PREPARATION AND CONCENTRATION OF STABLE WHEY MICRO-AGGREGATES

A Project Paper

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by

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ABSTRACT

During the trials to produce whey micro-aggregates, it was found that heating 6% whey protein isolates with 0.45% of caseins produced higher yield, and that with pH 5.6 to pH 6.1, the yield ranged from 67% to 76%. Different yield measurement methods were tested and it was found that centrifuging and drying the samples in an oven at 100°C for at least 24 hours produced the most consistent and reliable results. The bench-top trials were successfully scaled up to pilot plant trials as long as the conditions were kept the same. For the concentration of the microaggregates by membrane filtration, good retention and yield were obtained from the pilot plant trials when a 100,000 Molecular Weight Cut-Off Ultrafiltration Spiral Wound Membrane was used, resulting in a 4-fold concentration factor.

BIOGRAPHICAL SKETCH

Li Ann Yin Wai is part of the Olga Padilla-Zakour lab. She obtained her undergrad in food and nutritional science at the University of Hong Kong in 2018 and worked in the industry for more than 2 years before coming to Cornell to study the Master of Food Science.

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LIST OF ABBREVIATIONS

Abbreviation	**Meaning**
WPI	Whey protein isolates
WMA	Whey micro-aggregates
DI	De-ionized
BLG	Beta-lactoglobulin
NHS	Nestle Health Sciences

INTRODUCTION

1.1 Bovine Milk Proteins

Whey is a protein found in bovine milk and it makes up around 20% of the total protein, where casein makes up the other 80%. Whey proteins are globular proteins supported by intramolecular disulfide bonds and have an isoelectric point at pH 5.2 (Bovetto et al., 2005b). Beta lactoglobulins (BLG), alpha lactalbumin (ALA), bovine serum albumin (BSA), and immunoglobulins (IG) are some significant proteins and peptides found in whey. Under heat treatment, around 70°C, BLG unfolds and exposes a free thiol group along with hydrophobic residues. These groups interact with other molecules via covalent and hydrophobic bonds, which leads to aggregation. The aggregation of whey protein molecules is influenced by factors such as protein concentration, other protein species, temperature, and pH (Edwards and Jameson, 2014). Caseins form micelles where protein molecules are connected by calcium phosphate by ionic bridges. The isoelectric point for casein micelles is at pH 4.6 and a decrease in pH to this value causes precipitation (Horne, 2015). This precipitate is called acid casein and when hydroxide is added, water soluble caseinates are formed. Under heat treatment, casein micelles slightly dissociate, whereas whey proteins aggregate. It has been shown that whey proteins and caseins interact with each other when heated due to BLG forming disulfide bonds with kappa casein (Grindrod and Nickerson, 1967). Depending on the ratio of whey to casein, different sized aggregates can form, ranging from dimers to large heterogeneous aggregates (Cho et al., 2003). Similar to whey protein aggregation, this interaction depends on many factors, such as temperature, time, pH, and heating rate (Anema, 2014).

1.2 Formation of Whey Micro-aggregates

Whey proteins are desirable as an ingredient in the beverage industry for their nutritional composition and functionality. As stated previously, whey proteins tend to polymerize when heated. In beverages that are heat treated, which most are for safety reasons, whey protein aggregation could lead to undesirable changes in viscosity (Vardhanabhuti and Foegeding, 1999). One way to combat this is to separately heat the whey to create aggregates and add them as an ingredient. When aggregated, whey forms into strands or porous, cross-linked spheres that are more stable under heat treatment. These porous spheres can be referred to as micro-aggregates and have a lower volume fraction, thus are less thickening than strands when included in a beverage (Nicolai and Durand, 2013). Whey micro-aggregates (WMA) are typically smaller than 1000 nm in diameter and have a net charge. These characteristics give WMA a higher colloidal stability and the ability to withstand more heat, hence their desirability in beverages (Schmitt et al., 2009). Nestle Health Sciences (NHS) has proprietary technology to produce WMA and is continuing research before their use in products. The goal of this MFS capstone project was to work with NHS first by recreating WMA at the benchtop level. Then move up to pilot plant production at the Cornell AgriTech campus in Geneva, New York to produce a stable WMA.

1.3 Scope of Work

Formulation of our protein samples consisted of whey protein isolate and potassium caseinate in the ratios 10:1, 13.3:1, and 20:1. It has been shown in previous studies that casein acts as a chaperone protein for whey by binding to it and preventing reversible aggregation (O'Kennedy and Mounsey, 2006). These studies suggest that the whey to casein ratio 10:1 was effective in protecting whey aggregates from further aggregation and maintaining stability. For our purposes,

aggregation of whey protein is desired, but not to the extent of gelation. Thus, we chose to test the previously described ratios to replicate what the literature suggests and to see the lower limits of this functionality. Next, a 5% citric acid solution was used to adjust the pH from 5.6-6.4. It has been found that whey forms spherical aggregates at a pH slightly above the protein's isoelectric point where there are between 3 to 5 charges per protein (Nicolai, 2016). When the charge density of the proteins are higher, strands form and increase the solution's viscosity. We know whey has an isoelectric point of 5.2, thus our range was chosen to produce stable WMA and test the pH upper limit. The last step of WMA production includes heat treatment. During heating, whey aggregates into WMA in a two step process (Bovetto et al., 2005b). The first step is where the denatured monomers attach to one another via disulfide bridges. When the small aggregates are concentrated, they form the larger polymers that make up the WMA. We followed NHS parameters for heat treatment, which included heating the sample to about 90°C in under a minute and holding it for 15 minutes. To analyze how much of the whey formed into WMA, centrifugation at 26,900 g for 15 minutes at 20 °C will sediment any aggregates larger than 100 nm (Schmitt et al., 2011). Oven drying and weighing, both the pellet and supernatant, gives an accurate representation of WMA yield. Once lab samples proved the best protein concentration, whey to casein ratio, and pH, these conditions were used in pilot plant trials. Processing parameters, such as time and temperature, were replicated using a continuous pasteurizer and holding tube. Lastly, WMA were concentrated using membrane ultrafiltration.

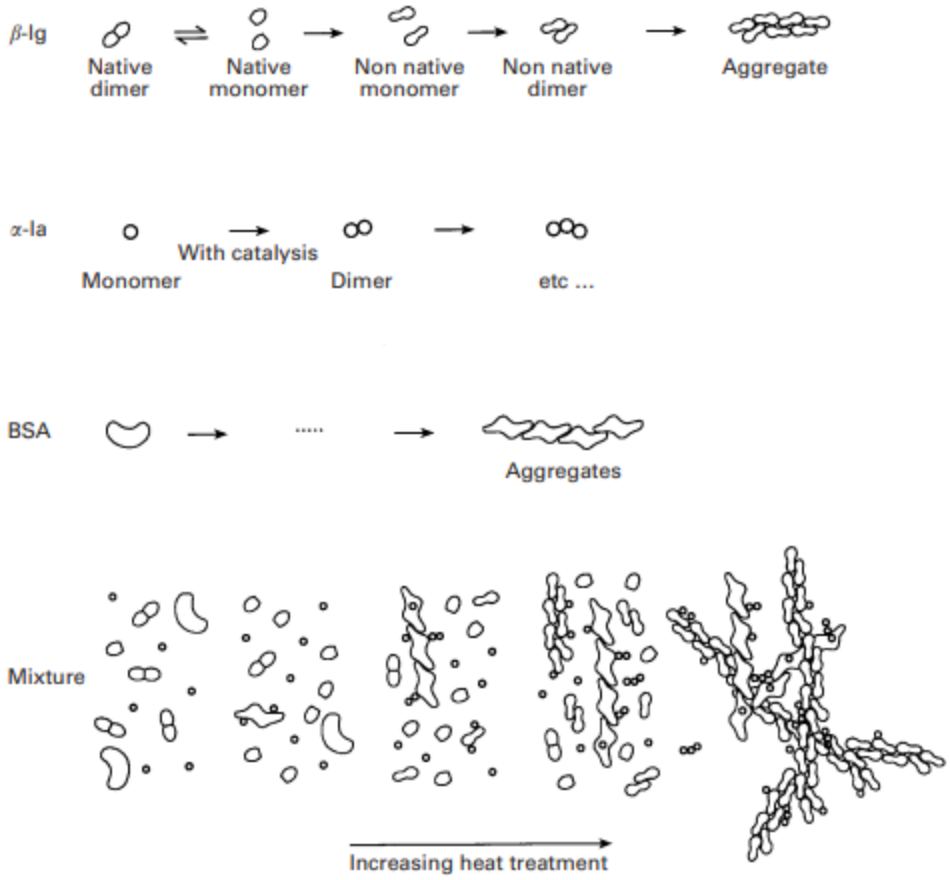


Figure 1.1: Mechanism of aggregation in beta-lactoglobulin (BLG), alpha-lactalbumin(ALA), bovine serum albumin (BSA), and their mixture (Havea *et al.,* 2001)

MATERIALS AND METHODS

2.1 Bench-top Experiments

2.1.1.1 Materials

Whey protein isolate (WPI), caseinate, and citric acid were obtained from Nestlé Health Science. pH 7 buffer solutions that were needed to perform the experiments were obtained from the lab.

2.1.1.2 Equipment and Analysis Kit

Equipment required for whey micro-aggregates (WMA) formulation and product formation is listed in table 2.1. Equipment and analysis kit used for determination of protein concentration and yield are listed in table 2.2.

Table 2.1: Equipment for whey micro-aggregates formulation and product formation

Device	**Manufacturer, City**
VWR Professional Series 7 x 7, ceramic Hotplate-Stirrer with Probe Kit and glassware	VWR International, Radnor
Thermo Scientific™ Orion™ Versa Star Pro™ Advanced Electrochemistry Meter	Thermo Fisher Scientific, Waltham
Thermo Scientific® S194925 Cimarec® Basic Economy Analog Magnetic Stirrer	Thermo Fisher Scientific, Waltham
Fisher Scientific Isotemp 220 Digital Water Bath	Thermo Fisher Scientific, Waltham
Sorvall® RC-5B Refrigerated Superspeed Centrifuge	Thermo Fisher Scientific, Waltham
Fisher Scientific Isotemp Oven	Thermo Fisher Scientific, Waltham

Table 2.2: Measurement devices and analytical kits

Device	**Manufacturer, City**
Thermo Scientific™ GENESYS™ 20 Visible Spectrophotometer	Thermo Fisher Scientific, Waltham
Quick Start™ Bradford Protein Assay	Bio-Rad Laboratories, Hercules
VWR Turbidity Meter KT1 (Serial No. 3048536)	VWR International, Radnor
Malvern Mastersizer 2000 with Hydro 2000s Particle Size Analyzer	Malvern instruments Ltd., Westborough, MA

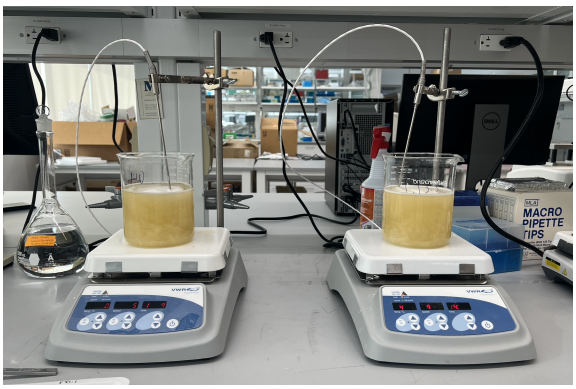
2.1.2 Whey Micro-Aggregate Production

The protein concentration, WPI to casein ratio, and pH for the WPI formulation are listed in table 2.3. The WPI and casein were weighed and added to deionized (DI) water at 40°C under constant stirring. The WPI solutions were hydrated overnight in refrigerated conditions before pH adjustment. The pH of the WPI solutions were adjusted by slowly dropping 5% citric acid solution under constant stirring with the magnetic stirrer at room temperature. Fifteen ml of samples were transferred to glass tubes and sealed completely with a screw cap. They were preheated to 50°C, then added to a water bath set at 95°C to adequately heat samples to 85°C within 1 minute with manual agitation. Samples were immersed in the water bath for 15 minutes in total, and cooled down immediately in an ice bath for 20 minutes.

Samples were diluted in half by adding 10 ml of DI water to 10 ml of the samples in the centrifuge tubes. Samples were then centrifuged at 26,900g for a total of 70 minutes at 20°C to get a clear supernatant. The supernatant was then separated from the pellet. Both the supernatant and the pellet were placed in the aluminum drying pans half-filled with dried sand. The samples were dried in the oven at 100°C for at least 24 hours to get dry protein weight. Figure 2.1 shows the set-up of the WMA production.

Table 2.3 Experimental design for the preparation of whey micro-aggregates

Whey Protein Isolate (WPI)/casein solutions (%)	**WPI Concentration (%)**	**Casein (%)**	**Ratio (Casein:WPI)**	**pH**
4.4	4	0.4	1:10	5.9, 6.0, 6.1
6.6	6	0.6	1:10	6.1, 6.2, 6.3
6.45		0.45	1:13.3	5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3
6.3		0.3	1:20	6.1, 6.2, 6.3
8.8	8	0.8	1:10	6.1, 6.2, 6.3, 6.4
8.6		0.6	1:13.3	6.2, 6.3, 6.4
8.4		0.4	1:20	6.1, 6.2, 6.3, 6.4
11	10	1.0	1:10	6.1, 6.2, 6.3, 6.4
10.75		0.75	1:13.3	6.1, 6.2, 6.3, 6.4
10.5		0.5	1:20	6.1, 6.2, 6.3, 6.4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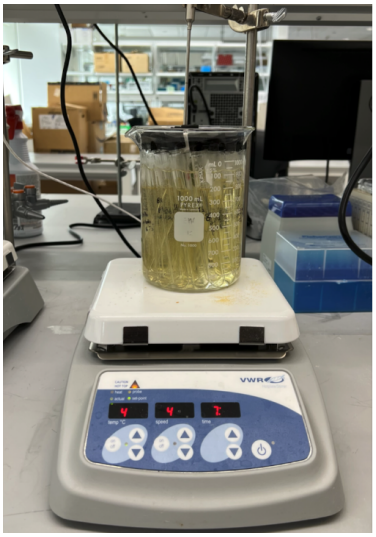
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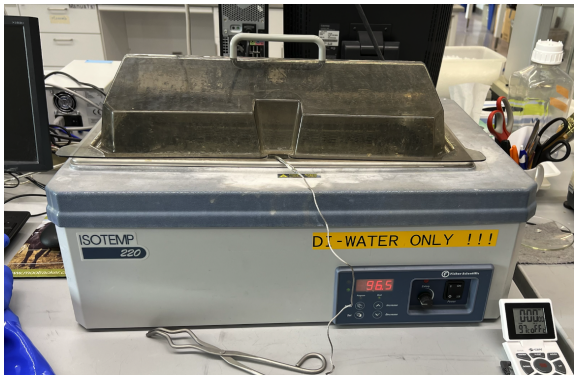
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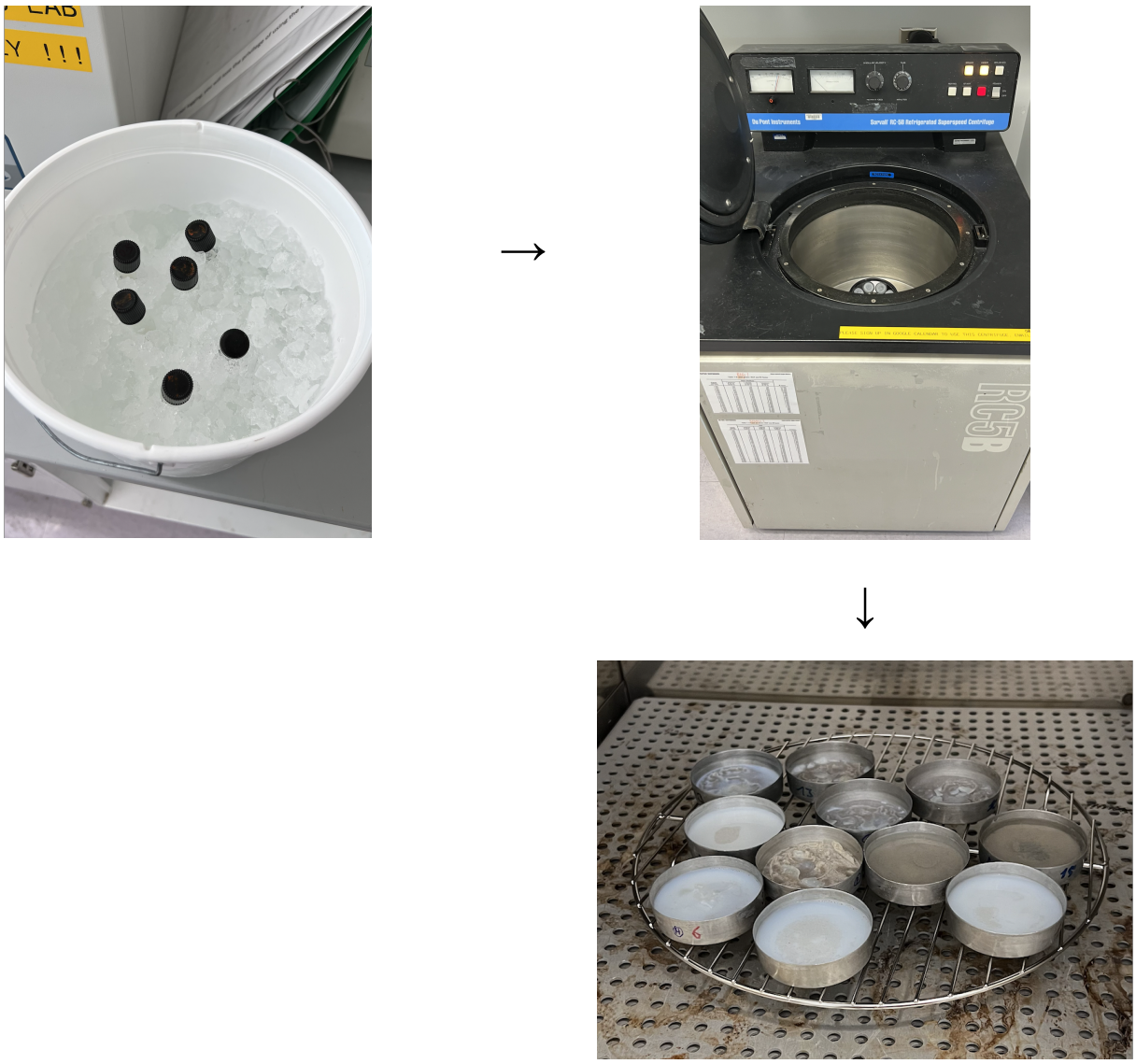

Figure 2.1: Set-up of the whey micro-aggregate production

2.1.3 Yield Measurement

The weight of both the supernatant and the pellet in the samples centrifuged at 26,900g for a total of 70 minutes at 20°C were measured before and after drying in the oven at 100°C for at least 24 hours. The WMA yield was calculated with the formula: (Dry weight of pellet)/(Dry weight of supernatant + pellet).

2.1.4 Turbidity

Turbidity was measured on the heated samples diluted 1:50 with a turbidity meter.

2.2 Pilot Plant Trials

2.2.1.1 Materials

All ingredients were the same as those used in the bench-top experiments and were obtained from Nestlé Health Science.

2.2.1.2 Equipment

Equipment required for WMA formulation and product formation in the pilot plant is different from the ones used in the bench-top experiments and is listed in table 2.4.

Table 2.4: Pilot plant equipment used for scale-up trials

Device	Manufacturer, City
MicroThermics® UHT/HTST Lab-25 HVHW Homogenizer (Serial No.: 3216806.1)	MicroThermics, North Carolina
Alfa Laval Membrane Filtration Module (Type: LabStak® M39L/H-1-1)	Alfa Laval Nakskov A/S, Denmark
Alfa Laval Ultrafiltration Spiral Wound Membrane UF GR40PP-3838/48 (Size: 100,000 MWCO)	Alfa Laval Nakskov A/S, Denmark

2.2.2 Whey Micro-Aggregates Production

120 kg of 6.45% WPI/Casein solution was used to make pH 6.1 and 5.8 samples for pilot plant production. Protein solutions were made in accordance with previous benchtop methods, except that mixing was done in large steam kettles from *Lee Industries* with immersion blenders to heat up the sample to 50°C. A Microthermics continuous pasteurizer attached to a holding tube was used to get the sample to 90 °C and hold for 15 minutes. A flow rate of around 1 L/min was achieved and the holding apparatus proved to work well as it was a tube-in-tube set up with hot water recirculating in the outer tube at 85 °C. Once finished, the sample circulated back through the cooling section of the pasteurizer to reach room temperature. Figure 2.2 and 2.3 show the set-up of the microthermics and holding tube, and the microthermics display screen showing the conditions that the samples were processed on.

Figure 2.2: Microthermics and holding tube set-up

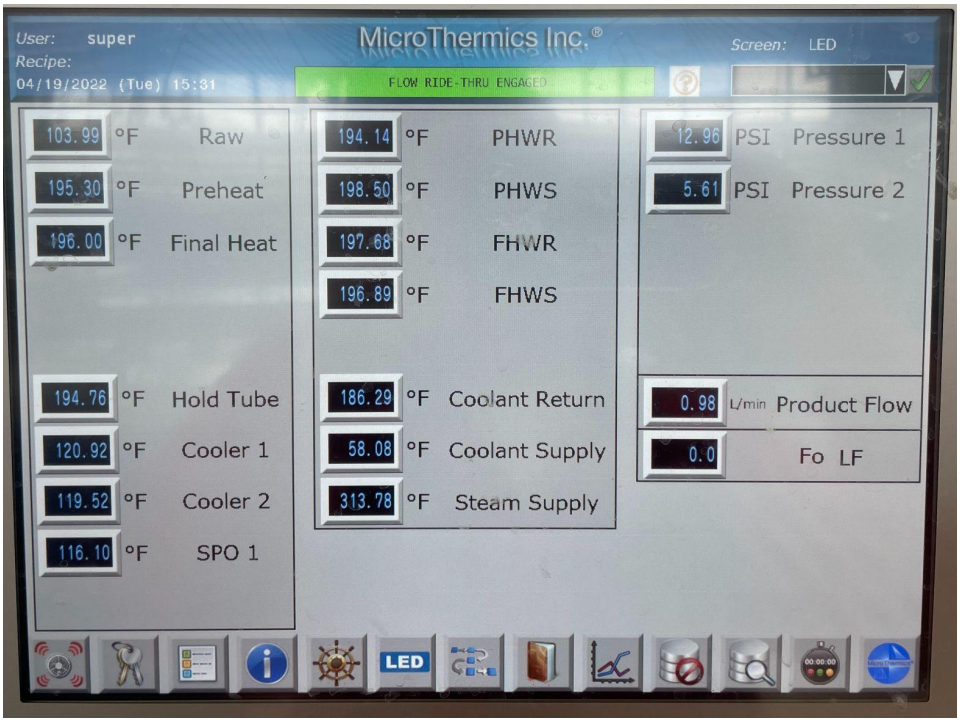

Figure 2.3: Microthermics display screen during heat treatment

2.2.2.1 Whey Micro-Aggregate Concentration

After heating, the WMA solution underwent membrane ultrafiltration to further concentrate the aggregates. Prior to filtration, potassium hydroxide (KOH) was used to standardize the pH of the samples up to 6.7. A size 100K spiral wound membrane was used and the product was run until flow rate stopped. Specific parameters of the membrane filtration process can be found in table 2.5 and in figure 2.4.

Table 2.5: Processing conditions for membrane ultrafiltration to concentrate the whey micro-aggregates

	Trial 1 (pH 6.1)	Trial 2 (pH 5.8)
Retentate Flow Rate	ave. 2800 L/h	ave. 3400 L/h
Pressure	5 bar	5 bar
Time	1.5 h	1.4 h
Temperature	25 °C	25 °C

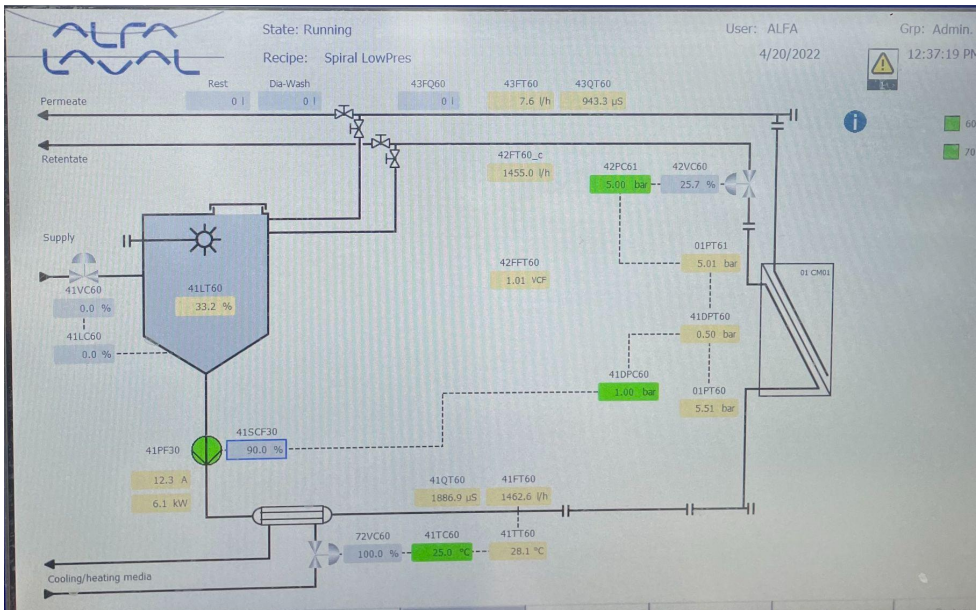

Figure 2.4: Display screen during membrane ultrafiltration

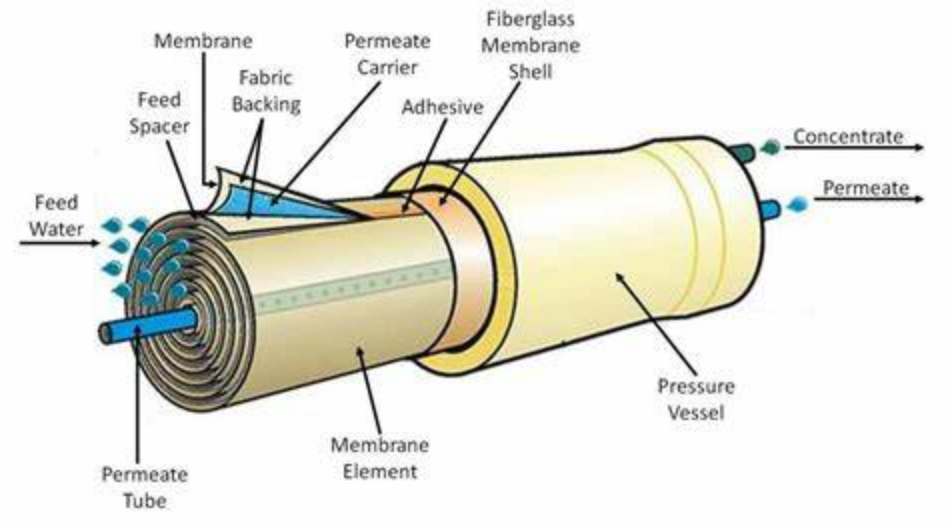

Figure 2.5: Diagram of spiral wound membrane filter
(www.porex.com)

2.2.3 Yield Measurement

Samples were taken from the pilot plant to the lab for analysis of the yield. The samples were centrifuged and dried using the same method as that of the bench-top experiments as explained in 2.1.3.

RESULTS AND DISCUSSIONS

3.1 Bench-top Experiments

3.1.1 Physical Observations

We conducted many different bench top experiments to test various parameters on the protein solutions. First, a noticeable observation could be the samples turning from a translucent yellow tint to a milky white once heated to 90 °C (Figure 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7). As seen in the experimental design, a wide range of protein concentrations and pH values were tested. We know that far from the isoelectric point of whey protein, aggregates can become unstable and form a viscous liquid due to interactions between strong ionic charges on the proteins. We wanted to test the upper and lower limits of pH values and found an upper limit for failure. However, we tested down to pH 5.6 and still did not see an undesirable viscosity. This is likely due to whey having an isoelectric point of 5.2, thus we were still quite far from being at the lower limit. Our partners at NHS were not planning on using this ingredient at a pH value that low so we did not continue. The upper limit occurred at pH 6.4 in the 11% WPI/Casein solution where we saw gelation of the proteins during heating (Figure 3.8). NHS parameters for centrifugation to sediment WMA called for 26,900 g for 15 minutes at 20°C. However, during our benchtop experiments, we were unable to fully attain a clear supernatant and solidified pellet with these parameters (Figure 3.9). We had to dilute the heated protein solutions by half with DI water and increase the time to 70 minutes to see proper separation (Figure 3.10). In all of our calculations following, this dilution factor was taken into consideration. Another observation regarding the pellet after centrifugation is its consistency varying between pH values. Figure 3.12 shows the 11% WPI/Casein pellet sample at pH 6.1 and Figure 3.11 shows the same protein concentration at pH 6.3. The pellet in the pH 6.1 sample was much more firm and rigid while the pellet in the pH 6.3 sample was more

gooey and did not hold together as well. One reason for this could be that since pH 6.1 is closer to the isoelectric point of whey protein, more stable WMA formed. Adding to this is that more complete WMA formed, as opposed to smaller strand aggregates that are known to increase viscosity. It would make sense that a higher yield of larger WMA would sediment into a more dense pellet during centrifugation. Lastly, differences observed in the dried samples between pellet and supernatant were consistent. Pellets were extracted from the centrifuge tubes in chunks and those dried to show solid caramel colored pieces throughout the can. On the other hand the supernatant was simply poured into the can and when dried, created a caramel crust over the sand (Figure 3.13).

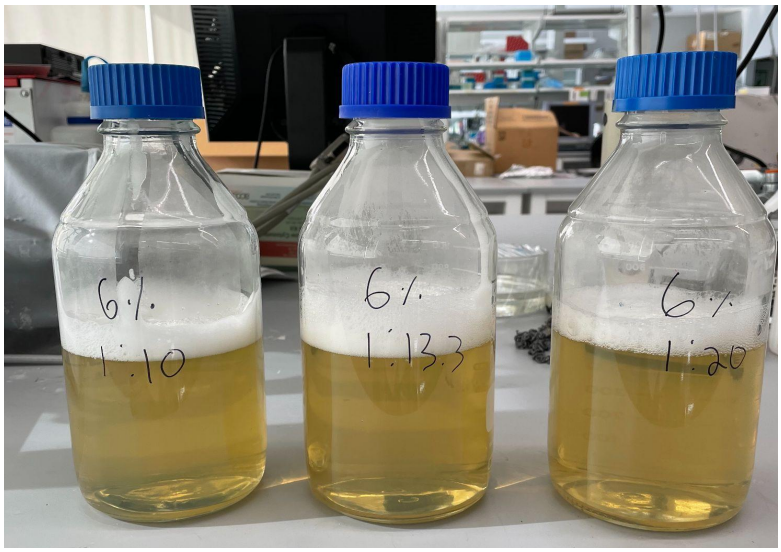

Figure 3.1: (from the left) Unheated WPI/Casein solutions of 6.6%, 6.45%, 6.3% before pH adjustment

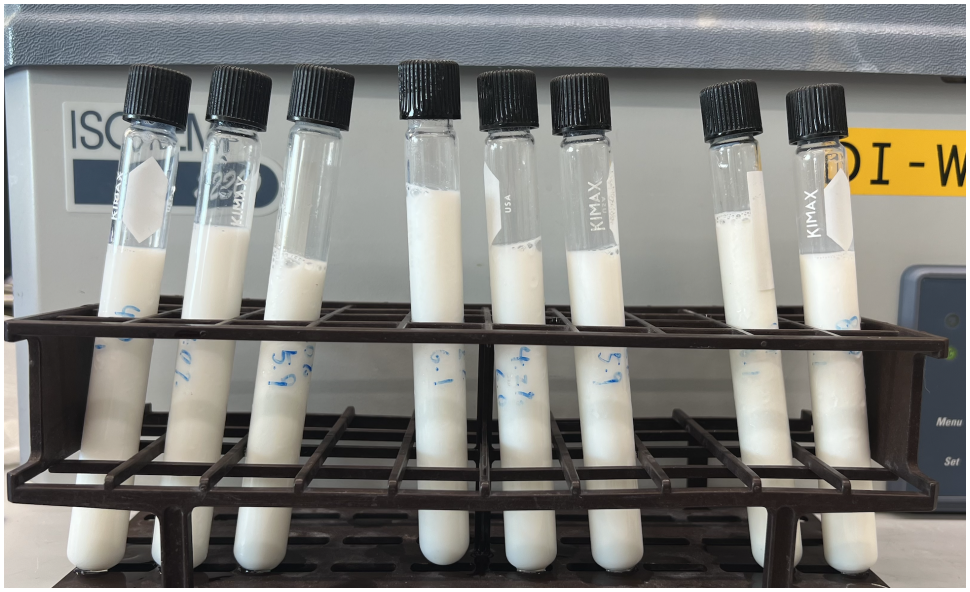

Figure 3.2: (from the left) Heated WPI/Casein solutions of 4.0% pH 6.1, 4.0% pH 6.0, 4.0% pH 5.9, 4.2% pH 6.1, 4.2% pH 6.0, 4.2% pH 5.9, 8.4% pH 6.1, 8.8% pH 6.1

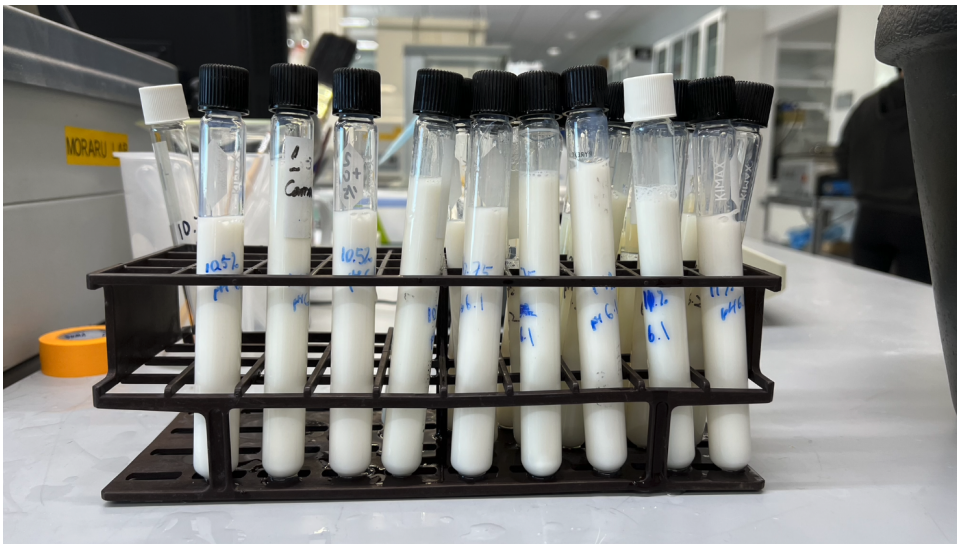

Figure 3.3: (from the left) Heated WPI/Casein solutions of 10.5% pH 6.1, 10.5% pH 6.2, 10.5% pH 6.3, 10.5% pH 6.4, 10.75% pH 6.1, 10.75% pH 6.2, 11% pH 6.1

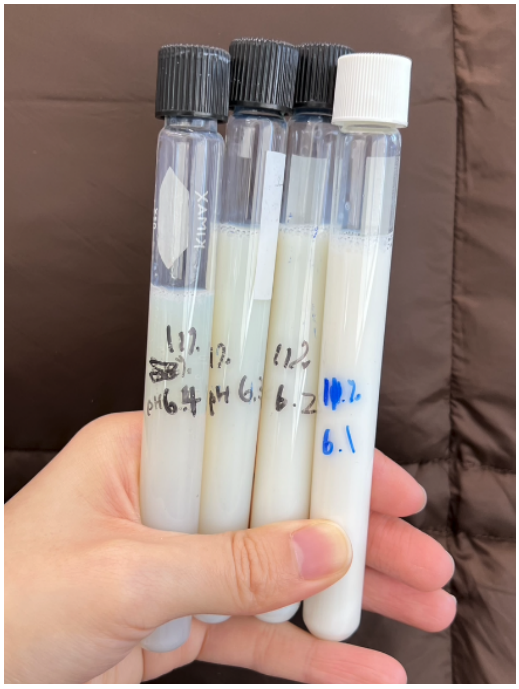

Figure 3.4: (from the left) Heated WPI/Casein solutions of 11% pH 6.4, 11% pH 6.3, 11% pH 6.2, 11% pH 6.1

Figure 3.5: (from the left) Unheated WPI/Casein solutions of 6.45% pH 5.6, 6.45% pH 5.7, 6.45% pH 5.8, 6.45% pH 5.9, 6.45% pH 6.0, 6.45% pH6.1, 6.45% pH 6.1 (20 ml), 6.45% pH 6.1 (10 ml)

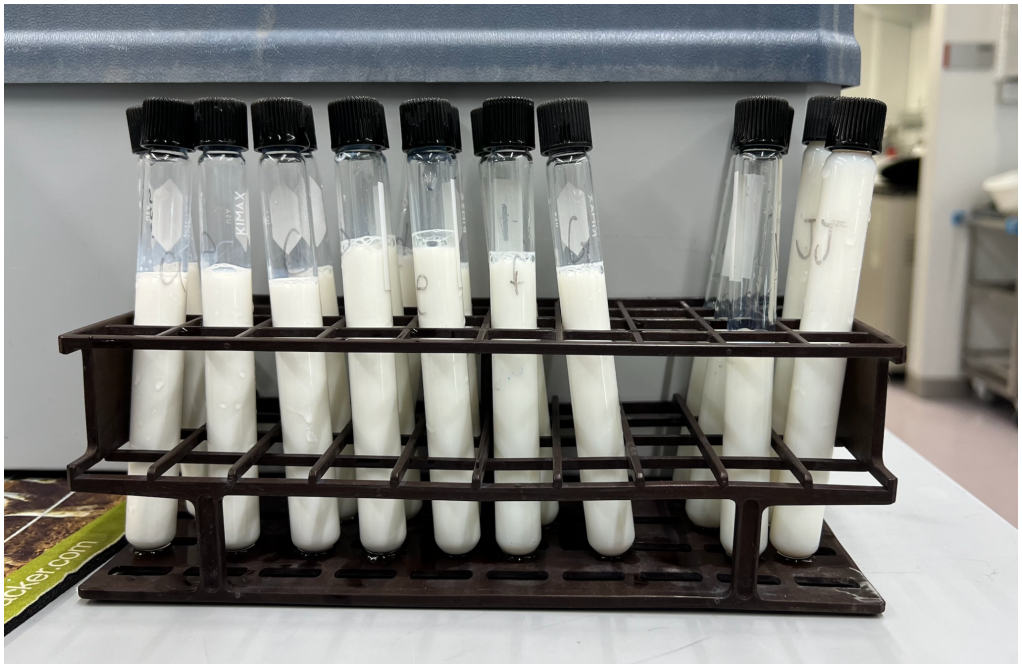

Figure 3.6: (from the left) Heated WPI/Casein solutions of 6.45% pH 5.6, 6.45% pH5.7, 6.45% pH5.8, 6.45% pH5.9, 6.45% pH6.0, 6.45% pH6.1 (heated at 85°C), 6.45% pH6.1 (heated at 90°C), 6.45% pH6.1 (10 ml, heated at 85°C), 6.45% pH6.1 (20 ml, heated at 85°C)

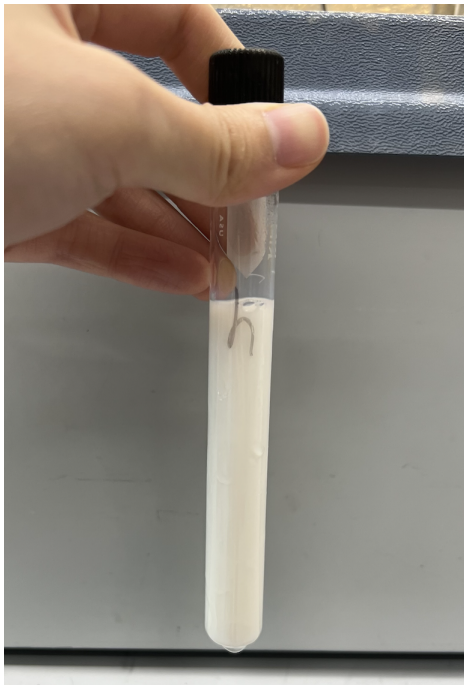

Figure 3.7: Heated WPI/Casein solutions of 6.45% pH 6.1 (15 ml, heated at 95°C)

Figure 3.8: Failure of heated WPI/Casein solutions of 11%, pH 6.4

Figure 3.9: Centrifugation failure

Figure 3.10: WPI/Casein solutions after successful centrifugation

Figure 3.11: pH 6.3 11% WPI/Casein pellet

Figure 3.12: pH 6.1 11% WPI/Casein pellet

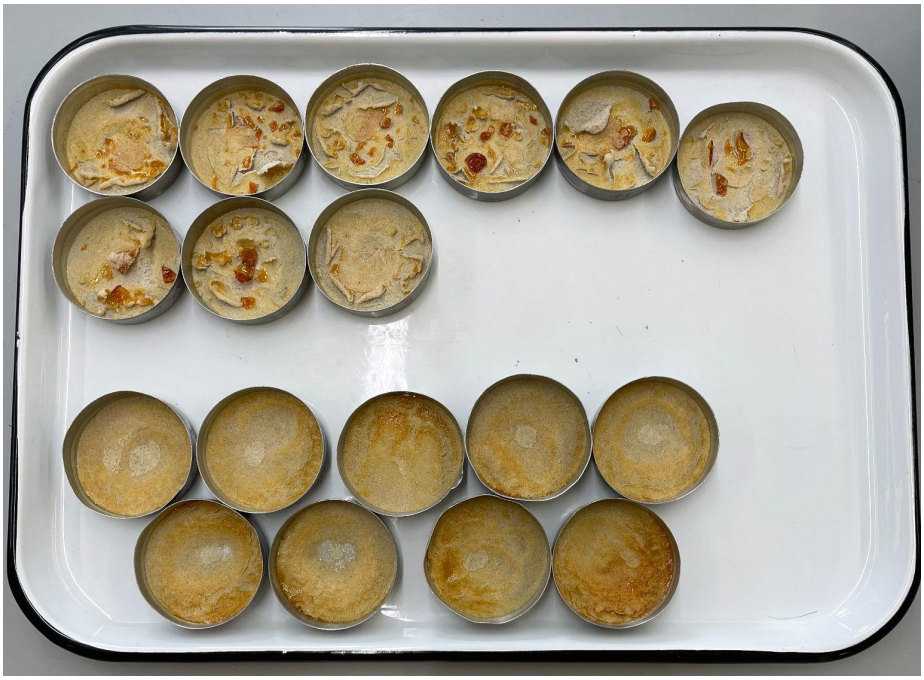

Figure 3.13: Samples after drying at 100°C for at least 12 hours. Top is pellet and the bottom is supernatant.

3.1.2 Yield

3.1.2.1 Whey Micro-Aggregate Yield of WPI/Casein solutions heated at 85°C

The WMA yield of the WPI/Casein solutions are shown in table 3.1. It was found that 6.45% WPI/Casein solutions with pH 5.6 to pH 6.1 achieved the highest yield when heated at 90°C. Across all protein concentrations, we found that pH values on the lower end of our range gave us better WMA yield than the range of upper pH values. This is consistent with our findings from physical observations in section 3.1.1, specifically in the instance of pellet consistency. A more dense and firm pellet was observed at lower pH values, which seems to indicate a higher WMA yield.

Table 3.1: Yield (% w/w) of micro-aggregates from different concentrations and pH of WPI/Casein solutions

Protein	**4.0%**	**4.2%**	**6.3%**	**6.45%**	**6.6%**	**8.4%**	**8.6%**	**8.8%**	**10.5%**	**10.75%**	**11%**
pH=5.6				75							
pH=5.7				76							
pH=5.8				72							
pH=5.9	68	59		72							
pH=6.0	66	61		67							
pH=6.1	59	60	67	68	67	67		66	67	67	67
pH=6.2			63	63	62	63	63	58	63	62	61
pH=6.3			56	58	55	55	58	45	59	54	63
pH=6.4						48	44	35	54	62	

3.1.2.2 Effect of pH on yield

As mentioned in section 3.1.1, samples had to be diluted by half with DI water before centrifugation to get sufficient separation. A concern was that this dilution with DI water was affecting the protein's charge and altering the yield. Since NHS plans to have this product standardized to pH 6.7-6.8, we used a pH 7 buffer for these dilutions to achieve this pH range in samples. After heating, 6.45% WPI/Casein solutions with pH 5.6, 5.8, 6.0, 6.1 were adjusted to around pH 6.8 by the addition of pH 7 buffer to the solutions according to the ratio in table 3.2. The table shows the pH before and after dilution, along with the WMA yield after oven drying. The yield of 6.45% WPI/Casein solutions with pH 5.6 and 5.8 were higher than that with pH 6.0 and 6.1. This is consistent with the data shown in table 3.1, thus it can be inferred that the sample's pH standardization does not affect the trends for WMA yield.

Table 3.2: The pH of 6.45% WPI/Casein solutions with pH 5.6, 5.8, 6.0, 6.1 before and after pH adjustment, the pH7 buffer : sample ratio, and the yield

pH before adjustment	**pH7 buffer : sample ratio**	**pH after adjustment**	**Yield (%)**
5.6	40/60	6.8	62
5.8	50/50	6.79	64
6.0	50/50	6.83	56
6.1	60/40	6.82	55

3.1.2.3 Effect of thermal variance on the yield

To test how sensitive WMA yield is to heating temperature, we used multiple samples of 6.45% WPI/Casein at pH 6.1 and heated it at 85°C, 90°C, and 95°C. Table 3.3 shows the WMA yield at each temperature tested. We found that there is a specific point where yield significantly increases somewhere between 85°C and 90°C. However, from 90°C to 95°C there is little difference in yield, thus 90°C seems to be the optimal temperature.

Table 3.3: Effect of thermal variance on the yield of 6.45% WPI/Casein solution with pH 6.1

Heating Temperature of 6.45% WPI/Casein solution with pH 6.1 (°C)	**Yield (%)**
85°C	59%
90°C	67%
95°C	68%

3.1.2.4 Effect of tube filling on the yield

Another concern in our benchtop experiments was if tube fill was a significant factor in WMA yield. Since we used 20 mL glass capped tubes submerged in a water bath, we wanted to see if filling the tube half way versus fully would impact yield. Our results (table 3.4) show that there was no significant effect from tube fill.

Table 3.4: Effect of tube filling on the yield of 6.45% WPI/Casein solution with pH 6.1

Tube filling of 6.45% WPI/Casein solution with pH 6.1	**Yield (%)**
Half filled (10ml)	57
Fully filled (20ml)	56

3.1.3 Yield Measurement

3.1.3.1 Absorbance

We diluted the unheated and heated WPI/Casein solutions to a concentration of 1% (Wprotein/W), then centrifuged the heated WPI/Casein solutions at 26,900g for 20 mins (including 5 mins warm up time for the centrifuge machine) at 20 ℃. The supernatant of the heated solution and the unheated WPI/Casein solution was then diluted by 1/10. The absorbance of both unheated WPI/Casein solutions and diluted supernatant of heated WPI/Casein solution were measured at 280 nm to find out the yield. The yields obtained were inconsistent and were not aligned with the yields obtained by NHS, thus we determined that absorbance was not a reliable method for yield measurement for WPI/Casein solution.

3.1.3.2 Bradford Protein Assay

The samples that were prepared in section 3.1.3.1 were also analyzed using Bradford Protein Assay. However, the yields obtained were inconsistent and were not aligned with the yields obtained by NHS, thus we determined that absorbance was not a reliable method for yield measurement for WPI/Casein solution.

3.1.3.3 Drying in Oven

We found that drying in an oven is the most accurate and consistent method for measuring yield, thus this method had been used for yield measurement throughout the whole project. After centrifuging the samples, we separated the supernatant from the pellet, then placed both of them separately in aluminum pans half-filled with dried sand in an oven at 100°C for at least 12 hours to get dry protein weight and calculated the yield.

3.1.4 Turbidity

We diluted 1 ml of each of the heated solutions with 49 g DI water, and measured turbidity with the turbidity meter. Turbidity follows a similar trend as that of the yield and thus, can be used as a rapid indicator of yield The turbidity values of the WMA are shown in table 3.5.

Table 3.5: Turbidity value of different WPI/Casein solution concentration and pH at different heating temperature and tube fill

WPI/Casein solution Concentration (%)	**pH**	**Heating Temperature (°C)**	**Tube Fill (ml)**	**Turbidity**
6.3	6.1	85	15	253
	6.2	85	15	237
	6.3	85	15	190
6.45	5.6	85	15	690
	5.7	85	15	563
	5.8	85	15	438
	5.9	85	15	352
	6.0	85	15	300
	6.1	85	20	232

	6.1	85	10	223
	6.1	85	15	239
	6.1	90	15	238
	6.1	95	15	240
6.6	6.1	85	15	233
	6.2	85	15	201
	6.3	85	15	157
8.4	6.2	85	15	354
	6.3	85	15	231
	6.4	85	15	203
8.6	6.2	85	15	286
	6.3	85	15	217
	6.4	85	15	150
8.8	6.2	85	15	266
	6.3	85	15	169
	6.4	85	15	128
10.5	6.1	85	15	658
	6.2	85	15	545
	6.3	85	15	329
	6.4	85	15	228
10.75	6.1	85	15	627
	6.2	85	15	482
	6.3	85	15	314
	6.4	85	15	263
11	6.1	85	15	730

	6.2	85	15	338
	6.3	85	15	266

3.1.5 Particle Size Distribution (PSD)

Particle size distribution was done on samples after heating and before centrifugation. The typical size for WMA is between 100-1000 nm (0.1-1 μm in the PSD charts). Our results show that all samples have the majority of aggregates that fall within that range. However, curves do show that there is a significant amount of particles that fall below this range. This could be due to not all the whey forming into WMA, rather forming into shorter strand aggregates. Our previous results support this since these samples ranged from 68-76% WMA yield, thus there was still a significant amount of protein in the supernatant. These protein aggregates would be too small to have sedimented during centrifugation. Yet, PSD analysis shows our benchtop experiments were successful in producing WMA.

Our analysis of different pH values for WPI/Casein concentration 6.45% (figure 3.14) shows that higher pH samples lead to a more even and narrow size distribution. On the other hand, a lower pH leads to a more uneven particle size distribution. This can be observed by the shorter peak and the shoulders formed for pH 5.8, 5.7, and 5.6 in the figure. While typically a more homogenous PSD is desirable, as seen in the higher pH range, the yield for these samples was still lower. The samples in the lower pH range show that there is a higher proportion of larger aggregates, approaching 1 μm (1000 nm). This could also help to explain our observations in section 3.1.1 where the pellet from the higher pH sample was less dense. It is possible that since

there are larger aggregates in the lower pH samples, they sediment more readily during centrifugation, leading to a denser pellet.

Other findings from PSD analysis show that the ratio of WPI to casein (figure 3.15) and tube fill (figure 3.16) do not have a significant effect on particle size. PSD for the thermal variance trials (figure 3.17) follow the same trends as this variable's impact on yield. Heating at 85°C has the lowest curve peak while the peak for 90°C increases and slightly more at 95°C.

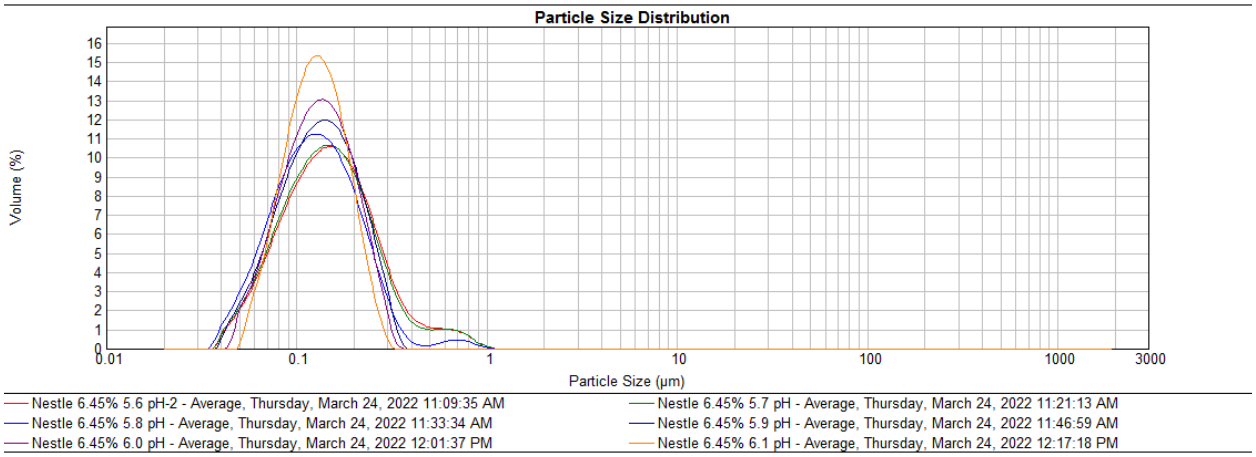

Figure 3.14: Particle size distribution for 6.45% WPI/Casein concentration from pH 5.6 to 6.1

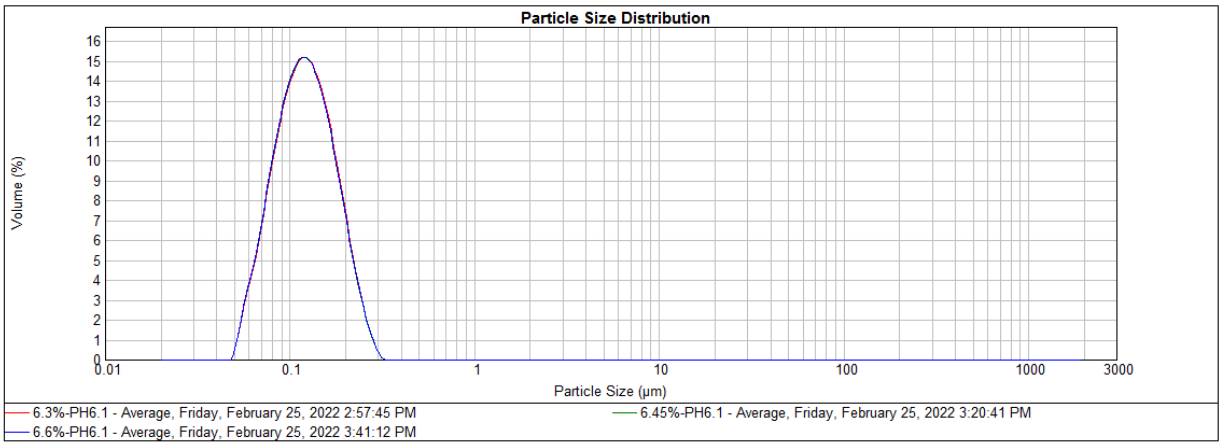

Figure 3.15: Particle size distribution of 6.3% , 6.45% and 6.6% protein solutions at pH=6.1

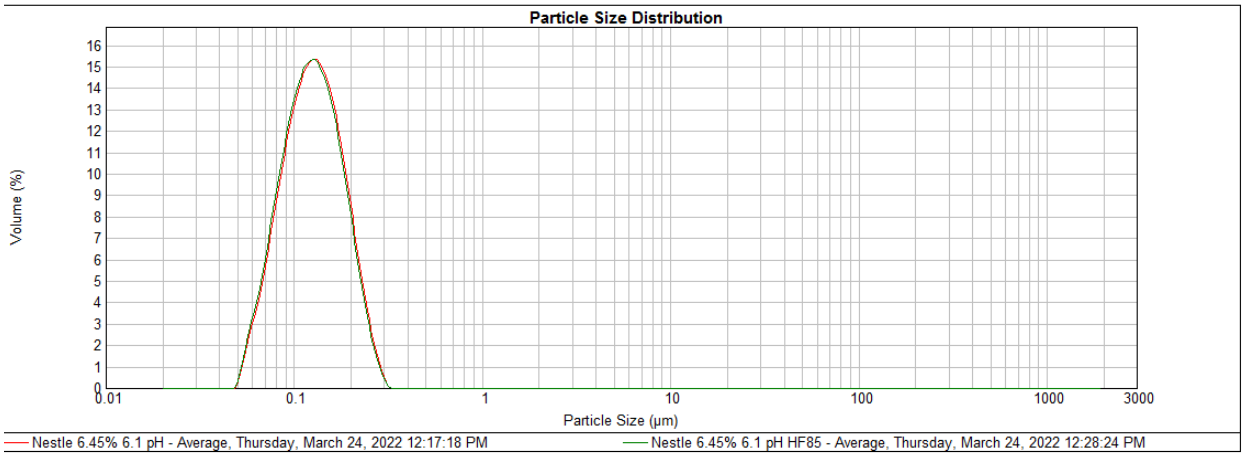

Figure 3.16: Effect of tube fill on particle size distribution

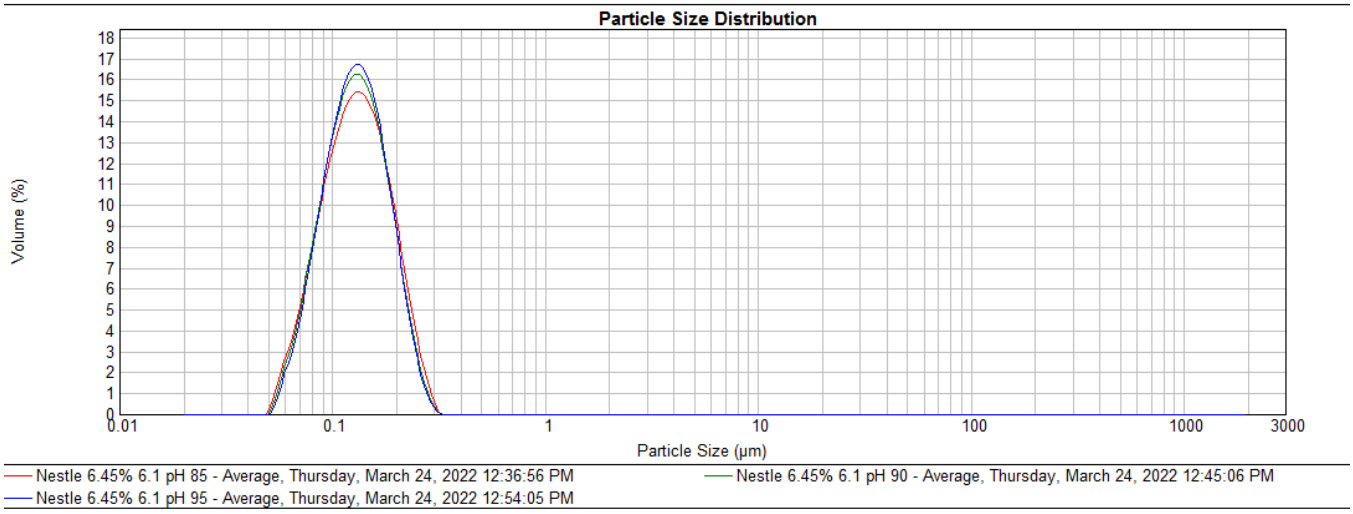

Figure 3.17: Effect of thermal variance (85°C, 90°C, and 95°C) on particle size distribution

3.2 Pilot Plant Trials

3.2.1 Physical Observations

Benchtop experiments proved to scale up very effectively at the pilot plant level. No issues were encountered when producing the WMA solution during both heat treatment and concentration. Figure 3.18 shows the milky white WMA solution after heat treatment. Figure 2.5 and 2.6 shows the retentate and permeate after membrane ultrafiltration. Retentate retained the milky white color while permeate looked similar to the unheated WMA solution.

Figure 3.18: Sample after heat treatment in Microthermics pasteurizer

Figure 3.19: Retentate

Figure 3.20: Permeate

3.2 Concentration factor of finished Whey Micro-Aggregate solution

After production of the WMA solution via thermal treatment, we wanted to concentrate it to the highest protein content possible. As described in section 2.1.3 we used membrane ultrafiltration with a size 100K spiral wound membrane. Product was run until flow rate stopped and we were able to achieve a concentration factor of 3.77 times for pH 6.1 and 4.12 times for pH 5.8 using Brix measurements (table 3.6). Based on this concentration factor of ~4, we tested to see if turbidity would show the same concentration. We diluted the unconcentrated samples 1:50, like previous turbidity measurements, and the concentrated solution by 1:200 (50 x 4). Thus, the turbidity measurement, in NTU, should have been similar between concentrated and concentrated. We saw this in pH 5.8 where unconcentrated was 660 NTU and concentrated was 710 NTU. However, in pH 6.1 measurements for unconcentrated were about 300 NTU lower than concentrated. This could be because the dilution factor was so extreme, a small addition or exclusion of the product could cause a large impact. For Brix, dilution was not needed so we feel that this result is more reliable.

Table 3.6: Measurements of concentration factors for whey micro-aggregate samples

	pH 6.1	pH 5.8
Brix of original WMA solution at pH 6.7 (Brix)	7.5	7.5
Final Brix of concentrated WMA solution (Brix)	28.3	31
Concentration factor based on Brix measurements	3.77	4.12
Initial turbidity of micelle solution with a 50 fold dilution (NTU)	328	660
Final Turbidity of concentrated micelle solution with 200 fold dilution (NTU)	690	710
Turbidity of final permeate (NTU)	3.71	0.47
Brix of final permeate (Brix)	0.6	0.7

3.3 Lab Analysis Results

We brought samples back from the pilot plant trials to run similar lab tests as what we did in benchop experiments. Table 3.7 shows the dilution factor we used for centrifugation of our samples. Since we diluted all other samples from previous experiments by half, we did the same with the unconcentrated samples. The concentrated samples were diluted by eight to account for the estimated 4 times concentration. Using the same method and formula for oven drying previously, we were able to calculate the yield of both unconcetrated and concentrated samples. As expected, the yields were consistent with one another and with previous data. We also used the samples from oven drying to measure concentration based on dry weight. Table 3.8 displays the concentration factor based on dry weight of solids from both the supernatant and pellet. Table 3.9 shows the same, except only dry weight from pellets was used to best estimate WMA

concentration. These results were consistent with the concentration measurements from Brix since all were around 4 times concentrated and pH 6.1 was consistently lower.

Table 3.7: Dilution factor for centrifugation and yield from oven drying

Sample	**Dilution Factor**	**Yield**
Unconcentrated		
pH 6.1	1/2	67%
pH 5.8	1/2	73%
Concentrated		
pH 6.1	1/8	72%
pH 5.8	1/8	76%

Table 3.8: Concentration factor based on total solids from oven drying

pH 6.1	3.72 times
pH 5.8	4.27 times

Table 3.9: Concentration factor based on solids in pellet from oven drying

pH 6.1	4 times
pH 5.8	4.47 times

3.4. Particle Size Distribution of Whey Micro-Aggregates produced in the pilot plant

PSD of the samples derived from pilot plant production was promising as curves looked similar to benchtop experiments. All curves for unconcentrated and concentrated samples had a significant amount of aggregates in the 0.1-1 µm range (100-1000 nm). Figure 3.21 shows expected trends in pH differences where the higher pH, 6.1, had a curve with a taller peak and smaller aggregates. However, it is interesting to note that the curves for concentrated and unconcentrated are more closely aligned in pH 5.8, whereas in pH 6.1 there is a noticeable difference. This could be due to the lower pH having a larger proportion of bigger aggregates that were retained in the retentate.

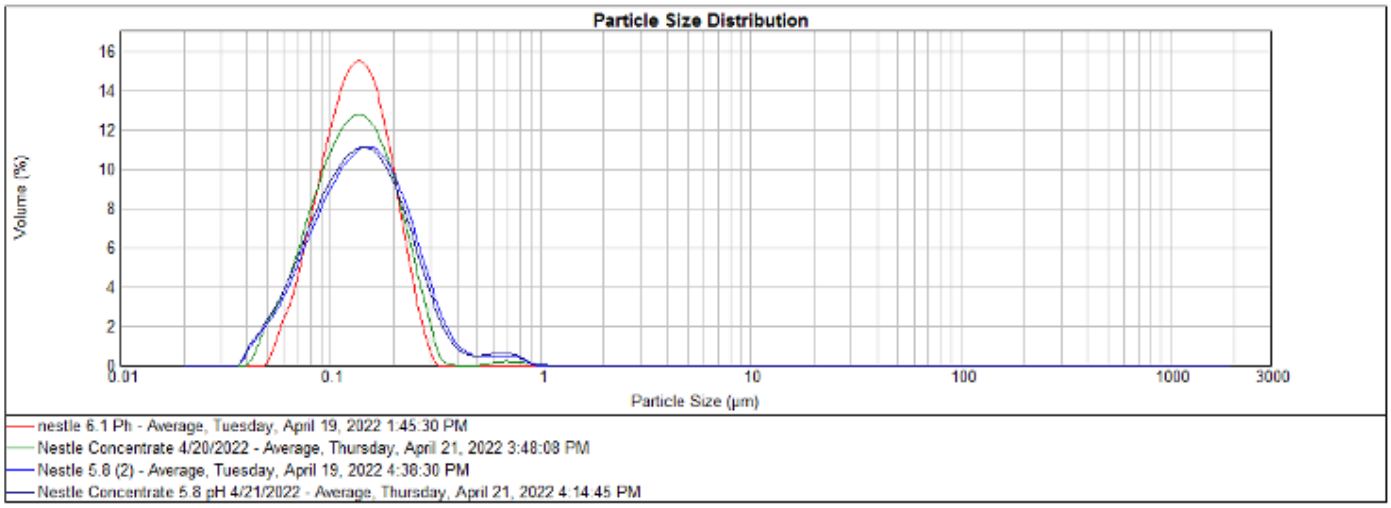

Figure 3.21: Particle size distribution of 6.45% WPI/Casein solutions at pH=6.1 (unconcentrated:red, concentrated:green) and pH=5.8(unconcentrated:blue, concentrated:purple)

CONCLUSIONS AND RECOMMENDATIONS

It was found that the concentration of WPI, WPI: casein ratio, and pH all have significant effects on the yield. Among the three factors, pH has the greatest impact on the yield. It was found that 6.45% WPI/Casein solutions at pH 5.6-6.1 produced the highest yields, and thus 6.45% WPI/Casein solutions at pH 5.8 and pH 6.1 were tested in the pilot plant trials. When measuring the yield, centrifuging and drying the samples in the oven to obtain the dry weight of the supernatant and pellet, along with measuring turbidity worked best among all the methods that had been tested and had yielded consistent results. It is important to note that the bench-top trials could be successfully scaled up to the pilot plant trials as long as conditions are kept the same. The conditions can be better controlled in pilot plants with the help of selected machines, compared to bench-top trials. It is likely that the methods for commercial production of whey micro-aggregates can be reproduced with the same conditions. As the permeate obtained from the pilot plant trials were clear and good retention has been achieved, it can be concluded that the 100 K Ultrafiltration Spiral Wound Membrane UF is an appropriate size for whey micro-aggregate production. It is possible that larger membranes can further optimize the production of whey micro-aggregate, as larger membrane size can increase the flow rate, thus it is suggested that larger membranes can be tested during pilot plant productions in the future.

APPENDIX

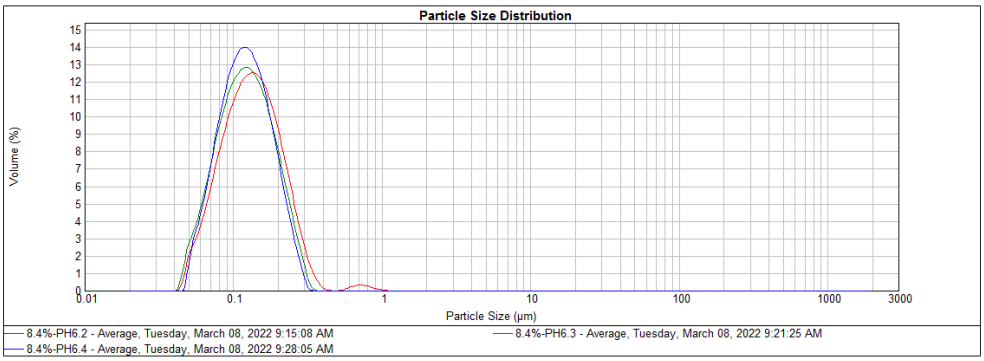

Figure 6.1: Particle size distribution of 8.4% protein solutions at pH=6.2, 6.3, 6.4

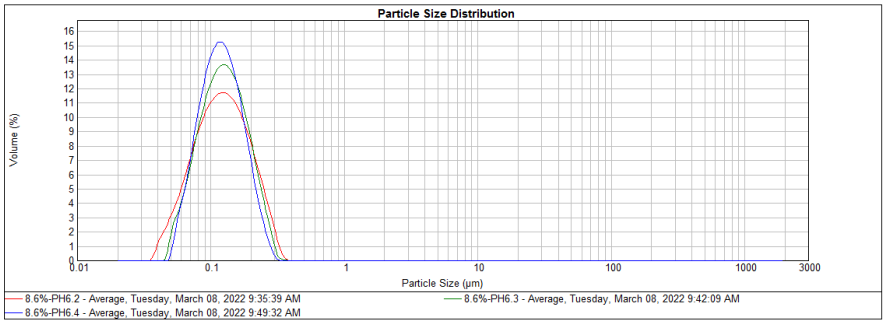

Figure 6.2: Particle size distribution of 8.6% protein solutions at pH=6.2, 6.3, 6.4

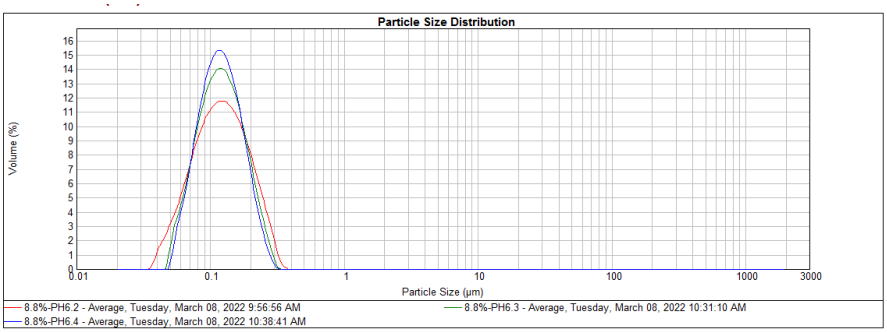

Figure 6.3: Particle size distribution of 8.8% protein solutions at pH=6.2, 6.3, 6.4

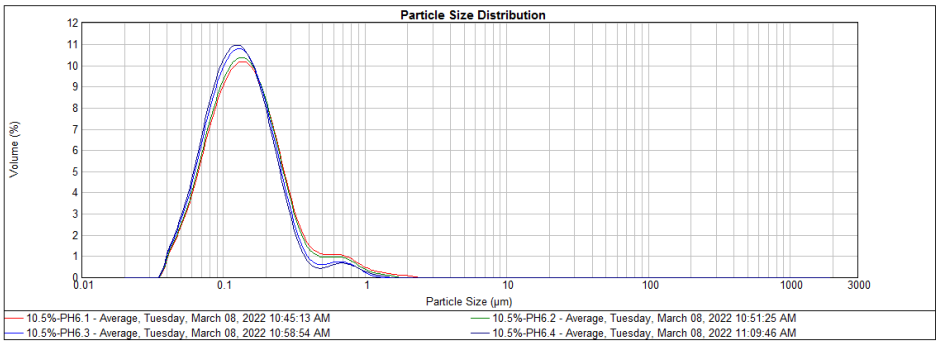

Figure 6.4: Particle size distribution of 10.5% protein solutions at pH=6.1, 6.2, 6.3, 6.4

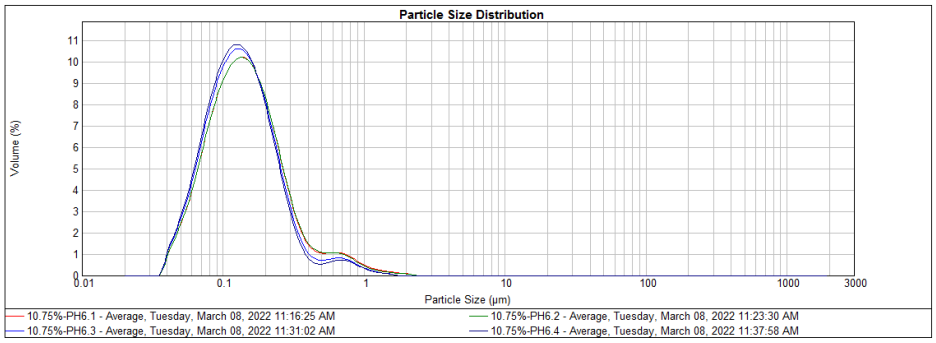

Figure 6.5: Particle size distribution of 10.75% protein solutions at pH=6.1, 6.2, 6.3, 6.4

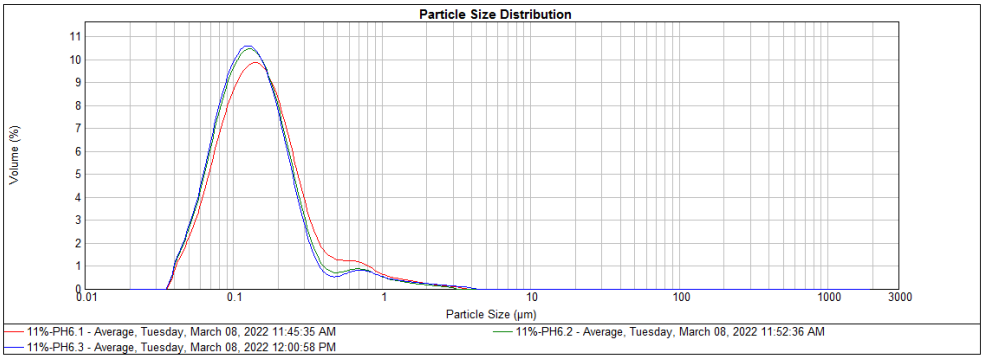

Figure 6.6: Particle size distribution of 11% protein solutions at pH=6.1, 6.2, 6.3

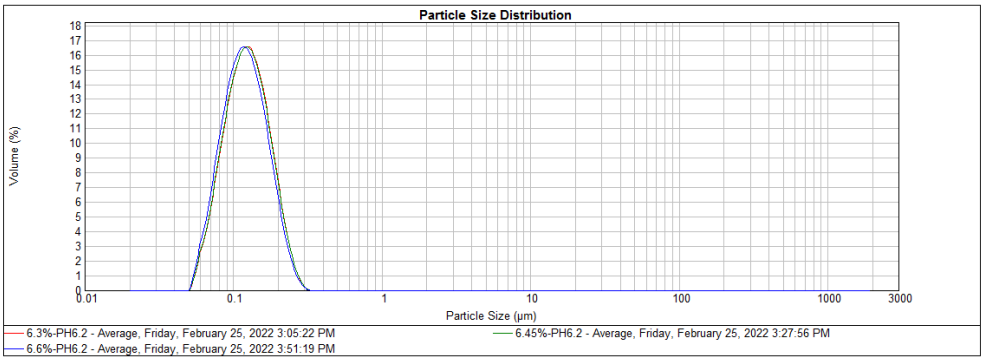

Figure 6.7: Particle size distribution of 6.3% , 6.45% and 6.6% protein solutions at pH=6.2

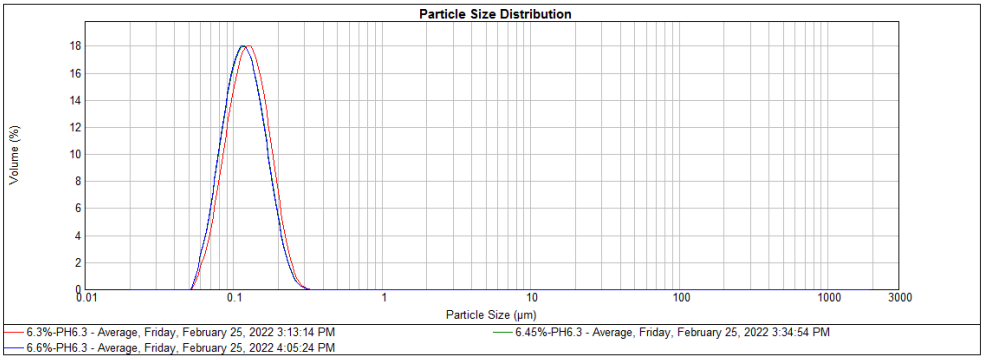

Figure 6.8: Particle size distribution of 6.3% , 6.45% and 6.6% protein solutions at pH=6.3

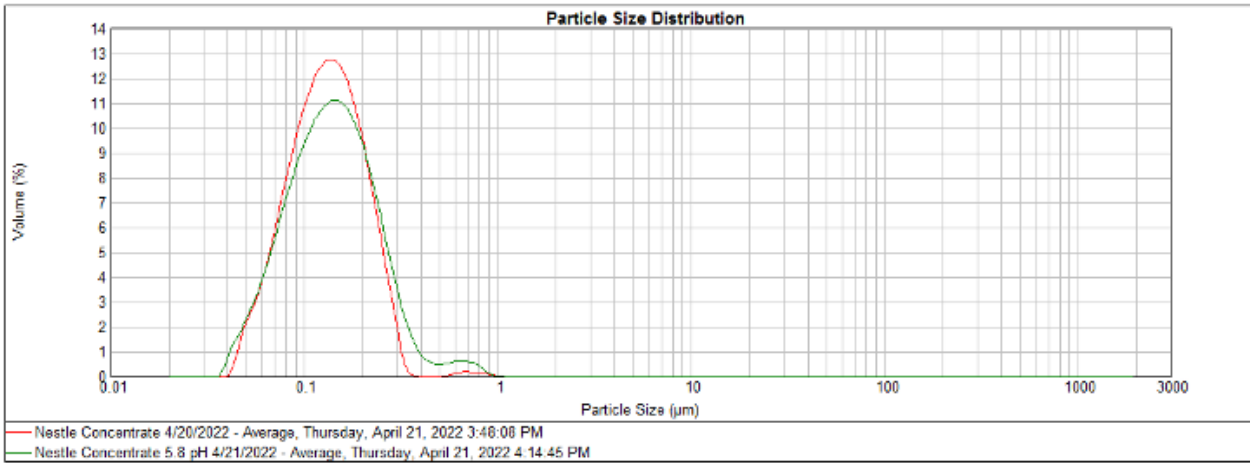

Figure 6.9: Particle size distribution of Nestle concentrates at pH=6.1 (red) and pH=5.8 (green)

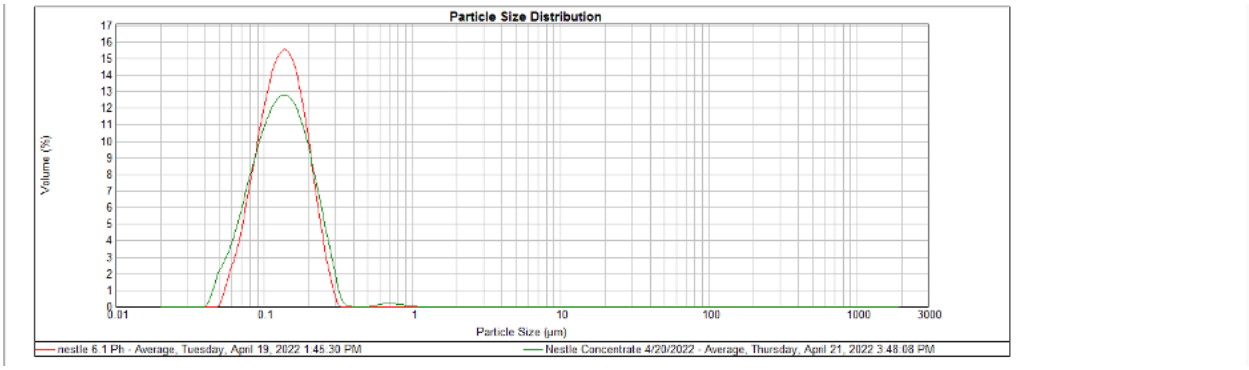

Figure 6.10: Particle size distribution of 6.45% WPI/Casein solutions at pH=6.1 before (red) and after (green) adjusting to pH 6.7 for membrane concentration

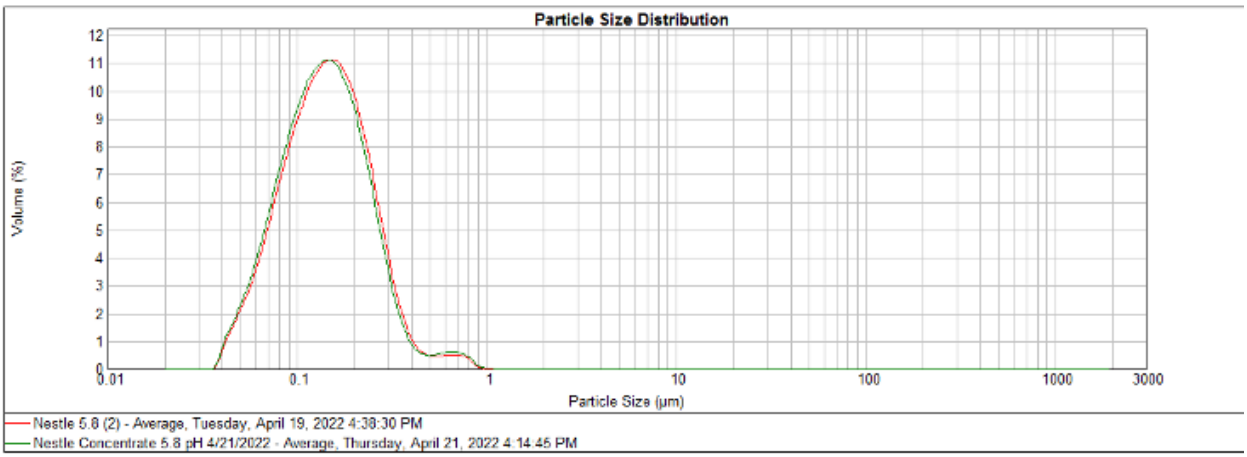

Figure 6.11: Particle size distribution of 6.45% WPI/Casein solutions at pH=5.8 before (red) and after (green) adjusting to pH 6.7 for membrane concentration

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