sarcopenia (muscle loss with aging) among
older adults (Paddon-Jones et al., 2008). Among all classes of proteins, milk proteins are very popular, since they have a very good image with consumers, a clean taste, and have been associated with many health benefits (Saito, 2008). Besides traditional sources of dairy proteins such as yogurt and cheese, consumers are more open than ever to new types of foods, ideally products that are minimally processed, have high nutritional value, flavor, and texture, and have a minimal environmental impact (Pasha et al., 2014; Hartman Group, 2016).

Some of these characteristics can be obtained using nonthermal technologies, such as membrane filtration and high pressure processing (HPP), which are increasingly replacing traditional processing methods because they allow foods to better retain their freshness, color, flavor, and nutrients. While the main utilization of nonthermal technologies has been so far microbial inactivation, for food safety and shelf life extension, some of these technologies can also achieve new and unique functionalities and product characteristics.

Opportunities for using HPP to create new dairy products

Many popular dairy products such as yogurt or cheese are based on the gelling properties of milk proteins. Traditionally, milk protein gelation is achieved either by fermentation, enzymatic coagulation (i.e. renneting in cheese making), or a combination of these two processes. Both of these methods require long times (from tens of minutes for renneting to several hours for fermentation). In recent years, several research groups, including ours, have shown that HPP can be used to induce gel formation of milk proteins, at high enough protein concentrations and pressures (Cadesky et al., 2017; Devi et al., 2013; Bravo et al., 2013). For example, work conducted in our research group, with funds from an USDA grant, showed that HPP of 10% micellar casein concentrate (MCC) and milk protein concentrate (MPC) at 450 MPa for 15 min resulted in weak protein gels (Cadesky et al., 2017). While this was an important step, the gels created at the native pH (around 6.6) have limited applicability for actual products, because this pH facilitates microbial growth. To lower the pH, when we used direct acidification with glucono-δ-lactone (GDL), and observed that at pH below 5.5 the structure becomes too weak (Fig. 1), probably because of the dissolution of calcium from the casein micelles.

To correct for this, we added calcium chloride (typical practice in cheese making), which allowed us to obtain strong, cheese-like samples (Fig. 2).

As a note, the samples shown in Fig. 1 and Fig. 2 were processed at 600 MPa and 4°C, for 3 min, which is a typical HPP treatment used in the food industry for nonthermal pasteurization.
As an alternative to this approach, we conducted one run where we used acid whey from Greek yogurt (GAW) as an acidulant, since GAW has high acidity and a high Calcium content. The results were remarkable, and we obtained a very firm product, with color and appearance resembling soft style Latin cheeses (Fig. 3).

To further increase the strength and stability of these samples, rennet can also be added. It has been shown that HPP treatment can lead to firmer gels (de Castro Leite Júnior et al., 2016). Our own preliminary work showed that the addition of rennet prior to HPP treatment leads to products similar to soft style cheeses.

**Opportunity for using all milk solids for making HPP cheese like products**

Although the results presented above are very promising, the gels shown in Fig. 1 and Fig. 2 were obtained by reconstituting milk protein powders, which is not the most practical and economical way of making such products. Ideally, it would be best if: a) we could use all milk solids instead of protein concentrates as a starting material; b) the entire process is nonthermal, to maximize the freshness and retention of sensory and nutritional properties of the final products.

Milk solids are abundantly available on the current market, and the dairy industry is trying to find new utilizations for skim milk powder and skim milk solids. Therefore, there is an opportunity to apply the approach described above to make novel, cheese-like products from skim milk rather than milk concentrates. However, as discussed above, these gel structures require a high concentration of milk proteins. This can be achieved by the concentration of skim milk. The traditional method used for milk concentration is thermal evaporation. While thermally evaporated milk is used sometimes as an ingredient in cheese making, to standardize the protein content of cheese milk, it cannot be used as the sole ingredient because the thermal evaporation process leads to cooked taste, loss of freshness, and has a negative effect on the functionality of milk proteins. Additionally, thermal evaporation is highly energy-intensive (Petrotos and Lazarides, 2001).

The most common alternative to thermal evaporation is reverse osmosis (RO). In an earlier study, Barbano and Bynum (1984) used RO to reduce the volume of heat treated,
standardized, whole milk by 0, 5, 10, 15, and 20% prior to Cheddar cheese manufacture. The RO cheeses had proximate compositions comparable to control cheeses, and the water removal from whole milk resulted in increased productivity and cheese yield. The highest milk solids achieved in that study was 15.05%. In general, RO can only achieve a solids level in milk of ~ 20° Brix, due to concentration polarization and membrane fouling by the high pressures (Rastogi, 2016). This is below the solids level that will allow the formation of a cheese-like product, without further removal of whey. HPP makes most sense to be used on products that have been formulated at the final composition and are pre-packaged, and thus a whey removal step is undesirable.

For the past two years our group, with funding from the National Dairy Council, showed that we can use Forward Osmosis (FO), a membrane process based on the difference in concentration between milk and a draw solution, to obtain concentrated milks with ~ 40°Brix (compared to ~ 20° Brix for RO). These concentrates were obtained at cold temperatures (4-15°C), and they perfectly preserves the color, sensory attributes, and functionality of milk proteins.

**Proposed work**

We are proposing to process skim milk solids using a combination of FO (as a nonthermal concentration method) and HPP (as a quick, nonthermal structure formation method) to create safe, high quality cheese-like foods, in a feasible and sustainable way. The pressure treatment used will also serve as a cold pasteurization step of the final product, and will help reduce the microbial content of the product, with food safety and shelf life extension benefits. The initial milk will still receive the legal minimal pasteurization treatment.

A preliminary trial conducted by our group by HPP treatment of FO concentrated milk showed that gels with the color and appearance of fresh dairy products and no cooked flavor can be obtained. The pH of these products can be lowered by adding an acidulant (i.e. GDL or acid whey from Greek yogurt), and the texture can be controlled by adding calcium chloride (or acid whey from Greek yogurt).

**Specific objectives** are:

**Obj. 1:** Evaluate the effect of processing conditions (pressure and holding time) and product composition (skim milk concentration, pH) on the structure and texture of the nonthermally processed novel cheese-type product.

**Obj. 2:** Evaluate the effect of rennet addition on the properties (texture, appearance) and stability of the final products (texture, proteolysis).

The microbiological load of the samples will also be evaluated, prior and after the HPP treatment, in all cases.

**Experimental methods**

**Concentrated milk** will be obtained by forward osmosis (FO) of skim milk (from Cornell Dairy), at cold temperatures, using an FO unit (Ederna, France) available in our laboratory, and experimental protocols established by our research group. Based on our preliminary data, we plan to use skim milk concentrates of about 40 °Brix, but if needed we
can increase this concentration to higher levels using a combination of RO and FO (also based on work done in our group).

**Acidification** with glucono-delta-lactone (GDL) or Greek yogurt acid whey (GAW) will be used to further promote gelling and extend product shelf-life. GDL will be added at levels established experimentally.

**Renneting.** A commercial rennet preparation will be used, and renneting will be conducted according to the manufacturer instructions and our own methodology, described by Cadesky et al (2017).

**High pressure processing (HPP).** The packaged pre-acidified, pre-renneted product will be HPP treated. We plan to conduct most HPP treatments at 600 MPa for 3 min, at refrigeration temperature, in a 55 L Hiperbaric HPP unit, using standard practices developed in our laboratory. We will also explore lower pressures in case the rennet is inactivated under the HPP conditions mentioned above.

**Chemical, physico-chemical and microbiological evaluation.** After processing, all samples will be evaluated for physico-chemical and microbiological properties.

Chemical composition (% total protein, % fat, % minerals, % lactose, % moisture) will be determined at the Dairy One Laboratory (Ithaca, NY), using the methodology described by Beliciu et al (2012). Proteolysis during refrigerated shelf life will be also evaluated for select samples, using the methodology described by Griep et al. (2018).

Physico-chemical characteristics (pH, titratable acidity, color) and mechanical properties (texture) will be evaluated using methodologies routinely used in our research lab. The textural properties will be determined with a TAXT2 texture analyzer and color attributes with a chroma meter (CR-400, Konica Minolta). The whiteness index (WI) of the samples will be calculated as: \( WI = 3.388 \times Z - 3 \times Y \).

Microbiological evaluation will consist in determining total aerobic counts (Tandon et al., 2003), and for the final (optimal) processing conditions yeast and mold counts as well (Splittstoesser and Churey, 1989).

**Structure evaluation** by Scanning electron microscopy (SEM) will be conducted to obtain a qualitative evaluation of sample structure. 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 CO\(_2\), coated with evaporated carbon, and imaged using a Zeiss LEO 1550 SEM.

**Replication and statistical analysis of data.** All experiments will be performed in triplicate, and data analyzed with appropriate statistical methods.

**References cited**


*Funding is requested for 50% of a graduate student (the other 50% is covered from an USDA grant), 25% of a technician, supplies, and fees (for pilot plant usage, maintenance, external analyses)*

Project 10: UHT Shelf life – The application of lactose oxidase to control *Pseudomonas* and improve UHT milk.

Project PI’s: Samuel Alcaine, Cornell University

Duration: January 1st, 2019 – December 31st, 2020 (year 1 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, does 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:

UHT milk represents nearly 26.3% of global liquid milk consumption, with a current growth of 5%. Not only does UHT milk represent an export opportunity for NY dairy producers, but single-serve UHT milk drinks also represent a convenient product for US families on the go. These products are expected to last a year, but heat-stable proteolytic enzymes produced by psychrotropic bacteria, can result in production gelation and significantly shorten the shelf life (Stoeckel et. al, 2016).

*Pseudomonas* spp. have been shown to be able to produce heat stable proteolytic enzymes (Law et. al, 1977) and are known to be common contaminants in raw milk (De Jonghe et. al, 2011). In fact, *Pseudomonas* levels have been shown to grow several orders of magnitude under the storage and transportation conditions used for raw milk (De Jonghe et. al, 2011). Currently, raw milk microbiological testing is done when storage tanks are filled, but the levels are not further monitored during storage and if they were, there is little room for intervention prior to pasteurization leaving processors at the mercy of tank and tankers logistics to minimize time for growth.

Lactose oxidase is GRAS enzyme (Ahmad et al., 2004), 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. Our previous work has shown that lactose oxidase is quite effective against *Pseudomonas* at refrigeration temperatures. The enzyme comes in a liquid form, so it could be easily added to either a raw milk silo or tanker truck, and because the milk will subsequently see a heat step, downstream activity of the lactose oxidase is eliminated. We believe this enzyme could be used to develop a novel, easy-to-implement method to improve raw milk microbiological quality prior to pasteurization, and reduce downstream defects associated with microbial growth during raw milk storage. This research represents an opportunity to prove that such a scheme can enhance UHT milk shelf life and quality, thus improving the marketability of US dairy product for both export and local markets.

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. Heat-treated and non-heat treated aliquots of these overnight cultures will be spotted onto D-BHI milk media and monitored for zones 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 with 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 evaluated for differences in taste and appearance by the trained milk sensory panel.

References


Project 10, Year 1: Request from NYS Milk Promotion Board for the period January 1st, 2019 – December 31st, 2019 - $73,855.

Project 11: Process development for conversion of skim milk concentrate and powder into extruded products.

Project PI: Sy Rizvi, Cornell University

Duration: January 1st, 2019 – December 31st, 2021 (year 1 of 2)
Summary:

The proposed work will build a new route for direct conversion of skim milk concentrate 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, called supercritical fluid extrusion or SCFX, involves injection of high-pressure carbon dioxide to produce expanded, microcellular extrudates at low temperatures to minimize any detrimental effects on product quality and offers an economically attractive opportunity to create a new platform for utilization of skim milk via a new generation of products. **Second**, non-fat dry milk (NFDM) contains over 50% lactose by weight, which creates a big problem 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 consumption conducive. **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 also be conducted to establish the maximum limit on utilization of NFDM in formulations containing milk protein concentrate, starch and rice flours to make consumer acceptable products. By creatively combining the unique capabilities of novel technologies, we will help create both new food products and manufacturing processes that are more economical and industrially attractive. Developing futuristic products and product categories, all of which will be designed with the goal of helping to address major utilization issues related to milk, would require the melding of advances in new food processing technologies, consumer needs and sustainability. Success of this work will not only promote value-added utilization of skim milk but it will also provide a new example of a commercially viable product based on extrusion technology, which has not been traditionally used by the dairy industry. In it new form, high pressure carbon dioxide based extrusion technology is very amenable to making products from heat sensitive ingredients like milk solids. We have shared our preliminary results of the proposed work with industrial partners and received enthusiastic support towards promoting such products in markets. This would provide skim milk-based extruded products the much-needed traction that it needs and would demonstrate a route to making nutritious, clean, expanded products that would be attractive to today’s discriminating consumers. Through our product, we hope to serve both the goals of enhancing the use of NFDM in current markets through novel and unique products as well as creating a value chain using sustainable and eco-friendly technology.

Background:

Traditionally, over 75% of U.S. nonfat dry milk and skim milk powder is exported without any value addition. However, the recent changes in trading policies of the European Union and Canada have exacerbated the issues and created hefty government stockpiles of skim milk powder. On the other hand, 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(8). 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\(^{(12)}\). 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. In addition, the category is positioned well for future growth, propelled by a strong demand to convert fun-for-you snacks into better-for-you snack and cereals. However, milk proteins are conspicuous by their absence in this segment due to the lack of a technology to preserve their quality and nutritional value when puffed to achieve the desirable texture that consumers desire. This offers a unique opportunity to introduce 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\(^{\circ}\)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 \((4-9,11)\) 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 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 process can be used to generate porous extrudates for use as snack foods, breakfast cereals, etc. 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. This what this proposal is designed to address, based on the following specific objectives.

**Preliminary Result**

Preliminary results have been obtained to demonstrate that NFDM mixed with a starch or protein matrix are amenable to expanded, porous extrudates of unique architecturally different morphology with expansion characteristics similar to steam-based extrudate but at lower temperatures by using supercritical carbon dioxide based extrusion, which helps preserve the original color and other heat sensitive qualities. The interior of the expanded extrudate is porous with very smooth skin, ideal for surface coating. The color of the NFDM and milk protein concentrate-containing product is well preserved through SCFX when compared with steam-expanded counterpart, Fig. 1.

**Objectives**
(a) Establish the kinetics of lactose hydrolysis in skim milk concentrate using enzyme beta-galactosidase
(b) Determine the effects of adding lactose hydrolyzed skim milk concentrate and extrusion processing parameters on formation of galacto-oligosaccharides (GOS) in extrudates.
(c) Determine the textural attributes of extrudates made with skim milk concentrate and skim milk powder containing formulations.
(d) Compare the physico-chemical attributes of extruded products with selected commercial counterparts.

Experimental Approach:
(a) Hydrolysis of lactose in skim milk concentrate using beta-galactosidase

The following methodology outlines a protocol we have established for the hydrolysis of lactose in whey into glucose and galactose utilizing the enzyme beta-galactosidase isolated from Aspergillus oryzae. This enzyme source was used due to its stability at the low pH of whey. This protocol will be tested and, if necessary, modified for skim milk concentrate to use an enzyme with optimum efficiency at the pH of milk such as beta-galactosidase from Kluyveromyces lactis (14). Lactose, glucose, and galactose will be quantified via nuclear magnetic resonance (NMR).

Skim milk of the desired concentration factor (20-30% solids) will be analyzed via NMR to quantify the initial lactose, glucose, and galactose content. Trimethylsilylpropanoic acid (TMSP) will be dissolved in deuterium oxide and used as the standard. A Bruker AV500 NMR Spectrometer with an H1 quantification method will be used to obtain the spectra, which will be integrated via MNova software. The total lactose content will be calculated from the integrated areas.

The total grams of beta-galactosidase needed for the reaction will be calculated based on 600 U of enzyme activity/µmol of lactose. This enzyme concentration is based on the food grade beta-galactosidase Lactase 17MDP (Biocatalysts Ltd., United Kingdom) that yielded an average of 97.3% lactose hydrolysis (96.3% for 12°Bx and 88.7% for 18°Bx whey) over 12 hours in 6°Bx whey during previous studies in our lab. The lactase will be added into the desired quantity of skim milk concentrate and incubated for hydrolysis. The enzyme concentration may be adjusted to achieve the desired lactose hydrolysis in short time interval.

The lactose, glucose, and galactose content in the hydrolyzed samples will be quantified using the same method as described for the untreated skim milk concentrate. Based on data from batch system, attempt will also be made to develop a continuous lactose hydrolysis system to economize on the use of enzyme.

(b) Determination of galacto-oligosaccharides (GOS) in extrudates

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. Polymerization 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.
GOS Determination: A 0.2 M, pH 6.0 phosphate buffer will be prepared from potassium dihydrogen phosphate (KH2PO4) and dipotassium hydrogen phosphate (K2HPO4). GOS will be extracted from pulverized extrudate using hot phosphate buffer (80°C). The pH will be adjusted to 1 M NaOH or 1 M HCl. The GOS in the sample extract can then be quantified using the following methods:

- **HPLC:** The carbohydrate composition of the sample extracts will be determined via HPLC. A Bio-Rad Aminex HPX-87P carbohydrate column (300 mm x 7.8 mm) will be used in conjunction with a refractive index detector (RID). The mobile phase will be Milli-Q filtered and degassed water with a flow rate of 0.6 mL/min and a column temperature of 85°C. The standards used will be inulin, stachyose, raffinose, glucose, fructose, xylose, arabinose, and inositol (10).

- **NMR:** The extrudate sample extract will be fractionated on a Bio-Gel P-2 column. 5 mL fractions will be collected and the molecular weights analyzed via matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). The fractions that contain samples with a single degree of polymerization will be separated and analyzed via NMR. Trimethylsilylpropanoic acid (TMSP) dissolved in deuterium oxide will be the standard used. This analysis will occur on a Bruker AV500 NMR Spectrometer with an H1 quantification method at 295K. This method has the potential to both quantify total GOS and also the degree of polymerization of GOS components (15).

(c) **Determination of extrudate quality attributes**

A factorial design will be used to set up experiments with various levels of milk proteins and additives in the formulations and the operating parameters of the extruder will be optimized. The first parameter will be pressure, which will be varied between 1000, and 2000 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.

- **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 an Instron Universal Testing Machine. 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.

- **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.

- **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.

(d) **Comparison of physico-chemical attributes of extruded products with selected commercial counterparts**

Physico-chemical and sensory attributes of selected commercial samples will be also determined as indicated above and compared with NFDM-based samples for quality characteristics and consumer acceptability. The following quality parameters of the extruded samples will be quantified and results will be used to optimize the manufacture process and product quality.

- **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.

- **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 an Instron Universal Testing Machine. 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.

- **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 cellular 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.

- **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.

**Benefits to Dairy Industry**

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 NFDM offers both an opportunity and 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 inducing
production of galacto-oligosaccharides (GOS) during extrusion to enrich the extruded product with a soluble dietary fiber would go a long way to open up new and novel 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 NFDM-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 products via expanded, cellular foods like R-T-E cereals and snacks that can be effectively marketed and help increase consumption of milk.

References

**Project 11:** Budget request from NYS Milk Promotion Board, $98,530. (Year one of two)

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**Project 12:** – Development of a rapid method to determine raw milk protein and fat quality. As high milk protein beverage and other high milk protein products increase in commercial importance, the impact of variation in protein degradation my present challenges in the manufacture of high quality high protein products.

**Project PI's:** Dave Barbano, Cornell University, Ithaca, NY

**Duration:** January 1st, 2019 – December 31st, 2019 (year 1 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 fingerprint 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 fingerprint 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 industry. 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


Verdi, R.J. and D.M. Barbano. 1991. Effect of coagulants, somatic cell enzymes, and extracellular


Project 12: Budget request from NYS Milk Promotion Board, $121,498. (Year one of two)

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Previous Year Projects 2017 - 2018

Continuing Projects

Project 1 - $111,363 (Abbaspourrad) - Astringency reduction of proteins
Project 2 - $81,734 (Sam Alcane) – Lactose oxidase to reduce spoilage
Project 3 - $139,717 (Moraru) - Distribution of spores and vegetative bacteria
Project 4 - $51,625 (Barbano) continuing project (center technology transfer – on going)

Total Direct Costs $325,795 for 2017-2018 (Projects 1, 2, 3 and 4)
Total Indirect Costs (18%) $58,643 for 2017-2018 (Projects 1, 2, 3, and 4)
Total Continuing Projects $384,438 for 2017-2018 (Projects 1, 2, 3, and 4)

Proposed New Projects

Project 5 - $102,513 (Abbaspourrad) Conversion of Lactose to GOS
Project 6 - $127,588 (Rizvi) Functionalization of Whey Proteins
Project 7 - $76,110 (Alcaine) Evaluation of ElastSens System
Project 8 - $78,470 (Alcaine) Upcycling dairy by products
Project 9 - $ (not funded) (Stafford/Barbano) Undergraduate Leadership Development
Project 10 - $119,080 (Barbano/Drake/Grant) Feed related oxidized off flavors in milk

Total Direct Costs $426,915 for 2017-2018 (Projects 5 thru 8 and 10)
Total Indirect Costs (18%) $76,845 for 2017-2018 (Projects 5 thru 8 and 10)
Total New Projects $503,760 for 2017-2018 (Projects 5 thru 8 and 10)

Total Budget for the Northeast Dairy Foods Research Center for the period
Oct. 1, 2017 to December 31, 2018 = $888,198
Proposed Projects for 2019.

Proposed Continuing Projects

Project 1 - $ 89,444 (Abbaspourrad) - Conversion of lactose to GOS
Project 2 - $ 102,070 (Rizvi) – Functionalization of Whey protein
Project 3 - $ 65,005 (Alcaine) – Elasto-sense measurement of texture
Project 4 - $ 64,434 (Alcaine) – Upcycling of acid whey
Project 5 - $ 101,864 (Barbano/Drake/Grant) – Sources of autoxidized off flavor in milk
Project 6 - $ 51,625 (Barbano) continuing project (center technology transfer – on going)

Total Direct Costs $ 403,764 for 2019 (Projects 1, 2, 3, 4, 5, and 6)
Total Indirect Costs (18%) $ 72,678 for 2019 (Projects 1, 2, 3, 4, 5, and 6)
Total Continuing Projects $ 476,442 for 2019 (Projects 1, 2, 3, 4, 5 and 6)

Proposed New Projects

Project 7 - $ 80,240 (Abbaspourrad) Nutritious Spreads and Fillings using Milk Ingredient
Project 8 - $ 103,367 (Moraru) Vacuum Microwave treatments.
Project 9 - $ 67,398 (Moraru) Production of cheese like products
Project 10 - $ 73,855 (Alcaine) Improving UHT shelf-life
Project 11 - $ 98,530 (Rizvi) skim milk concentrates and milk powder extrusion.
Project 12 - $ 121,498 (Barbano) rapid method to determine raw milk protein and fat quality

Total Direct Costs $ 461,769 for 2019 (Projects 7 thru 12)
Total Indirect Costs (18%) $ 83,118 for 2019 (Projects 7 thru 12)
Total New Projects $ 544,887 for 2019 (Projects 7 thru 12)

Total Budget Request for the Northeast Dairy Foods Research Center for the period January 1, 2019 to December 31, 2019 = $ 1,021,328.
### Continuing Projects

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#### Total Direct Costs

- **F & A (18%)**: $13644 15570 9916 10134 15539 7875 $72,678
- **Budget Totals (2019) - continuing projects**: $89,444 102,070 65,005 66,434 101,864 51,625 $476,442

Page 59
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<tr>
<td>Business Services</td>
<td>0</td>
<td>3000</td>
<td>3000</td>
<td>0</td>
<td>0</td>
<td>6000</td>
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<tr>
<td>Services from individuals</td>
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<tr>
<td>Interdepartmental Services (proteomics, SEM)</td>
<td>0</td>
<td>1000</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Photocopying (within University)</td>
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<td>0</td>
<td>1000</td>
<td>0</td>
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<td>Publications</td>
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<td>1700</td>
<td>0</td>
<td>0</td>
<td>1800</td>
<td>5200</td>
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<td>Food Supplies</td>
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<td>0</td>
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<td>0</td>
<td>2000</td>
<td>2000</td>
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<tr>
<td>Laboratory Supplies</td>
<td>9000</td>
<td>10000</td>
<td>6000</td>
<td>6000</td>
<td>5000</td>
<td>4000</td>
<td>400000</td>
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<tr>
<td>Office Supplies</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Other (Sensory Testing &amp; Pilot Plant)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9000</td>
<td>0</td>
<td>0</td>
<td>9000</td>
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<tr>
<td>subcontract</td>
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<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Total Other Than Personnel Service</td>
<td>9000</td>
<td>26700</td>
<td>19300</td>
<td>15000</td>
<td>9500</td>
<td>9300</td>
<td>$88,800</td>
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<tr>
<td>Total Direct Costs</td>
<td>12240</td>
<td>15768</td>
<td>10281</td>
<td>11266</td>
<td>15030</td>
<td>18534</td>
<td>$83,118</td>
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### Budget Totals (2019) - New Projects

<table>
<thead>
<tr>
<th>Project 7</th>
<th>Project 8</th>
<th>Project 9</th>
<th>Project 10</th>
<th>Project 11</th>
<th>Project 12</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Budget Totals (2019) - New Projects</td>
<td>$80,240</td>
<td>$103,367</td>
<td>$67,398</td>
<td>$73,855</td>
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<td>$121,498</td>
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### Northeast Dairy Center (2019) - total budget request

<table>
<thead>
<tr>
<th>Project 7</th>
<th>Project 8</th>
<th>Project 9</th>
<th>Project 10</th>
<th>Project 11</th>
<th>Project 12</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northeast Dairy Center (2019) - total budget request</td>
<td>$169,684</td>
<td>$205,437</td>
<td>$132,403</td>
<td>$140,289</td>
<td>$200,394</td>
<td>$173,123</td>
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