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The Influence of Protein Concentration and Homogenization on Moisture Content, Curd Yield, and Fat Retention of Model Cheese Made from Microfiltered Skim Milk Recombined with Cream

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THE INFLUENCE OF PROTEIN CONCENTRATION AND HOMOGENIZATION ON MOISTURE CONTENT, CURD YIELD, AND FAT RETENTION OF MODEL CHEESE MADE FROM MICROFILTERED SKIM MILK RECOMBINED WITH CREAM

by

Richard Byron Geslison

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of

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ABSTRACT

Influence of Protein Concentration and Homogenization on Moisture Content, Curd Yield, and Fat Retention of Model Cheese made from Microfiltered Skim Milk Recombined with Cream

by

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Utah State University, 2020

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In the US, cheese production efficiency is commonly improved through the use of UF (ultrafiltration), which is a pressure-driven membrane separation technique to concentrate caseins– the key component of cheese – to about 3.5% in fluid milk. Microfiltration (MF) concentration is an attractive alternative to UF because of the potential to collect milk serum proteins (whey protein) from milk rather than from cheese run off. This research investigated the use of highly concentrated MF retentates with 3 different casein concentrations to make cheese curds and the resulting effects on curd moisture, curd yield, and fat retention.

Micellar casein concentrate (MF concentrated skim milk) was mixed with cream and UF permeate to obtain recombined concentrated milks (RCM) of 3.5%, 7%, and 10.5% casein with casein to fat (C/F) ratios of 0.60-0.75. These RCMs were then used in a cheese making model specifically modified for this research. Glucono-δ-lactone (GDL) and rennet proportional to the casein protein level of the RCM were added to provide

acidification and coagulation activity, respectively. After cut, curds were agitated using the inverting motion of a tube rotator, and the curds were heated up to 40°C. Whey was drained and collected followed by more agitation. Final whey separation was accomplished by centrifuging of the RCM samples at 250 g for 30 min. Homogenization of 7% casein RCM samples using a microfluidizer prior to cheese making was also investigated to determine if fat retention and curd yield could be improved without increasing curd moisture.

Increasing RCM concentration to 10.5% casein increased ($P < 0.05$) fat retention to 84.3% compared to 64.4% and 62.0% for RCM with 3.5% and 7.0% casein, respectively. RCM of 10.5% casein also had higher ($P < 0.05$) relative dry curd yields, 9.5% versus 8.8% and 7.4% respective to 7.0% and 3.5% casein. Lower moisture was also achieved ($P < 0.05$) with increased RCM concentration, with 44.6% for 10.5% RCM compared to 47.9% and 48.5% for 7.0% and 3.5% casein, respectively.

Homogenization of RCM increased ($P < 0.001$) fat retention from 66.2% at control to 95.0% at 0.41 MPa and increased ($P < 0.01$) curd yield from 18.8% at control to 23.2% at 0.41 MPa gauge pressure (GP). Moisture was lowered from 48.0% to 45.0% ($P < 0.01$) from control to 0.41 MPa GP RCM. Homogenization, therefore, has the potential to improve the cheese making performance of RCM without adversely increasing curd moisture levels. Our cheese model was manageable by a single person and could be implemented with minimal upgrades in a dairy lab. We obtained curd moisture levels similar to standard pre-press cheddar curds.

(103 pages)

PUBLIC ABSTRACT

Influence of Protein Concentration and Homogenization on Moisture Content, Curd Yield, and Fat Retention of Model Cheese made from Microfiltered Skim Milk Recombined with Cream

Richard Geslison

This project was funded by the Western Dairy Center at Utah State University as part of a multi-pronged approach to improve the current understanding of using concentrated milks in cheese making. Concentrated milk for this study was provided by South Dakota State University.

This study compared the effect of different concentration factors of milk on curd moisture levels, fat content, and cheese curd yields. To see if these results could be improved (i.e. remove more moisture and retain more fat) milk samples were also subjected to limited pressure homogenization (microfluidization) treatments.

It was found in the course of this study that limited homogenization treatment of concentrated milks before cheese making did indeed cause curds to retain more fat and less moisture. Also, the amount of cheese curds made was increased due to increased fat retention, thus providing another potential benefit to implementing this practice in the cheese industry. Our method of cheese making required very little additional equipment beyond what is normally available in dairy laboratories. Additionally, it was performed by a single person, which further simplifies using this method in future research projects.

Dedicated to my wife and daughter, whose cheery fascination with life bring out the very best in me

ACKNOWLEDGMENTS

This thesis would not have been possible without the patience, expertise, and wisdom of Dr. Donald J. McMahon whose tutelage was unceasing, patience was indefatigable, and insights very invaluable. Ever kind and thoughtful, he calmly guided me through every stop and barrier in this project. I would also like to thank the other members of my master's committee, Dr. Marie Walsh and Dr. Almut Volmer, for all the work they have done to improve this research and aid in my progression. I would like to thank Dr. Xin Dai for her assistance in running my data through SAS as well as her advice on trouble shooting on my attempts at using SAS myself. I am appreciative to all the members of the Utah State University Creamery: Dave Irish, David Campbell, Dan Combe, and Megan Armstrong, who took additional time beyond their regular duties to support my research. I am very grateful for the financial support obtained through the Western Dairy Center and Dairy West via the Build Dairy program, and to all those who administered it including, but not limited to, Dr. Donald J. McMahon, Dr. Eric Bastian, Kim Rasmussen, and Tara B. Johnson. And last, but certainly not least, I would like to thank my dear wife who has kindly but firmly kept me on track to complete this work.

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LIST OF ABBREVIATIONS

ANOVA = Analysis of Variance

CA= Casein

C/F = Casein to Fat ratio

FDB = Fat on Dry Basis, fat as a percent of all total non-water composites as measured by weight

GDL = glucono-δ-lactone

GP= Gauge Pressure

HC-MCC = Highly-Concentrated Micellar Casein Concentrate

IR = Infrared

LAB = Lactic Acid Bacteria

MCC = Micellar Casein Concentrate

MF = Microfiltration

NF = Nanofiltration

RCM = Recombined Concentrated Milk

RO = Reverse Osmosis

UF = Ultrafiltration

INTRODUCTION

Cheese making dates back in Europe at least as far as 5,000 BC (Gillis et al., 2017). Clay pots with straining holes and residual dairy protein show the early ingenuity used to separate cheese curds and whey. Cheese production has advanced much as stainless steel vats and automated production facilities have replaced those clay pots. Other changes to cheese making now include the importance of whey collection and purification. Previously considered a waste product, whey proteins are now an important food ingredient. Cheese and whey production now go hand in hand. After curd formation the runoff whey is collected and used (Damodaran et al., 2008; Patel 2015). Multiple filtration and drying steps are employed to produce a concentrated protein powder, which can then be sold (Henning et al., 2006). However, filtering out and collecting whey proteins from milk before the cheese is made would prevent cheese process contaminants from entering the whey production stream. Microfiltration (**MF**), filtrating with membranes with pore sizes from 10^{1} to 10^{-1} µm, is able to do this (Brandsma et al., 1999; Nelson et al., 2005; Holland et al., 2011).

Ultrafiltration (**UF**), a related process with membranes between 10^{-1} to 10^{-2} µm, has been in use in the cheese industry for several decades to remove excess water and lactose from the milk prior to cheese making (Henning et al., 2006). The ultimate goal of this is to increase the amount of cheese made in a cheese vat. Each cheese vat has a set volume. However, increasing the protein in that vat by concentrating the milk will yield more cheese per vat. Said another way, throughput is increased. Theoretically, MF provides these same benefits but with the additional benefit of removing milk serum (whey) protein before coloring, enzyme treatments, salting, and microbial treatments are

employed, all common in cheese making. This is advantageous as removing these substances, i.e., by chemical bleaching to remove colors, could damage the protein powder product (Qiu et al., 2015).

Meaningful changes to processes and flow of cheese is required to incorporate a MF process. First, to be effective, MF treatment requires that skim milk is used. Next, cream can be added back into the milk at a controlled rate before or during vat filling. In many facilities, this would only require minimal upgrades as separating and cream addition equipment are already common in the cheese industry.

In order to maximize serum (whey) protein collection, MF retentate needs to be concentrated to 4X, and can then be further concentrated using evaporation to a thick product referred to as highly-concentrated micellar casein concentrate (**HC-MCC**) (Lu et al., 2015). This can be mixed with cream, forming recombined concentrated milk (**RCM**) from which cheese can be made. However, without modifying the cheese making procedures, HC-MCC coagulates differently and loses an excessive amount of fat (Lu et al., 2016) which is detrimental to the texture, functionality, and legal definition of the final cheese. To realize the benefits MF has for whey production, MF cheese production issues need to be addressed to encourage investment, development, and use by cheese manufacturers.

The purpose of this study was to assess the effects of MF concentration levels on cheese curd fat retention, moisture retention, protein retention, and curd yield. We also investigated if these values would improve with the application of limited homogenization via microfluidization of the RCMs prior to cheese making giving direction in addressing cheese-making issues from using MF concentrates.

HYPOTHESIS AND OBJECTIVES

Hypothesis of this study:

1. Higher concentrations of caseins in recombined cream and MF concentrated milk lead to greater water retention of cheese curds resulting in complications to attain proper cheddar moisture levels leading to a need for process modification
2. Greater fat losses will result from higher concentrations of recombined HC-MCC with cream leading to the need for limited homogenization to attain acceptable fat retention

Objectives of this study are:

1. Develop a laboratory cheese making model system of renneted HC-MCC recombined with cream to simulate moisture and fat losses by whey expulsion from curds both before and after whey is drained
2. Determine the effect of HC-MCC concentration level on curd moisture content from renneted HC-MCC recombined with cream
3. Determine the effect of HC-MCC concentration level on fat in whey, i.e. fat loss from curd formed from renneted HC-MCC recombined with cream
4. Determine the effect of HC-MCC concentration level on protein in whey, i.e. protein loss from curd formed from renneted HC-MCC recombined with cream
5. Determine if modifications to the cheese making procedures (i.e. homogenization of the HC-MCC) will prevent fat losses to whey

LITERATURE REVIEW

Overview

In the United States, cheddar cheese production is often streamlined by concentrating milk via UF (Govindasamy-Lucey et al., 2004; Lu et al., 2017). Cheese vats are fixed in volume, but if the milk is concentrated, the manufacturer may in effect fill each vat with a greater amount, at least in terms of milk solids and fat. To put another way, throughput is increased. Furthermore, much of cheese making consists of steps to remove moisture (whey draining, temperature control, cheddaring, salting, pressing, etc.), and membrane concentration provides cheese manufacturers with an opportunity to remove some moisture before cheese making begins. When optimized, the overall effect can present a great improvement to process and production efficiency.

However, certain problems in the cheese making may arise when milk concentrations are too high with inferior texture, increased cheese losses, increased whey protein retention, and flavor changes having been reported (Creamer et al., 1987; Bech, 1993; Karlsson et al., 2007). Currently, UF technology is employed in the United States cheese industry, but limited to only lower concentration factors of up to about 1.5X or approximately 3.9% casein, assuming 2.6% casein in standard milk. Using higher concentrations has been shown to result in increasing problems with losses in cheese yield (Henning et al., 2006). Because of the potential boost in processing efficiency interest remains strong for the use of highly concentrated milk provided the above mentioned problems can be solved.

Microfiltration has gained attention as it provides possible benefits to the cheese industry either used with UF or in place of UF to concentrate milk (Schreier et al., 2010,

Lu et al., 2015, Eshpari et al., 2015). The advantage of MF lies in its ability to remove milk serum proteins (whey protein) from milk prior to cheese making. The benefits are two-fold: first, collecting serum proteins from milk rather than from whey prevents contamination with cheese run-off components, such as hydrolyzed peptides that can impart bitter flavors on the final product, enzymes (such as coagulants, proteases, and lipases), cheese inclusions, salt, and colors (Nelson et al., 2005). Second, less whey protein ends up in the cheese vat (Neocleous et al., 2002a) preventing excess curd retention and aged flavor retardation effects (Creamer et al., 1987; Neocleous et al., 2002b).

An important observation is that milk that was highly concentrated by MF had different physical attributes when compared to standard, unfiltered milk. For example, it only become liquid at 40°C or higher, it coagulated at different rennet levels, and formed a much firmer curd requiring upgraded tooling to process (Schreier et al., 2010; Lu et al., 2016; Lu et al., 2017). Cheeses made with milk that was highly concentrated by MF was characterized by having slower or delayed proteolysis leading to altered functional properties (Ardisson-Korat and Rizvi, 2004). In addition, the buffering capacity in milks concentrated by either UF or MF has been shown to be increased which further complicates acidification and cheese development (Mistry and Kosikowski 1986; Ardisson-Korat and Rizvi, 200; Bulbul, 2018,).

Cheeses of acceptable characteristics have been made using MF in milks concentrated up to 1.6X or 4.15% casein (Neocleous et al., 2002a, 2002b) which is similar to current industrial practices with UF. However, if the benefits to the cheese and whey manufacturing processes are to be fully realized, milk concentration needs to be

pushed further into the medium (3.5 to 15% casein) and, if possible, high concentration factor ranges (above 15% casein).

Cheese can be described as the casein protein matrix enclosing fat, water, and other dissolved/suspended molecules (such as minerals, peptides, acids, and residual lactose). Each of these composites have an impact on cheese flavor and functionality (Barden et al., 2015). Thus, understanding cheese curd retention and loss of these composites can be an important step in addressing issues that arise with using more highly concentrated milk.

Rennet Coagulation

Rennet-induced coagulation of milk is attributed to the behavior and form of milk casein proteins (Damodaran et al., 2008). Caseins form irregular, spheroidal structures commonly referred to as casein micelles with a median size of 100 to 200 nm. These proteins interact with minerals forming a lattice network (McMahon and Oommen, 2008, Figure 1). Hydrophobic interactions, calcium bridging, hydrogen bonding, and other electrostatic/entropic interactions stabilize this network leading to curd formation.

The casein micelles stay suspended in the water phase of the milk due to the hydrophilic surface on protein segments of the κ-caseins. Hydrolysis of the outside κ-caseins leads to coagulation. Rennet, or other coagulation enzymes, cleave off these hydrophilic κ-caseins leaving hydrophobic sections exposed on the surface of the micelles. Hydrophobic micelle sections begin to associate together and form clusters. This leads to cross-linked protein matrices that capture fat, moisture, minerals, and lactose inside. As moisture is removed and heat applied to this matrix (called a coagulum), it hardens and contracts, becoming curds and eventually cheese.

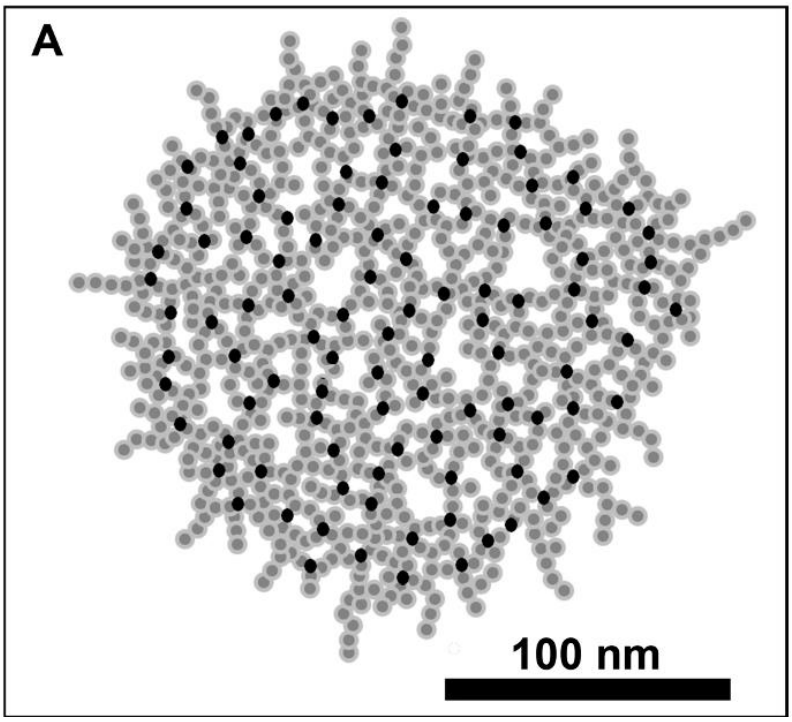

Figure 1. Cross-sectional schematic of the casein micelle with caseins in grey and calcium phosphate nanoclusters in black illustrating the assumed form of caseins in milk pre-renneting. From McMahon and Oommen, 2008.

Cheese Identity

Milk composition and cheese processing parameters play critical rolls in the cheese industry. Many cheeses have a standard of identity that specify allowable processes and end cheese parameters that must be met in order to sell the final product under a legally protected name. United States law dictates that "Cheddar cheese is the food prepared … which produces a finished cheese… [the] minimum milkfat content is 50 percent by weight of the solids, and the maximum moisture content is 39 percent by weight" (FDA, 2018). The law further outlines acceptable practices such as cutting, stirring, heating, draining, matting, other cuttings, stacking, further draining, washing, salting, pressing into forms, and/or by any other procedures which yield a cheese mass of

acceptable qualities (FDA, 2018). In order for a cheese maker to sell their cheese as cheddar, the cheese must retain enough milk fat and expel enough moisture to meet the above-mentioned criteria and may use any process or practices allowed.

The legalities of cheese naming as it relates to allowable process and final cheese composition is not the only concern cheese makers have with their products. Nájera et al., (2003) noted how many of the common treatments (temperature, pH, $CaCl_2$, and coagulation enzymes) of milk affect key cheese forming properties. As these properties change, so too does the final cheese product, which may cause the cheese to not perform as a consumer has come to expect from a flavor, texture, or functional standpoint. The cheese industry therefore must not only pay attention to applicable laws that govern their processes, but also how those processes might affect product acceptance by their customers.

Effects of Milk Concentration

Milk concentration via filtration (UF and MF) has many complicating effects on cheese making including shortening rennet-induced coagulation times (Holland et al., 2011; Lu et al., 2017) and increasing curd stiffness/brittleness (Holland et al., 2011; Sandra et al., 2011; Lu et al., 2017). Further complications in retarded flavor development and inferior texture have been noted in other research (Bech 1993; Ardisson-Korat and Rizvi, 2004; Karlsson et al., 2007) It has been shown that cheeses with acceptable flavors and functionalities can be made using filtration-concentrated milk (Neocleaous et al., 2002a; Govindasamy-Lucey et al., 2007, 2011). It has to be noted, however, that such cheeses were made with lower range concentrations (<7% casein), the range in which UF is currently being used in industry (Henning et al., 2006).

As the concentration increases, there are fundamental changes to the way the milk coagulates to form cheese curd. Gelation of the milk happens at a lower level of casein micelle hydrolysis than in standard whole milk and the hydrolysis rate of κ-casein slows as well (Karlsson et al., 2007; Gaygadzhiev et al., 2009). Electron micrographs of milk coagulum from concentrated milk gels showed far more caseins clumping and thicker interlinking branch chains in a relatively smaller area (Lu et al., 2016, 2017) when compared to caseins in gels formed from unconcentrated milk. Lu et al. (2017) even found that aggregation of casein micelles began before the addition of a coagulation agent (rennet) in MF concentrated recombined milks, perhaps contributing to some of these differences.

All of these issues will affect the final cheese product from a flavor, texture, functional, or legal standpoint. To make cheese of acceptable characteristics from higher concentrated milks (>7% casein) will require process and equipment modifications. These modifications will need to be gentler on the curds than what is currently in practice and will need to introduce new steps and treatments to correct the issues resulting from concentration (Orme, 1998; Brandsma and Rizvi, 2001; Lu et al., 2017).

Filtration Technologies: Ultrafiltration and Microfiltration

While filtration technology encompasses a larger selection of membranes and functions, for the purposes of this research, we will only consider UF and MF systems. The primary difference between UF and MF systems is the overall size of the pores in their membranes which affect what is retained and what is permitted to pass through (Figure 2).

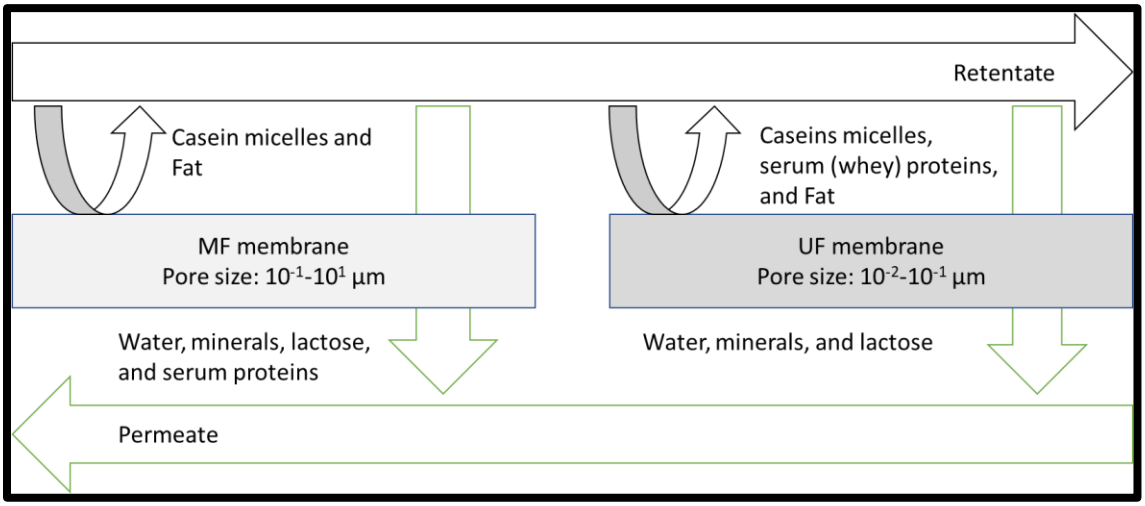

Figure 2. Ultrafiltration (UF) and microfiltration (MF) comparison of retained and permeated milk components. The top flow arrow represents the flow of the milk over the membrane becoming the retentate, or that which is retained by the membrane. The bottom flow arrow is the flow of materials that have successfully passed through the membrane to become permeate.

As it relates to milk and cheese making, MF membranes have a pore size that allow for the separation of milk serum proteins from the caseins (Brandsma et al., 1999; Nelson et al., 2005; Holland et al., 2011). This is facilitated by the nature of these proteins in milk; serum proteins do not form large size structure with one another as opposed to the caseins, which form the very large (in comparison) previously mentioned casein micelles (McMahon and Oommen, 2008). This gives MF concentration a potential advantage over UF concentration in the cheese industry. It provides an opportunity to not only remove excess water and lactose from cheese milk prior to cheese making but to also remove serum proteins (whey proteins) from milk rather than from cheese runoff.

This can be very beneficial to food manufacturers as whey proteins are subjected to proteases (such as rennet), heat treatments (cooking), and salting as part of the cheese

making process. Whey is also contaminated with any ingredient added to cheese, such as food inclusions (peppers for pepper jacks), colors (Annatto), bacteria (cheese cultures and others), enzymes (rennet, bacterial, and even some added for flavor), etc. Normally, each contaminate would need to be removed from or inactivated in the whey to prevent degrading the whey protein product. By removing the serum/whey protein prior to cheese making however, all of these potentially damaging treatments and contaminates are avoided in one preparatory step.

Microfluidizer and Homogenization

Microfluidizers achieve particle size reduction in emulsions through different means than the more conventional two-stage valve homogenizers. Product is fed from the inlet into an intensifier pump that increases the pressure and forces the product into an interaction chamber (Olson et al., 2004, Anonymous, 2016, 2019,). A stream or streams of product enter the interaction chamber and is accelerated to very high velocities. Product particles then collide, either with each other or with the interaction chamber itself (Anonymous, 2016). The impact and shear of these interactions breaks down particle size in the product emulsion.

Microfluidizers are typically operated at much greater pressures than valve homogenizers (Olson et al., 2004; Anonymous, 2016, 2019). These two processes may yield similar results in terms of particle size reductions in emulsions, but they achieve these through different mechanical actions. As such, operational pressure parameter differences between valve homogenization and microfluidization can be misleading, as pressure alone does not fully describe what happens to an emulsion run through these two processes.

Recombined Concentrated Milk

To prevent fat fouling on MF membranes, separation of casein from whey proteins should be performed with skim milk (Brandsma et al., 1999; Nelson et al., 2005) rather than with whole milk. Cream needs to be reintroduced as part of the post-concentration standardization step, and UF permeates of milk should be used as a diluting agent rather than water so as to prevent a decrease in soluble mineral and lactose content (Lu et al., 2015, 2016, 2017).

Lu et al., (2015, 2016, 2017) performed a large amount of work with preparing and using HC-MCC to make RCM. Using HC-MCC with very high levels of casein (~18% or about 5X concentrated), Lu et al., (2016) demonstrated that HC-MCC remains in a gel state until warmed to 50°C with gentle agitation. The HC-MCC could then be mixed with cream and other diluents to attain the desired casein concentration and casein to fat (**C/F**)ratios (an important indicator in standardizing cheese milk).

Ultrafiltration permeate is an optimal diluting agent when preparing RCM (Nelson et al., 2005) from MF concentrates as it reintroduces lactose, calcium, and other trace minerals. Calcium is a known contributor to cheese coagulation and texture (Nájera et al., 2003) and lactose is essential in lactic acid formation. Using water as a diluting agent in RCM would dilute calcium and lactose affecting cheese texture and limiting the development of lactic acid.

Small Lab Scale Model for Cheese Making

Laboratory and small-scale cheese making models are diverse and plentiful in literature. These models are used to test cheese-making modifications without the cost in resources, time, and risk entailed in a full-scale trial. Milk volumes range from a few

milliliters to several thousand liters and cheese-making vessels can comprise beakers, flasks, and purpose-built micro-vats with customized tools (Bachmann et al., 2009; Cipolat-Gotet et al., 2013).

Cipolat-Gotet et al. (2013) made use of specialized stainless steel 1.5-L micro-vats in water baths to make scaled-down, open-vat cheese. Temperature was controlled by keeping these vats partially submerged in water baths and whey was drained by removing the curds and placing them into a mold suspended over the vat. The matting curds were turned periodically to allow whey to drip. This was followed by pressing and brining to complete their model, yielding cheeses that are similar enough in process and treatments to full-scale cheese to be adequate stand-ins for research purposes.

The Bachmann et al. (2009) model focused on the ability to manufacture many samples simultaneously; hundreds of tests could be run at once in a microplate with each cell serving as a single treatment of cheese. Custom tooling was devised to stir and cut the curds while incubators and a climate stove with humidity controls were used for temperature treatments. Due to the small size of each sample, a centrifuge was used in place of more traditional pressing to remove whey.

When selecting or designing a cheese model, a researcher must keep needs and available resources in mind, especially when customized tools may have no other use other than in the specific model. Concentrated RCM gels are brittle (Brandsma and Rizvi, 1999; Lu et al., 2017) and require handling that is gentle in addition to temperature control, allowing for whey drainage, and controllable stirring.

Brown et al. (2012) made cheese in conical centrifuge tubes with glucono-δ-lactone (**GDL**) and a 30-min holding time to acidify the forming gel. The curds were cut

with a lab spatula in a repeatable fashion inside the vials and centrifuging was used for pressing. No customized tooling was needed in the Brown model; centrifuge, conical centrifuge tubes, lab spatulas, incubators, and cheese making agents like GDL are common in many labs and can fill multiple needs beyond a single cheese model. This keeps the cost of running and maintaining the Brown cheese model low, all attractive aspects for the purposes of this research.

For this research, we selected a model that is a slight modification to the one used by Brown et al. (2012). We run the entire modified model with the equipment and supplies we had on hand. We used GDL as a standard for acid development in our cheese. This eliminated the complicating effects milk concentration has on cheese culture activity, an issue beyond the scope of this research. Using 50-mL centrifuge tubes provided an elegant solution to assure adequate temperature control as the small size of the tubes facilitates quick heat exchange in both, a water bath and in an incubator. In addition, we were able to conduct the entire cheese making model in the same conical tube; there was no need to transfer curds from container to container. The primary modification to the model used by Brown et al. (2012) was the use of a tilt rotator located inside an incubator to provide agitation and heat. The spin disk of the tilt rotator was set vertical (the disk being perpendicular to the floor) and each vial was clipped to the face of the disk. The effect was that when activated, each turn of the disk caused a gentle end-over-end inverting stir motion for each vial. Everard et al. (2008) noted how stirring could affect curd fines and fat losses, and if stirring agitation is too harsh, fines and fat losses are inevitable. Our method of inverting each tube lacks any sort of aggressive mechanical handling of the curd and represents an ideal scenario of curd handling.

MATERIALS AND METHODS

Recombined Concentrated Milk Preparation

The HC-MCC, 17.0% casein, 1.4% whey protein, 0.7% fat (Lu et al., 2017) was manufactured at the Institute for Dairy Ingredient Processing at South Dakota State University (Brookings, SD) and was shipped and stored frozen at -29°C. A sufficient amount of HC-MCC for testing was transferred to storage at 4°C until malleable. Sections of partially thawed HC-MCC were placed in a covered glass beaker and then melted in a water bath set to 50°C as shown by Lu et al. (2015) to allow complete melting and solubilization of the HC-MCC gel.

Portions of HC-MCC were mixed with UF permeate (obtained from the creamery at Utah State University) to give 600-mL skim milk aliquots containing approximately 3.5, 7.0, and 10.5% casein. Sufficient amounts of cream (obtained from the university creamery or purchased at a local retail store) were added to each skim milk aliquot to produce RCM with a target casein-to-fat (C/F) ratio of 0.60 to 0.70. Each RCM was mixed for 10 min at 32°C and then sampled for fat and protein measurement.

Model Curd Manufacturing

To 600-mL aliquots of RCM (at 32°C) was then added glucono-δ-lactone (GDL) in proportion to the casein content of the RCM (8 g for 3.5% casein, 16 g for 7% casein, 24 g for 10.5% casein) and the RCM mixed to start acidification in a similar fashion as shown in Brown et al. (2012). After mixing for 2 to 3 min, chymosin (Chy-Max 2X, Chris Hansen, Milwaukee, WI) was added in proportion to protein concentration (i.e., 300 µL for 3.5% casein, 600 µl for 7% casein, and 900 µl for 10.5% casein) and stirred by hand for about 30 s. Next, 45 mL of renneted RCM was poured out into each of

twelve 50-mL screw-cap centrifuge tubes (Thermo Fisher Scientific, Rochester, NY) and placed in a water bath at 32°C for 30 min to coagulate. The coagulum in each tube was cut using a small stainless steel spatula by making three parallel cuts across the curd and then three cuts perpendicular to the first three (making sure the spatula extended to the bottom of the tubes on each cut).

After cutting, the tubes were capped tightly, dried with paper towels, and then were clipped to the face of the turning plate of an adjustable tilt rotator (Roto-Torque, Model 47874, Cole Parmer, Vernon Hills, Il.) that was located inside a large incubator set at 50°C. The rotator's tilt was angled at 90° from horizontal with the face of the turning plate perpendicular to the floor, and the rotation speed was initially set at ~12 rpm (speed setting "low 3"). After 15 min, the speed was then increased to ~24 rpm (speed setting of "high 3"). At 30 min, the curd and whey temperature reached 40°C (simulating a typical 30-min cook and stir step typical in cheddar cheese making) and the incubator temperature was lowered to 40°C to maintain this temperature.

After another 30 min of stirring, the tubes were opened, and the whey was decanted. The tubes were recapped, the curds kept inside, and the rotation started again at ~24 rpm and 40°C (simulating continued stirring of the curd during and after whey draining to allow further whey expulsion). After another 30 min, whey was again decanted and the re-capped tubes placed in a centrifuge at 250 g for 30 min at ambient temperature (~22°C). Any further expelled whey was decanted one final time, then the curds were removed from the tubes (Figures 3 and 4).

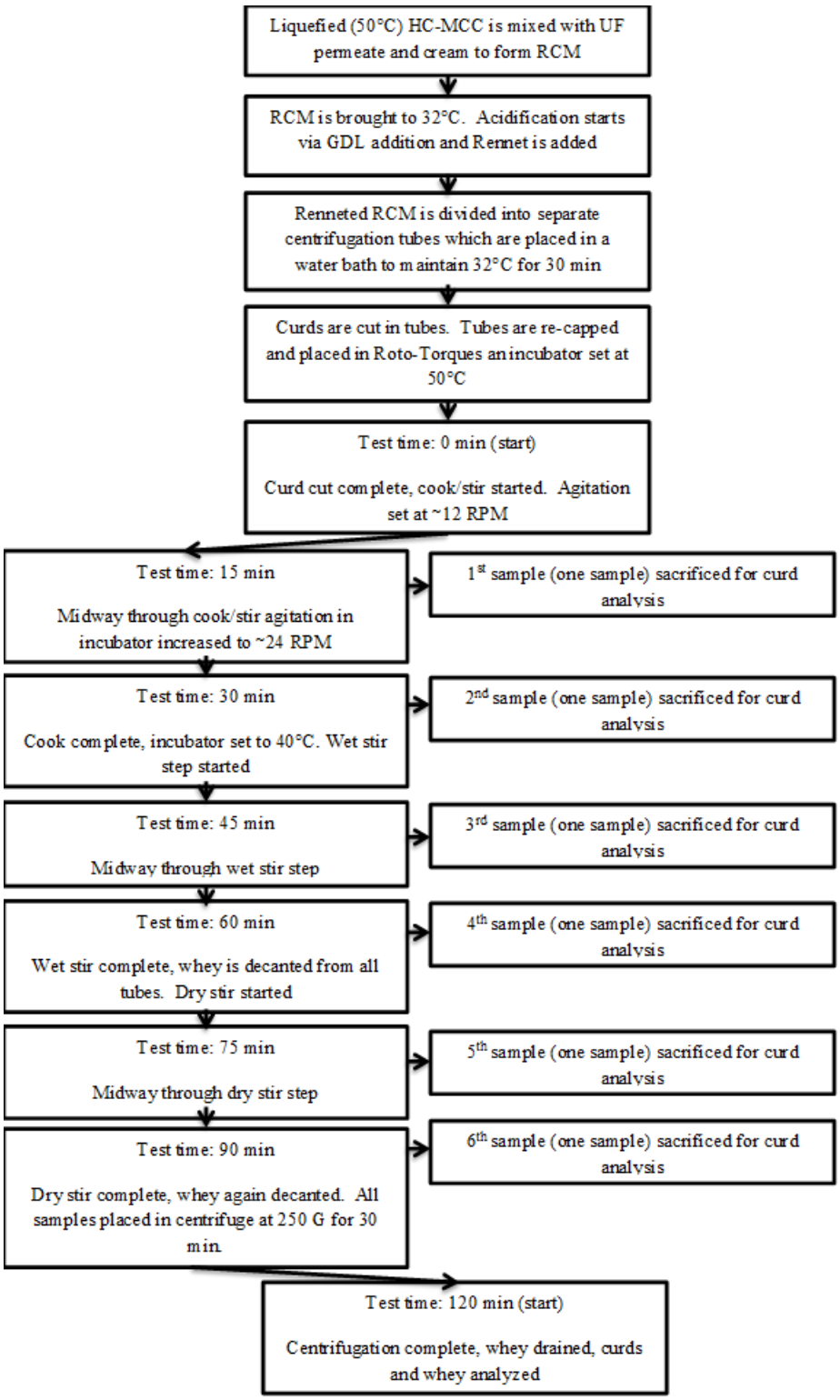

Figure 3. Outline of cheese model and sample collection using recombined concentrated milk (RCM) made from highly-concentrated micellar casein concentrate (HC-MCC).

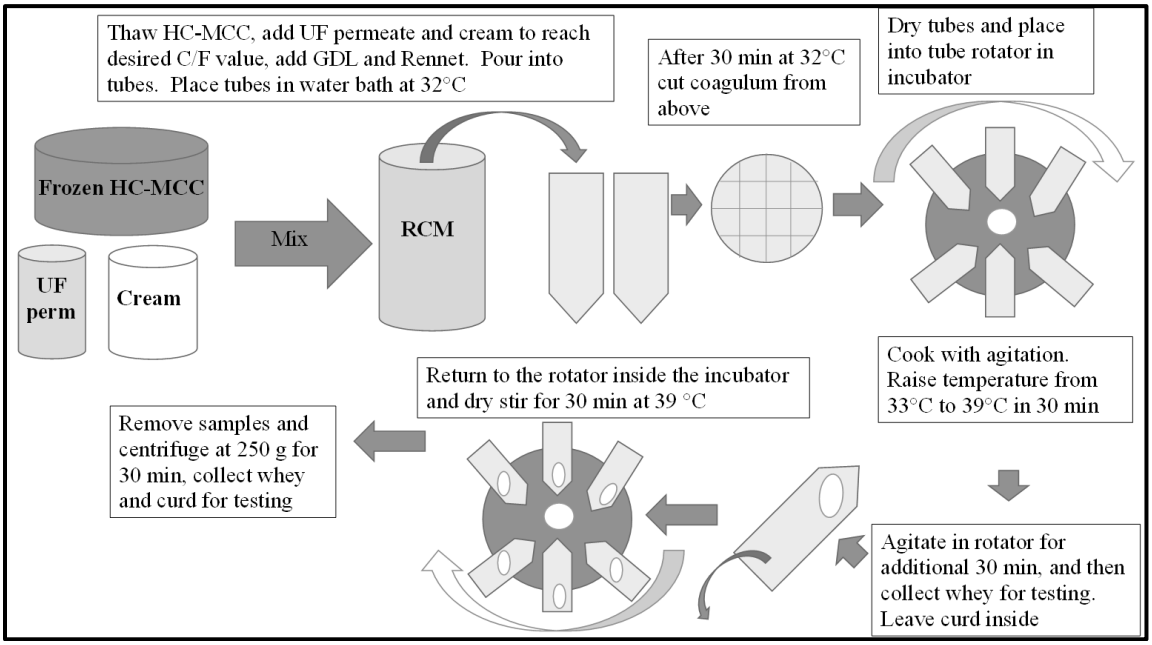

Figure 4. Graphical outline of cheese model using recombined concentrated milk (RCM) made from highly-concentrated micellar casein concentrate (HC-MCC).

Curd Moisture and Whey Expulsion during Stirring

The 45-mL aliquots of RCM curd in centrifuge tubes were sampled periodically starting 15 min into the cooking/agitating step of the cheese model, and then every 15 min until centrifuging (Figure 3). After centrifuging, all remaining aliquots were sampled. Samples were collected by first removing the tubes from the tilt rotator or centrifuge, the whey was decanted, and then the curds were removed from the centrifuge tubes with a lab spatula.

Samples of curd were also obtained during the manufacture of a standard cheddar cheese made in the university creamery from non-concentrated milk (see Appendix P). The sample points included milk, curd after cutting, curd before cooking, curd after cooking, curd before draining, curd after draining, curd during cheddaring, curd before milling, curd after salting, and lastly, cheese after pressing.

Low Pressure Homogenization of RCM

Recombined skim milk containing 7% casein was homogenized. Three 1-L aliquots of RCM (at 32°C) were passed through a Microfluidizer 110S (Microfluidics Corp. Newton, MA) with three different gauge pressure (**GP**) treatments: 0.14, 0.41, and 0.69 MPa GP (20, 60, and 100 psi GP, respectively). According to the equipment manufacturer's manual, the total pressure treatment on the RCM was 32.1 MPa (4.6×10^3 psi), 96.4 MPa (1.4×10^4 psi), and 160 MPa (2.3×10^4 psi), respectively. For ease of reporting, treatments will be referenced as 0.14, 0.41, and 0.69 MPa GP, respectively. The pressure-treated RCM aliquots were sampled and then used to make laboratory model cheese as previously described.

For comparison, whole milk 2X UF retentate (approximately 7% casein, referred to as 7% casein UF, obtained from the university creamery) was pressure-treated with the university creamery's two-stage valve homogenizer. Samples of 7% casein UF retentate were collected from the homogenizer after the following treatments: control (collected prior to homogenization), 1.72 MPa (250 psi), 3.45 MPa (500 psi), 5.17 MPa (750 psi), 6.89 MPa (1000 psi), 10.3 MPa (1500 psi), and 17.2 MPa (2500 psi).

Fat, Protein, and Moisture Analysis

Gross composition of milk, RCM, and whey was determined by Fourier-transform infrared spectroscopy using a Bentley Instruments Dairy Spec FT (Bentley Instruments Inc., Chaska, MN) at Rocky Mountain Dairy Herd Improvement Laboratories (Logan, UT). Moisture content of curd made from RCM was measured using a vacuum oven at 100°C with ≤13 kPa pressure (Nielsen, 2010) for 4 h. About 3 g (+/- 0.05g) of RCM curd samples were accurately weighed and placed into previously weighed flat-bottom

aluminum dry pans with an additional metal dish used as a loose cover over the sample. After 4 h of heating, desiccated air was carefully readmitted to the oven interior, and samples were removed and weighed. Curd and milk moisture from the standard university cheddar cheese make was measured using a force-air drying oven at 100°C (Bulbul, 2018). Samples were prepared in triplicate by weighing out sample onto previously weighted flat-bottom aluminum dry pans. After 18 h of heating, samples were removed and weighed.

Fat Droplet Particle Size

Oil droplet size distributions were determined in 7% casein RCM and 7% casein UF milk samples using Beckman Coulter particle characterization equipment (LS20 Version 3.19, Beckman Coulter Inc., Brea, California). Oil droplet distributions were reported as volume percent of total oil droplets respective to droplet diameter, i.e. the volume/surface mean, also called the Sauter mean (Tippetts et al., 2012; Lee, 2018).

Calculation of Values: Yield and Retention

Wet curd yield was obtained by comparing the weight of the RCM curds after centrifuging and whey decanting to the initial weight of the RCM aliquots. Dry curd yield was calculated by finding the moisture content of the RCM curd via the vacuum oven test. The moisture values found were used to determine how much of the wet curd weight was moisture and how much was dry matter with the dry matter faction of the wet curd yield becoming the dry curd yield.

Relative dry curd yield was calculated by dividing the dry curd yield by the concentration factor of each RCM. Concentration factor was calculated by averaging the increase in fat and protein of the RCM compared to that of 3.5% casein.

Fat and protein retention were calculated based upon amount lost in the whey. The percent retained was then the difference in their content in whey compared to the initial RCM aliquots and initial serum RCM content of these factors with drained whey content of these factors.

Experimental Design and Statistical Analysis

For assessing the effects of concentration factor, two trials were performed with each trial consisted of 12 aliquots of renneted RCM. Six samples were sacrificed during whey expulsion (as described above) for moisture monitoring. Six samples were collected after centrifuging. The effects of homogenization were assessed in the same manner.

Linear regression was performed to investigate treatment effects on curd moisture, whey fat, whey protein, curd fat retention, curd protein retention, wet curd yield, dry curd yield, and relative dry curd yield. Significance was declared at $P < 0.05$ using Proc ANOVA and Glimmix procedures on statistical analysis software (SAS version 9.3, SAS Institute Inc., Cary, NC). Post-hoc analyses were performed using Tukey-Kramer adjustments to obtain differences of least square means based on *P*-values ($\alpha = 0.05$).

RESULTS

RCM Composition

Fat and protein content of the RCM was within the expected range based upon the target casein concentration of 3.5, 7.0 and 10.5% (Table 1). Casein content was estimated based on 94% of the protein in HC-MCC being casein, while that in cream was at the typical proportion in milk of about 82% of protein. Estimated casein; fat ratios ranged from 0.58 to 0.71.

Typical fresh cows milk is expected to have about 5% lactose (Damodaran et al., 2008) while lactose content of our RCM was much lower, 2.7-3.5%. This was expected, as lactose can pass through MF membranes (Figure 2). This highlights the ability to reduce lactose content in RCM; additional diafiltration steps in HC-MCC preparation could further reduce the lactose levels if needed.

Table 1. Composition of recombined concentrated milk (RCM) used to make cheese

Target casein Concentration	Rep	Fat	Protein	C/F[1]	Lactose
(%)		(%)	(%)		(%)
3.5	1	5.2	3.8	0.69	2.7
3.5	2	5.0	3.6	0.67	2.9
7	1	10.1	8.1	0.75	3.1
7	2	11.5	7.1	0.58	3.2
10.5	1	15.7	11.9	0.71	3.3
10.5	2	16.7	11.0	0.62	3.5

[1]Casein:Fat ratio, as calculated based on estimating 94% of protein in RCM as being casein

Moisture Loss during Cheese Making

Initial moisture levels in the starting milk/RCM samples were 87.7% for the university cheddar milk, and 87.9%, 77.5%, and 67.9% for 3.5% casein RCM, 7% casein RCM, and 10.5% casein RCM, respectively (Figures 5 and 6). The cheese-making model with the RCM samples was completed within 120 min at which point the average moisture was 48.5%, 47.9%, 44.6% for 3.5% casein, 7% casein, and 10.5% casein, respectively (Table 2). This compares to an average moisture level of the university cheddar curds of 61.7% at time 103 min (whey draining complete), 48.4% at time 149 min (during cheddaring step), and 38.8% (legal cheddar) at time 480 min (after pressing).

Curds from RCM formed from our cheese-making model had an initial faster moisture loss than university cheddar curds. By the end of both processes however, only the university cheddar curds reached sufficiently low moisture to be called cheddar.

Final Moisture. Analysis of the final moisture results found curds from 10.5% casein RCM to be the lowest in moisture at 44.6% and were statistical significant in difference from both 3.5% casein and 7% casein curds, with values of $P < 0.01$ and $P < 0.05$ in respective comparisons (Table 2). Curds from 3.5% casein and 7% casein RCMs were not significant in difference from each other with mean moisture contents of 48.5% and 47.9% respectively.

Final Curd Yields. Each wet curd yield mean was statistically significantly different from each other mean ($P < 0.05$) with the yield increasing with initial RCM casein content (Table 2). Further testing by determining curd yield on dry basis (dry curd yield) resulted in statistically significance differences remaining between all means ($P < 0.05$) for dry curd yield with the same trend of increasing yield with increasing casein

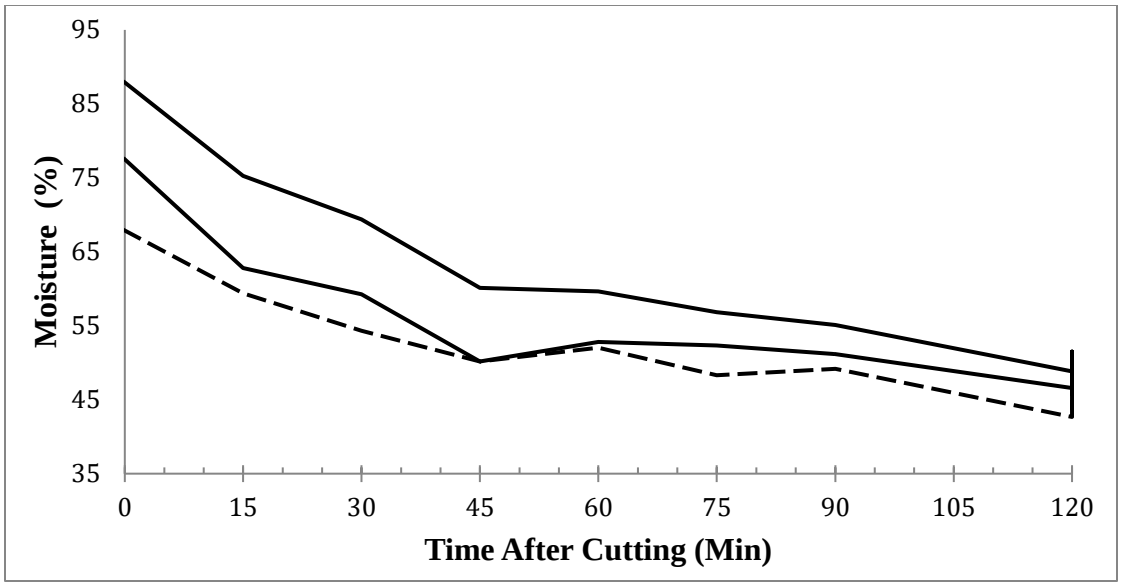

Figure 5. Curd moisture content as a percent (%) of renneted recombined concentrated milk with cream at 3.5% casein concentration (solid line), 7% casein concentration (dotted line), and 10.5% casein concentration (dash line) during acidification using glucono-δ-lactone and a model cheese making process starting at 30°C initial milk (0 min), post cutting cook to 40°C (0 to 30 min), whey draining at 60 min with continued agitation of curd, and then centrifuging at 250 g at 90 to 120 min.

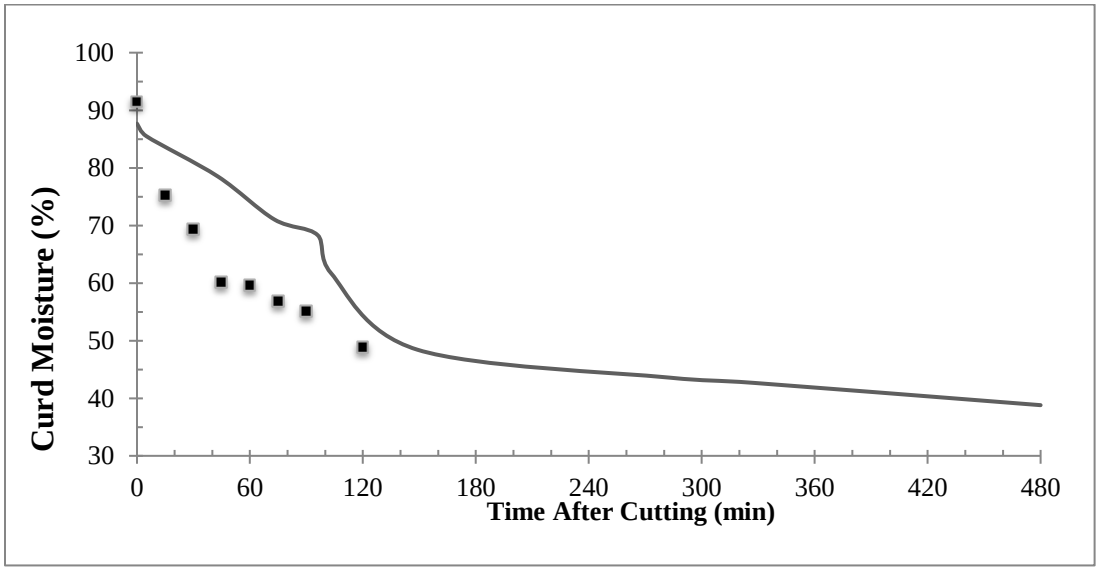

Figure 6. Curd moisture content from (solid line) a typical cheddar cheese make, with milk of 3.8% fat, 3.3% protein, and 5.2% other solids and (■) recombined concentrated milk with 3.5% casein. Curd was sampled 5 min after cutting, before cook starting at 31°C (43 min), after cook up to 39°C (73 min), before whey drain (96 min), after whey drain (103 min), before milling (283 min), after salting (328 min), and after pressing (480 min).

Table 2. Effect of casein concentration in RCM (Recombined Concentrated Milk) used to make model cheese on levels of cheese curd final moisture, whey fat, cheese curd fat retention, whey protein, cheese curd protein retention, and curd yields.

Target casein Concentration	Final Moisture	Wet curd Yield	Dry curd Yield	Relative Dry curd Yield	Whey Fat	Fat Retention	Protein in Whey	Protein Retention
	(%)							
3.5	48.5^{A}	14.4^{A}	7.4^{A}	7.4^{A}	1.9^{B}	62.0^{B}	0.2^{C}	96.0^{A}
7.0	47.9^{A}	36.2^{B}	18.8^{B}	8.8^{B}	4.2^{A}	64.4^{B}	0.6^{B}	92.4^{B}
10.5	44.6^{B}	55.0^{C}	30.5^{C}	9.5^{C}	2.6^{AB}	84.3^{A}	2.0^{A}	82.3^{C}

$^{A-C}$ Means with the same superscript letter within the same column were not significantly different, p=0.05

content. Ong et al. (2013) showed an increase in both yield and dry matter yield correlated with an increase in starting cheese-milk protein achieved through the addition of UF concentrate. These results demonstrate MF concentration has a similar effect.

Effects of Concentration

Correcting dry curd yields by the relative concentration of each sample, called the relative dry curd yield, found statistically significant differences ($P < 0.05$) between each treatment, with increasing concentration correlating with increasing relative dry curd yield (Table 2). Concentration factors in all three observed yield variants (wet curd yield, dry curd yield, and relative dry curd yield) showed a positive correlation between RCM concentration factor (casein content) and increasing curd yield.

Whey Fat and Retained Curd Fat. Comparing mean whey fat values, we found statistically significant differences ($P < 0.02$) only between the 7% casein and 3.5% casein whey samples (Table 2). The 10.5% casein samples were not statistically different

from either the 7% casein or 3.5% casein. Furthermore, the 10.5% casein RCM whey fat value was in-between the 7% casein and 3.5% casein samples, breaking the trend from the 3.5% and 7.0% caseins RCMs of increasing fat in whey with increasing concentration of RCM.

The fat retention of 10.5% casein curds, 84.3%, was statistically significant in differences from the curds of 3.5% casein and 7% casein, 62% and 64.4% respectively (Table 2), with $P < 0.01$ and $P < 0.02$ in respective comparisons. The magnitude of difference was also large with the 10.5% casein RCM retention values being approximately 20-percentage points greater than the other two RCMs, showing a trend of greatly increased fat retention once a higher concentration level (somewhere between 7% and 10% casein) is reached.

Whey Protein and Retained Curd Protein. In our results, there were statistically significant differences ($P < 0.001$) between each of the three concentration factors: 3.5% casein, 7% casein, and 10.5% casein with mean values of 0.15%, 0.61%, and 1.95% protein in respective concentrated RCM effluent whey (Table 2). The increasing protein lose to whey from increasing concentration of RCM is greater than the concentration difference between samples, 7% casein mean protein is about 4 times greater than 3.5% casein and 10.5% casein is about 3 times greater than 7% casein.

Analyzing the retention of protein for 3.5% casein, 7% casein, and 10.5% casein RCM had mean values of 96.0%, 92.4%, and 82.3% respectively. Each concentration factor was statistically significantly different ($P < 0.005$) from each other factor showing a trend of decreasing protein retention with increasing concentration factor (Table 2).

Homogenization

Oil Droplet sizes: Microfluidization and Homogenization of 7% Casein Concentrate.

Effects on the resulting fat droplet size distributions were tested and recorded graphically for MF concentrated 7% casein RCM treated with different pressures in a microfluidizer (Figure 7) and samples of 7% casein UF concentrated treated at different pressures in a two-stage homogenizer (Figure 8). Initially in the MF 7% casein RCM, the fat droplet sizes were multimodal and uneven, peaking, at about 0.6 µm, 2 µm, and 5 µm with large and uneven distributions (Figure 7a). The 0.14 MPa GP pressure treatment yielded a tri-modal distribution, with distinct peaks at 1 µm, 2 µm, and 4 µm (Figure 7b). At the 0.41 MPa GP and treatments, the distributions become bimodal, the central peak and left most peak seen in the previous two treatments having converged into one large peak centered between 1 µm and 2 µm and the diminished right most peak remaining close to 5 µm (Figures 7c and 7d). These peaks, however, remained broad.

The process of concentrating whole milk to 7% casein via UF had a limited homogenizing effect. The distribution of oil droplets prior to homogenization treatment had three distinct peaks at 0.6 µm, 2 µm, and at 5 µm. The application of pressure to 7% casein UF concentrate changed the multi peak nature of the dispersion, becoming 1 peak by 6.89 MPa (1,000 psi), and 1 peak with a very tight distribution centered on 1 µm, with a stretch from about 0.5 µm to 2 µm at 10.3 MPa (1,500 psi). We continued to test up until 17.2 MPa (2500 psi) (Data not shown in text, see Appendix Q) but the results were very similar to the 10.3 MPa results, with a single tight peak centered on 1 µm, with a stretch from about 0.5 µm to 2 µm.

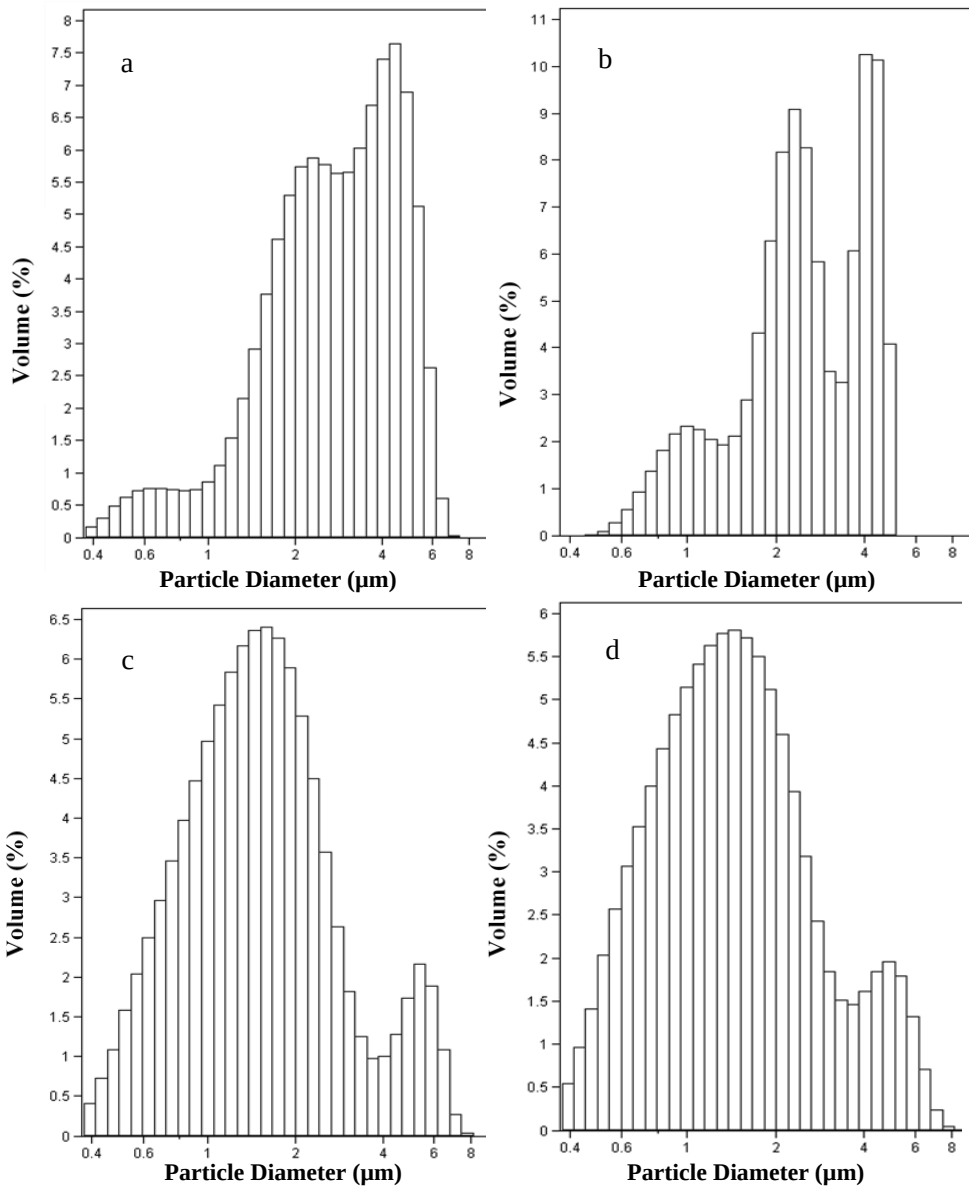

Figure 7. Recombined concentrated milk, from combining micro-filtered milk retentate and cream, concentrated to 7% casein homogenized with a microfluidizer. Resulting fat particle size distributions, in µm as a percentage of total fat volume, when treated with the following gauge pressures: a- control non-homogenized, b- 0.14 MPa (20 psi) gauge pressure, c- 0.41 MPa (60 psi) gauge pressure, d- 0.69 MPa (100 psi) gauge pressure.

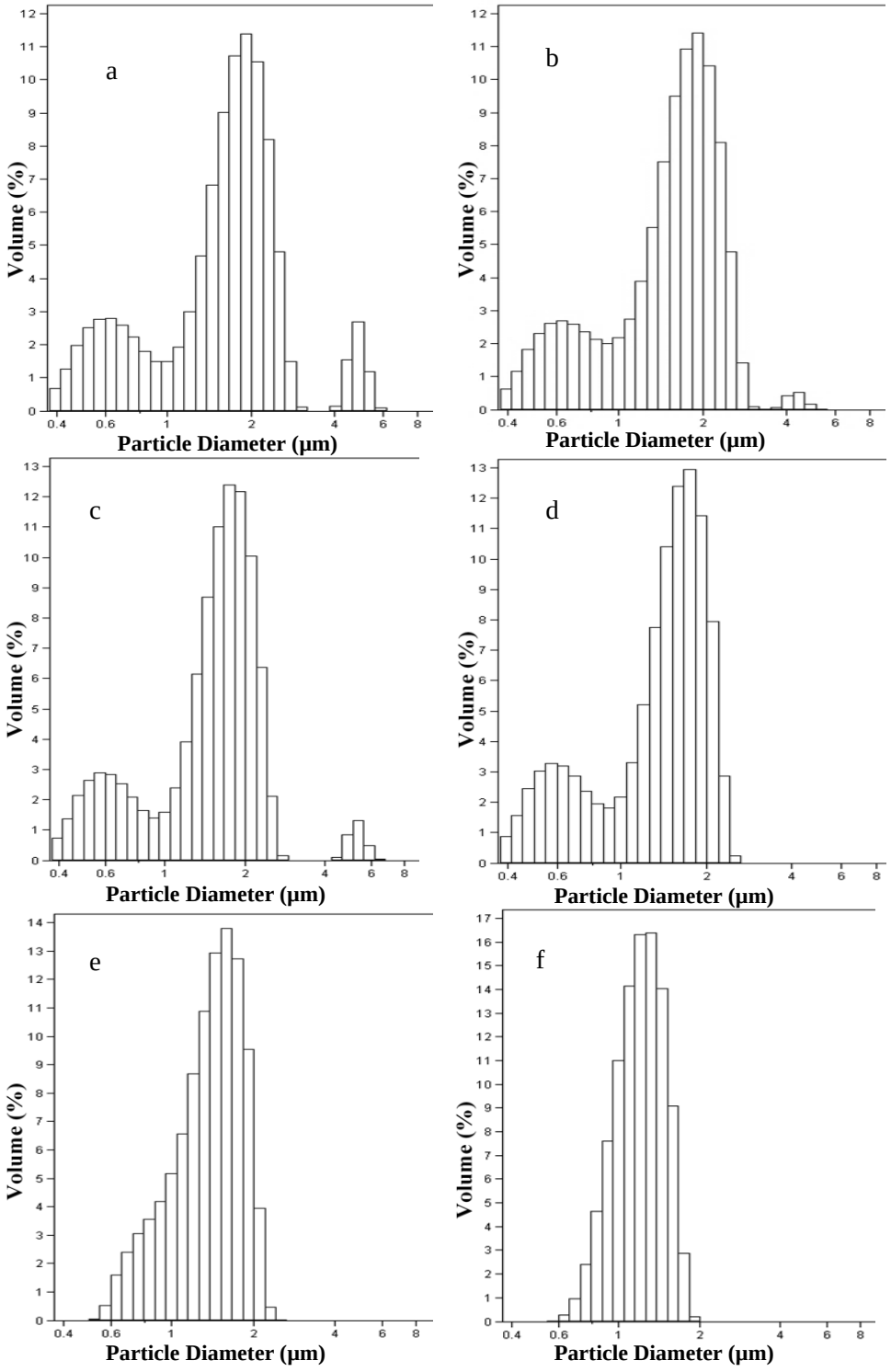

Figure 8. Ultra-filtered milk concentrated to 7% casein homogenized with a 2-stage valve homogenizer. Resulting fat particle size distributions, in µm as a percentage of total fat volume, when treated with the following pressures: a- control non-homogenized, b- 1.72 MPa (250 psi), c- 3.45 MPa (500 psi), d- 5.17 MPa (750 psi), e- 6.89 MPa (1,000 psi), and f- 10.3 MPa (1,500 psi).

Table 3. Effect of microfluidizer homogenization (gauge pressure) on recombined concentrated milk (RCM) used to make model cheese on levels of cheese curd final moisture, whey fat, cheese curd fat retention, whey protein, and cheese curd protein retention

Microfluidizer Gauge Pressure	Final Moisture (%)	Whey Fat (%)	Fat Retention (%)	Protein in Whey (%)	Protein Retention (%)	Wet Curd Yield (%)	Dry Curd Yield (%)
0	48.0^{A}	3.9^{A}	66.2^{B}	1.4^{A}	80.2^{B}	36.2^{B}	18.8^{B}
0.14 MPa	46.0^{AB}	4.6^{A}	57.3^{B}	1.3^{B}	81.7^{A}	31.6^{C}	17.1^{C}
0.41 MPa	45.0^{B}	0.6^{B}	95.0^{A}	1.3^{B}	82.2^{A}	42.3^{A}	23.2^{A}
0.69 MPa	47.8^{A}	0.5^{B}	95.6^{A}	1.2^{B}	82.7^{A}	43.5^{A}	22.7^{A}

$^{A-C}$ Means with the same superscript letter within the same column were not significantly different, $P = 0.05$

Microfluidization

Microfluidization Final Moisture. From analysis of the final moisture of curds from microfluidized RCM, the 0.41 MPa GP treated RCM curds were the lowest at 45.0% moisture and were statistically significant in difference ($P < 0.001$) from the final curd moistures of control and 0.69 MPa GP treated RCM. The curds from 0.14 MPa GP treated RCM were not significant in difference of moisture composition from any other treatment. Restated, the results were that moisture in curds were higher at the lowest and at the highest microfluidized treatments, with the lowest curd moisture levels being found in between, optimized at about 0.41 MPa GP.

Microfluidization Curd Yields. Wet curd yields were affected by microfluidization pressure treatments with statistically significant ($P < 0.01$) differences found between every treatment except in one comparison: 0.41 MPa GP and 0.69 MPa

GP. Analyzing the dry curd yield also found statistically significant differences ($P <$ 0.01) between all samples except 0.41 MPa GP and 0.69 MPa GP, which again were not significant in difference from each other. Both wet and dry curd yields show a trend of increasing curd yield with increasing microfluidizer pressure until a maximum is reached at approximately 0.41 MPa GP, after which no additional benefit to curd yield was found.

Microfluidization Fat Retention. From analysis of the whey fat and fat retention of RCM curds results, the treatments were categorized into two groups. First, the low-pressure high-fat loss group consisting of the control and 0.14 MPa GP treatments. The second, the high-pressure low-fat loss group consisted of the 0.41 MPa GP and 0.69 MPa GP treatments (Table 3). Differences between these groups (high pressure versus low pressure) were statistically significant ($P < 0.001$) in each treatment-to-treatment comparison. Within each grouping (0.41 MPa GP versus 0.69 MPa GP and control versus 0.14 MPa GP) there was no statistically significant difference ($P>0.05$). The trend was that until a pressure of 0.41 MPa GP was reached, there was no benefit observed to increased fat retention and lower whey fat. At the 0.41MPa GP a benefit of increased fat retention and lower fat in whey was observed with no further benefit observed by increasing pressure to 0.69 MPa GP.

Microfluidization Protein Retention. Protein in whey and subsequent protein retention were not as contrasting in effect as previously mentioned factors. Our analysis of whey protein and RCM curd protein retention found statistically significant differences ($P < 0.01$) only between control and all other treatments for both factors, with microfluidization having a minor increase in protein retention of RCM curds.

DISCUSSION

Moisture Removal

The 3.5% casein RCM curds moisture development was similar and in close time with the standard university cheddar cheese curds tested (Figures 4 and 5). Starting moisture levels after cutting were close to each other, 87.7% and 87.9% for 3.5% casein RCM and the university curds respectively, and at the 120 min (model complete) 3.5% casein was 48.5%, which was very similar to the standard cheddar's 48.4% at 149 min, (cheddaring step). Using our model, we were unable to remove enough moisture to achieve legal cheddar (Table 2). The model is hampered in comparison to standard cheddar making by needing to use sealed vessels (centrifuge tube) rather than open air stirring, having periodic rather than continual whey drainage, no addition of salt, and the need to press (via centrifuge) in sealed non-draining vessels. Even with these limitations, however, using the model we were able to compare curd moisture levels in all tested RCM concentrations similar to help predict their effect on cheese manufacture.

Using this model, we were also able to make cheese curds in small scale without much complication. Only one operator was needed, and all the tools and equipment were such as could be readily acquired in a dairy research laboratory such as standard conical centrifuge tubes instead of customized vats. Additionally, we achieved gentile agitation and handling, fulfilling this need as recommended by previous works (Brandsma and Rizvi, 1999; Lu et al., 2017) again without customized equipment. The process was also quick and cost effective, only required limited amounts of concentrated milk and lab supplies. Some modification to the model is needed though if curds of cheddar moisture level are to be made.

Concentration Factor

Final Moisture. We found that RCM concentration on final curd moistures levels was not statistically different between curds from 3.5% casein and 7% casein RCM. We did find that 10.5% casein RCM curds were statistically different and were lower in moisture than both 3.5% casein and 7% casein curds. These results are seemingly contradictory to Orme (1998) who found a positive trend of final curd moisture with increasing UF milk concentration.

Orme (1998) concluded that syneresis was increasingly problematic with increases in concentration thus causing higher final curd moisture from milks of higher UF concentration levels. However, Panthi et al. (2019) found an inverse relationship between curd moisture level at any given stage of curd making and the starting milk protein level although protein level did not seem to have an effect on the rate of moisture loss itself. Rather, it seems that the moisture is lower in curds from higher concentrated milks due to the fact there was less moisture present initially. Panthi et al. (2019) further concluded that curd cut size had a more dramatic effect on final curd moisture than did the milk protein or concentration level. Increasing moisture loss rate with shrinking curd size resulted from a combination of increasing surface area of curds with decreasing distance from curd centers to edge. This may explain some of our inconsistencies with Orme, (1998) who used a single large curd cut size (15 mm) compared to the size used in this research (<6 mm) and to the size range used by Panthi et al. (2019) (6 mm, 12 mm, and 18 mm). In addition, the cheese-making model used by Orme (1998) was a cheddaring process that included matting, channeling, and milling of his curds post whey drainage. Panthi et al. (2019) and this research followed a stirred curd method of curd

handling which does keeps curds in gentle motion, a process which may improve whey syneresis.

Industry will undoubtedly be concerned at the prospect of reducing curd cuts down to the level of Panthi et al. (2019), i.e., 6 mm or to our even smaller cut sizes in tubes due to anticipated increase in fines losses. Also switching processes to a stirred curd method may further exacerbate the issue by not allowing curd matting, which may have otherwise recaptured curd fines. Significant process changes are needed to scale up the usage of MF RCM to make cheese. To counter concerns of industry, more research must be done to address the needs of handling finer and more brittle curds.

Final Curd Yields. The cheese industry currently uses limited concentration via filtration to increase per vat cheese curd yield (Govindasamy-Lucey et al., 2004; Henning et al., 2006; Lu et al., 2017), or said another way, throughput increase. Our results support this practice, with wet curd and dry curd yields having statistically significant increases tied to increases in concentration factor. Though reported 3.5% casein, 7% casein, and 10.5% casein concentrations are approximates in relative comparison, we expected curd yields in 7% casein to be two times greater than 3.5% casein, and 10.5% casein to be about three times greater than 3.5% casein in relative comparisons. However, 3.5% casein results of 14.4% and 7.4% in wet/dry curd yields respectively were more than doubled by 7% casein results of 36.2% and 18.8% wet/dry curd respective yields, and then more than tripled by the 10.5% casein results of 55.0% and 30.5% wet/dry curd respective yields. Indeed, in each case, the proper multiples of 3.5% casein results were less than 80% of the actual higher concentration yields in both wet and dry analysis. This suggested that perhaps the rate of curd yield per unit of milk might

have also increase with MF concentration factor, an increase in efficiency in addition to throughput.

To compare each sample directly to each other, a relative concentration factor was calculated for each concentration tested in terms of each samples starting relation to the initial 3.5% casein sample's fat and protein content. This factor was applied to each dry curd yield result to make a relative dry curd yield data point. Statistically significant differences were found between each result of 7.4%, 8.8%, and 9.5% relative dry curd yields from concentrations of 3.5% casein, 7% casein, and 10.5% casein, respectively, showing an increasing curd yield with an increasing concentration of RCM. Under the right treatment conditions, manufacturing of curds from concentrated milk does improve efficiency of retention of non-water milk composites (fat, protein, and etc.) into cheese thus not only improving increases per vat cheese yields (throughput increase), but also increases of cheese per unit of original milk used. Scale-up and equipment modification may complicate industrial application, but further research into this possibility of cheese efficacy improvement may prove worth the effort.

Whey Fat and Retained Curd Fat. Improving fat retention of curds is another area that is of interest to the cheese industry, not just due to curd yield increases that would result from better retention, but also because whey cream is of limited usefulness. Some manufacturers recycle whey cream back into the start of their process, using whey cream to increase their cheese milk fat. However, the Standards of Identity do not list whey fat as an acceptable ingredient in any defined cheeses (FDA, 2018), thus forming the potential for a regulatory issue with this practice. From our work we did find a problem: RCM made from previously frozen HC-MCC and cream does not retain fat

well, with 3.5% casein and 7% casein having 62% and 64.4% fat retentions, respectively. Fat retention in standard non-concentrated cheese should fall between 91% to 93% (Orme, 1998). Higher concentrated samples retained more fat, with 10.5% casein at 84.2% fat retention, an obvious improvement, and very similar to Orme (1998) observations of 4X and 5X UF concentrated samples with fat retention of around 80%. Even so, our RCM fat losses are still too high and demonstrated the need to consider more aggressive mechanical mixing beyond simple stirring to improve fat retention via better distribution of caseins and fat. Our fat retention results may, however, be overestimating fat losses. Our retention results came from comparing whey fat percentage to initial RCM fat percentage, the total volume and thus the total amount of fat lost to whey could not be determined accurately. Prolonged dripping or straining would have would have dried out our curds interfering with those data points. In addition, the small size of samples in our model made limited amount of curd, our preference was to use curds for moisture analysis rather than fat analysis, and thus we were unable to obtain a total fat loss data point. Even so, there is reason to believe that more mechanically aggressive agitation may be needed to better disperse fat in the RCM to discourage excess loss to whey run off.

Whey Protein and Retained Curd Protein. In addition to the importance of fat retention, protein retention of curd is a vital characteristic that affects curd yields and was monitored to understand the previously mentioned relative curd yield increases. In addition, as approximately 70% of whey protein has been removed via MF filtration prior to making the RCM (Lu et al., 2016), there was also an opportunity to compare with UF concentration methods that retain whey proteins. Our results demonstrated an increasing

loss of protein retention of curds of 96%, 92.4%, and 82.3% protein retained of 3.5% casein, 7% casein, and 10.5% casein concentrated curds, respectively, with all results being statistically significant in difference from each other. This shows that the relative curd yield increases mentioned previously were the result of increases in fat retention in the concentration tests offsetting the increasing protein losses as concentration factors increased. This also suggests that RCM of these concentrations needs better mechanical agitation in preparation to better disperse caseins and fat globules in an effort to better retain both.

Orme (1998) found in his UF 4X and 5X concentrated milk curds protein retention of 82.6% and 84.3%, respectively, with no statistical difference between them, values, it should be noted, that are quite similar to our RCM concentration of 10.5% casein. This points to a problem: 10.5% casein RCM made with reduced whey protein skim milk (Lu et al., 2016) has whey of comparable protein content to UF 4X and 5X. While our RCM has had cream add some protein into the RCM, including more whey protein, there may be cause for concern that whole caseins, and not just cleaved κ-caseins, are being lost to whey. Orme (1998) noted significant syneresis problems in his 4X and 5X UF concentrated milk curds likely causing the curds to be retaining a significant portion of whey proteins. There is the possibility that both UF and MF concentrated milks at these higher ends are losing an increased amount of caseins due to clusters of amalgamated caseins such as those found by Lu et al., (2016) not being fully renneted and not incorporating into forming curd matrixes during coagulation. This again suggests more mechanically aggressive agitation should be attempted to see if protein retention rates could be improved.

Homogenization

Oil Droplet sizes: Homogenization versus Microfluidization of 7% Casein Concentrate. Fox et al. (2006) and Walstra et al. (1999) showed that in unhomogenized whole milk the oil droplet size distributions peak and center around 4 to 5 µm with a wide distribution curve stretching from just under 1 µm to about 10 µm. As homogenization pressure was increased, these distributions shifted left toward much smaller oil droplet sizes, and the distribution curves themselves tightened dramatically, the oil droplets becoming more uniform. At about 18 MPa, the oil droplet distribution peaked at about 1 µm with tight edges that reached from 0.5 µm to about 2 µm.

Whole milk concentrated to 7% casein via UF showed a very similar pattern in peaks, particle sizes, and distributions for the same homogenization treatments as homogenized whole milk. The only difference between the UF concentrate and whole milk was a weak initial homogenizing effect seen in 7% casein control (Figure 8a). This suggests that the pumping and shearing action of concentrating milk with a UF membrane does have a limited homogenizing effect. Otherwise the results match very well with the graphical data of whole milk homogenized up to 18 MPa (2611 psi) provided by Fox et al. (2006) and Walstra et al. (1999).

Microfiltered RCM treated with microfluidization had some interesting similarities and differences to homogenized whole milk and UF concentrated whole milk. Starting with the control RCM, the sample displayed a lot less homogenization effect than the UF control. The cream used in the RCM was non-homogenized and as it was added after the skim milk was concentrated, it further shows that membrane filtration does indeed have an effect on fat particle sizes.

The RCM treated with 0.14 MPa GP resulted in a tri-modal distribution of particle sizes as did the 7% casein UF homogenized control and 1.72 MPa treated samples. The peaks for each of these three were also similar in location on the particle size scale, though the magnitude of the microfluidized 0.14 MPa GP far right peak was much larger, showing a greater volume of larger particles than found in the UF homogenized samples. The center peaks for all three were similar in magnitude (9 to 11% peaks), and similar in position around 2 µm. The final left peaks were also similar in magnitude (about 2% peaking), but the UF treated samples smallest particles center peaked around 0.5 µm, while the microfluidized samples were a bit larger at around 1 µm. The 0.14 MPa GP (32.1 MPa total treatment) samples therefore have particles of slightly larger size and have a greater volume of the largest particles than UF concentrated whole milk treated up to1.72 MPa in a valve homogenizer.

Microfluidized RCM samples of 0.41 MPa GP and 0.69 MPa GP (96.4 and 160 MPa total pressure respectively) where comparable in mean/median oil droplet sizes to those found in 7% casein UF whole milk homogenized to 10.3 MPa (Figure 8f) and 3.5% casein whole milk homogenized at 18 MPa (Fox et al., 2006), each being around 1 µm. However, the ranges and peaking behavior of particle sizes of the microfluidized RCM were quite different from the homogenized UF whole milk samples. Microfluidized RCM retained a bi-modal distribution of particle sizes, with the right peak even shifting slightly more to the right (meaning a volume of larger particles) as the pressure increased from 0.14 MPa GP to 0.41 MPa GP and 0.69 MPa GP. The magnitude of this far right peak did diminish (meaning there was a lesser volume of these large particles) but the slight shift right shows the development of some large particles even larger than those

found in the lower and untreated samples. These results may seem counterintuitive; while particles are getting smaller, higher microfluidization pressures on 7% casein RCM is making some particles larger, albeit in a small amount. Olson et al. (2004) found the same effect in whole milk treated in a microfluidizer between 100 and 200 MPa (total pressure) and cream treated above 50 MPa. Increasing microfluidization treatment pressures on milk, cream, and RCM beyond a certain point starts forming larger particles, some can be even larger than those originally found in control. This effect is not seen in 2-stage valve homogenization.

In addition to larger large particles, our data also shows that microfluidization of RCM at 0.41 MPa GP and 0.69 MPa GP (Figure 7 a and b) created even smaller particles than what was seen in the homogenized UF samples of 10.3 MPa (Figure 8f). The left side tails of the two highest microfluidized continue off the graphs, particles even smaller than 0.4 μm are present, in the UF particles smaller than 0.4 μm disappeared after homogenization treatments greater than 5.17 MPa where applied (Figure 8d). Increasing microfluidization pressure on concentrated RCM samples continues to make smaller and smaller particles. Olson et al. (2004) and Strawbridge et al. (1994) also showed this in their findings with Strawbridge et al. (1994) even finding additional peaks in microfluidized milk even below 100nm, well below our setup's detection range. Valve homogenization, however, creates tighter and tighter peaks as the pressure increases, with particles larger than the center becoming smaller and particles smaller than the center becoming larger. Figure 5.4 in Fox et al. (2006) also show a similar effect of valve homogenization on whole milk samples, greater pressure usage can reduce the amount of the very smallest particles. Taken all together, microfluidization creates larger and

smaller particles as the pressure treatment increases, generating a wide particle size distribution; while increasing a 2-stage valve homogenizer's pressure creates particle distributions that are more homogeneous, narrower in distribution.

Microfluidization

Microfluidization Final Moisture. While microfluidization and homogenization do have differences, there are similar effects on milk concentrates used to make curds. Orme (1998) made mention in his work that "High-pressure homogenization of milk produces … moisture retention." Orme (1998) further notes that by lowering pressure homogenization (5.5 MPa), some of the issues he faced were corrected. In our own tests a single statistically significant lower curd moisture was found between microfluidizer GP of 0.41 MPa GP versus control and 0.69 MPa GP. The difference was also significant in size, about 3 percentage points lower than both control and 0.69MPa GP, an amount that could help curds meet legal requirements at the end of process. The 0.14MPa GP treatment was not statistically different from either group, perhaps showing the development of the beneficial effect on whey expulsion which is optimized close if not at 0.41MPa GP for 7% casein RCM. Our findings concur with the findings of Orme (1998) that limited homogenization or microfluidization treatment of RCM or milk concentrate can help overcome some issues with excessive moisture retention of the curds. However, if taken to far the issue rebounds and moisture retention again rises. We suggest further research to see if 0.41 MPa GP is indeed optimal for 7% casein and other concentrations of RCM.

Microfluidization Curd Yields, Protein Retention, and Fat Retention. Both wet and dry curd yield data showed statistically significant differences in curd yields

correlating with microfluidizer homogenizations compared to 7% casein RCM control. There was first a statistically significant dip in yields, about 5% and 2% difference in total wet and dry respective curd yields, versus 7% casein RCM treated at 0.14 MPa GP. Then there was a statistically significant rise in curd yields in comparing 7% casein RCM control and 0.41 MPa GP RCM, about 6% and 4.4% difference in total wet and dry respective curd yields. The higher treated 0.69 MPa GP 7% casein RCM curd yields were very similar to 0.41 MPa GP treated 7% casein RCM suggesting a leveling off of the curd yield increasing effect.

Protein retention differences between control samples and all treated samples were minor, even with statistically significant differences only found between control and all other treated samples of RCM, these differences did not exceed 2.5%, thus they cannot explain the decrease and then increase in curd yields. Further, there disparity shown in protein retention data between the concentration tests (Table 2) and the homogenization tests (Table 3) may show that protein retention is affected by other factors such as handling of the RCM in this cheese model. Larger scale tests may be more appropriate not only for better representation of large-scale cheese making, but also by better uniformity in RCM handling. Either way, our results do not demonstrate a meaningful improvement of protein retention by 7% casein RCM curds when the RCM is homogenized via microfluidization.

Fat retention results are the most useful at explaining the observed curd yield effects (Table 3). Indeed, fat yield data show the same dip and then rise in respective treatments as was found in the curd wet and dry yield results. Furthermore, fat retention improved to 95% by 0.41 MPa GP treated 7% casein RCM, significant especially when

we consider that the concentration tested a maximum of 84.3% in 10.5% casein RCM (Table 2). Orme (1998) noted an increasing amount of fat-protein complex that formed when homogenizing UF milk prior to cheese making leading to increased fat retention. Increasing the pressure to much, however, had a negative effect on cheese texture due to finer dispersions of fat in the resulting cheese matrix (i.e. smaller fat globules). Orme (1998) further suggested a mid-range two-stage valve homogenization treatment of about 5.5 MPa to increase fat retention while avoiding oil droplets from becoming too small.

We have already shown that microfluidizer GP and total pressure do not correlate directly with two-stage valve homogenization. From our data, however, it seems that an indicating GP of about 0.41 MPa GP in a microfluidizer may be another option to that proposed by Orme (1998). Benefits of increased fat retention may be explained by oil droplet diameter dispersion similarities between 0.41 MPa GP treated 7% casein RCM and 7% casein UF treated at 6.89 MPa and 5.17 MPa (Figures 6 and 7), all having at least one peak at around the range of 0.5 to 2 μm. While the microfluidized 7% casein RCM does have a broader primary peak and a trailing secondary peak close to 6 μm, this may actually be an advantage of microfluidization considering that poor textural behavior of curds was linked to numerous excessively small fat globules (Orme 1998). Microfluidization may be mending some of these issues by generating particles of larger sizes, perhaps explaining fat retention. There is a need for further research into optimized treatment parameters; increasing microfluidization pressures generates smaller sized fat particles (Strawbridge et al., 1994; Olson et. al., 2004), which may negate any gains to fat retention (and maybe cheese texture improvements) if the pressure is too high.

CONCLUSIONS

Concentrated recombined cream milk cheese curds can be made in small scale for research purposes using our methods. Our model cheese method is gentle on curds and can be operated by a single individual with laboratory tools and supplies that are widely available. While we were not able to reach cheddar moisture levels in our model, the model is simple, quick, and cost effective. Results from using our model could be tested with other models, such as used by Orme (1998) in which proper moisture levels at larger scales were reported.

Concentration of casein did improve curd yield both in terms of total amount and relative to concentration factor, thus presenting the possibility of not only throughput increases in manufacturing cheese using MF concentration, but also potentially improving curd yields per unit of starting milk by better retention of fat.

In terms of moisture retention concerns, our results showed that 10.5% casein, the highest concentration tested, had better (lower) final curd moisture than control and 7% casein. This demonstrates that even if syneresis is hampered in higher concentrations of RCM, any removal of moisture or whey from the system has a cumulatively greater effect on lowering moisture, thus higher concentrated RCM curds are still able to achieve lower moistures in our cheese-making model.

Microfluidization of 7% casein RCM did have a positive impact on curd fat retention, curd yield, and end moisture level, with treatment of 0.41 MPa GP being optimal for all three. Fat retention of the curds grew from an initial 66% in control to 95% in 0.41 MPa GP treated samples which impacted curd yields which increased from under 19% to about 23% in control and 0.41 MPa GP respectively. The higher 0.69 MPa

GP treated 7% casein RCM was very comparable to the 0.41 MPa GP results in curd yield and fat retention benefits. However, 0.41 MPa GP proved to be optimal, having a statistically significant lower (better) moisture level of 45.0% compared to the 47.8% of the 0.69 MPa treatment.

Microfluidization of 7% casein RCM did reduce the median/mean sizes of contained oil droplets similar to the effects of a two-stage homogenizer of 7% casein UF whole milk. However, pressure-to-pressure effects are off set: 0.41 MPa GP on a microfluidizer generates smaller and larger particle sizes than 7% casein UF whole milk treated from 5.15 MPa to 10.3MPa on a two-stage valve homogenizer. It is conceivable that this optimum range of microfluidization treatment might not only improve compositional make-up but also textural properties of the eventual cheese due to the generation of limited larger particles.

This research does show that retentates derived from MF concentration of skim milk, HC-MCC, combined with cream, forming RCM, indeed form a cheese with characteristic fat retention, protein retention, and moisture losses forming the basic functionalities of a standard cheese curd with some of these improved by the application of mid-ranged microfluidization. Further research can use our optimal microfluidization pressure treatments of RCM to capitalize on these gains and further research into using RCM for larger scale cheese making.

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APPENDICES

APPENDIX A: PROC ANOVA PROCEDURE ON RELATIVE DRY CURD YIELDS, DRY CURD YIELDS, AND WET CURD YIELDS FROM 3.5% CA, 7% CA, AND 10.5% CA CONCENTRATIONS OF RCM MADE INTO CHEESE

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for yield

Alpha	**0.05**
Error Degrees of Freedom	11
Error Mean Square	0.146221
Critical Value of Studentized Range	3.81958

Comparisons significant at the 0.05 level are indicated by *.**

Concentration Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
3 - 2	0.6830	0.0576	1.3084	***
3 - 1	2.0980	1.3438	2.8522	***
2 - 3	-0.6830	-1.3084	-0.0576	***
2 - 1	1.4150	0.6847	2.1453	***
1 - 3	-2.0980	-2.8522	-1.3438	***
1 - 2	-1.4150	-2.1453	-0.6847	***

APPENDIX B: PROC ANOVA PROCEDURE ON DRY CURD YIELDS FROM 3.5% CA, 7% CA, AND 10.5% CA CONCENTRATIONS OF RCM MADE INTO CHEESE

The ANOVA Procedure
Tukey's Studentized Range (HSD) Test for yield

Alpha	**0.05**
Error Degrees of Freedom	11
Error Mean Square	0.886044
Critical Value of Studentized Range	3.81958

Comparisons significant at the 0.05 level are indicated by *.**

Concentration Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
3 - 2	11.6783	10.1389	13.2178	***
3 - 1	23.0900	21.2334	24.9466	***
2 - 3	-11.6783	-13.2178	-10.1389	***
2 - 1	11.4117	9.6140	13.2093	***
1 - 3	-23.0900	-24.9466	-21.2334	***
1 - 2	-11.4117	-13.2093	-9.6140	***

APPENDIX C: PROC ANOVA PROCEDURE ON WET CURD YIELDS FROM 3.5% CA, 7% CA, AND 10.5% CA CONCENTRATIONS OF RCM MADE INTO CHEESE

The ANOVA Procedure

Alpha	**0.05**
Error Degrees of Freedom	11
Error Mean Square	3.047538
Critical Value of Studentized Range	3.81958

Tukey's Studentized Range (HSD) Test for yield

Comparisons significant at the 0.05 level are indicated by *.**

Concentration Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
3 - 2	18.767	15.912	21.622	***
3 - 1	40.521	37.077	43.964	***
2 - 3	-18.767	-21.622	-15.912	***
2 - 1	21.753	18.419	25.087	***
1 - 3	-40.521	-43.964	-37.077	***
1 - 2	-21.753	-25.087	-18.419	***

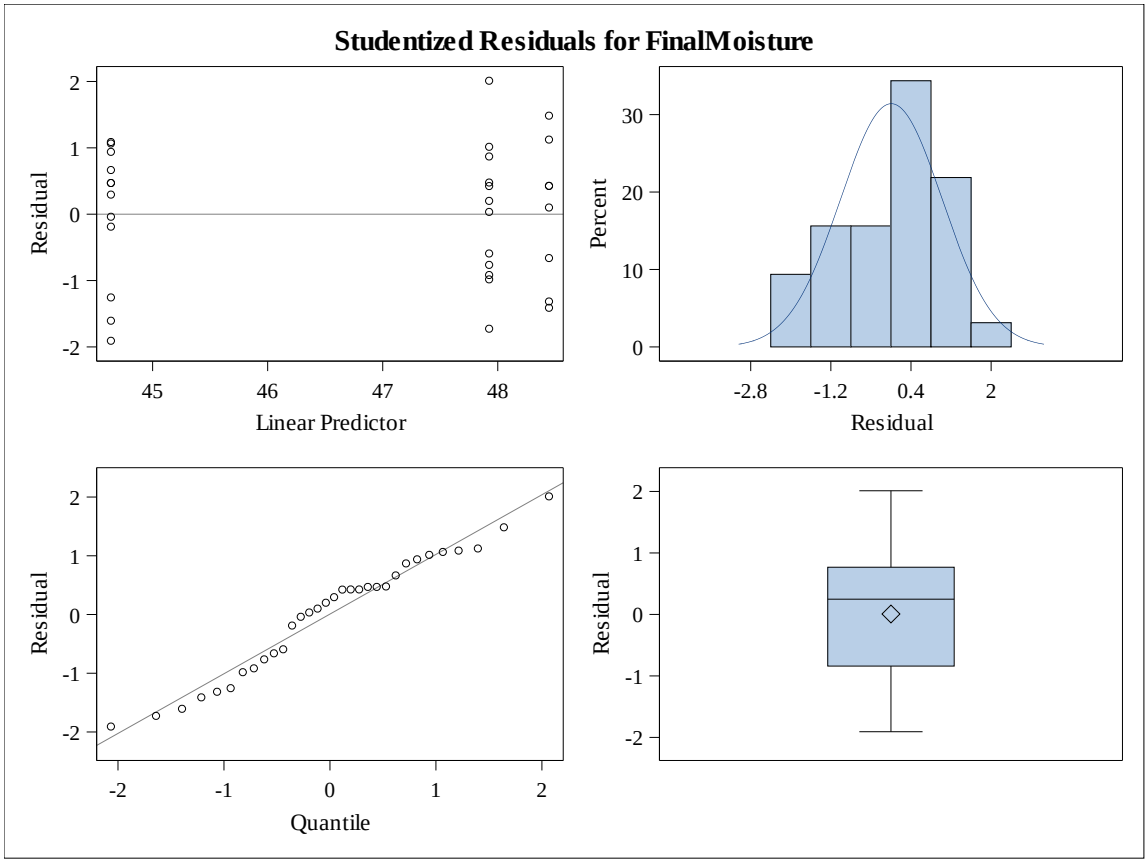
APPENDIX D: GLIMMIX ANOVA PROCEDURE– FINAL MOISTURE FROM CURDS MADE FROM 3.5% CA, 7% CA, AND 10.5% CA RCM

The GLIMMIX Procedure

Class Level Information		
Class	**Levels**	**Values**
milkID	6	1 2 3 4 5 6
Concentration	3	1 2 3
Rep	12	1 2 3 4 5 6 7 8 9 10 11 12

Differences of Concentration Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer							
Concentration	**Concentration**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adj P**
1	2	0.5204	0.5515	3	0.94	0.4149	0.6545
1	3	3.8077	0.5010	3	7.60	0.0047	0.0097
2	3	3.2873	0.5720	3	5.75	0.0105	0.0212

Tukey-Kramer Grouping for Concentration Least Squares Means (Alpha=0.05)		
LS-means with the same letter are not significantly different.		
Concentration	**Estimate**	
1	48.4452	A
		A
2	47.9248	A
3	44.6375	B


Studentized Residuals for FinalMoisture
Residual
Linear Predictor
Percent
Residual
Residual
Quantile
Residual

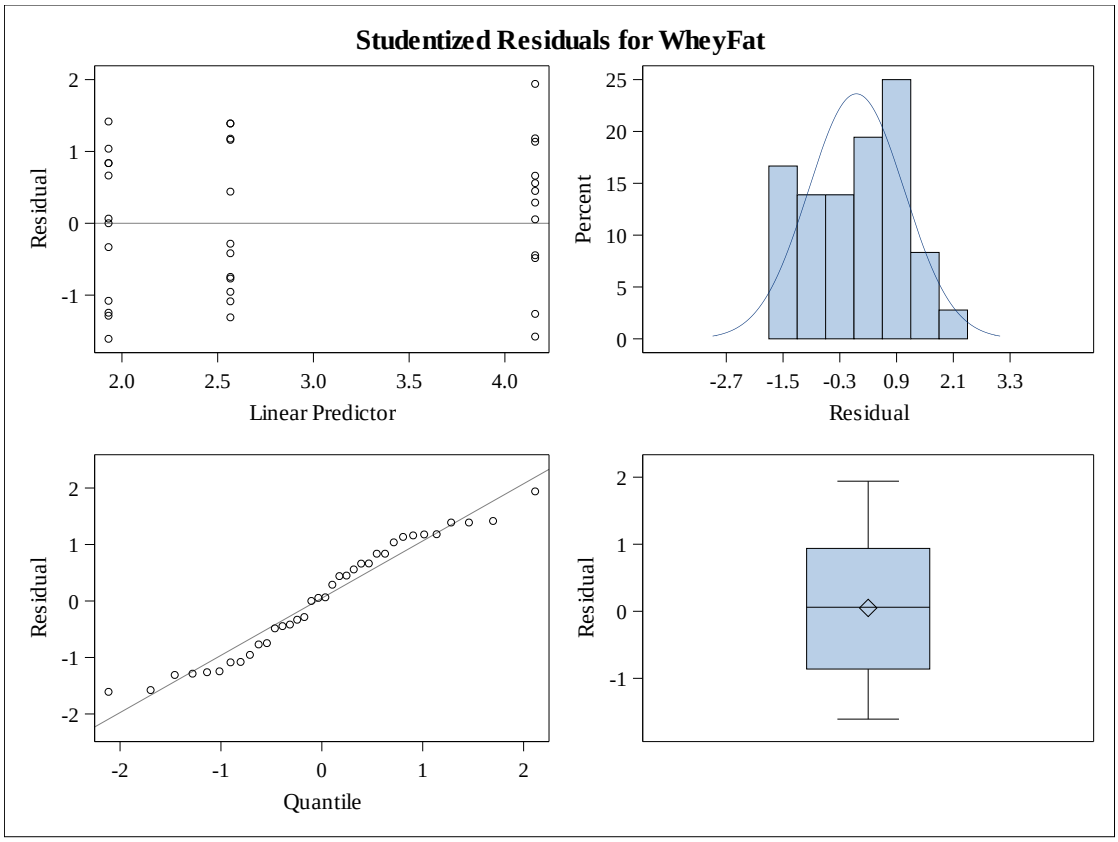
APPENDIX E: GLIMMIX ANOVA PROCEDURE–WHEY FAT FROM CURDS MADE FROM 3.5% CA, 7% CA, AND 10.5% CA RCM

Whey Fat

Class Level Information		
Class	**Levels**	**Values**
milkID	6	1 2 3 4 5 6
Concentration	3	1 2 3
Rep	12	1 2 3 4 5 6 7 8 9 10 11 12

Differences of Concentration Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer							
Concentration	**Concentration**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adj P**
1	2	-2.2281	0.3114	3	-7.15	0.0056	0.0115
1	3	-0.6364	0.3028	3	-2.10	0.1263	0.2365
2	3	1.5917	0.4270	3	3.73	0.0336	0.0669

Tukey-Kramer Grouping for Concentration Least Squares Means (Alpha=0.05) LS-means with the same letter are not significantly different.			
Concentration	**Estimate**		
2	4.1580		A
			A
3	2.5663	B	A
		B	
1	1.9299	B	


Studentized Residuals for WheyFat
Residual
Linear Predictor
Percent
Residual
Residual
Quantile
Residual

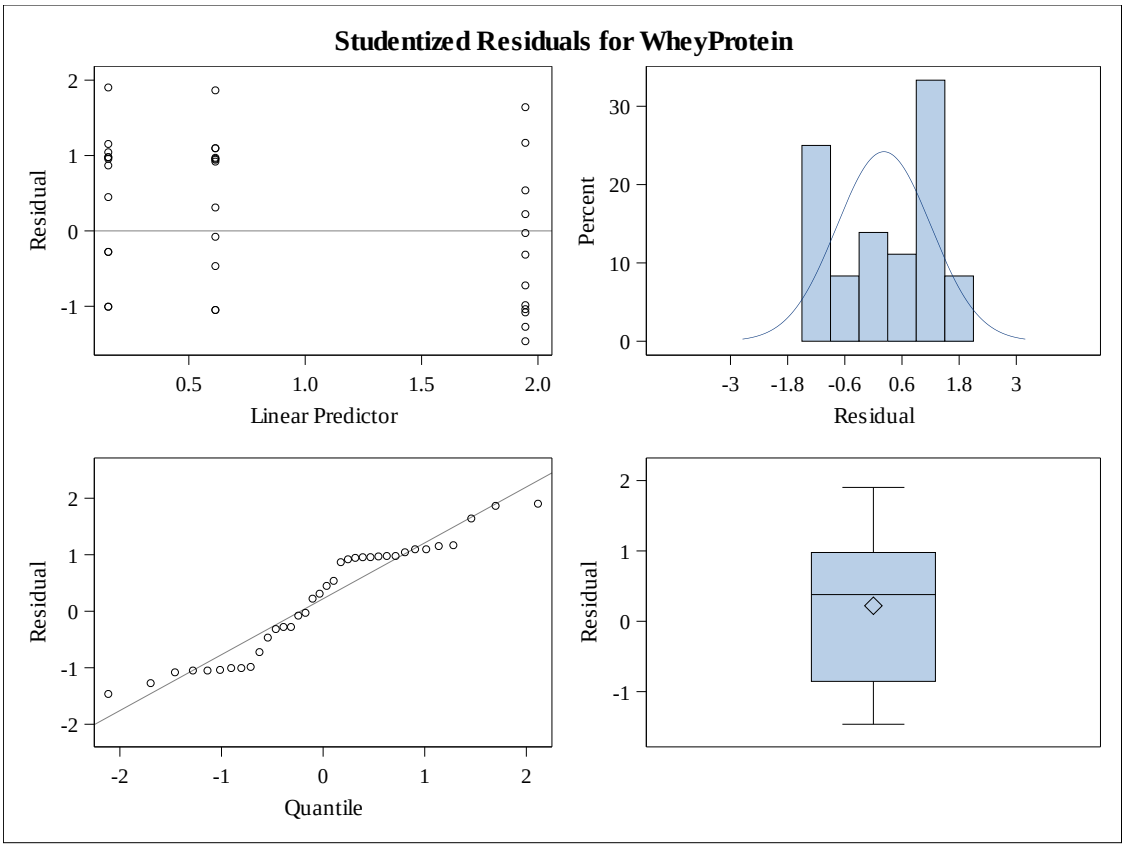
APPENDIX F: GLIMMIX ANOVA PROCEDURE–WHEY PROTEIN FROM CURDS MADE FROM 3.5% CA, 7% CA, AND 10.5% CA RCM

Whey Protein

Class Level Information		
Class	**Levels**	**Values**
milkID	6	1 2 3 4 5 6
Concentration	3	1 2 3
Rep	12	1 2 3 4 5 6 7 8 9 10 11 12

Differences of Concentration Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer							
Concentration	**Concentration**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > t|**	**Adj P**
1	2	-0.4602	0.02376	3	-19.37	0.0003	0.0006
1	3	-1.7920	0.02741	3	-65.38	<.0001	<.0001
2	3	-1.3319	0.03522	3	-37.82	<.0001	<.0001

Tukey-Kramer Grouping for Concentration Least Squares Means (Alpha=0.05)		
LS-means with the same letter are not significantly different.		
Concentration	**Estimate**	
3	1.9459	A
2	0.6140	B
1	0.1538	C


Studentized Residuals for WheyProtein
Residual
Linear Predictor
Percent
Residual
Residual
Quantile
Residual

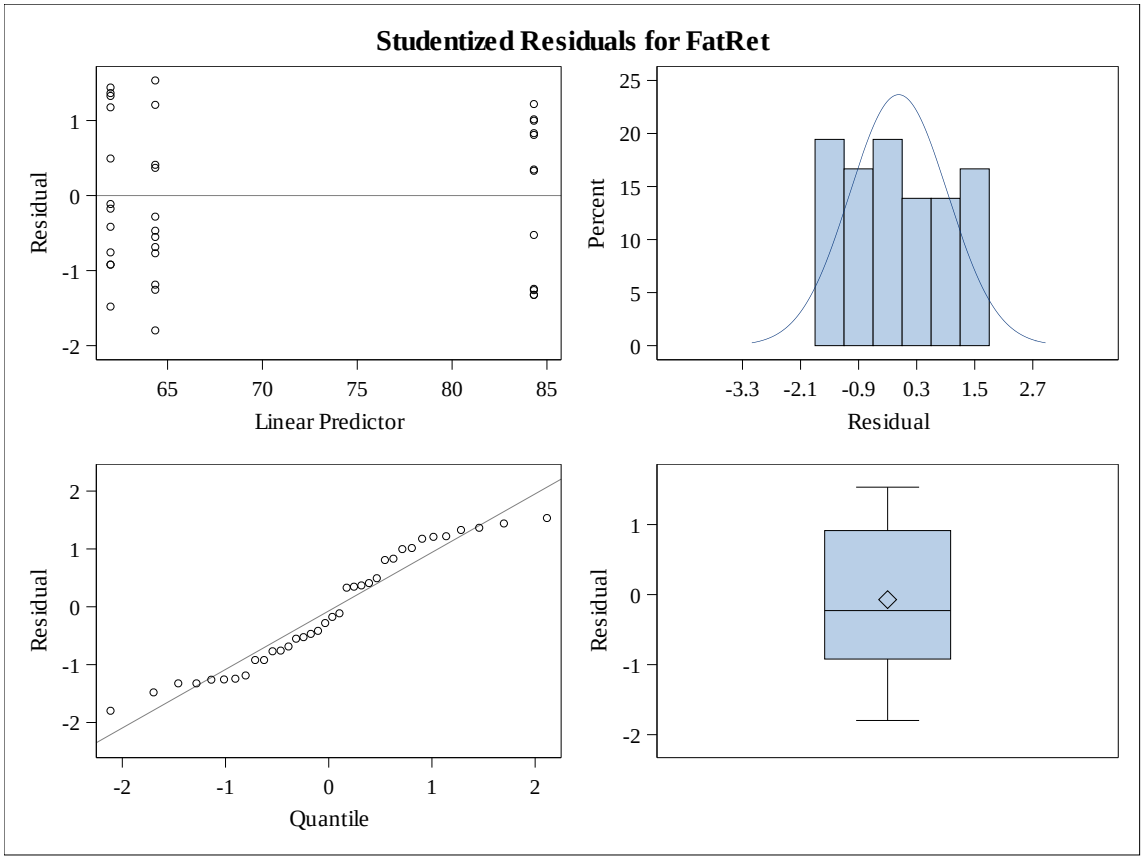
APPENDIX G: GLIMMIX ANOVA PROCEDURE– (CURD) FAT RETENTION, FROM CURDS MADE FROM 3.5% CA, 7% CA, AND 10.5% CA RCM

(Curd) Fat Retention

Class Level Information		
Class	**Levels**	**Values**
milkID	6	1 2 3 4 5 6
Concentration	3	1 2 3
Rep	12	1 2 3 4 5 6 7 8 9 10 11 12

Differences of Concentration Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer							
Concentration	**Concentration**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adj P**
1	2	-2.3581	2.9645	3	-0.80	0.4845	0.7311
1	3	-22.3205	2.1638	3	-10.32	0.0019	0.0040
2	3	-19.9624	3.2812	3	-6.08	0.0089	0.0181

Tukey-Kramer Grouping for Concentration Least Squares Means (Alpha=0.05)		
LS-means with the same letter are not significantly different.		
Concentration	**Estimate**	
3	84.3118	A
2	64.3494	B
		B
1	61.9913	B


Studentized Residuals for FatRet
Residual
Linear Predictor
65
70
75
80
85
1
0
-1
-2
Percent
Residual
-3.3
-2.1
-0.9
0.3
1.5
2.7
25
20
15
10
5
0
Residual
Quantile
2
1
0
-1
-2
Residual
1
0
-1
-2

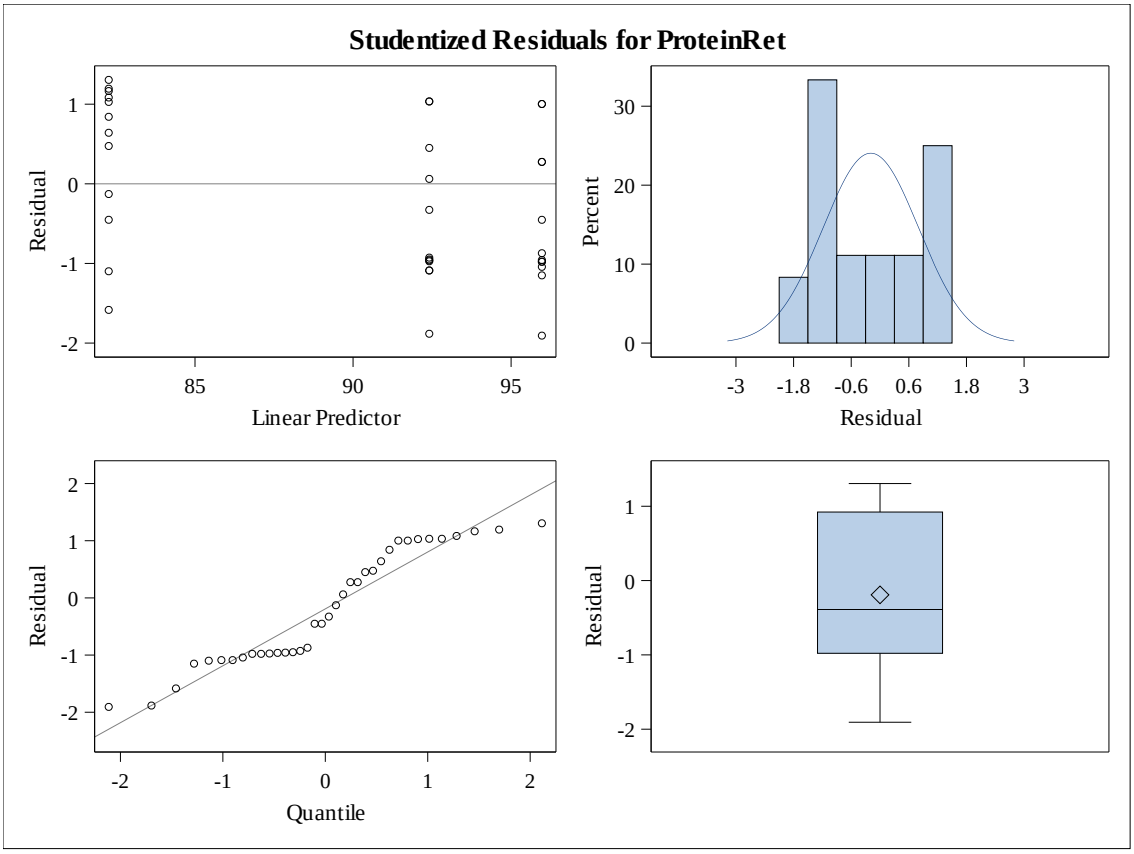
APPENDIX H: GLIMMIX ANOVA PROCEDURE– (CURD) PROTEIN RETENTION FROM CURDS MADE FROM 3.5% CA, 7% CA, AND 10.5% CA RCM

(Curd) Protein Retention

Class Level Information		
Class	**Levels**	**Values**
milkID	6	1 2 3 4 5 6
Concentration	3	1 2 3
Rep	12	1 2 3 4 5 6 7 8 9 10 11 12

Differences of Concentration Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer							
Concentration	**Concentration**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adj P**
1	2	3.5631	0.3264	3	10.92	0.0016	0.0034
1	3	13.7041	0.2934	3	46.71	<.0001	<.0001
2	3	10.1410	0.3753	3	27.02	0.0001	0.0002

Tukey-Kramer Grouping for Concentration Least Squares Means (Alpha=0.05)		
LS-means with the same letter are not significantly different.		
Concentration	**Estimate**	
1	95.9742	A
2	92.4111	B
3	82.2701	C

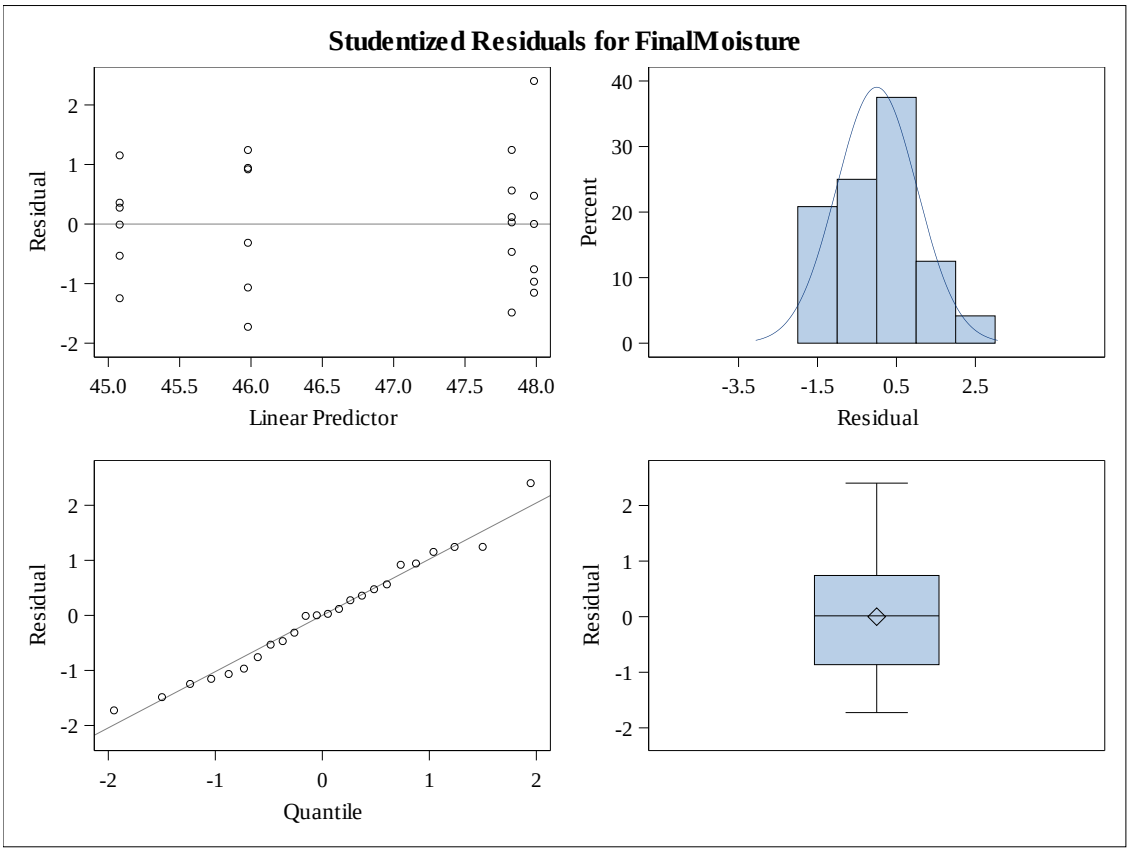

Studentized Residuals for ProteinRet
Residual
Linear Predictor
Percent
Residual
Residual
Quantile
Residual

APPENDIX I: GLIMMIX ANOVA PROCEDURE – FINAL MOISTURE FROM CURDS MADE FROM 7% CA RCM TREATED WITH MICROFLUIDIZER GAUGE PRESSURES OF 0 MPA (CONTROL), 0.14 MPA (20 PSI), 0.41 MPA (60 PSI) , AND 0.69 MPA (100 PSI)

Class Level Information		
Class	**Levels**	**Values**
milkID	4	1 2 3 4
Concentration	1	2
Pressure	4	0 20 60 100
Rep	6	1 2 3 4 5 6

Differences of Pressure Least Squares Means Adjustment for Multiple Comparisons: Tukey							
Pressure	**Pressure**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adj P**
0	20	2.0053	0.9325	20	2.15	0.0439	0.1716
0	60	2.9042	0.9325	20	3.11	0.0055	0.0258
0	100	0.1563	0.9325	20	0.17	0.8686	0.9983
20	60	0.8989	0.9325	20	0.96	0.3466	0.7710
20	100	-1.8490	0.9325	20	-1.98	0.0613	0.2274
60	100	-2.7479	0.9325	20	-2.95	0.0080	0.0368

Tukey Grouping for Pressure Least Squares Means (Alpha=0.05)			
LS-means with the same letter are not significantly different.			
Pressure	**Estimate**		
0	47.9837		A
			A
100	47.8274		A
			A
20	45.9784	B	A
		B	
60	45.0795	B	

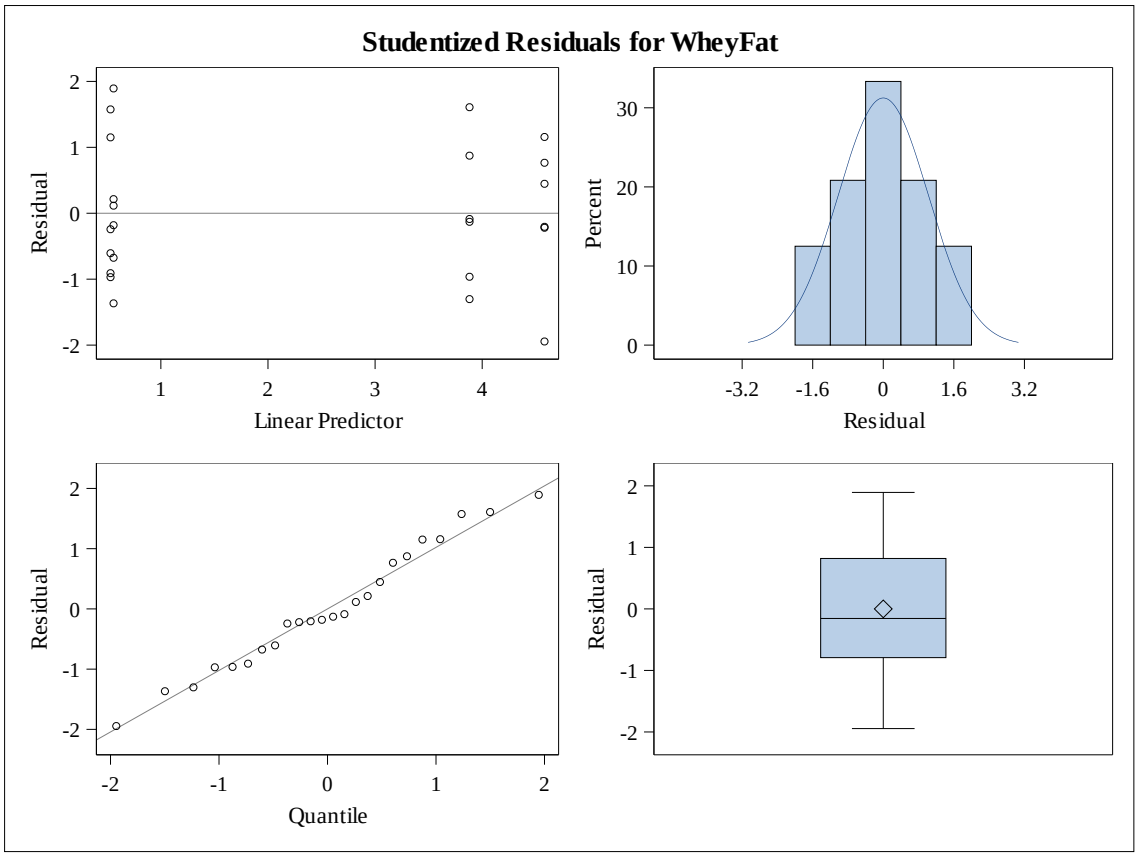

Studentized Residuals for FinalMoisture
Residual
Linear Predictor
Percent
Residual
Residual
Quantile
Residual

APPENDIX J: GLIMMIX ANOVA PROCEDURE –WHEY FAT FROM CURDS MADE FROM 7% CA RCM TREATED WITH MICROFLUIDIZER GAUGE PRESSURES OF 0 MPA (CONTROL), 0.14 MPA (20 PSI), 0.41 MPA (60 PSI), AND 0.69 MPA (100 PSI)

Class Level Information		
Class	**Levels**	**Values**
milkID	4	1 2 3 4
Concentration	1	2
Pressure	4	0 20 60 100
Rep	6	1 2 3 4 5 6

Differences of Pressure Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer							
Pressure	**Pressure**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adj P**
0	20	-0.7000	0.4419	20	-1.58	0.1289	0.4096
0	60	3.3233	0.3197	20	10.40	<.0001	<.0001
0	100	3.3517	0.3250	20	10.31	<.0001	<.0001
20	60	4.0233	0.3118	20	12.90	<.0001	<.0001
20	100	4.0517	0.3172	20	12.77	<.0001	<.0001
60	100	0.02833	0.08658	20	0.33	0.7469	0.9875

Tukey-Kramer Grouping for Pressure Least Squares Means (Alpha=0.05)		
LS-means with the same letter are not significantly different.		
Pressure	**Estimate**	
20	4.5817	A
		A
0	3.8817	A
60	0.5583	B
		B
100	0.5300	B

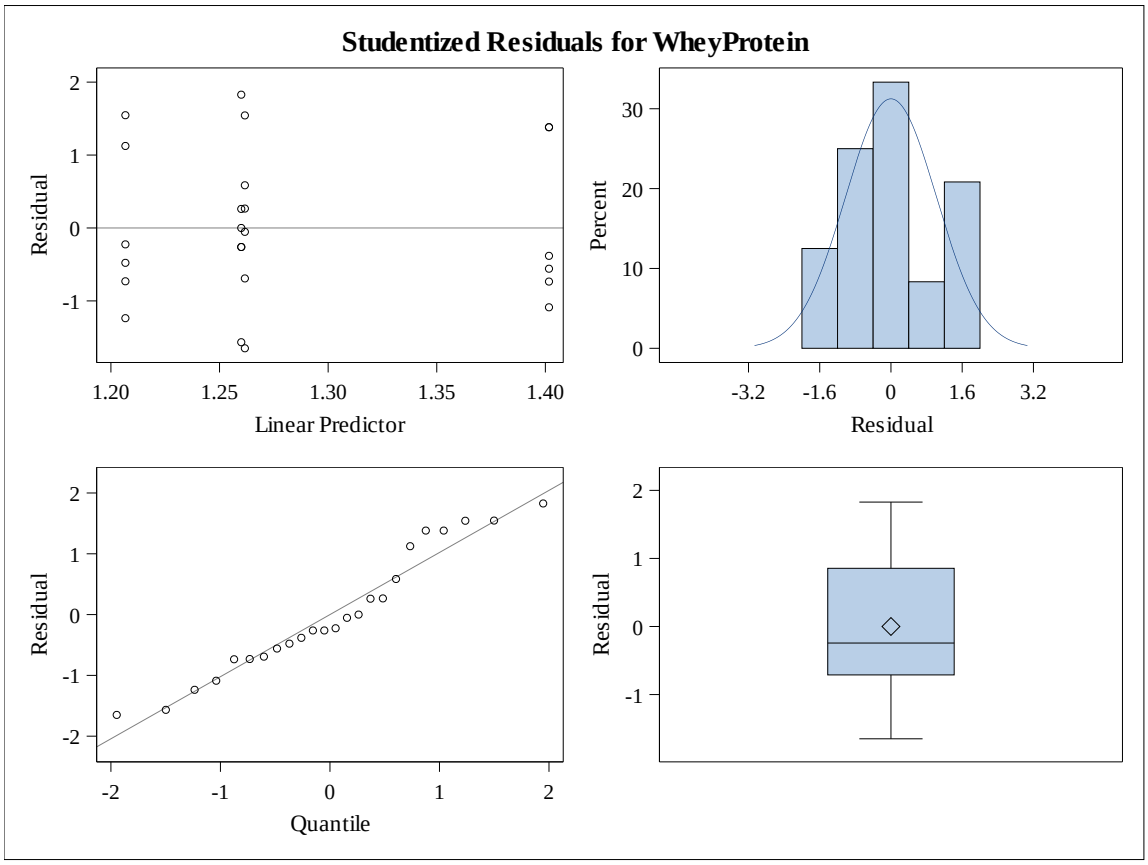

Studentized Residuals for WheyFat
Residual
Linear Predictor
Percent
Residual
Residual
Quantile
Residual

APPENDIX K: GLIMMIX ANOVA PROCEDURE –WHEY PROTEIN, FROM CURDS MADE FROM 7% CA RCM TREATED WITH MICROFLUIDIZER GAUGE PRESSURES OF 0 MPA (CONTROL), 0.14 MPA (20 PSI), 0.41 MPA (60 PSI), AND 0.69 MPA (100 PSI)

Class Level Information		
Class	**Levels**	**Values**
milkID	4	1 2 3 4
Concentration	1	2
Pressure	4	0 20 60 100
Rep	6	1 2 3 4 5 6

Differences of Pressure Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer							
Pressure	**Pressure**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adj P**
0	20	0.1400	0.02896	20	4.83	0.0001	0.0005
0	60	0.1417	0.03060	20	4.63	0.0002	0.0009
0	100	0.1950	0.05877	20	3.32	0.0034	0.0166
20	60	0.001667	0.02212	20	0.08	0.9407	0.9998
20	100	0.05500	0.05484	20	1.00	0.3279	0.7495
60	100	0.05333	0.05572	20	0.96	0.3499	0.7747

Tukey-Kramer Grouping for Pressure Least Squares Means (Alpha=0.05)		
LS-means with the same letter are not significantly different.		
Pressure	**Estimate**	
0	1.4017	A
20	1.2617	B
		B
60	1.2600	B
		B
100	1.2067	B

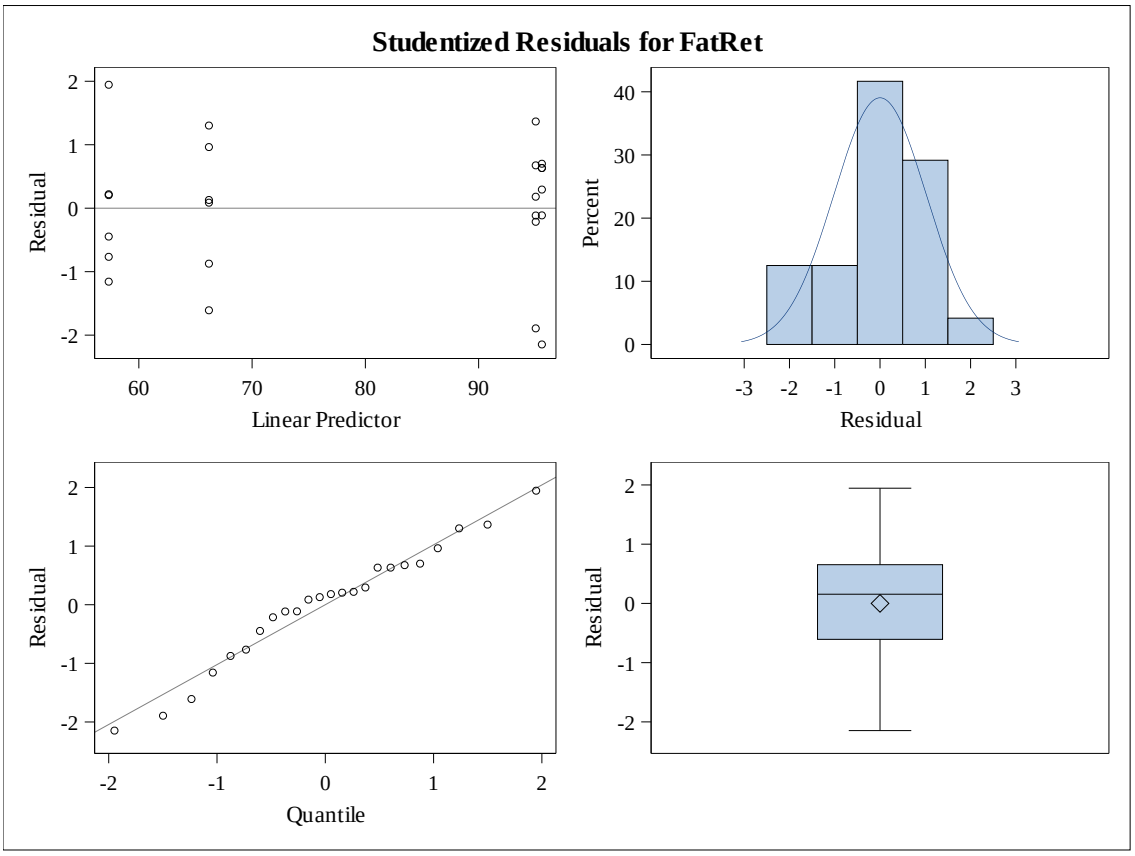

Studentized Residuals for WheyProtein
Residual
Linear Predictor
1.20
1.25
1.30
1.35
1.40
Percent
Residual
-3.2
-1.6
0
1.6
3.2
Residual
Quantile
Residual

APPENDIX L: GLIMMIX ANOVA PROCEDURE - (CURD) FAT RETENTION, FROM CURDS MADE FROM 7% CA RCM TREATED WITH MICROFLUIDIZER GAUGE PRESSURES OF 0 MPA (CONTROL), 0.14 MPA (20 PSI), 0.41 MPA (60 PSI), AND 0.69 MPA (100 PSI)

Class Level Information		
Class	**Levels**	**Values**
milkID	4	1 2 3 4
Concentration	1	2
Pressure	4	0 20 60 100
Rep	6	1 2 3 4 5 6

Differences of Pressure Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer							
Pressure	**Pressure**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adj P**
0	20	8.8474	3.9810	20	2.22	0.0380	0.1513
0	60	-28.8539	2.7858	20	-10.36	<.0001	<.0001
0	100	-29.3970	2.8245	20	-10.41	<.0001	<.0001
20	60	-37.7013	2.9002	20	-13.00	<.0001	<.0001
20	100	-38.2444	2.9374	20	-13.02	<.0001	<.0001
60	100	-0.5431	0.7350	20	-0.74	0.4685	0.8802

Tukey-Kramer Grouping for Pressure Least Squares Means (Alpha=0.05)		
LS-means with the same letter are not significantly different.		
Pressure	**Estimate**	
100	95.5846	A
		A
60	95.0414	A
0	66.1876	B
		B
20	57.3402	B

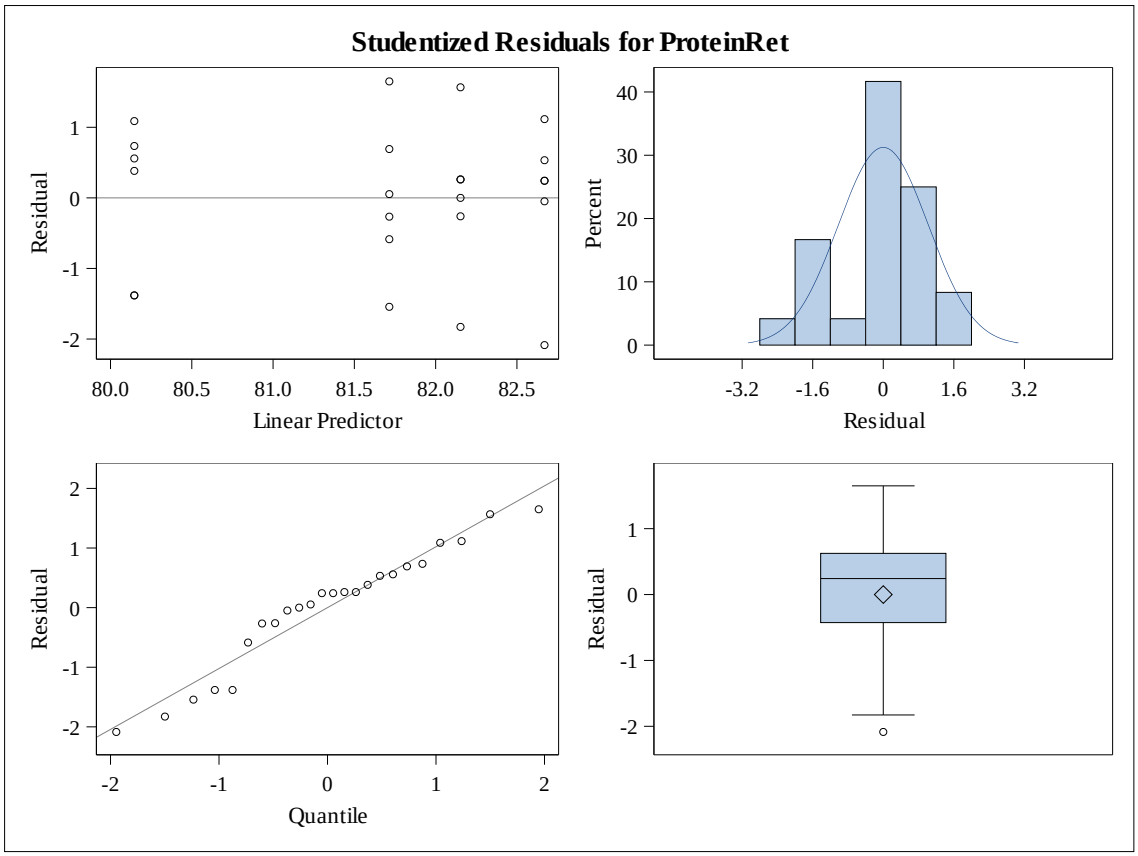

Studentized Residuals for FatRet
Residual
Linear Predictor
Percent
Residual
Residual
Quantile
Residual

APPENDIX M: GLIMMIX ANOVA PROCEDURE – (CURD) PROTEIN RETENTION, FROM CURDS MADE FROM 7% CA RCM TREATED WITH MICROFLUIDIZER GAUGE PRESSURES OF 0 MPA (CONTROL), 0.14 MPA (20 PSI), 0.41 MPA (60 PSI), AND 0.69 MPA (100 PSI)

Class Level Information		
Class	**Levels**	**Values**
milkID	4	1 2 3 4
Concentration	1	2
Pressure	4	0 20 60 100
Rep	6	1 2 3 4 5 6

Differences of Pressure Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer							
Pressure	**Pressure**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adj P**
0	20	-1.5686	0.4125	20	-3.80	0.0011	0.0057
0	60	-2.0066	0.4334	20	-4.63	0.0002	0.0009
0	100	-2.5233	0.7689	20	-3.28	0.0037	0.0180
20	60	-0.4380	0.3163	20	-1.38	0.1814	0.5228
20	100	-0.9546	0.7096	20	-1.35	0.1935	0.5462
60	100	-0.5166	0.7219	20	-0.72	0.4825	0.8897

Tukey-Kramer Grouping for Pressure Least Squares Means (Alpha=0.05)		
LS-means with the same letter are not significantly different.		
Pressure	**Estimate**	
100	82.6696	A
		A
60	82.1530	A
		A
20	81.7150	A
0	80.1464	B

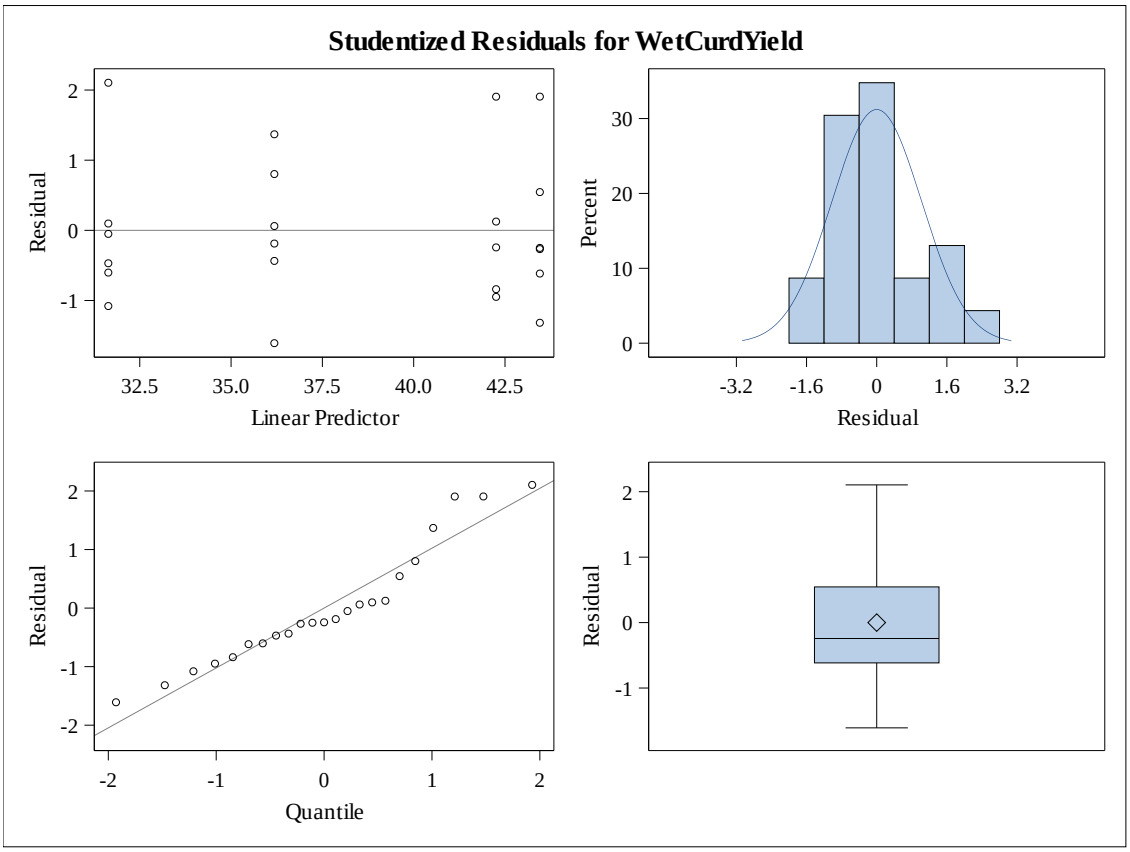

Studentized Residuals for ProteinRet
Residual
Linear Predictor
Percent
Residual
Residual
Quantile
Residual

APPENDIX N: GLIMMIX ANOVA PROCEDURE –WET CURD YIELD FROM CURDS MADE FROM 7% CA RCM TREATED WITH MICROFLUIDIZER GAUGE PRESSURES OF 0 MPA (CONTROL), 0.14 MPA (20 PSI), 0.41 MPA (60 PSI), AND 0.69 MPA (100 PSI)

Class Level Information		
Class	**Levels**	**Values**
milkID	4	1 2 3 4
Concentration	1	2
Pressure	4	0 20 60 100
Rep	6	1 2 3 4 5 6

Differences of Pressure Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer							
Pressure	**Pressure**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adj P**
0	20	4.5457	1.0366	19	4.39	0.0003	0.0017
0	60	-6.0712	1.0872	19	-5.58	<.0001	0.0001
0	100	-7.2693	1.0366	19	-7.01	<.0001	<.0001
20	60	-10.6170	1.0872	19	-9.77	<.0001	<.0001
20	100	-11.8150	1.0366	19	-11.40	<.0001	<.0001
60	100	-1.1980	1.0872	19	-1.10	0.2842	0.6927

Tukey-Kramer Grouping for Pressure Least Squares Means (Alpha=0.05)		
LS-means with the same letter are not significantly different.		
Pressure	**Estimate**	
100	43.4540	A
		A
60	42.2560	A
0	36.1848	B
20	31.6391	C

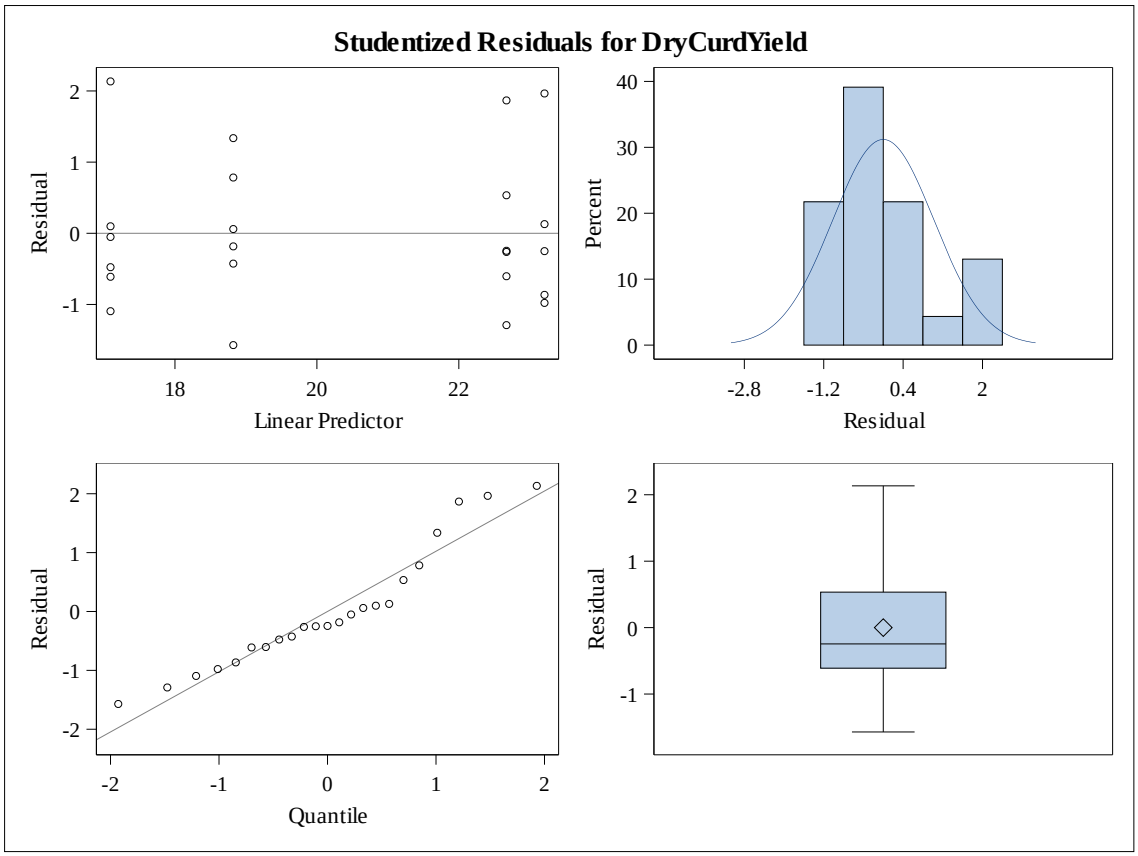

Studentized Residuals for WetCurdYield
Residual
Linear Predictor
32.5
35.0
37.5
40.0
42.5
Percent
Residual
-3.2
-1.6
0
1.6
3.2
Residual
Quantile
Residual

APPENDIX O: GLIMMIX ANOVA PROCEDURE –DRY CURD YIELD FROM CURDS MADE FROM 7% CA RCM TREATED WITH MICROFLUIDIZER GAUGE PRESSURES OF 0 MPA (CONTROL), 0.14 MPA (20 PSI), 0.41 MPA (60 PSI), AND 0.69 MPA (100 PSI)

Class Level Information		
Class	**Levels**	**Values**
milkID	4	1 2 3 4
Concentration	1	2
Pressure	4	0 20 60 100
Rep	6	1 2 3 4 5 6

Differences of Pressure Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer							
Pressure	**Pressure**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adj P**
0	20	1.7301	0.5521	19	3.13	0.0055	0.0258
0	60	-4.3852	0.5791	19	-7.57	<.0001	<.0001
0	100	-3.8491	0.5521	19	-6.97	<.0001	<.0001
20	60	-6.1153	0.5791	19	-10.56	<.0001	<.0001
20	100	-5.5792	0.5521	19	-10.10	<.0001	<.0001
60	100	0.5361	0.5791	19	0.93	0.3662	0.7915

Tukey-Kramer Grouping for Pressure Least Squares Means (Alpha=0.05)		
LS-means with the same letter are not significantly different.		
Pressure	**Estimate**	
60	23.2072	A
		A
100	22.6711	A
0	18.8220	B
20	17.0919	C


Studentized Residuals for DryCurdYield
Residual
Linear Predictor
Percent
Residual
Residual
Quantile
Residual

APPENDIX P: UNIVERSITY CHEDDAR CHEESE MANUFATURE AND SAMPLING

Transcribed Cheddar Cheese Make Record for Standard University Cheddar Cheese

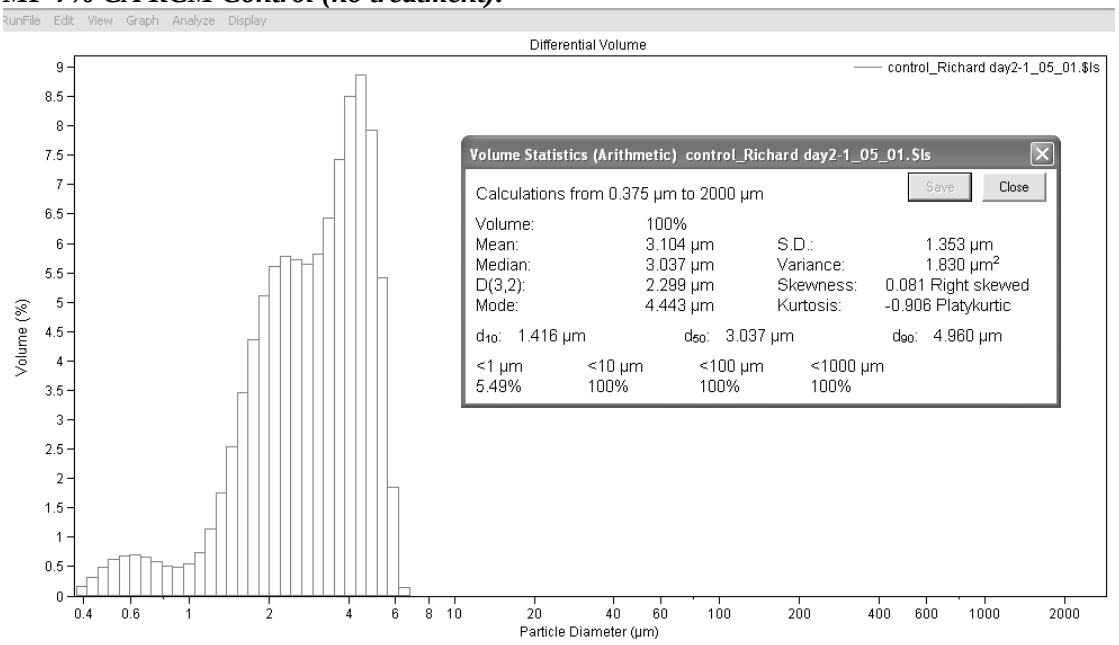
Milk in vat	lbs. milk	% Fat	% Protein	P/F	C/F	pH	Date
	300	3.76	3.30	0.878	0.711	6.67	11/15/2018

Curds Sampled #	Cheese making steps	Target Times	Actual Time	Target Temp (°F)	Actual (°F)	Target pH	Actual	Comments
1	Add Starter	-0:30	11:07	88	88		6.67	USU mixture of DVS and adjuncts
	Add Rennet	0:00	11:38	88	88.5		6.61	DS Chymosin, 12 ml diluted 1:20 with water
2	Cut	0:30	12:17	88				1/4" knifes, healed 10 min
3	Start Cook	1:05	1:00	88			6.50	Heat slowly by schedule, stir
4	End Cook	1:40	1:30	102			6.40	Increase stirring rate
5	Start Draining	2:25	1:53	102	103	6.30	6.32	Drain whey to just below curd level. Gently push curd to top half of vat, form trench in the middle and continue to drain, allow curd to matt together.
	End Draining	2:30		102				
6	Pack Curd	2:35	2:00			6.20	6.27	
	Cut and Turn	2:40		98				Cut curd matt into 6-7" wide slabs, turn every 20-30 min
7	Stack 2 High	3:20	2:46	95		5.85	5.97	turn every 20-30 min
	Stack 3 High	4:10	3:45	94	90	5.55	5.74	Turn every 20-30 min
8	Mill	4:35		91		5.40	5.47	Mill curd
9	Salt	4:40	5:00					Add 380 g salt over three applications 5 min apart
	Hoop	4:55	5:25	88				Hoop curd, 25 lbs. per hoop
	Press	5:15	5:45					30 psi pressure
10	Final Cheese		7:40					Curds sampled from cheese blocks

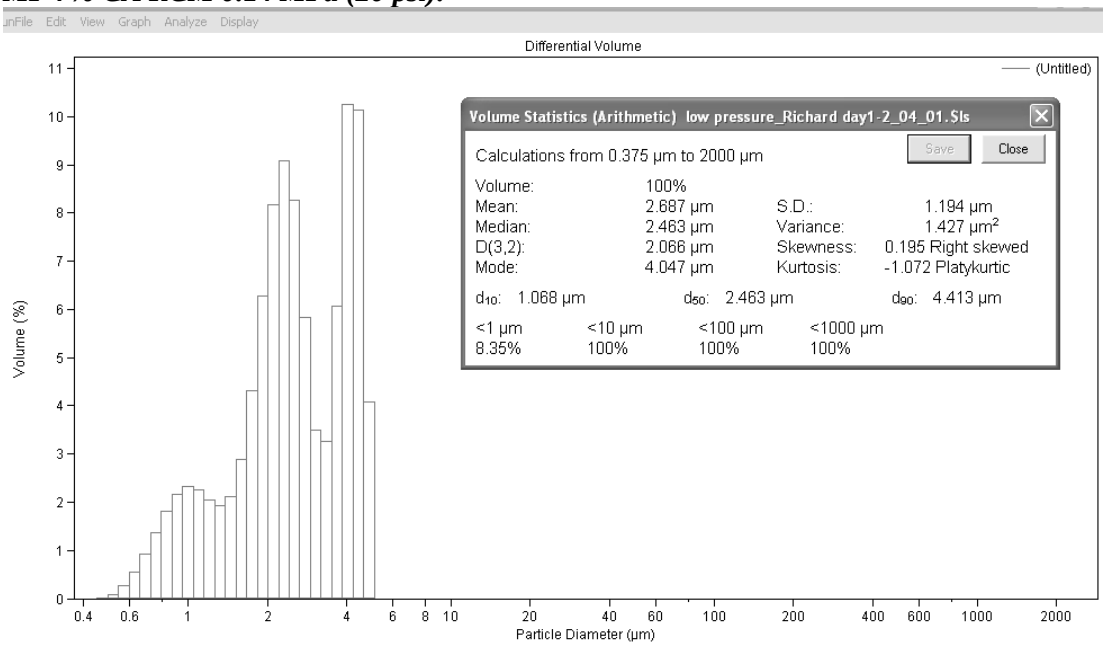
APPENDIX Q: INDIVIDUAL GRAPHICAL DATA OF HOMOGENIZATION EFFECTS ON FAT PATICLE SIZES DISTRUBUTIONS AS A PERCENT OF ALL FAT PARTICLES IN SAMPLE

Microfluidized 7% CA MF RCM at Gauge Pressure of:

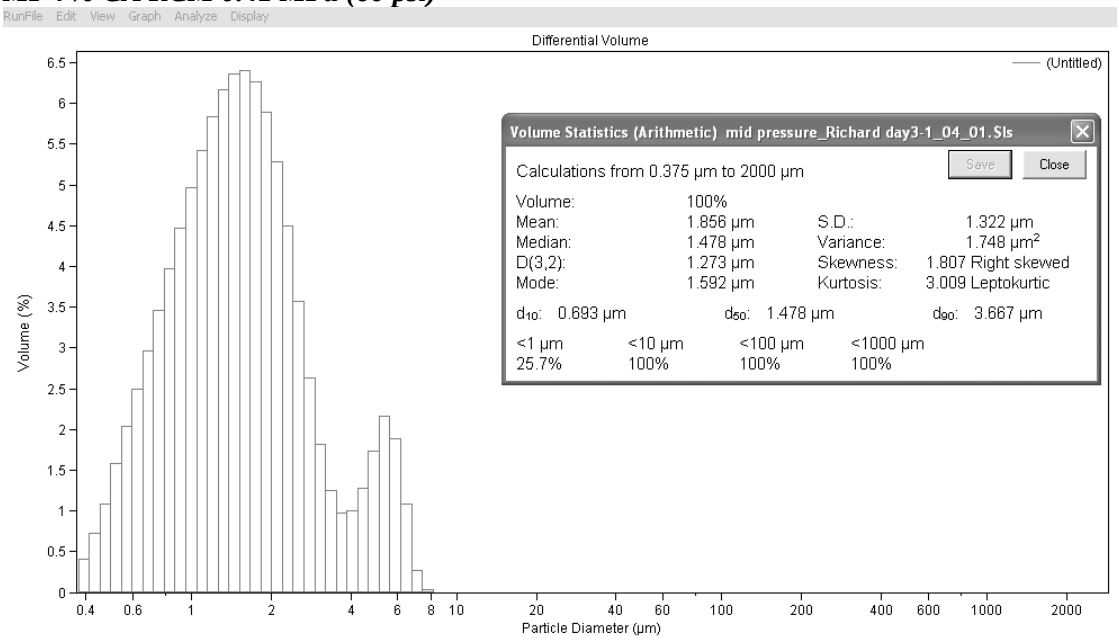
MF 7% CA RCM Control (no treatment):



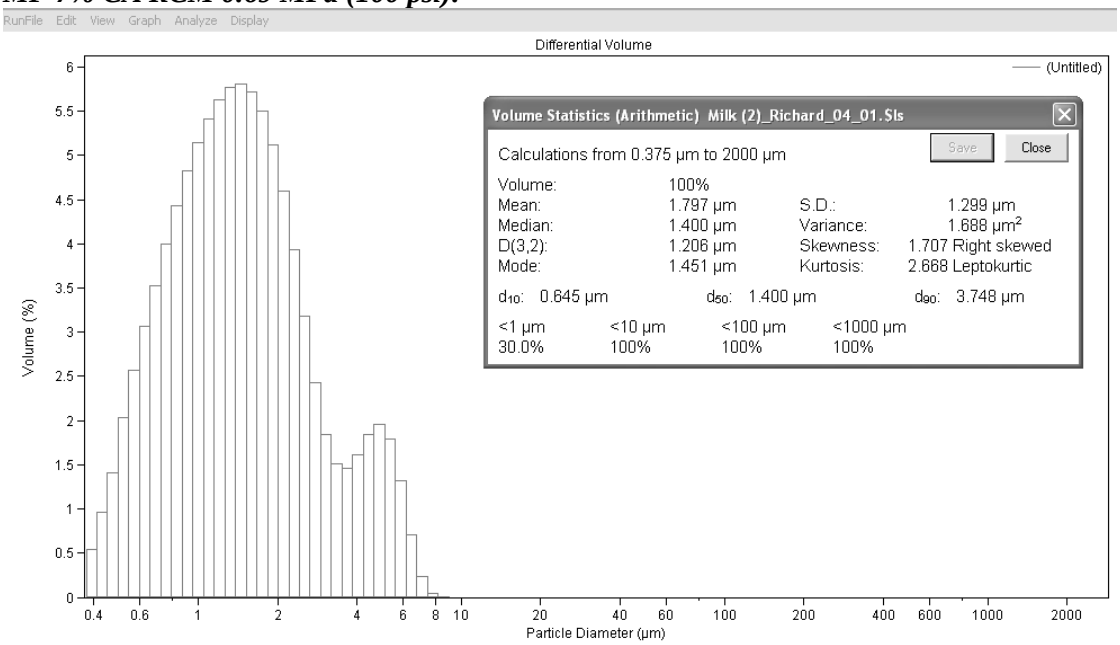
MF 7% CA RCM 0.14 MPa (20 psi):



MF 7% CA RCM 0.41 MPa (60 psi)

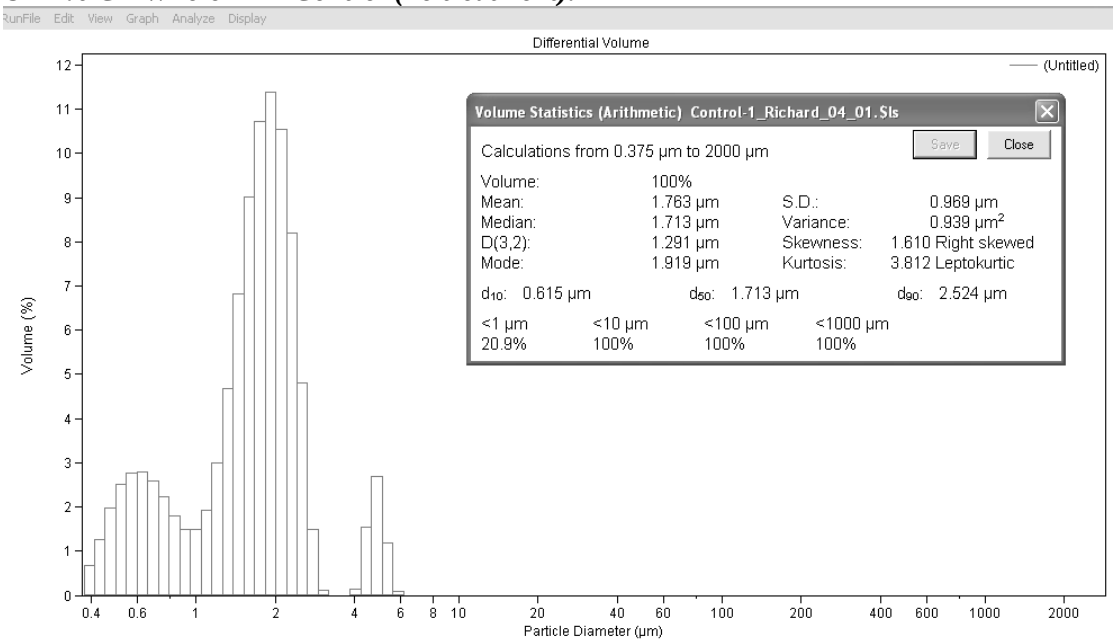


MF 7% CA RCM 0.69 MPa (100 psi):

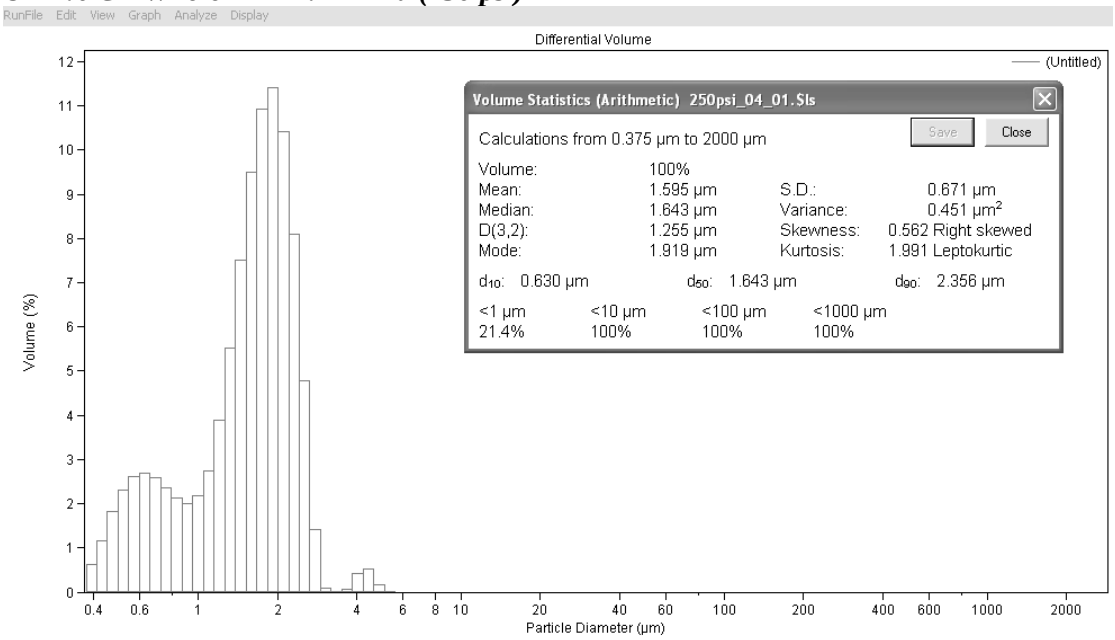


Homogenized (via 2-stage homogenizer) 7% CA UF whole milk at the following pressures:

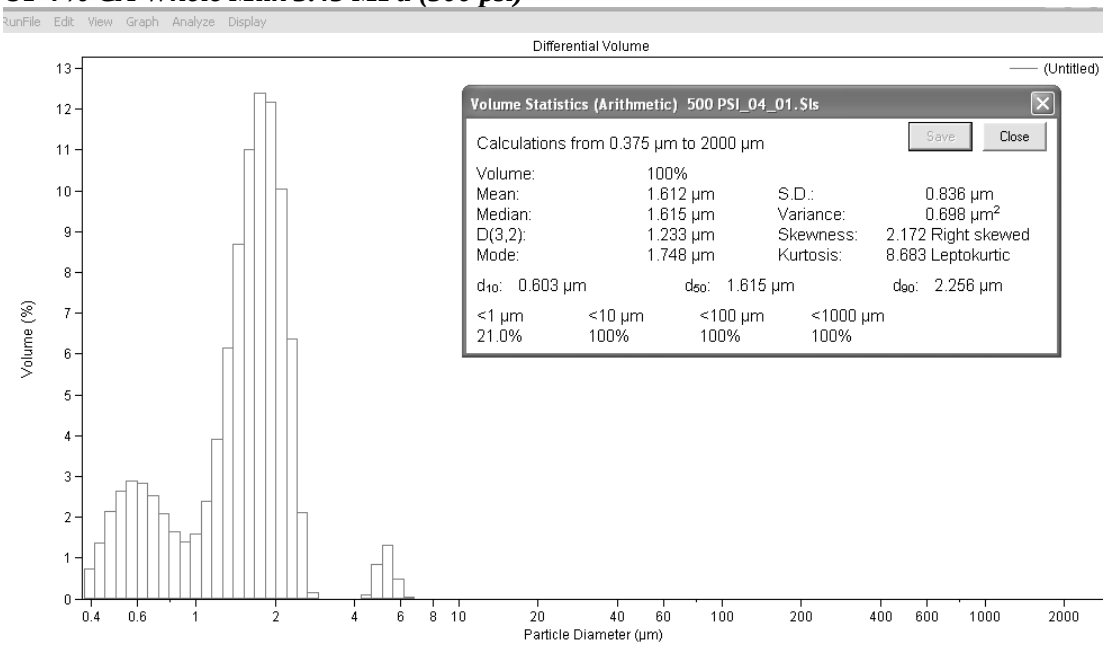
UF 7% CA Whole Milk Control (no treatment):



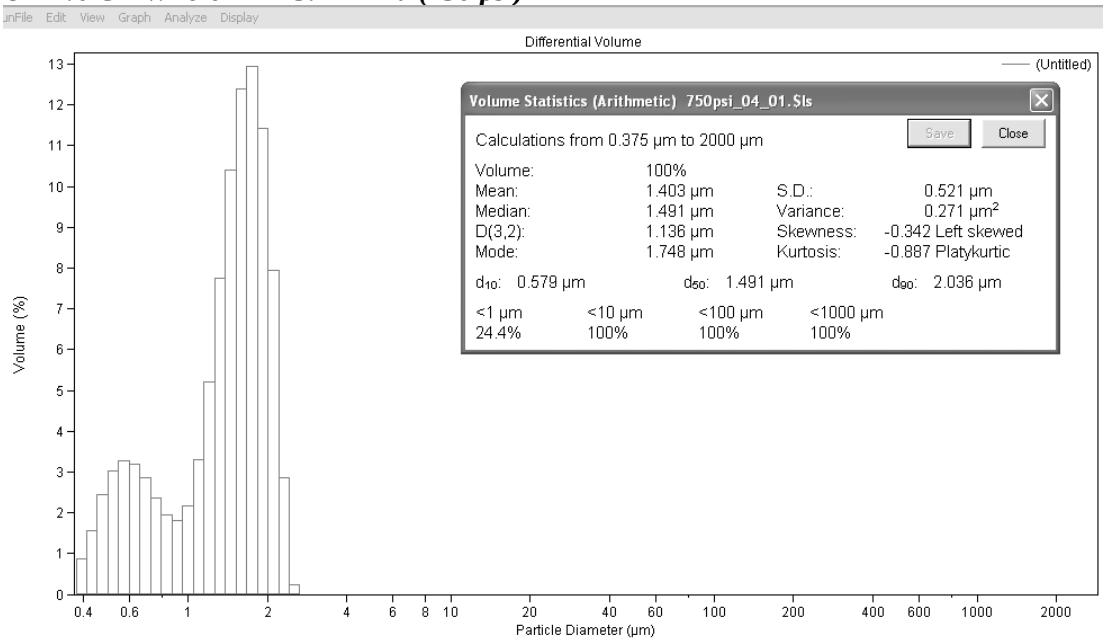
UF 7% CA Whole Milk 1.72 MPa (250 psi)



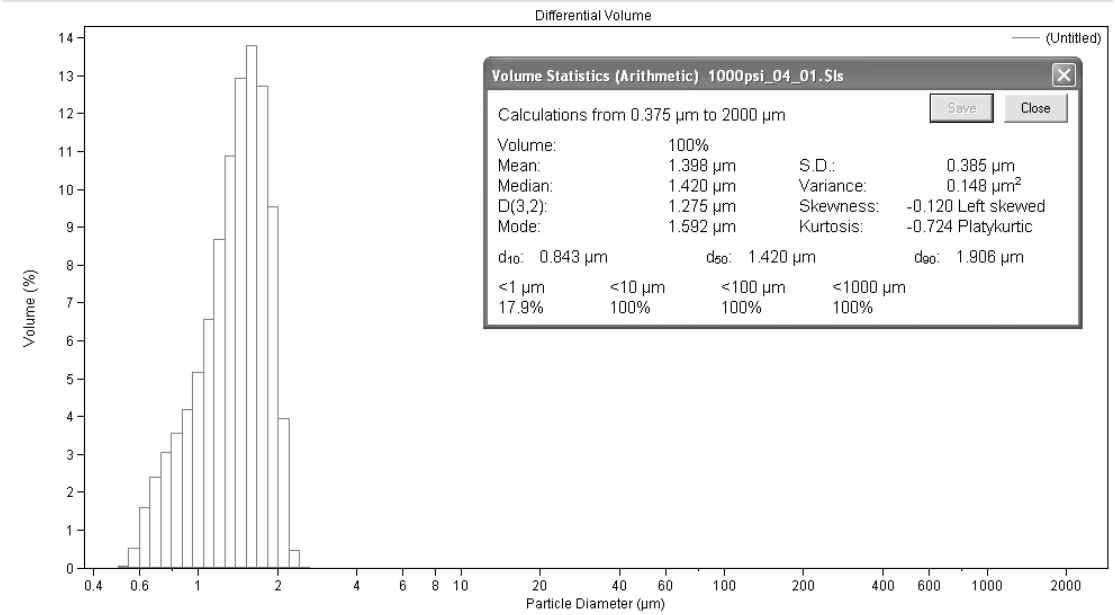
UF 7% CA Whole Milk 3.45 MPa (500 psi)



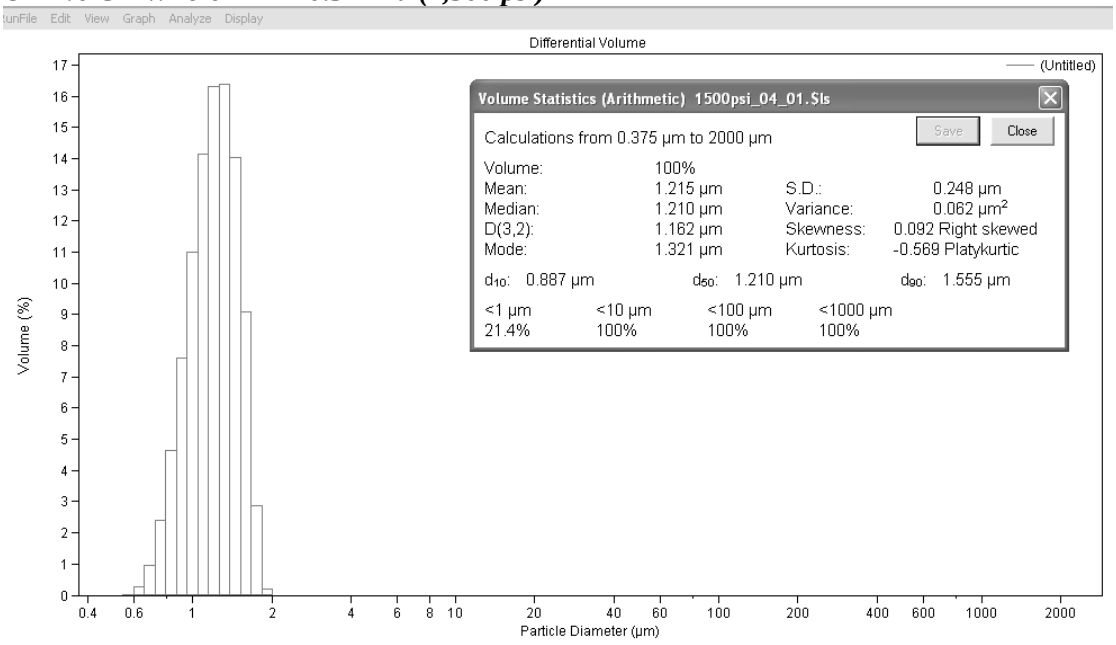
UF 7% CA Whole Milk 5.17 MPa (750 psi)



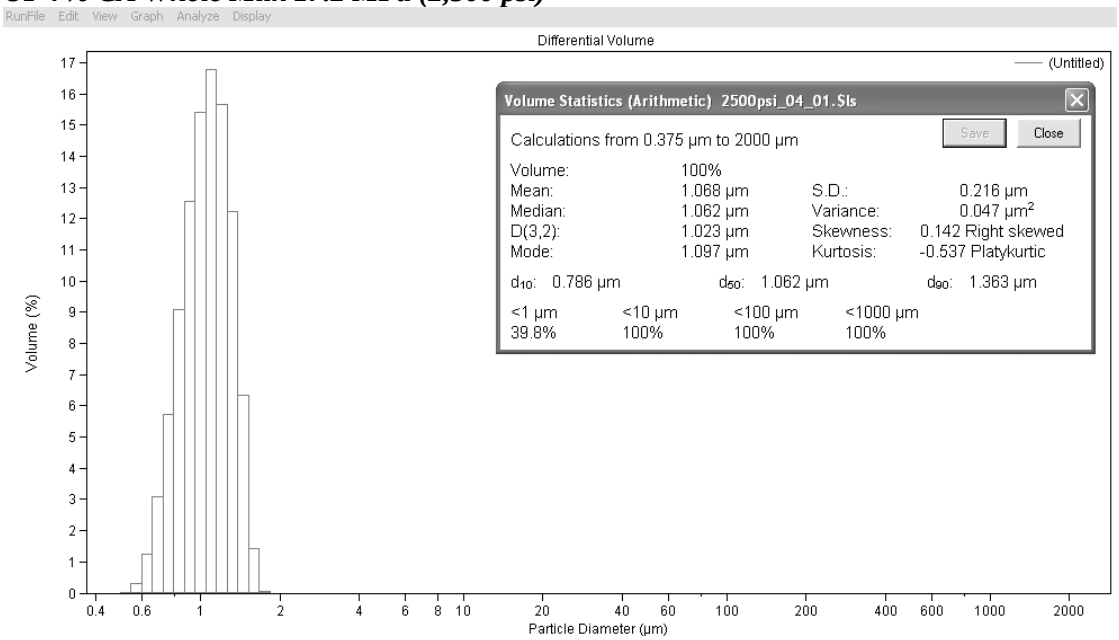
UF 7% CA Whole Milk 6.89 MPa (1,000 psi)



UF 7% CA Whole Milk 10.3 MPa (1,500 psi)



UF 7% CA Whole Milk 17.2 MPa (2,500 psi)



APPENDIX U: COMPOSITION OF RECOMBINED CONCENTRATED MILK (RCM) SAMPLES CONCENTRATED TO 7% CA THAT WERE TREATED WITH MICROFLUIDIZER GAUGE PRESSURES OF 0 MPA (CONTROL), 0.14 MPA (20 PSI), 0.41 MPA (60 PSI), AND 0.69 MPA (100 PSI)

RCM Sample treated with: Microfluidizer Gauge Pressure[1] of:	**Microfluidizer Total Pressure[1]**	**Protein (%)**	**Milk Fat (%)**	**C/F**
0	0	7.1	11.5	0.58
0.14 MPa	32.1 MPa	6.9	10.7	0.60
0.41 MPa	96.4 MPa	7.1	11.3	0.59
0.69 MPa	160 MPa	6.8	10.7	0.59

[1] Per the manufacturer, gauge pressure (GP) must be multiplied by a factor of 233 to find the total internal product treatment pressure inside the microfluidizer