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Abstract

Aqueous 6.86% whey protein concentrate 35 (WPC 35) solution was heated at the temperature of 70°C for 10, 15 and 20 minutes respectively to quantify the amount of native protein, soluble protein aggregates and insoluble protein aggregates in the heated samples. The serums of the emulsions formed from the different heated samples were also analyzed for percentages of native proteins and aggregated proteins using Dumas combustion method. The aim of this work was to improve the knowledge on the functionality of WPC 35 with a heat treatment of 70°C at varied period of time. The results show that there was native protein fraction of 76.6%, 14.4% soluble protein fraction and 9% insoluble protein aggregates when aqueous 6.86% WPC 35 solution was heated at 70°C for 10 minutes. Emulsion formed from the heated sample contains 78.2% native protein in its serum after centrifugation. With a heat treatment of the same sample at 70°C for 15 minutes, the content of native protein fraction was 73%, 8.3% soluble protein fraction and 18.7% insoluble protein aggregates. The resulting emulsion from this heat treatment had 66.9% native protein in its serum after centrifugation. Heat treatment of aqueous 6.86% WPC 35 at 70°C for 20 minutes contains 80.43% of native proteins, 5.6% of soluble protein and 13.97% insoluble proteins. The emulsion prepared from the heated sample has 81.7% native proteins in its serum. Effect of some other processing conditions such as homogenization and length of storage of whey protein dispersion were also examined.

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This work is dedicated to my daughter, “My little angel”, Mercy Ibukun Ayomipo ONI.

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Lists of Abbreviations

Aq. – Aqueous

Homo - Homogenized sample

HPSEC - High-performance size exclusion chromatography

SDS- PAGE - Sodium dodecyl sulfate polyacrylamide gel electrophoresis

WP - Whey protein

WPC - Whey protein concentrate

WPC 35 - Whey protein concentrate 35

1 Introduction

The Canadian dairy food market had been more concerned about consumers' satisfaction and wellbeing in the recent times (Peng et al., 2006). One of the numerous attempts of the dairy food market in achieving its goal is an idea of producing and/or reformulating a dairy-based food, which is more nutritious, healthy, more appealing to consumers, yet economical to both buyers and producers. In addition to these, the functionality of the new food product and its components become of great interest for effective end use (Mangino et al., 1987; Schmidt et al., 1984; De Wit, 1998). In an ongoing study at the University of Guelph, Ontario, Canada, it was discovered that there is a possibility of making a butter oil-based recombined dairy cream with partially heat-denatured commercial whey proteins (Kethireddipalli & Hill, ongoing). The butter oil-based creams would have similar globule size distribution as that of natural milk cream with other additional advantages.

Conventionally, recombined creams are prepared by homogenizing butter oil with skim milk but then, the resulting fat globules are small, coated with caseins. Caseins are directly involved in cheese gel formation. This is undesirable in some cheese production, especially in hard and semi-hard types, as it is often responsible for the tough, rubbery texture, poor melting and stretching of such cheeses (Gaygadzhiev et al., 2009; Raikos, 2010). The new recombined dairy cream would have an advantage of better fortification, reformulation, modification, taste, stability and better cheese-making property, in relative to the natural cream milk. In addition to the advantages mentioned above, the new product also provides an opportunity of dairy food consumption especially in areas/countries where there is no/not enough production of fresh milk (De Wit, 1998). It could therefore be summarized that the new recombined dairy cream would have a lot of economic advantages resulting from enhanced food product innovation, development and diversification (Capon, 2009; Trott, 2012).

The present competitive and largely globalised business environment places the need on different sectors and organizations to constantly innovate. This is because innovation is of key importance to the success and economic growth of a business (Landsperger & Spieth, 2011; Trott, 2012). The term innovation is surrounded by several phenomenon such as problem identification, knowledge, new idea, idea development, market research, consumers' needs and wants, new technology, new product or services, new process, amidst others (Earle, et al., 2001; Earle & Earle, 2008; Capon, 2009; Trott, 2012). For the purpose of this study, innovation could be defined as a clever way of maximizing an identified business opportunity, which gives an organization a competitive advantage over other competitors (Earle et al., 2001; Capon, 2009; Trott, 2012). This is achieved by transforming residual and new knowledge into new product or services that would be acceptable by the target market (Capon, 2009). The results of innovation assessments help in the identification of the gap between innovation capabilities and innovation goals of the innovator (Lawson & Samson, 2001). The enhanced knowledge and clarity therefore helps in bridging the gap between capabilities-to-goals for improved innovation performance (Rush et al., 2007).

The newly formulated recombined dairy cream is intended to be fortified with nutritional components such as fat-soluble vitamins and healthy omega oils, with a better cheese-making property. Then, some of the desired attributes in the new recombined dairy cream are the presence of non-interacting fat globules, which would enhance better product stability before and after fortification. Whey fractions of different protein compositions had therefore been used as emulsifiers in these formulations due to their characteristic attributes, both when denatured and in cheese-making. One of these attributes is the ability of whey proteins to form and stabilize oil-in-water emulsion. In this study, focus would be on whey protein concentrate 35% (WPC 35). Another major attribute of WPC 35, which qualifies it for this project, is that WPC having 35% protein content could act as a replacer of skim milk in food industries (De Wit, 1998).

Whey proteins are generally acceptable food ingredients, as they are well known for their good nutritional and functional properties such as their ability to form and stabilize oil-in-water emulsions (Roufik et al., 2005; Manion & Corredig, 2006; Ye & Taylor, 2009). This might be due to their characteristic structure and biological attribute, which when modified, could enhance their functionalities (De Wit, 1998; Sajedi et al., 2014). To further justify the utilization of WPC, more knowledge about the functionality of whey proteins and the different factors that affect them must be acquired. Schmidt et al. (1984) reported that whey heat treatment, heating during ingredients' application, storage conditions and sanitation factors are some of the processing factors that directly and/or indirectly affect whey protein functionality. A typical protein concentration for commercial WPC such as WPC 35 ranged from 29 - 60 %. This is because there is a limit to attainable protein purity in WPC manufacture due to economic reasons. For example, increased total solids decrease the rate of protein denaturation (Schmidt et al., 1984). From the perspective of whey proteins' nutritional value, increased usage of whey proteins in dietetics and also in the production of infant foods and other health foods shows its effectiveness (De Wit, 1998; Roufik et al., 2005).

Aqueous solutions and emulsions would be made from WPC 35 after which there is a need to identify and quantify the type and amount of proteins at the interfaces of whey protein dispersions and the resulting emulsions. As part of the preliminary study in the ongoing project, some exploratory researches are expected. This is to clearly understand what proportion of whey proteins, both at the native and aggregated forms, gives the best sample for required product stability, cheese-making and fortification. The measure of the particle size distribution of each sample also gives enhanced knowledge of potential functionality of such sample, both in the food and pharmaceutical industries (Lam & Nickerson, 2013; Shakeel et al., 2012).

Native proteins are those proteins that still retain their original conformation or structure. They are often referred to as folded proteins with their native three-dimensional structure undisrupted, thereby retaining the ability of such protein to continually carry out its biological functions (Levinthal, 1968; Visschers & de Jongh, 2005; Boutin et al., 2007; Anandharamakrishnan et al., 2008). Native proteins are often retained when the configurational energy acting on it is at the barest minimum (Levinthal, 1968; Visschers & de Jongh, 2005). A common food processing condition which leads to protein denaturation from its native state is heating (Pelegrine & Gasparetto, 2005; Gulzar et al.,

2011; Dissanayake et al., 2013). Protein denaturation through heat-treatment could be reversible or irreversible, depending on conditions such as the intensity of heat treatment. Mild heat treatment might cause random unfolding of the protein structure which could be restored to its folded metastable state, having its biological functions completely restored (Levinthal, 1968; Visschers & de Jongh, 2005). Both folded and refolded proteins are described as being in their native states as long as the biological functionalities of such proteins are not negatively affected (Visschers & de Jongh, 2005). Aggregated proteins on the other hand are the denatured or unfolded protein, which results from the disruption in the protein structure during food processing conditions (Levinthal, 1968; Visschers & de Jongh, 2005). Protein denaturation could be heat-induced, acid-induced, high pressure-induced and other denaturants-induced (Visschers & de Jongh, 2005). During protein denaturation, the structural disruption of protein leads to hydrophobic interaction and then the formation of disulphide bond, thereby leading to protein aggregation (Boutin et al., 2007; Raikos, 2010). Protein solubility is one of the measures taken to know how much of protein remains at its native state and how much is aggregated after protein denaturation (Pelegrine & Gasparetto, 2005; Lim et al., 2008).

Some of the processes involved in this study include preparation of sample solutions, protein denaturation, homogenization, mastersizing (to measure the particle size distributions of samples), centrifugation, protein quantification in samples using Dumas, amidst others.

1.1 Aim and Objectives

The objectives of this study are:

1. To understudy the effect of some processing conditions such as sample preparation, storage conditions, duration of storage, duration of heating and homogenization on particle size distribution of both heated and unheated aqueous WPC 35 solutions and the resulting emulsions.
2. To quantify the amount of native and aggregated protein fractions in different aqueous WPC 35 dispersions heated at 70°C for 10,15 and 20 minutes respectively.
3. To quantify the amount of native and aggregated protein fractions in different aqueous phases (serum) of butter oil and whey protein-based emulsions prepared with the heat treatment of 70°C for 10, 15 and 20 minutes respectively.

1.2 Delimitation of Study

This study does not in any way involve assessment on the shelf life of the aqueous whey protein concentrates (both when heated and unheated), shelf life assessment of the resulting emulsions and the stability of the formed emulsions.

2 Theoretical Background

2.1 Whey proteins

Food proteins such as whey proteins are known for their great emulsifying properties (Raikos, 2010; Singh & Sarkar, 2011; Lam & Nickerson, 2013). Several authors had reported about the compositional, nutritional, functional and economical properties of whey proteins with respect to their importance in the food industry (Mangino et al., 1987; Schmidt et al., 1984; De Wit, 1998; Roufik et al., 2005; Liu et al., 2005). This means that whey proteins are able to compete with functional vegetable proteins in the market, ability to replace egg proteins in bakeries and confectioneries, ability to replace milk in dairy products such as ice cream, suitability for usage in dietetics and production of infant formula (Roufik et al., 2005; De Wit, 1998). Apart from the nutritional and functional benefits of whey proteins, the different types and wide range of whey protein compositions broaden their level of utilization. For example, there are whey protein isolates (Hunt & Dalgleish, 1994; Manion & Corredig, 2006), whey protein concentrates (Dickow et al., 2012; Roufik et al., 2005; Mangino et al., 1987) and whey protein hydrolysates, which are as well known as predigested whey proteins. According to the findings in Roufik et al. (2005), the report showed that whey protein concentrates ranged from 32% - 81% in protein contents. Lim et al. (2008) also reported some attributes of WPC 35 containing approximately 35% protein. This suggests that the different protein contents available in each whey protein concentrate could determine its usefulness.

Other researchers focused on the several factors that might be modified to get the best of whey proteins' usage as a result of improved functionality (Lim et al., 2008; Liu et al., 2005; Mangino et al., 1987; Schmidt et al., 1984). For instance, heat treatments, sample concentration, cheese or casein manufacturing practices, storage conditions and sanitation aspects are some factors affecting whey protein functionality as discussed in Schmidt et al. (1984). Functionality of protein also depends on its hydrophobicity, which in turn influences its emulsion capacity (Liu et al., 2005). Lam and Nickerson (2013) defined emulsion as the dispersion of two or more immiscible liquids, in which one of the liquids is dispersed in the other as small droplets which ranged from 0.1 – 100μm and ≤100nm in the case of nanoemulsions. The ability of a protein acting as an emulsifier in a mixture, to lower interfacial tension of either oil-water and/or water-oil mixture is defined as emulsion capacity of such protein. This same ability is identified with whey proteins, to reduce interfacial tension in an oil-water interface especially in an oil-in-water emulsion (O/W) as described in this study, which enhances their usefulness in the formation of emulsions (Lam & Nickerson, 2013).

2.2 Effect of heat treatment on whey proteins' functionality

Heat treatment is one of the ways how protein denaturation could be carried out (Pelegrine & Gasparetto, 2005; Gulzar et al., 2011; Dissanayake et al., 2013). To start with, thermal processing of milk is one of the numerous methods adopted in the dairy industry. Some of the reasons for heat processing of milk include extension of products' shelf-life, quality improvement of products, reduction in the risk of food poisoning,

improvement in the organoleptic properties of food products and modification of functional properties (Raikos, 2010).

Protein denaturation occurs when there is a disruption in its original structure (Anandharamakrishnan et al., 2008; Boutin et al., 2007). The structural disruption of protein via heat treatment might therefore be either intentional or as a result of processing (Raikos, 2010). For instance, when liquid whey protein undergoes pasteurization, a level of denaturation could have occurred depending on the pasteurization temperature (Dickow et al., 2012). Also, reversible structural unfolding of protein or irreversible structural disruption could have occurred as a result of intentional heat treatment to modify proteins' functionality such as in induced gelation of whey proteins (Manion & Corredig, 2006; Boutin et al., 2007; Ye & Taylor, 2009; Sajedi et al., 2014). Again, in the manufacture of whey protein concentrates, the conversion of liquid whey into powdered form requires heat treatment in form of drying. In this process, depending on the method of drying and the drying temperature, there could have been a level of disruption in the protein structure. Although spray drying is the most preferred method of manufacturing powdered whey protein, significant thermal denaturation still occurs. (Gulzar et al., 2011; Anandharamakrishnan et al., 2008).

When there is structural disruption of whey protein for instance as a result of thermal denaturation, two stages are involved; there is an exposure of the hydrophobic group and then the sulphydryl group. This unfolding and exposure enhances protein aggregation due to protein hydrophobicity and disulphide bond formation. During the first stage, there is attractive interaction of protein polymers thereby forming aggregates. The second stage on the other hand involves strengthening of the gel matrix as a result of disulphide bonds formation (Boutin et al., 2007; Raikos, 2010). Depending on the functional end use of such whey protein, the denaturation could be considered as desirable or detrimental (Raikos, 2010).

2.3 Protein Solubility and its functionality

Protein solubility in the context of this study could be defined as the ability of protein to be retained in the supernatant of its solution after being centrifuged under a given condition. It simply identifies the extent to which whey protein is denatured (Pelegrine & Gasparetto, 2005; Lim et al., 2008). Protein solubility could also be measured as the concentration of proteins in a dissolved liquid phase in relation to the total amount of protein, either dissolved or undissolved in the sample (Anandharamakrishnan et al., 2008). There is a level of interaction between temperature and pH in the context of factors that affect protein solubility (Dissanayake et al., 2013; Anandharamakrishnan et al., 2008; Pelegrine & Gasparetto, 2005). Generally, at protein heat treatments of between 40°C - 50°C, protein solubility increases, but with higher temperature, especially when sustained for a given time, the denaturation occurs (Pelegrine & Gasparetto, 2005). Decrease in protein solubility unfavorably affects its functionality as high solubility of protein is required for making good emulsions (Anandharamakrishnan et al., 2008; Pelegrine & Gasparetto, 2005; Manion & Corredig, 2006). The case of whey protein is not an exception. Whey protein solubility decreases with increase in the temperature of heat treatment, thereby leading to protein denaturation, either at pH of about 4.6 or 6.8. The higher the solubility of a protein the more suitable it is in its functionalities such as in

the formation of emulsion, gelation, foam and whipping properties (Pelegrine & Gasparetto, 2005).

2.4 Whey protein based emulsions

Emulsions are becoming more and more important in the recent times as they form a significant part of processed food formulations such as milk, cream and cheese (Kiokias et al., 2004; Singh & Sarkar, 2011). The formation of emulsions basically requires a scientific understanding of the nature and components of the immiscible liquids coming together to interact. Having known what constitutes the mixture with the knowledge of the desired end result, the choice of emulsifiers to be used becomes very important. In the preparation of emulsion, proteins are usually used as emulsifier to stabilize oil droplets (Sünder et al., 2001). In a case of a partially heat-denatured whey protein concentrate such as used in this study, emulsification temperature is very important as it is a major determinant of emulsion consistency (Sünder et al., 2001; Boutin et al., 2007). Oftentimes, the higher the protein levels of an emulsion, the better the behaviour of such emulsion. This is because the smaller oil droplets resulting from higher protein content, increases the stability of the emulsion (Sünder et al., 2001). Gaygadzhiev et al. (2009) further reported that regardless of the type of protein used in stabilizing the oil droplets of an emulsion, increasing amount of protein gradually reduces the average droplet size.

Milk proteins generally known for their emulsifying ability are broadly categorized into two groups: the caseins and the whey proteins (Singh, 2011; Raikos, 2010). Some of the numerous advantages of whey protein based emulsions therefore are observed over emulsions stabilized with skim milk powder (SMP) protein. This might be related to the economic advantage associated with the use of whey protein. For instance, more SMP protein is required to stabilize an emulsion to get a particular average droplet size in relative to whey protein used in getting the same result (Liu et al., 2005; Gaygadzhiev et al., 2009). Also, solubility is an important attribute of whey protein as soluble proteins positively influence the formation, stability and consistency of emulsion (Anandharamakrishnan et al., 2008). Again, the functional end result of the intended new recombined dairy cream necessitates and/or justifies the preference for using whey proteins as emulsifier over other food emulsifying agents. Looking at other food emulsifying agents such as skim powder and milk caseins for instance, they contribute directly to cheese gel formation, which is not desirable in the new product in view (Gaygadzhiev et al., 2009).

2.5 Homogenization

Homogenization of an emulsion is the act of inducing mechanical shear to the mixture of immiscible liquid, to produce uniformity in the mixture by making small droplets of one of the liquids to be dispersed in the other (Kiokias et al., 2004; Lam & Nickerson, 2013). Proper homogenization of an emulsion leads to proper stability of such emulsion, depending on what is desirable in each emulsion (Kiokias et al., 2004). This fact was further established by Sünder et al. (2001) and Raikos (2010), stating, that the parameters of homogenization are some of the major tools that determine the physico-chemical properties of an emulsion. The process of homogenization aids the dispersal of one phase of the immiscible liquids in the other phase by stretching and breaking the coarse droplets thereby leading to enhanced emulsion stability (Kiokias et al., 2004).

3 Materials and Method

All experiments except stated were carried out at room temperature.

3.1 Preparation of WPC 35 solution

WPC 35 powder (Prodel 35, Parmalat, London, ON, Canada) usually kept at 4°C was prepared into an aqueous solution of 6.86% w/w in a glass beaker with 0.02% sodium azide added. The sodium azide was added as a biocide; a preservative to inhibit the growth of microorganisms in the prepared sample (Manion & Corredig, 2006; Gaygadzhiev et al., 2009). The resulting dispersion was brought to stirring for one hour while covered with film to avoid evaporation and contamination of the prepared sample. After stirring for one hour, the solution was stored in a refrigerator overnight. The day of solution preparation is reported as unheated Day 1, the solution that was refrigerated overnight is reported as unheated Day 2 solution, while the sample refrigerated till the third day is reported as unheated Day 3.

3.2 Heat treatments of aqueous WPC 35 solution

The aqueous WPC35 solution prepared and refrigerated the previous day was brought to room temperature before heating. The process of pre-heating WPC 35 solution is important to the formation and the resulting properties of whey protein gels (Ye & Taylor, 2009). The solution was dispensed into several but uniform test tubes (as much as needed), using pipette, before heating to ensure even heat penetration into solutions being heated. The already filled test tubes were arranged in test tube racks and then covered with aluminum foil to prevent any form of evaporation or condensation. The water bath (Isotemp 3016H, Fisher Scientific Incorporation, USA) was pre-set for 70°C before the solutions to be heated was placed. This process was done for all the samples heated at 70°C for 10, 15 and 20 minutes respectively, except that samples experimented at 70°C for 10 and 15 minutes were heated on Day 2 while sample 70°C for 20 minutes was heated on Day 3. The heated samples still in test tubes were then transferred into ice for about 3-5 minutes to cool (Manion & Corredig, 2006). Later, the heated samples were collected back into a bigger beaker for further experiment.

3.3 Homogenization of samples

All homogenizations in this study were done using Emulsiflex – C5 homogenizer by Avestin, Canada, at the pressure of 175 - 200 bars. The homogenizer was always ensured to be thoroughly clean with no trace of fat, oil, dirt, soap, or any other particles in the outlet, hose or pump of the homogenizer. This was to get just the accurate particle size of the homogenized samples without any external influence. In this study, there were 3 categories of samples homogenized. First, the unheated but homogenized WPC 35. Second, the heated and homogenized WPC 35. And lastly, the butter oil and whey protein based emulsion. All the homogenized samples from these categories were ensured to be at a temperature of 60°C at homogenization.

3.4 Preparation of Emulsion

The butter oil and whey protein- based emulsion was prepared using butter oil melted from a butter fat (Laetania Clarified butter, Parmalat, London, ON, Canada) and aqueous 6.86% WPC 35 solution. The mixture was prepared at ratio 70:30, where aqueous 6.86% WPC 35 solution has the 70% portion and the butter oil has the 30% portion of the mixture. In the case where emulsion is prepared from heated aqueous 6.86% WPC 35 solution and butter oil, 30% butter oil that was melted from butter fat in a water bath at 60°C was added to 70% heated aqueous 6.86% WPC 35 solution to make a total of 100%. Throughout the process of making the emulsion, all samples were kept at 60°C. The mixture at 60°C was thereafter pre-homogenized/blended using high speed shear dispersing tool (Polytron Kinematica AG dispersing and mixing technology, PT 1300D, Fisher Scientific, Mississauga, Ontario) to have a single phase mixture since the two components were immiscible liquids. This was also done to avoid phase-separation of the mixture, as it is undesirable in samples to be homogenized. The pre-homogenization and/or blending were carried out at the speed of 10 000 rpm for 3 minutes. After the blending, the blended mixture was warmed up in the water bath to 60°C before being homogenized to obtain an emulsion. The obtained sample of emulsion was always covered with aluminum foil to avoid contaminations and dryness of surface of the emulsion.

3.5 Measure of particle size distribution

All the prepared samples were analyzed for particle size distribution using mastersizer, which is a static light scattering instrument (Mastersizer 2000, Malvern Instruments Limited, Worcestershire, United Kingdom). The Small Volume Sample Dispersion Unit (Malvern Instruments, Hydro 2000SM) attached to the mastersizer was always ensured to be thoroughly cleaned using low foam chlorinated detergent and rinsing with distilled water. This was to get rid of any residual particle from previous usage in the mastersizer that might influence the new result. Depending on the sample to be analyzed in the mastersizer, there are different standard operational procedures (SOP), which could be used in the measurements. For instance, whenever the particle size distribution of an emulsion was to be assessed, SOP for butter-oil was selected and whenever whey protein solution of any kind was to be analyzed, SOP for WP-aggregate was used. The dispersant in which the samples were dispensed for analysis was water with refractive index of 1.33, refractive index of butter oil was 1.455, while the refractive index of WP was 1.53. The analysis of the different samples was based on these values. The laser speed was always set to 1000 rpm and laser obscuration of the dispersed sample at between 11-15% was always ensured before readings were taken. To plot the graphs of particle size distributions of samples as shown in the result and discussion section, the mastersizer values that ranged from columns “result between user sizes” through “operator notes” were used.

3.6 Separation of native and aggregated protein fractions in samples

In this study, all the samples that were analyzed were separated into native protein fraction and aggregated protein fraction according to the method adopted in Roufik et al. (2005) with the process of centrifugation.

3.6.1 WPC 35 samples

For the unheated, heated and "heated and homogenized" samples of 6.86% aqueous WPC 35 solution in each case of varied duration of heat treatment, there was a 50% dilution thereby reducing the concentration of samples to 3.43% aqueous WPC 35 solutions. Two samples each of diluted unheated, diluted heated and diluted "heated and homogenized" WPC 35 solutions were prepared making 6 samples altogether. One out of the two samples in each group was acidified with 0.1N HCL to a pH of approximately 4.6 from an initial pH range of 6.50 - 6.52, using pH meter (Accumet AR15 pH meter, Fisher Scientific, Mississauga, Ontario). This is to say that one sample of heated 3.43% aqueous WPC 35 solution was acidified while the other sample was not acidified. This scenario holds for the unheated and "heated and homogenized" samples as well. All the prepared samples were constantly brought to stirring to avoid sedimentation of samples especially for the acidified ones. The constant stirring was not a rigorous stirring but a mild one, so as not to leave the prepared sample in a total state of rest. This condition of stirring was also consistent for all the concerned samples. The samples were thereafter weighed (~88 g) into centrifuge bottles (~31 g) of an ultracentrifuge (Sorvall WX Ultra series ultracentrifuge, Thermo Fisher Scientific, Germany).

The filled centrifuge bottles were carefully arranged in the F37L – 8 x 100 rotor to be placed in the rotor chamber of the ultracentrifuge. The centrifugation was carried out at 48 000 g for 1 hour at 20°C. After the centrifugation was completed, the samples were carefully brought out of the ultracentrifuge and the supernatants of all the centrifuged samples were collected into well-label separate containers. For each of the three categories of the WPC 35 solutions which are unheated, heated and "heated and homogenized" samples, acidified, unacidified, supernatant of acidified and finally supernatant of unacidified samples were analyzed for total protein contents. There were pellets formed in all the centrifuged WPC 35 samples but the amount of aggregate protein fractions in the pellets were accounted for in the results and discussion section. A diagram of the process of protein separation is shown in the Figure 1.

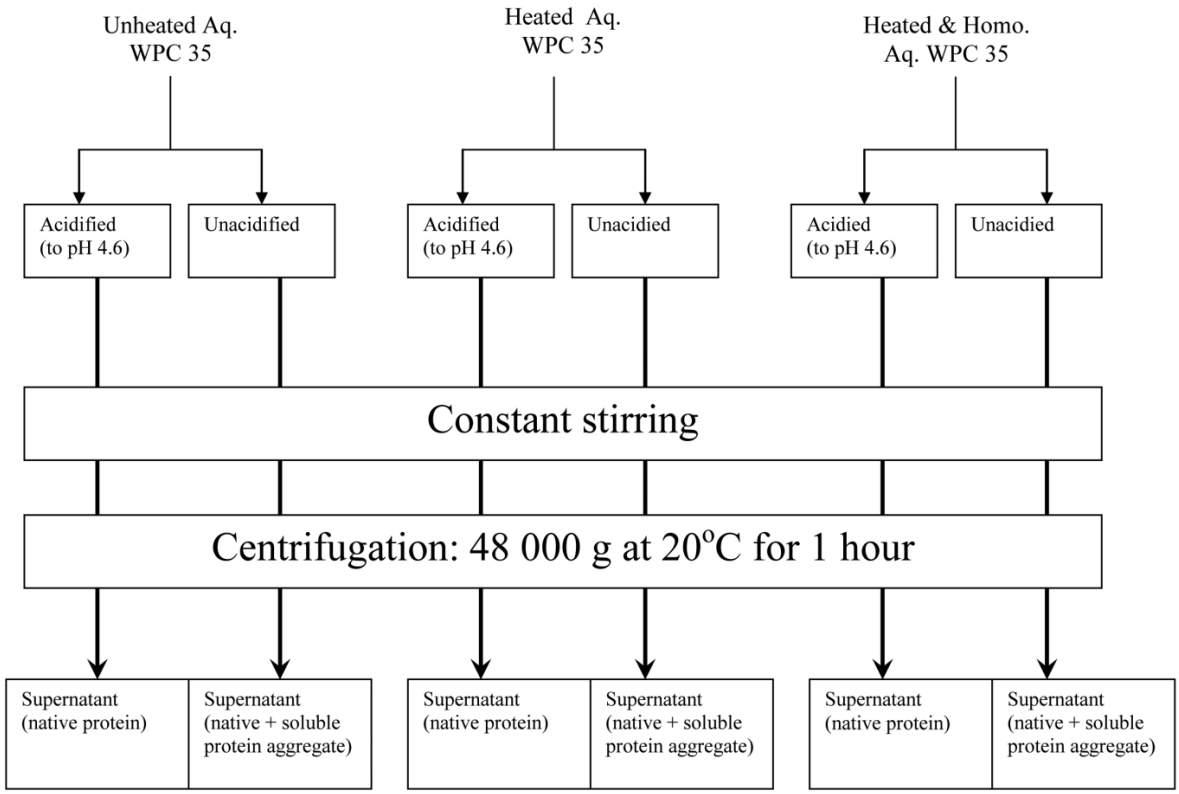

Figure 1: A chart of WPC 35 protein separation process used in this study

3.6.2 Emulsions

The emulsions prepared from varied durations of heat treatment of aqueous 6.86% WPC 35 solutions at 70°C for 10, 15 and 20 minutes respectively were also centrifuged in order to quantify the total protein content of the aqueous phase/serum of the emulsions. The emulsions of the 3 samples; 70°C for 10 minutes, 70°C for 15 minutes and 70°C for 20 minutes, were carefully weighed into centrifuge bottles of the F37L – 8 x 100 centrifuge rotor and carefully places in the rotor chamber of the ultracentrifuge. The centrifugation of the emulsions was carried out at 6 000 g for 45 minutes at 4°C. At this condition, there was no pellet formed in the centrifuged samples and in samples where there were appearances of pellets, the amount was negligible. The serum of the different samples was carefully collected from the centrifuged samples into well-labeled containers, avoiding the top cream layers. Again, the pH of the serum were adjusted to approximately 4.6 with 0.1N HCL from the initial pH range of 6.3 - 6.4. The acidified serum was re-centrifuged following the same procedure at 48 000 g for 1 hour at 20°C. The supernatants of the re-centrifuged serum of different samples were also carefully collected and both the original serum with the supernatant of the acidified samples was analyzed for total protein contents. A diagram of the process of protein separation is shown in Figure 2.

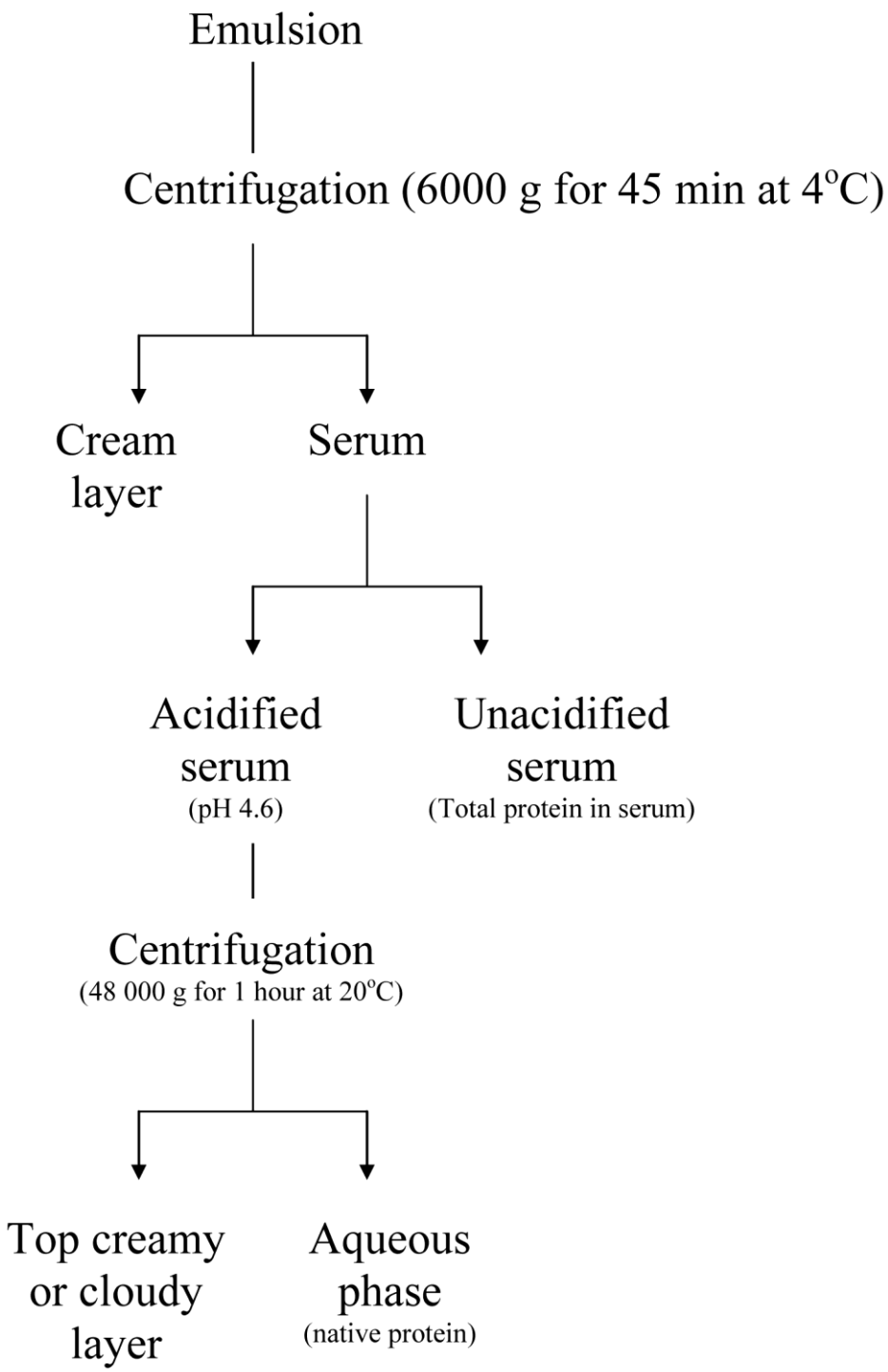

Figure 2: A chart of protein separation process of an emulsion

3.7 Quantification of total protein in samples

The different samples of aqueous WPC 35 solutions heated at 70°C for 10, 15 and 20 minutes respectively, with the serum of their resulting emulsions were collected into separate well labeled containers after centrifugation. The total protein contents quantification of samples were carried out using Dumas combustion method (Leco FP – 528, Mississauga, ON, Canada) and the obtained nitrogen values were multiplied by a conversion factor of 6.28. The obtained values were further computed as the case may be for each sample as shown in the most tables in the results and discussion section. All experiment would be done in replicates and all samples would be analyzed for an advanced identification and quantification of proteins using High-performance size exclusion chromatography (HPSEC) and Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

4 Results

4.1 Measure of particle size distributions of the different samples

The graph of comparison in the particle size distribution of the different samples of unheated aqueous 6.86% WPC 35 solutions assessed on the Day 1, Day 2 and Day 3 of sample preparations is shown in Figure 3. This is an important step in the study as there is a close relationship between protein structures and their functionalities (Sajedi et al., 2014). The distribution shows that the prepared sample at Day 1 has the smallest particle size, followed by Day 2, with Day 3 sample having the largest. The particle size distribution of 3 samples of aqueous 6.86% WPC 35 heated at 70°C at different durations of 10, 15 and 20 minutes respectively is shown in Figure 4. The different samples of heated aqueous WPC 35 solutions observed (Figure 4) were used to prepare emulsions. The particle size distributions of the resulting emulsions were also assessed (Figure 5).

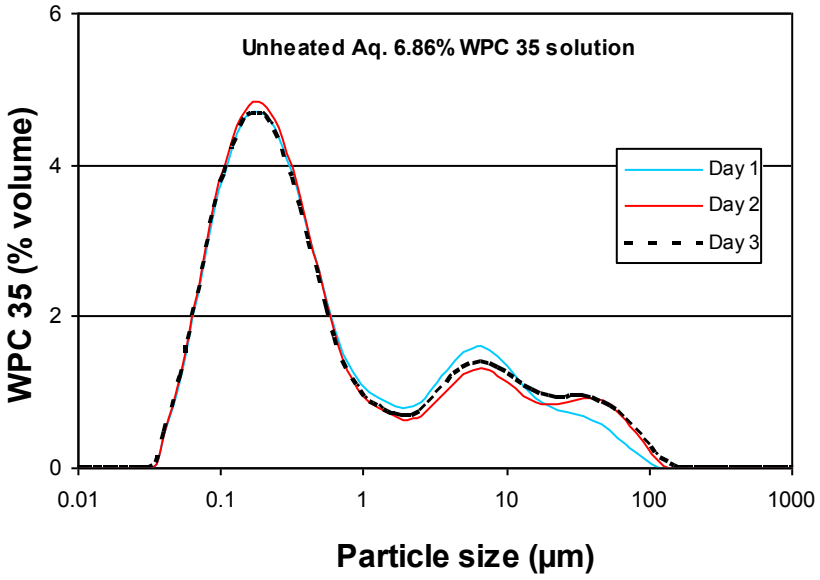

Figure 3: Comparison between the particle size distributions of unheated aqueous 6.86% WPC 35 solutions assessed on the Day 1, Day 2 and Day 3.

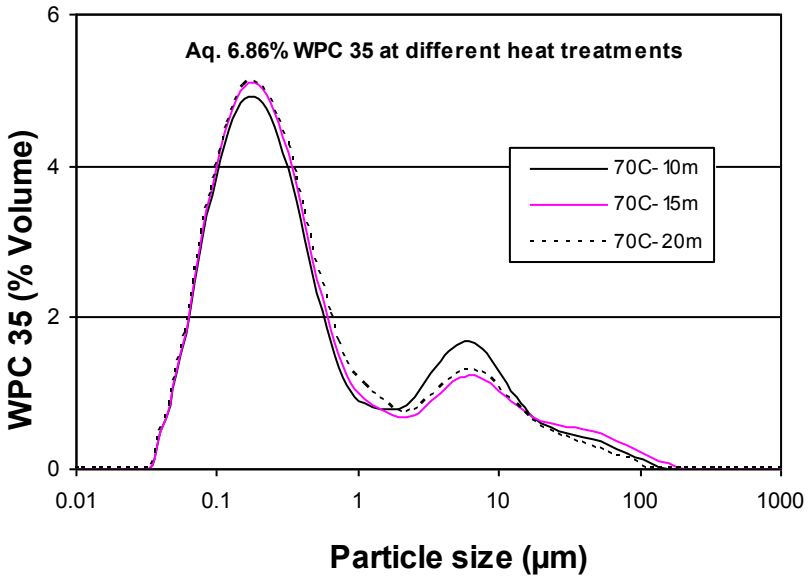

Figure 4: Comparison of particle size distribution between heated aqueous 6.86% WPC 35 solutions assessed at 70°C for 10, 15 and 20 minutes.

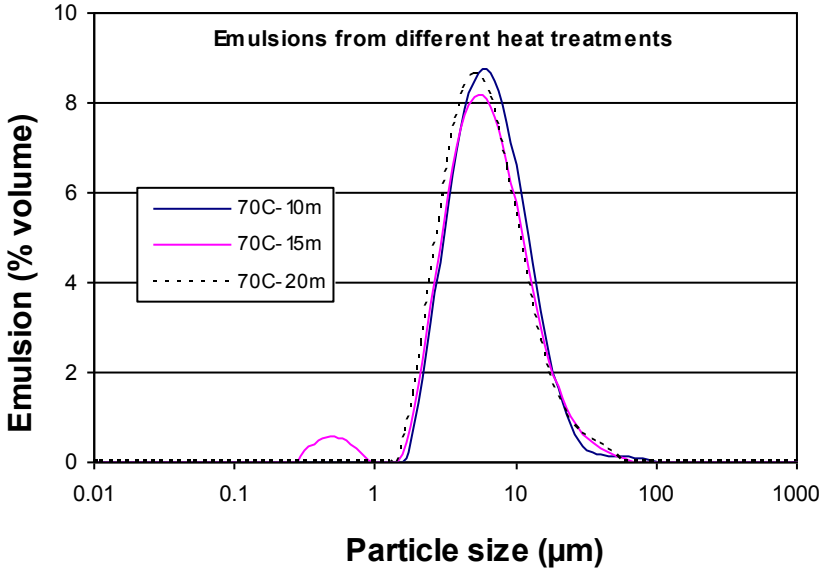

Figure 5: Comparison between the emulsions prepared from samples in Figure 4.

Table 1: Mean droplet sizes [$D_{3,2}$] (± standard deviation) of the different prepared samples from aqueous 6.86% WPC 35 solution

Different attributes of aq. 6.86% WPC 35 solution observed	**Samples**	**Mean droplet sizes [$D_{3,2}$] of samples (µm)**
Unheated aq. 6.86% WPC 35 solution	Day 1	0.228±1.343
	Day 2	0.223±1.359
	Day 3	0.230±1.317
Aq. 6.86% WPC 35 solution at different heat treatments	70°C for 10 minutes	0.219±1.397
	70°C for 15 minutes	0.212±1.441
	70°C for 20 minutes	0.212±1.464
Emulsions from different heat treatments of aq. 6.86% WPC 35 solution	70°C for 10 minutes	5.936±2.344
	70°C for 15 minutes	4.253±2.191
	70°C for 20 minutes	5.433±2.293

In order to further have the knowledge of the different samples of aqueous WPC 35 solutions and the different resulting emulsions for the best optimization of their functionalities, the prepared samples were further analyzed (Sajedi et al., 2014; Lam & Nickerson, 2013; Shakeel et al., 2012; Gaygadzhiev et al., 2009; Roufik et al., 2005). The results of the varied attributes and parameter are reported in Figure 6 and Table 1. The samples whose results are shown in Figure 6 were produced from the prepared aqueous 6.86% WPC 35 solutions, refrigerated until Day 2 and heated at 70°C for 15 minutes. Figure 6a shows the relationship in the particle size distribution of unheated and heated aqueous 6.86% WPC 35 solution. Figure 6b shows the relationship between the particle size distributions of emulsions made from heated and unheated aqueous 6.86% WPC 35 in Figure 6a. Figure 6c shows the characteristic particle size distributions of heated and homogenized aqueous 6.86% WPC 35 solution in relation to unheated but homogenized aqueous 6.86% WPC 35 solution. Figure 6d reveals the relationship in the particle size distributions of emulsions of samples shown in Figure 6c. Figure 6e and 6f shows the relationship between heated and "heated and homogenized" aqueous 6.86% WPC 35 solutions and the resulting emulsions from the two samples.

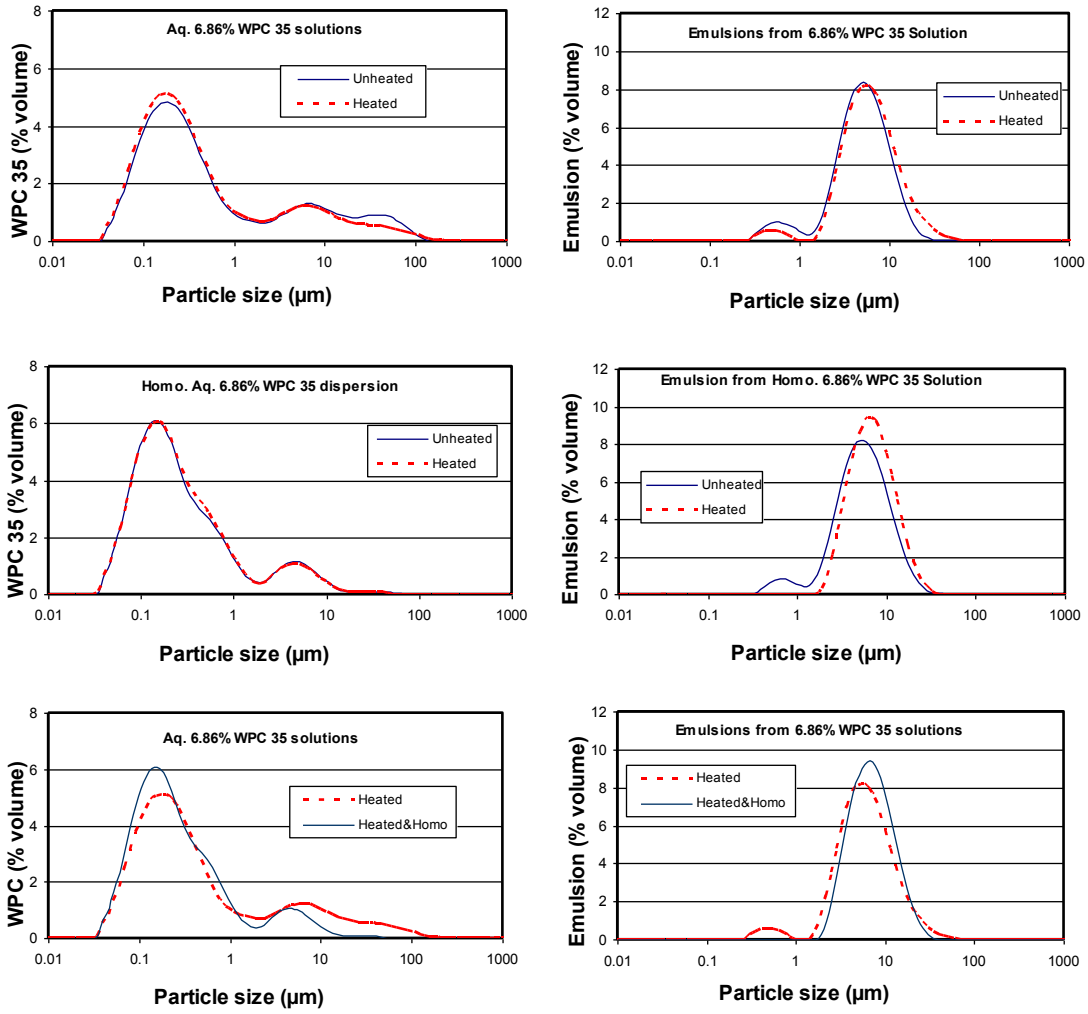

Figure 6: Comparison of the particle size distribution of unheated/heated aqueous 6.86% WPC35 solution (a) and its emulsion (b), as well as homogenized unheated/heated aqueous 6.86% solution (c) and its emulsion (d). Comparison of heated and heated/homogenized aqueous 6.86% WPC 35 solution and its emulsion are shown in plot (e) and (f). All samples in this figure were prepared on Day 2 at 70°C for 15 minutes.

Table 2: Mean droplet sizes [$D_{3,2}$] (± standard deviation) of the different prepared samples from aqueous 6.86% WPC 35 solution experimented at 70°C for 15 minutes

Different attributes of Day 2 aq. 6.86% WPC 35 solutions heated at 70°C for 15 minutes	**Mean droplet sizes [$D_{3,2}$] of samples (µm)**			
	Aq. WPC 35 solution	**Homo. aq. WPC 35 solutions**	**Emulsion from WPC 35 samples**	**Emulsion from homo. WPC 35 samples**
Unheated	0.223±1.359	0.174±1.681	3.109±2.181	3.725±2.183
Heated	0.212±1.441	0.176±1.696	4.253±2.191	6.394±2.453
Heated and homo	0.176±1.696	_	6.394±2.453	_

4.2 Quantification of total protein contents of samples

Table 3: Quantification of protein contents for 3.43% aqueous WPC 35 solutions with heat treatment at 70°C for 10 minutes

Samples of WPC 35 solution	**Total protein content (%)**	**Amount of protein in the supernatant (%)**	**Amount of protein in the pellet (%)**
Unheated	1.300	1.137 (+ soluble aggregates)	0.163
Heated	1.262	1.149 (+ soluble aggregates)	0.113
Heated and homo	1.287	1.143 (+ soluble aggregates)	0.144
Unheated + HCL	1.225	1.093 (native)	0.132
Heated + HCL	1.212	0.967 (native)	0.245
Heated and homo + HCL	1.212	0.917 (native)	0.295

The Table 3 above show the percentage total protein content obtained for each of the sample listed in the table by multiplying the total nitrogen value obtained from Dumas analysis by a conversion factor of 6.28. The amount of percentage protein contents in the supernatant of each of the samples was also generated directly from Dumas. The acidified samples would have just the percentage native proteins in their supernatants while the unacidified samples would have both the native and soluble protein aggregates in their supernatants, according to Roufik et al. (2005). All other protein contents less of the percentage total protein content of each of the samples was assumed to be the percentage amount of insoluble protein aggregates in each of the sample, which is residual in their pellets. Percentage soluble protein aggregates were obtained by subtracting the percentage amount of protein in the supernatant of acidified unheated sample (native protein) from the percentage amount of protein in the supernatant of unacidified unheated

sample (native and soluble protein aggregates) (Anandharamakrishnan et al., 2008; Pelegrine & Gasparetto, 2005).

This process was repeated for the heated and also "heated and homogenized" samples to obtain the percentage values of the soluble protein aggregates present in them. Table 4 shows the percentage fractions native, soluble and insoluble protein aggregates of the different samples of unheated, heated and "heated and homogenized" WPC 35 solutions. The percentage total protein content of each of the samples, less of the amount of native and soluble protein aggregates gives percentage amount of insoluble protein aggregates present in each sample (Roufik et al., 2005). Table 5 shows the values for native protein fraction, soluble protein aggregate fraction and insoluble protein aggregate fraction in a total of 100% solutions of unheated, heated and "heated and homogenized" samples of aqueous 6.86% WPC 35 solutions. This discussion also holds for quantification of protein contents in 3.43% WPC 35 heated at 70°C for 15 minutes shown in Tables 6, 7 and 8, and for the samples heated at 70°C for 20 minutes shown in Tables 9, 10 and 11.

Table 4: Percentages of native, soluble and insoluble protein aggregates of unheated, heated and "heated and homogenized" aqueous WPC 35 heated at 70°C for 10 minutes

Samples of WPC 35 solution	**Total protein content (%)**	**Native (%)**	**Soluble aggregates (%)**	**Insoluble aggregates (%)**
Unheated	1.300	1.093	0.044	0.163
Heated	1.262	0.967	0.182	0.113
Heated and homo	1.287	0.917	0.226	0.144

Table 5: Native, soluble and insoluble protein fractions in a 100% solutions of unheated, heated and "heated and homogenized" 6.86% WPC 35 solution at 70°C for 10 minutes

Protein contents in aq. WPC 35 solution	**Unheated**	**Heated**	**Heated and homo**
Total	100%	100%	100%
Native	84.1%	76.6%	71.3%
Soluble aggregates	3.4%	14.4%	17.5%
Insoluble aggregates	12.5%	9%	11.2%

% protein in the pellet = Total protein content (%) – protein in the supernatant (%); % soluble aggregates = (% of protein in the supernatants for 'Unheated', 'Heated' and 'Heated and homo') – (% of protein in the supernatants for 'Unheated + HCL', 'Heated +HCL' and 'Heated and homo + HCL') respectively; % insoluble aggregates = Total protein contents (%) – native (%) + soluble aggregates (%)].

Table 6: Quantification of protein contents for 3.43% aqueous WPC 35 solutions with heat treatment at 70°C for 15 minutes

Samples of WPC 35 solution	**Total protein content (%)**	**Amount of protein in the supernatant (%)**	**Amount of protein in the pellet (%)**
Unheated	1.507	1.281 (+ soluble aggregates)	0.226
Heated	1.514	1.231 (+ soluble aggregates)	0.283
Heated and homo	1.551	1.275 (+ soluble aggregates)	0.276
Unheated + HCL	1.407	1.168 (native)	0.239
Heated + HCL	1.915	1.105 (native)	0.81
Heated and homo + HCL	1.262	1.086 (native)	0.176

Table 7: Percentages of native, soluble and insoluble protein aggregates of unheated, heated and "heated and homogenized" samples heated at 70°C for 15 minutes

Samples of WPC 35 solution	**Total protein content (%)**	**Native (%)**	**Soluble aggregates (%)**	**Insoluble aggregates (%)**
Unheated	1.507	1.168	0.113	0.226
Heated	1.514	1.105	0.126	0.283
Heated and homo	1.551	1.086	0.189	0.276

Table 8: Native, soluble and insoluble protein fractions in a 100% solutions of unheated, heated and "heated and homogenized" 6.86% WPC 35 solution at 70°C for 15 minutes

Protein contents in aq. WPC 35 solution	**Unheated**	**Heated**	**Heated and homo**
Total	100%	100%	100%
Native	77.5%	73%	70%
Soluble aggregates	7.5%	8.3%	12.2%
Insoluble aggregates	15%	18.7%	17.8%

Table 9: Quantification of protein contents for 3.43% aqueous WPC 35 solutions with heat treatment at 70°C for 20 minutes

Samples of WPC 35 dispersion	**Total protein content (%)**	**Amount of protein in the supernatant (%)**	**Amount of protein in the pellet (%)**
Unheated	1.043	0.999 (+ soluble aggregates)	0.044
Heated	1.124	0.967 (+ soluble aggregates)	0.157
Heated and homo	1.143	1.024 (+ soluble aggregates)	0.119
Unheated + HCL	1.068	0.986 (native)	0.082
Heated + HCL	1.005	0.904 (native)	0.101
Heated and homo + HCL	1.043	0.936 (native)	0.107

Table 10: Percentages of native, soluble and insoluble protein aggregates of unheated, heated and "heated and homogenized" samples heated at 70°C for 20 minutes

Samples of WPC 35 solution	**Total protein content (%)**	**Native (%)**	**Soluble aggregates (%)**	**Insoluble aggregates (%)**
Unheated	1.043	0.986	0.013	0.044
Heated	1.124	0.904	0.063	0.157
Heated and homo	1.143	0.936	0.088	0.119

Table 11: Native, soluble and insoluble protein fractions in a 100% solutions of unheated, heated and "heated and homogenized" 6.86% WPC 35 solution at 70°C for 20 minutes

Protein contents in WPC 35 solution	**Unheated**	**Heated**	**Heated and homo**
Total	100%	100%	100%
Native	94.53%	80.43%	81.89%
Soluble aggregates	1.25%	5.60%	7.70%
Insoluble aggregates	4.22%	13.97%	10.41%

Table 12: Summary of the percentage native, soluble and insoluble aggregate protein fractions in unheated, heated and "heated and homogenized" aqueous 6.86% WPC 35 solutions prepared at 70°C for 10, 15 and 20 minutes

Temperature and durations of samples' heat treatments	**Samples**	**Protein contents in 6.86% WPC 35 solution**			
		Total	**Native protein**	**Soluble aggregate protein**	**Insoluble aggregate protein**
70°C for 10 minutes	Unheated	100%	84.1%	3.4%	12.5%
	Heated		76.6%	14.4%	9%
	Heated and homo		71.3%	17.5%	11.2%
70°C for 15 minutes	Unheated	100%	77.5%	7.5%	15%
	Heated		73%	8.3%	18.7%
	Heated and homo		70%	12.2%	17.8%
70°C for 20 minutes	Unheated	100%	94.53%	1.25%	4.22%
	Heated		80.43%	5.6%	13.97%
	Heated and homo		81.89%	7.7%	10.41%

Table 13: Summary of the total amount protein contents in the serum of the resulting emulsions from samples experimented at 70°C for 10, 15 and 20 minutes

Total	**Protein contents in 6.86% WPC 35 - based emulsions**	**Samples**	**Emulsions**		
			70°C for 10 minutes	**70°C for 15 minutes**	**70°C for 20 minutes**
		Serum	1.790	1.746	1.576
		Supernatant of acidified serum	1.400 (native)	1.168 (native)	1.287 (native)
		Aggregated protein content	0.390	0.578	0.289
100%	Native protein fraction		78.2%	66.9%	81.7%
100%	Aggregated protein fraction		21.8%	33.1%	18.3%

5 Discussions

5.1 Measure of Particle size distributions

The results of the measures of particle size distributions of the prepared samples of both aqueous 6.86% WPC 35 solutions and the resulting emulsions is shown as plots in Figures 3, 4, 5 and 6. These results is also presented as mean droplet sizes in Tables 1and 2. The measure of particle size distribution of each prepared sample becomes very necessary in this project as it a quick measure to assess the stability, functionality and acceptability of the different samples of WPC 35 and the resulting emulsions (Nakashima et al., 2000; Huang et al., 2001; Coupland & McClements, 2001; Chanamai & McClements, 2001; Bouchemal et al., 2004). Literature reports that despite the rising awareness of whey proteins (WP) and whey protein concentrates (WPC) in the food industry, some undesirable changes may occur in the physico-chemical properties of both WP and WPC, which must be adequately handled (Carbonaro et al., 1998). For instance, the process of pre-heating WPC often aggravates protein denaturation and aggregation (Carbonaro et al., 1998; Pelegrine & Gasparetto, 2005; Gulzar et al., 2011; Dissanayake et al., 2013). The measure of particle size distribution is therefore a control measure to understudy if samples' particles are still within the food manufacturers' target.

All the samples explored in this project are prepared from WPC 35 and the heat treatments of aqueous 6.86% WPC 35 solutions are carried out at 70°C. This is because WPC 35 has the capability to be heat-treated at different degrees of temperatures without its functionalities being negatively affected. The fact is further established as Lim et al. (2008) also reported that heat treatment temperature greater than 75°C usually negatively affect the functional properties of proteins except for WPC 35.

5.2 Quantification of native and aggregated protein contents

A summary of the different percentages of native, soluble and insoluble aggregate protein fractions of unheated, heated and "heated and homogenized" aqueous 6.86% WPC 35 solution prepared at 70°C for 10, 15 and 20 minutes is given in Table 12. At heat treatment 70°C for 10 minutes, there is 76.6% native protein fraction in the heated sample while there is a total 23.4% aggregated protein fraction. The heated and homogenized sample at this heat treatment has 71.3% protein in the native state, with 17.5% protein as soluble aggregate protein and 11.2% protein as insoluble aggregate protein fraction. The result obtained from the heated and homogenized sample shows a little less value compared to the value obtained from ordinary heated sample at its native state and a little more value than the values at the aggregated state of heated sample. This might be due to improved solubility of the homogenized samples (Lam & Nickerson, 2013; Schmidt et al., 1984). Moreover, smaller sized aggregates have the tendency to diffuse to the interfaces higher than the larger aggregates (Sajedi et al., 2014). The unheated sample at this preparation has more native protein of 84.1% and total denatured protein content of 15.9%. Since there was no heat treatment of any form on this sample, the aggregated protein fraction was assumed to be due to the heat treatment the WPC 35 powder was subjected to in the process of drying during manufacture (Dickow et al., 2012; Anandharamakrishnan et al., 2008; Mangino et al., 1987).

Also for the set of samples heated at 70°C for 15 minutes, the results show that there are more aggregated protein fractions in relative to the samples heated at 70°C for 10 minutes. This could obviously be due to longer period of heating (Boutin et al., 2007; Mangino et al., 1987; Schmidt et al., 1984). Also, Raikos (2010) reported that with heat treatment of between 60°C - 90°C for about 1000 seconds on whey protein solution, large protein aggregates are formed. The more aggregated protein fraction obtained in a sample, the less native protein fraction remaining in such sample. For all the samples, there were more native protein contents in the unheated samples. This was due to no laboratory heat treatment samples leading to less protein denaturation and less protein aggregate formation (Schmidt et al., 1984; Mangino et al., 1987). Again, for all the samples heated at 70°C for 10, 15 and 20 minutes, there were more soluble aggregate proteins in the heated and homogenized samples that there are in the ordinary heated samples. This might be as a result from the process of further homogenizing the heated WPC 35 solutions at a pressure of 175 – 200 bars, enhancing better protein solubility (Sajedi et al., 2014; Lam & Nickerson, 2013).

The slight differences in the values obtained for unheated samples prepared for heating at 70°C for 10minutes and 15 minutes could be due to experimental precision errors such as in sample weighing, pipetting, accuracy in pH adjustment of the two sets of samples and period of overnight refrigeration of prepared samples. The prepared WPC 35 dispersion must be refrigerated overnight for specific number of hours for consistency of results rather that just refrigerating overnight. As mentioned earlier in this report, the prepared samples used for 70°C for 10 minutes and 15 minutes experiment were heated on Day 2 while the prepared sample for 70°C for 20 minutes experiment was heated on Day 3. The results of different protein fractions obtained from the 70°C for 20 minutes experiment was quite different from the previous trend. The 3 samples show the highest values of percentage native protein fractions in their 3 sub-samples and the lowest percentage total aggregated protein fractions in relative to samples treated at 70°C for 10 and 15 minutes respectively. It was expected that the longer the duration of heat treatment obtained, the more the denatured protein obtained. Reverse in the case. The likely reason for this result might be the longer time of refrigeration of the prepared aqueous WPC 35 dispersion, which was for about 48 hours (Schmidt et al., 1984).

The values obtained for the native and aggregated protein fractions of the serum of resulting emulsions from aqueous 6.86% WPC 35 solution prepared at 70°C for 10, 15 and 20 minutes respectively was also reported in Table 13. Emulsion from WPC 35 sample prepared at 70°C for 20 minutes has the highest percentage of native protein fraction of 81.7%. Emulsion from 70°C for 10 minutes sample has 78.2% native protein fraction in its serum, while 70°C for 15 minutes sample has the least value of 66.9% as its native protein fraction. The reason for the range of differences in the three values might not be concluded until an advanced analysis on protein identification and quantification is conducted. The emulsions and all its components such as cream and serum parts, both before and after centrifugation would be analyzed in the course of the project. This analysis would be done using high-performance size exclusion chromatography (HPSEC) and Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

6 Conclusion

The results from quantification of the amount of protein present as native protein fractions and aggregated protein fractions in aqueous 6.86% WPC 35 enables an improved knowledge of the functionalities of the dispersion (Roufik et al., 2005). De Wit (1998) described the functionality of whey proteins as being "versatile". This is as a result of the adaptability, flexibility, multifacetedness and resourcefulness of whey proteins, thereby enhancing their modification to perform several functions in the interest of the manufacturer. This was also proven in this research work. As earlier discussed in the study, the different stages of the ongoing project, right from the preparation of the WPC 35 dispersion to the production of fortified cheese has specific desired attributes in focus. This project therefore has been able to study at a preliminary level some characteristics of aqueous 6.86% whey protein dispersion under different conditions and durations of heating, providing information for the next stage of the experiment.

In conclusion, this study emphasizes that heat treatment is of key importance in optimizing the flexible functionalities of whey protein. Appropriate heating temperature and period of heating for whey protein denaturation depends on the interest of the manufacturer per time. Different mean droplet sizes and particle size distributions of different emulsions are directly related to the type, functionality and stability of such emulsions. Although it is generally believed that heat treatment decreases protein functionalities (Dissanayake et al., 2013; Schmidt et al., 1987), however, the functionalities of whey proteins and their resulting emulsions could be well modified under controlled conditions (Sünder et al., 2001). The result of this study also shows that with heat treatment of aqueous 6.86% WPC 35 at 70°C for 10 minutes, there was 76.6% native protein fraction, 14.4% soluble protein fraction and 9% insoluble protein aggregates. The resulting emulsion from the heat treatment at 70°C for 10 minutes has 78.2% native protein in its serum after centrifugation. Also, with heat treatment of aqueous 6.86% WPC 35 at 70°C for 15 minutes, there was 73% native protein fraction, 8.3% soluble protein fraction and 18.7% insoluble protein aggregates. The resulting emulsion from the heat treatment at 70°C for 15 minutes has 66.9% native protein in its serum after centrifugation. Finally, with heat treatment of aqueous 6.86% WPC 35 at 70°C for 20 minutes, there was 80.43% native protein fraction, 5.6% soluble protein fraction and 13.97% insoluble protein aggregates. The resulting emulsion from the heat treatment at 70°C for 20 minutes has the highest percentage of native protein fraction. The serum sample of the emulsion contains 81.7% native protein fraction and 18.3% aggregated protein fraction in its serum after centrifugation.

A major limitation to this research work was the shortness of the period at which the project was done. This affected the number of times replicates of each sample analyzed could be done. However, future analysis of the different samples experimented in this research work would be done at least in triplicates to enable an advanced statistical analysis on the results. Also further analysis on protein identification and quantification of the different samples experimented could be done using high-performance size exclusion chromatography (HPSEC) and Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in the course of the project. Since this study is a part of an ongoing project, final conclusion might not be drawn on the results obtained from these experiments.

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Popular Scientific Summary

Consumers' satisfaction and well being is of key importance in the process of food innovation, of which product development and diversification are good novelty strategies that could be adopted in achieving this goal. This is because there are great economic values attached to new product development and/or product diversification, if these innovation strategies are properly implemented. More so, improved food product innovation enhances food availability, hence, global food security. In line with this, an ongoing project at the Food Science Department of the University of Guelph, Ontario, Canada, poses to conduct a study on recombined dairy creams. This project aims at improving the quality, taste, availability, nutrient, stability and acceptability of recombined dairy creams, cheeses and other dairy products, yet with feasible cost and selling prices of new products.

Usually recombined dairy creams are prepared by homogenizing butter oil with skim milk. This process, similar to homogenizing milk, produces small fat globules coated mainly with caseins (milk protein), which can be involved in the casein gel formed during cheese making thereby causing cheese gel formation. This is undesirable for most types of cheese because the resulting cheese texture is too tough and rubbery. The alternative that is being explored in this project is to make the recombined cream with partially denatured whey proteins. The globules produced when whey proteins are used are similar in size to native globules of natural milk and of course do not interact with caseins during cheese making. The presence of non-interacting fat globules in the new recombined dairy cream becomes desirable as it enhances better product stability before and after fortification. This also serves as an additional economic advantage of products, as a longer shelf-life of the new products would be guaranteed.

This study is therefore a preliminary work of exploration in the ongoing project, to study some processing parameters that might be best suitable for proper optimization of whey protein in producing the recombined creams. Despite the scientific evidences that whey proteins are great emulsifier with numerous nutritional, economic and functional properties, more knowledge is still required for best optimization of whey proteins. Factors such as heat treatment temperature for partial denaturation, period of denaturation, condition and period of storage, solubility of the denatured proteins, amidst others, directly contribute to the type, stability and functionality of the formed emulsions. For instance, fat globule size distribution of natural milk ranged between 1 - 15µm, any particle size value less or more than this range would no longer function as natural milk cream. The new recombined dairy cream and the resulting cheese from this project therefore have an advantage of better nutrient fortification, reformulation, modification, taste, stability and better cheese-making property, in relative to the natural cream milk. The results in this study show the variations in the particle size distributions of the partially denatured whey protein concentrate 35 (WPC 35) with the emulsions at heat treatment of 70°C for 10, 15 and 20 minutes respectively. This was to clearly understand what proportion of whey proteins after denaturation (both at the native and aggregated forms) gives the best sample for required product stability, cheese-making and fortification as solubility of emulsifying proteins play a vital role in the formation of good emulsions.