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Towards a Better Understanding of Solubility of Thermally Polymerized and Aggregated Whey Proteins.

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ABSTRACT

Whey proteins polymers and aggregates possess unique functional properties. However, limited solubility of protein polymers and aggregates limits the protein utilization. In this work, the solubility of whey protein polymers and aggregates resulting from two methods of thermal treatment and drying was evaluated. The solubility of aggregates at pH 3 was found to be much higher than that of polymers at pH 7 because intermolecular hydrogen bonds impeded the solubility of polymers and aggregate. The temperature of drying was found to be an important factor in protein solubility, where higher solubility was obtained at pH 3 when drying temperature was below the denaturation temperature.

Keywords: whey proteins, solubility, hydrogen bonds.

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INTRODUCTION

The solubility of powdered food ingredients is critically important. In many instances, solubilizing the ingredient is needed to prepare the food ingredient in a solution form. Whey proteins are important food ingredient that contains balanced amino acids (Mcintosh, Regester et al. 1995; Regster, Mcintosh et al. 1996). Whey proteins also possess specific physiological properties (Bounous, Gervais et al. 1989; Kennedy, Konok et al. 1995). When native whey protein molecules are hydrated in water under neutral pH and close to room temperature, they form spherical-like configuration. In order to exploit the potential functional properties of the proteins, molecules have to be denatured by changing the neutral conditions; either by heating or by changing the solvent quality. The denatured molecules aggregate and/or polymerize to form larger molecules of superior functional properties. In order to obtain the aggregated or polymerized whey proteins into easily handled food products, they have to be dried into soluble powdered form. However, one of the major problems of whey protein aggregates and polymers is their scarce solubility.

Polymerization and aggregation of whey proteins have been extensively investigated (Monahan, German et al. 1995; Elofsson, Dejmek et al. 1996; Mleko and Foegeding 1999). Usually, polymerization refers to chemical bonding, while aggregation is attributed to physical interactions. Polymerization of whey proteins proceeds via disulphide interchange reaction to form polymers when heated at a temperature greater than the protein denaturation temperature (Monahan, German et al. 1995; Mleko and Foegeding 1999). In addition, hydrogen bonds and hydrophobic interactions play an important role in protein aggregation according to reaction conditions.

Solubility of native whey protein has been investigated at different temperature and pH values (Pelegrine & Gasparetto, 2005). Solubility of heated whey proteins has also been addressed in literature (Hidalgo & Gamper, 1977). On the other hand, solubility of whey protein gels and films at different conditions has been addressed (Shimada & Cheftel, 1988) (Perez-Gago, Nadaud, & Krochta, 1999) (Perez-Gago & Krochta, 2001). It is still far from being understood what the role of each type of interactions in protein solubility is, and how to tailor polymerization conditions to yield a soluble protein macromolecules.

The aim of this work is to investigate the solubility of powdered polymerized and aggregated whey proteins prepared by two different methods. The role of both disulfide bonds and hydrogen bonds will be discussed. The drying temperature will also be investigated.

MATERIALS AND METHODS

Materials. Whey protein isolate (WPI) (BiPRO) prepared by an ion exchange process was supplied from Davisco Food International (LeSueur, Mn, USA) and used as received. Protein concentration was 91% by weight. Distilled water was used in all the experiments. Total Dissolved Solids (TDS) of distilled water was equal to ~ 25 PPM.

Preparation of Protein Solution. The protein solution was prepared by dissolving the native protein powder in distilled water by gentle magnetic stirring for 30 minutes. Protein concentration was adjusted to 5% w/w. The pH value of protein solution was then adjusted to 3, 7, and 10

Preparation of Polymerized and Aggregated Protein Powder. Polymerized and aggregated protein powder were obtained by two methods. In ***Method 1***, Protein solution (5% w/w) was poured into glass petri dishes and heated in a drying oven (at 110 ^{o}C for 1¼ hrs or 85 ^{o}C for 2 hrs). By the end of this period, protein solution turns into a film of polymerized or aggregated proteins. Film is then scratched off the petri dishes and ground using kitchen grind to produce the powder. In ***method 2***, protein solution (5% or 7% w/w) was heated at 95 ^{o}C in a sealed container in a water bath for different time intervals, then cooled to 6 ^{o}C for 24 hrs, then poured in glass petri dishes and dried in a tray drier at 50 ^{o}C and air velocity of ~ 1.5 m/s for 2 hours. Film is then scratched off and ground using similar technique of method 1.

Determination of Protein Concentration. Protein concentration was determined using Bradford method (Bradford, 1976)

Preparation of Protein Reagent. Coomassie Brilliant Blue G-250 (100 mg) was dissolved in 50 ml 95% ethanol. To this solution, 100 ml 85% (w/v) phosphoric acid was added. The resulting solution was diluted to a final volume of 1 liter. Final concentrations in the reagent were 0.01% (w/v) Coomassie Brilliant Blue G-250, 4.7% (w/v) ethanol, and 8.5% (w/v) phosphoric acid.

RESULTS AND DISCUSSION

Effect of pH and drying temperature on solubility in case of simultaneous reaction and drying procedure (*Method 1*).

Protein solutions (5%) at different pH values (3, 7 and 10) were heated and dried by *Method 1* (*simultaneous reaction and drying procedure*) and assessed for solubility. **Fig. 1** shows the effect of pH at two different drying temperatures. It is clear that solubility (as expressed by absorbance at 595 nm) at pH 3 is remarkably higher than both pH 7 and pH 10 at both drying temperature. The higher solubility at pH 3 is expected to be due to the absence of disulfide bonds.

The solubility at pH 7 and pH 10 appears to be similar. This can be attributed to the fact that at both pH values, polymerization proceeds primarily via disulphide interchange reaction (Damodaran & Anand, 1997) (Monahan, German, & Kinsellat, 1995) . On the other hand, the drying temperature appears also to have an influence on the solubility of the polymerized and aggregated proteins. We clearly see a modest enhancement in solubility when drying temperature is decreased from 110 to 85 ^{o}C. The effect of temperature can be explained on the light of surface hydrophobicity. The higher the temperature of thermal treatment, the higher the surface hydrophobicity. The increased surface hydrophobicity with heating temperature was observed by (Sava, Van der Plancken, Claeys, & Hendrickx, 2005). Heating at 110 ^{o}C is expected to create stronger hydrophobic intermolecular interactions that led to less soluble whey protein polymers and aggregates. The effect of temperature appears to be similar for the three investigated pH values, 3, 7 and 10. This observation indicates that intermolecular hydrophobic interactions proceed in a similar manner at the different pH values.

However, the absence of disulphide bonds at pH 3 causes the aggregated molecules via intermolecular hydrophobic interactions to be more susceptible to solvation by water. The higher solubility is probably due to more open conformational structure of the aggregated molecules, which enables water molecules to access the buried hydrophilic residues of the protein.

Role of hydrogen bond on solubility of polymers and aggregates.

Hydrogen bonds are abundant in proteins (Ippolito, Alexander, & Christianson, 1990). The aggregation of whey proteins involves intra and intermolecular hydrogen bonding. To assess the effect of hydrogen bonding on polymers and aggregates solubility, urea was used to dissociate the hydrogen bonds, and solubility results were compared for the three investigated pH values. Urea destabilizes proteins by forming hydrogen bonds to the peptide groups (Lim, Rösgen, & Englander, 2009).
From **Fig. 1,** we notice that that addition of urea increases the solubility of protein polymers and aggregates for all pH values. This increase in solubility indicates that the presence of intermolecular hydrogen bonds impedes the polymers and aggregates solubility.

Effect of concentration, pH and incubation time on solubility in case of two step heating and drying (Method 2) at low temperature.

At pH 7.

The solubility data of polymers and aggregates obtained by method 2 are show in **Fig. 2**. We clearly see that the period of heating affects the solubility at pH 7 up to 30 minutes. We clearly see as well that the solubility decreases with increasing protein concentration from 5% to 7%. Addition of Urea increases the solubility at both concentrations (5% and 7%), but the solubility at 5% remain higher. The lower solubility a 7% is most probably due to the higher population of disulfide boding at higher protein concentration. Although the population of hydrogen boding is expected to be higher as well at 7%, the addition of Urea should have dissociated the hydrogen bonding and the difference in solubility between 5% and 7% samples is due to disulfide bonds.

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At pH 3.

The samples prepared with *Method 2* appears to be completely soluble, with not effect of incubation time or concentration. Also, the addition of Urea caused almost no effect on solubility. The complete solubility at pH 3 is most probably due to the absence of disulfide bonds.

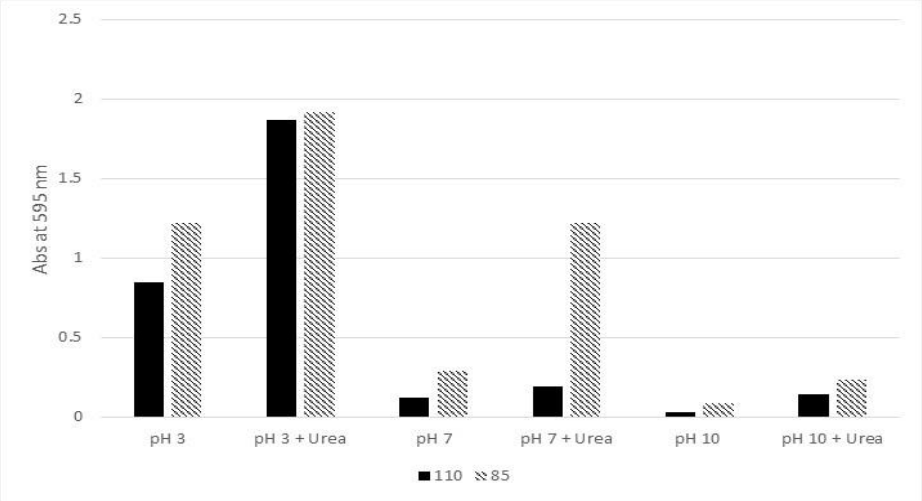

Figure 1. Solubility – as expressed by absorbance at 595 nm - of whey protein polymers and aggregates at different conditions as prepared via *Method 1*.

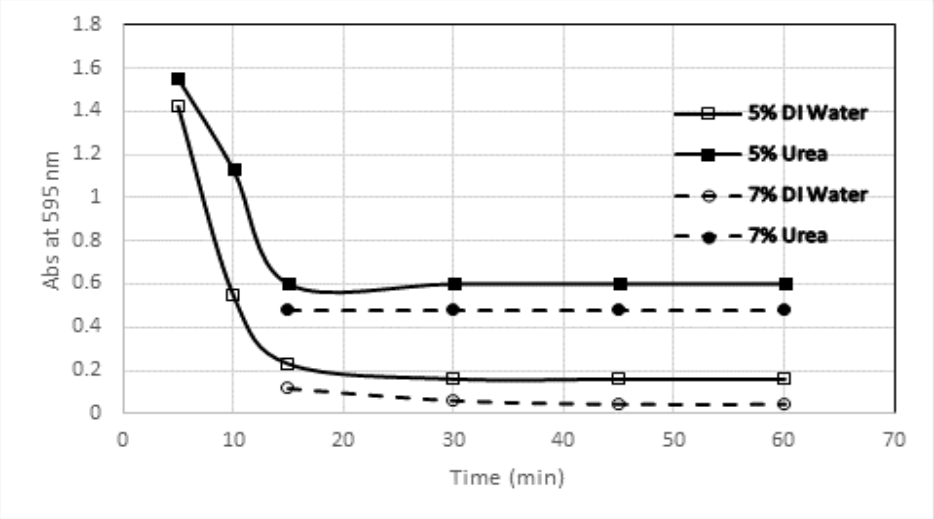

Figure 2. Solubility – as expressed by absorbance at 595 nm - of whey protein polymers and aggregates at different conditions as prepared via *Method 2*.

The complete solubility at pH 3 without Urea using *Method 2* is unlike the partial solubility using *Method 1* at both temperatures (110 ^{o}C and 85 ^{o}C). This difference in solubility can be attributed to the drying mechanism. In *Method 1*, drying takes place at a temperature higher than denaturation temperature, while in Method 2, drying takes place at much lower temperature. Drying using *Method 1* can be expected to cause more hydrophobic interactions and hydrogen bonding, leading to less soluble protein polymers and aggregates. However, upon using Urea, both methods, at pH 3, gives complete solubility.

It is worth mentioning that a similar solubility does not mean identical molecular structure. Hence, further probing using molecular characterization techniques may be necessary for deeper understanding.

CONCLUSIONS

Whey protein polymers and aggregates resulting from two methods of thermal treatment and drying were assessed for solubility. Aggregates at pH 3 show much higher solubility as compared with pH 7. Disruption of hydrogen bonding increased polymers and aggregate solubility. Drying at a temperature higher than denaturation temperatures adversely affect protein solubility in the absence of disulfide bonds. It is thus advised that in order to obtain soluble protein macromolecules, aggregation should take place under conditions that minimizes disulphide and hydrogen bonds by manipulating pH, reaction and drying conditions.

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