Technical Report:

Whey Protein Heat Stability

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As part of a growing health and wellness trend, food and beverage manufacturers are boosting the protein content of products. Many food and beverage manufacturers use whey proteins as the protein source of choice for product innovation. Expanded utilization of these ingredients holds great potential for creating even more new formulation possibilities through improvements in whey quality and performance.

This report summarizes recent research on improving the heat stability of whey protein ingredients, thus helping product developers utilize them in more applications. Whey, by definition from the Code of Federal Regulations, is the liquid substance obtained by separating the coagulum from milk or cream in cheese making. Whey proteins represent about 20 percent of the milk proteins, which remain in the serum phase during the process of making cheese and are later processed into many different ingredients. The primary whey proteins are beta-lactoglobulin (β-lg), alpha-lactalbumin (α-lac), bovine serum albumin, immunoglobulins and proteose peptones.[1]

A unique set of proteins with special physical properties, whey proteins are a component of many commercial ingredient blends that contribute to the flavor, texture and nutrition of a wide variety of food products. The high level of essential amino acids, especially branched-chain amino acids, makes whey protein a sought-after nutritional ingredient. The physical properties of whey protein ingredients give them versatility across many food applications. One specific aspect of whey protein that may be challenging for some formulations is sensitivity to heat. Consequently, the Dairy Research Institute®, established under the leadership of America's dairy farmers through the dairy checkoff program, has supported a variety of research that aims to improve whey protein's performance in higher heat processing.

Whey Protein Characteristics

Whey Protein Ingredient Composition

Whey protein ingredients include whey protein concentrates (WPC) and whey protein isolates (WPI), which typically range in protein from 25 to 90 percent protein.[2] Beta-lactoglobulin and α-lactalbumin are the predominant whey proteins, representing up to 70 percent of the total protein (Table 1). The characteristics of these two proteins account for many of the physical properties of whey protein ingredients.

Table 1. Whey Protein Composition[2]

Whey Protein	WPC %	WPI %
α-lactalbumin	12 to 16	14 to 15
β-lactoglobulin	50 to 60	44 to 69
Glycomacropeptide (GMP)	15 to 21	2 to 20
Serum albumin	3 to 5	1 to 3
Immunoglobulins	5 to 8	2 to 3
Lactoferrin	<1	Not reported

[1]Walstra P, Wouters JTM, Geurts TJ. *Milk Components, Dairy Science and Technology*. 2nd ed. CRC Press;2006:Chapter 2.
[2]Foegeding EA, Luck P, Vardhanabhuti B. *Encyclopedia of Dairy Sciences*. 2nd ed. Elsevier Ltd.;2011:Whey Protein Products.

Whey Protein Functionality

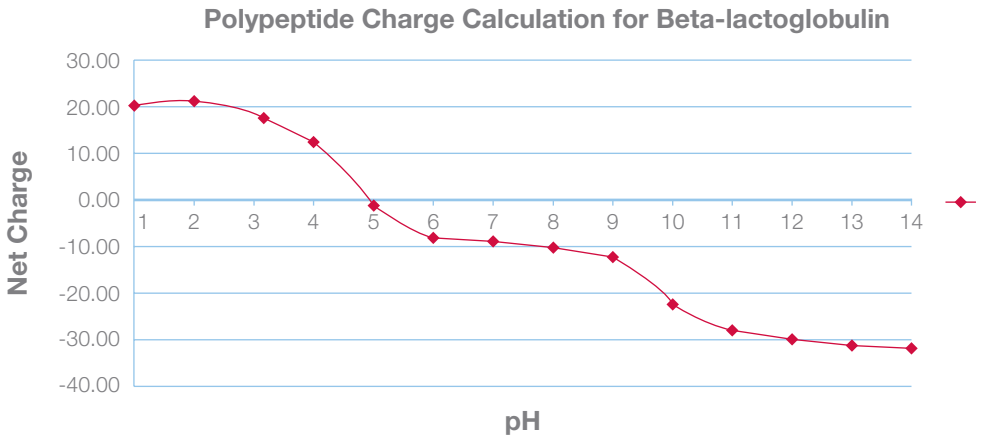
Whey protein ingredients are multifunctional ingredients providing properties such as gelation, water binding, solubility, foaming, viscosity and emulsification for foods. Of all the functional properties, solubility is thought to be one of the most important because good solubility is a necessity for all of the other functional properties. Protein functionality is demonstrated through interactions between protein molecules and the solvent (water), salts (ions) and other food components.[3] One of the unique properties of whey protein is good solubility in water over a wide range of pH (from pH 2 to 9), which is important for many beverage applications.[4] One challenge for whey protein is maintaining solubility during heat processing. Most foods are heat processed in some way, and whey proteins are susceptible to changes during heating, such as denaturation.

Understanding Heat Stability and Denaturation

Heat stability is the ability of proteins to survive heat processing without detrimental changes such as excessive turbidity, increased viscosity, phase separation, or precipitation or gelation. Beta-lactoglublin and α-lactalbumin are the major whey proteins responsible for the heat stability characteristics of ingredients such as WPC and WPI.[4] Heat denaturation of whey protein involves the unfolding of the protein followed by aggregation, which includes protein-protein interactions that are covalent (not reversible) and noncovalent (possibly reversible).[5]

One example of covalent interactions is the formation of disulfide bonds between two molelcules of β-lg. This can result from sulfhydryl oxidation or sulfhydryl-disulfide interchange. Thermal aggregation of whey protein is influenced by the temperature, ionic strength (salt concentration), ionic source and pH of the protein solution. Depending on these conditions, the proteins will show varying degrees and types of aggregate formation. In general, large, dense aggregates increase solution turbidity, whereas smaller and more linear aggregates can have a large effect on solution viscosity with minimal increase in turbidity. If the protein concentration is high enough, these protein aggregates will form either a turbid or a transparent gel network.[6] Protein aggregation increases in the presence of salt (e.g., divalent salts such as calcium chloride cause increased protein aggregation when compared to monovalent salts such as sodium chloride). The salt increases aggregation by either decreasing inter-protein charge repulsion (which is similar to the idea of bringing together the north poles of two magnets) or forming salt bridges between proteins. Because both mechanisms involve the electrostatic charge on protein molecules, it is modified at different levels of pH. The aggregation of whey proteins occurs readily in the pH range of 4.8 to 5.3, which is the range for β-lg and α-lac isoelectric points. The electrostatic interactions can be explained according to the relationship between net charge of β-lg and pH (Figure 1).

Figure 1.



Adapted from K. Higgs and G. Rhodes[7]

[3]Xiong YL. Influence of pH and Ionic Envirnoment on Thermal Aggregation of Whey Proteins. *J Agric Food Chem*. 1992;40:380-384.
[4]Vardhanabhuti B, Foegeding EA. Effects of dextran sulfate, NaCl, and initial protein concentration on thermal stability of β-lactoglobulin and α-lactalbumin at neutral pH. *Food Hydrocolloids*. 2008;22(5):752-762.
[5]Ryan KN, Vardhanabhuti B, Jaramillo DP, van Zanten JH, Coupland JN, Foegeding EA. Stability and mechanism of whey protein soluble aggregates thermally treated with salts. *Food Hydrocolloids*. 2012;27:411-420.
[6]Prabakaran S, Damodaran, S. Thermal unfolding of β-lactoglobulin: Characterization of initial unfolding events responsible for heat-induced aggregation. *J Agric Food Chem*. 1997;45:4303-4308.
[7]Adapted from Polypetide Charge Calculation for Beta-lactoglbulin chart developed by Gale Rhodes, University of Southern Maine. Available at spdbv.vital-it.ch/TheMolecularLevel/Goodies/PeptChg.xls. Accessed April 19, 2012.

Most of the foods we consume are between pH 3 and 7. At pH 3, β-lg has a very high net positive charge, which means the proteins in the solution will have a lot of repulsive forces between them. These repulsive forces will inhibit interactions between proteins, even when heat is applied — so heat stability and clarity (lack of turbidity) even at protein concentrations of 7 percent protein is high. Once the pH is increased to 4, the net charge is decreased, as is the repulsion. The whey protein solution will now increase in turbidity, even without heating, due to the attraction between proteins. When heat is applied at temperatures higher than the denaturation temperature of 78 C β-lg, protein aggregation and precipitation will occur unless some other ingredients are added to improve the heat stability of the proteins. At pH 5.2, the isoelectric point, the protein has a net charge of 0 and the potential for protein-protein interaction is at its highest point. The protein will precipitate out of solution readily at this pH. As the pH is increased up to pH 7, the net charge on the protein will shift to negative, but the net negative charge at pH 7 is similar to the net positive charge at pH 4.2.[8]

Commercial Heat-stable Ingredients

Techniques exist today to improve the heat stability of whey protein ingredients. Some of these techniques are used in commercial ingredients. Examples include protein hydrolysis, modifying the mineral composition or adding mineral chelators. These techniques and other new research are discussed in the next section.

New Research and Methods to Improve Heat Stability in Commercial Ingredients of the Future

Recent research funded by the Dairy Research Institute has focused on improving the heat stability of whey proteins to increase their use in an even wider range of food products. Researchers have chosen various approaches to improve whey protein heat stability including: controlling the size of protein aggregates through sugar addition, enzymatic cross-linking, mineral chelation and ultrasonication; or modifying whey protein to prevent aggregation through molecular chaperones, enzyme hydrolysis, electrostatic repulsion, conjugation with carbohydrates and protein encapsulation.

Controlling the Size of Protein Aggregates

Sugar Addition

Several researchers have studied the relationship between sugar addition and heat stability of whey protein ingredients. Sucrose addition was found to increase gelation temperature and gel strength of WPI and bovine serum albumin.[9,10,11,12,13] Adding glycerol improved heat stability of WPI and decreased turbidity and protein gelation.[10,14] The addition of sorbitol also increased thermal denaturation temperatures of WPI and was more effective than glycol. No gelation occurred after heating a 10 percent β-lg solution with the addition of 0 to 55 percent sorbitol at pH 7.0.[15] Many food applications contain sugars or sugar alcohols, and their presence can help to improve the heat stability of whey protein ingredients by preventing the formation of large aggregates and providing better clarity in applications such as beverages.

[8]Personal communication with Allen Foegeding.
[9]Foegeding EA, Davis JP, Doucet D, McGuffey MK. Advances in modifying and understanding whey protein functionality. *Trends Food Sci Technol*. 2002;13:151-159.
[10]Rich LM, Foegeding EA. Effects of sugars on whey protein isolate gelation. *J Agric Food Chem*. 2000;48(10):5046-5052.
[11]Baier S, McClements DJ. Impact of preferential interactions on thermal stability and gelation of bovine serum albumin in aqueous sucrose solutions. *J Agric Food Chem*. 2001;49(5):2600-2608.
[12]Baier SK, McClements DJ. Influence of cosolvent systems on the gelation mechanism of globular protein: Thermodynamic, kinetic, and structural aspects of globular protein gelation. *Compr Rev Food Sci F*. 2005;4(3):43-54.
[13]Baier SK, McClements DJ. The effect of binary cosolvent systems (glycerol-sucrose mixtures) on the heat-induced gelation mechanism of bovine serum albumin. *Int J Food Sci Technol*. 2006;41(2):189-199.
[14]Kulmyrzaev A, Bryant C, McClements DJ. Influence of sucrose on the thermal denaturation, gelation, and emulsion stabilization of whey proteins. *J Agric Food Chem*. 2000;48:1593-1597.
[15]Chanasattru W, Decker EA, McClements DJ. Modulation of thermal stability and heat-induced gelation of β-lactoglobulin by high glycerol and sorbitol levels. *Food Chem*. 2007;103:512-520.

Enzymatic Cross-linking

Enzymatic cross-linking of individual whey proteins and other proteins with transglutaminase (TG) also has been shown to increase heat stability.[16,17,18] Cross-linking of β-lactoglobulin and casein resulted in no gel formation at concentrations of 5 percent and a soft gel at 10 percent with water and heat treatments of 95 C for 30 minutes. Optimum heat stability was obtained at pH 8, but 90 percent solubility was maintained at pH 7 and heating conditions of 100 C with concentrations up to 5 percent.[16] Treatment of WPI solutions with TG at concentrations of 4 and 8 percent with water at pH 7.5 showed increases in gel temperature from 68 C to 94 C and decreases in gel strength. The cross-linking that resulted between α-lactalbumin and β-lactoglobulin was found to be extensive and thought to create large enough polymers to prevent formation of a gel network.[15] Cross-linking of whey protein concentrates (30 to 35 percent protein) with TG provided increased heat stability at 90 C for 30 minutes between pH 6.4 and 7.2 at concentrations of 3.5 percent protein.[17]

This research used the hypothesis of others that the improved heat stability of the cross-linked WPC primarily was due to cross-linking between α-lactalbumin and β-lactoglobulin. An additional recent study demonstrated the effects of cross-linking microbial transglutaminase with WPI on pH and heat stability of the proteins. The cross-linking significantly increased the denaturation temperature of β-lg from 71.84 C in the untreated sample to 78.50 C after the 30-hour reaction time with transglutaminase.[19]

Mineral Chelation

Minerals are naturally present in whey protein concentrate and isolate. Divalent cations, like calcium, are capable of forming an ionic bridge between two adjacent carboxyl groups from different peptide chains, whereas monovalent ions like sodium cannot. Sodium phosphate addition decreased protein-protein interactions in 10 percent WPI solutions that were heated up to 96 C.[3] Another study using N,N,N',N',-tetraacetic acid (EGTA) or ethylenediamine-tetraacetic acid (EDTA) as chelators was effective at reducing protein aggregation and gelation in WPC and WPI solutions (11 percent with water) at pH 7.0.[20] In other Dairy Research Institute-funded research conducted by Foegeding, which has not yet been published, sodium citrate, EDTA and protein hydrolysates were used to bind calcium, producing a product that was heat-stable at 90 C for five minutes at 5 percent protein in a neutral pH beverage.

Ultrasonication

Ultrasonication for different times (five and 15 minutes) and temperatures (20 C, 60 C and no temperature control) has been applied to whey solutions from 6.9 to 30.2 percent total solids and 13.5 to 88 percent protein. A 90 percent decrease in turbidity was achieved in a whey solution of 28.2 percent solids and 35.6 percent protein when ultrasonication was used at 15W of electrical power at 60 C and with no temperature control. Ultrasonication of solutions with 88 percent protein resulted in an increase in turbidity.[21] The mechanism of this new, patent-pending work is attributed to physical forces generated by acoustic cavitation, which yield a smaller particle size and whey proteins that have better heat stability and clarity. Another study found that combining ultrasonication with a preheat treatment of 80 C for 1 minute or 85 C for 30 seconds significantly improved the heat stability of whey protein concentrates. Heat stability was still maintained after spray-drying and reconstitution.[22]

Controlling Protein Aggregation

Molecular Chaperones

Molecular chaperones are compounds that help stabilize whey proteins and prevent them from unfolding, aggregating and precipitating. Casein has the ability to bind with other proteins to make them more chemically and heat-stable and

[16]Truong VD, Clare DA, Catignani GL, Swaisgood HE. Cross-linking and rheological changes of whey proteins treated with microbial transglutaminase. *J Agric Food Chem*. 2004;52:1170-1176.
[17]Tanimoto SY, Kinsella JE. Enzymatic modification of proteins: effects of transglutaminase cross-linking on some physical properties of β-lactoglobulin. *J Agric Food Chem*. 1988;36:281-285.
[18]Lorenzen PC. Effects of varying time/temperature-conditions of pre-heating and enzymatic cross-linking on techno-functional properties of reconstituted dairy ingredients. *Food Res Int*. 2007;40:700-708.
[19]Agyare K, Danodaran S. pH-stability and thermal properties of microbial transglutaminase-treated whey protein isolate. *J Agric Food Chem*. 2010;58:1946-1953.
[20]Kuhn P, Foegeding EA. Factors influencing whey protein gel rheology: Dialysis and calcium chelation. *J Food Sci*. 1991;56(3):789-791.
[21]Martini S, Potter R, Walsh MK. Optimizing the use of power ultrasound to decrease turbidity in whey protein suspensions. *Food Res Int*. 2010;43(10):2444-2451.
[22]Zisua B, Bhaskaracharyab R, Kentishb S, Ashokkumar M. Ultrasonic processing of dairy systems in large scale reactors. *Ultrasonics Sonochemistry*. 2010;17(6):1075-1081.

more resistant to aggregation; thus, casein acts as a molecular chaperone. Alpha-casein can interact with partially unfolded proteins through hydrophobic surfaces, preventing the normal thiol-disulfide interchange with other whey proteins and eventual aggregation. This casein protein also can solubilize hydrophobically aggregated proteins. And its chaperone ability increases with decreasing temperature.[23] Other researchers also have studied the ability of alpha-casein, beta-casein and kapa-casein to be molecular chaperones.[24]

Beta-casein forms a complex with proteins through electrical charges. Complex formation can occur with soluble or aggregated whey protein, and the resulting compound is smaller than a self-aggregated whey protein complex. Smaller complexes were more soluble and heat tolerant to 145 C, resulting in solutions with improved clarity. The chaperone effect of milk protein concentrate was evaluated in conjunction with whey protein solutions and found to be stable at 10 percent protein in a meal replacement beverage processed under retort heating conditions.[25,26]

Enzymatic Hydrolysis

A common method of improving heat stability is through the enzymatic hydrolysis of whey proteins. Commercial hydrolyzed whey proteins (WPH) designed for improved heat stability generally have a 5 to 10 percent level of hydrolysis. Changes in functional properties of WPH are due to the physical characteristics of the peptides. Generally, the peptides have a lower molecular weight, exposed hydrophobic groups and more ionic groups. Typically, WPH have increased solubility, decreased viscosity and modified foaming, gelling and emulsifying properties compared with unmodified proteins.[27] The hydrolysis process produces peptides that lack a secondary structure, resulting in minimal conformational changes during heat processing.[7] The physiochemical properties of WPH are related to the purity of the protein substrate; the pretreatment of the protein substrate; the specificity of the enzyme used for proteolysis; the physicochemical conditions (pH, temperature, ionic strength, activator) used during hydrolysis; the degree of hydrolysis; the technique used for enzyme inactivation (heat treatment, acidification or membrane filtration); and the use of post-hydrolysis treatments.[26] Recent research also showed that partial hydrolysis of whey proteins improved their heat stability and functionality, but excessive hydrolysis could reduce heat stability and functionality.[28]

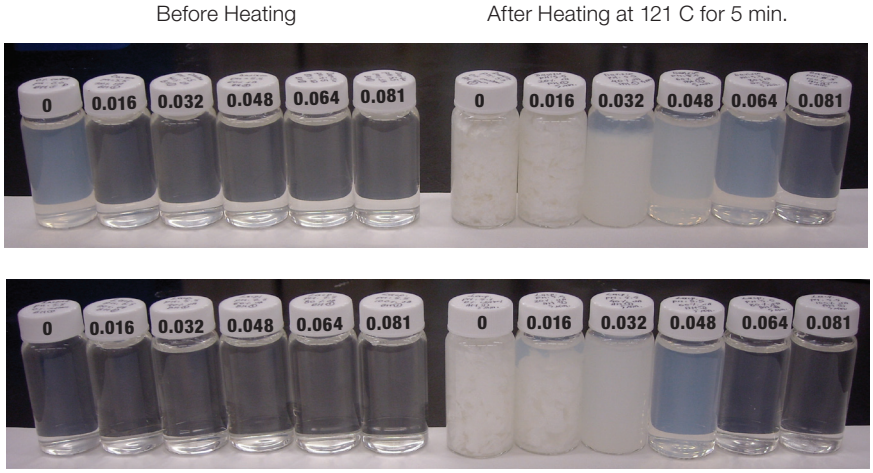
Another project utilized protein solutions containing 5 to 35g of protein per 8 oz. between pH 3.0 and 4.5. A centrifuge technique was used to remove traces of precipitated whey protein in solutions and adjusted to pH 4.6. Clarity was improved in the finished beverages using this technique, as well. This research then combined the use of enzymes to divide the protein into water-soluble and insoluble fragments followed by centrifugation or filtration at pH 4.6.[29] Researchers used trypsin to make macropeptide fragments that were too large to be bitter and too small to aggregate for the purpose of manufacturing whey proteins designed for acidic and neutral pH beverages. Beverages with improved clarity and heat stability were created even up to pH 4.6 using this method.

Electrostatic Repulsion

New research sponsored by the Dairy Research Institute combined enzymatic hydrolysis with electrostatic repulsion to improve the heat stability and clarity of whey proteins. Two approaches were used to modify the charge on the whey proteins. In one approach, the positively charged portions on the protein were blocked by a succinylation reaction (modification of primary amino groups) at pH 8. This reaction reduced the number of basic groups while increasing the acidity of the protein and enhancing the electrostatic charge repulsion. In the second approach, an amidation reaction blocks the negatively charged ω-carboxyl groups and converts them to uncharged amino acids. This research determined the minimum number of charges that should be modified to increase the electrostatic repulsion and prevent aggregation at pH 3.8 to 4.6.[30]

[23]Bhattacharyya J, Das KP. Molecular chaperone-like properties of an unfolded protein, α-casein. *J Biol Chem*. 1999;274:15505-15509.
[24]Morgan PE, Treweek TM, Linder RA, Price WE, Carver JA. Casein proteins as molecular chaperones. *J Agric Food Chem*. 2005;53:2670-2683.
[25]Zhang X, Fu X, Zhang H, Liu C, Jiao W, Chang Z. Chaperone-line activity of β-casein. *Int J Biochem Cell Biol*. 2005;37:1232-1240.
[26]Yong YH, Foegeding EA. Effects of caseins on thermal stability of bovine β-lactoglobulin. *J Agric Food Chem*. 2008;56:10352-10358.
[27]Gauthier SF, Pouliot Y. Functional and Biological Properties of Peptides Obtained by Enzymatic Hydrolysis of Whey Proteins. *J Dairy Sci*. 2003;86(13):E78-E87.
[28]Doucet D, Foegeding EA. Gel Formation of Peptides Produced by Extensive Enzymatic Hydrolysis of β-Lactoglobulin. *Biomacromolecules*. 2005;6(2):1140-1148.
[29]Zhu D, Damodaran S, Lucey JA. Physicochemical and emulsifying properties of whey protein isolate (WPI)-Dextran conjugate produced in aqueous solution. *J Agric Food Chem*. 2010;58:2988-2994.
[30]Yilmaz-Gemili A. Electrostatic repulsion enhancement for heat stable, clear whey protein beverages. M.S. thesis, 2012.

Figure 2.



Solution 1. Solutions were tested at pH 5.5, 6.0 and 7.0. As pH was increased from 5.5 to 7.0, less charge modification was needed to prevent protein aggregation and produce a clear beverage.

More charge modification was necessary to achieve a clear beverage for WPI 1 (top) solutions as compared with WPI 2. Modification of 77 percent of the epsilon amino groups was necessary to produce a clear beverage in WPI 1 at pH 5.5 (0.081g SA/g protein)

Solution 2. Modification of 65 percent of the epsilon amino groups was necessary to produce a clear beverage in WPI 2 (0.064g SA/g protein).

Figure 2 on this page represents the succinylation approach described above. Researchers blocked the negatively charged carboxyl group by adding succinic anhydride (SA) to two different, modified WPI solutions. As the number of charges increased, the heat stability and clarity of the whey protein improved in the control WPI before (left side) and after heating (right side) at pH 5.5. Values on the top of vials are the ratio of grams (g) of SA/g WPI. Control samples at different pH were prepared by using the same procedure without the addition of SA.

Conjugation With Carbohydrates

Several researchers have explored the conjugation of whey proteins and polysaccharides for the purpose of improving the heat stability of whey proteins in food applications. One study evaluated the effects of sodium chloride, dextran sulfate and protein concentration of β-lg and α-lac on thermal stability (85 C for 15 minutes) at pH 6.8. Sodium chloride had the most effect on increasing protein aggregation evidenced by increases in turbidity, molecular size and loss of protein solubility. Dextran sulfate provided a protective effect against protein aggregation at low concentrations.[4] Subsequent research using dextran sulfate and β-lg (6 percent with water) at pH 5.6 to 6.2 and heated at 85 C for 15 minutes decreased turbidity of solutions by decreasing the denaturation temperature of β-lg and altering its aggregation.[31] Other research explored the conjugation reaction between WPI and dextran in solution at the initial stage of the Maillard reaction. The conjugation reaction (24 hours) was optimal at pH 6.5 and a temperature of 60 C with a WPI concentration of 10 percent and a dextran concentration of 30 percent.[32] Later research examined the heat stability and emulsifying properties of WPI and dextran conjugates under similar conditions for 48 hours. The purified conjugate had significantly improved heat stability at 80 C for 30 minutes and maintained solubility at pH 3.2 to 7.5 and ionic strengths of 0.05 to 0.2 Molar as compared with control WPI. The emulsifying ability and stability were better than a gum arabic and WPI solution.[27]

Protein Encapsulation

Initial work included two different approaches prior to encapsulation. A 5 percent dispersion of WPI was cross-linked by transglutaminase prior to incorporation in microemulsions and heat treatment at 90 C for 20 minutes. In the second approach, WPI was cross-linked by transglutaminase within the microemulsions before heat treatment. The two approaches produced particles of different dimensions and heat stability. Heat stability was improved with increase in transglutaminase concentration and cross-linking duration.[33] In more recent research, heat-stable whey

[31]Vardhanabhuti B, Yucel U, Coupland JN, Foegeding EA. Interaction between b-lactoglobulin and Dextran Sulfate at Near Neutral pH and their Effect on Thermal Stability. *Food Hydrocolloids*. 2009;23:1511-1520.
[32]Zhu D, Damodaran S, Lucey JA. The formation of WPI-dextran conjugates in aqueous solutions. *J Agric Food Chem*. 2008;56:7113-7118.
[33]Zhang W, Zhong Q. Microemulsions as nanoreactors to produce whey protein nanoparticles with enhanced heat stability by sequential enzymatic cross-linking and thermal pretreatments. *J Agric Food Chem*. 2009;57:9181-9189.

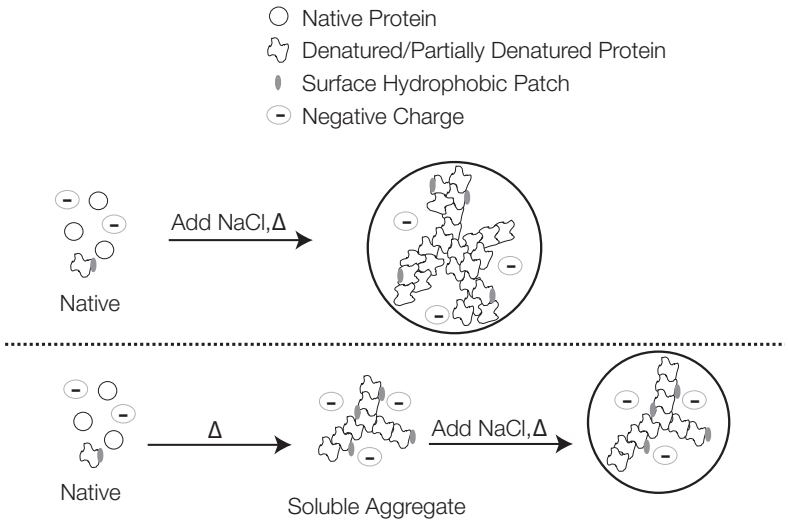
protein aggregates were formed using water/oil microemulsions. These nanoparticles were immersed in various surfactants to produce a heat-stable transparent dispersion even in the presence of a high concentration of salt.[34]

Currently, scale-up research funded by the Dairy Research Institute is using various protein encapsulation approaches to produce solutions with 10 to 15 percent whey protein that are clear and heat-stable under severe processing conditions including retort at both acidic and neutral pH.

Formation of Soluble Aggregates

Recently published research examined the formation of soluble aggregates from WPI and β-lg. Solutions of 7 percent protein (buffered to pH 6.8) were heated to 90 C for 10 minutes and cooled in an ice bath for 10 minutes.[5] The native solutions and heated solutions of soluble aggregates were evaluated for their salt stability by combining them with sodium chloride solution, diluting the protein concentration 3 percent. The salt/protein solutions were heat-treated again at 90 C for five minutes and cooled in an ice bath to room temperature. The whey-protein-soluble aggregates had increased heat stability in the presence of salt at neutral pH. The proposed mechanism is illustrated in Figure 3.

Figure 3.[5]



Denatured proteins are present in whey protein isolate due to spray drying. Denatured proteins have exposed hydrophobic groups due to unfolding. The negative charges shown reflect the average charge of the proteins. The researchers propose that using soluble aggregates increases heat stability by reducing the aggregate size and modifying the shape of the aggregate. These changes reduce the turbidity and viscosity and increase the solubility of the final ingredient. Heating in salt solutions also lowers the potential for secondary interactions due to their higher negative charge, more compact structure with less branching and small size.

Formulating for Heat-stable Applications

Improved whey protein heat stability can benefit multiple types of food and beverage applications. Many of the projects cited in this paper were designed with protein-enhanced beverages as a target for their work, and a basic understanding of formulating with whey proteins in beverages already has been published.[35] Despite this progress, protein beverage developers may have formulation challenges. It is the goal of whey protein researchers to develop technologies addressing stability issues created by whey proteins.

[34]Zhang W, Zhong, Q. Microemulsions as nanoreactors to produce whey protein nanoparticles with enhanced heat stability by thermal pretreatment. *Food Chem*. 2010;119:1318-1325.
[35]Rittmanic S, Burrington K. U.S. Dairy Export Council Whey Applications Monograph: U.S. whey proteins in Ready to Drink Beverages. 2006. Available at: www.usdec.org/files/Publications/BEVERAGESwebversion8-16-06.pdf. Accessed April 19, 2012.

Beverages probably pose the greatest challenge for protein stability due to the high concentrations of protein that some developers hope to achieve. One of the most important steps in achieving good heat stability is hydration of the whey protein ingredient. Whey protein ingredients are powders that require good hydration to achieve optimal performance during heat processing. Best practices for hydration include mixing the whey protein ingredient in water that is less than 60 C with a high-speed mixer and then allowing the whey to hydrate with slow or no agitation for a minimum of 30 minutes prior to heat processing.[34] Continuous mixing with high shear will create foaming and denature the whey proteins prior to heat treatment. This denaturation will lead to a cloudy or grainy/chalky texture and protein precipitation after heat processing.

Improvements in whey protein heat stability also will help in other food applications where protein enhancement is desired, such as retorted soups and sauces, confections such as caramels, protein-enhanced ice creams and other foods that undergo high-temperature processing to extend their shelf life. Research efforts supported by the Dairy Research Institute will continue to improve the functionality of whey protein ingredients that can be used for future foods and beverages.

For more information about whey protein and ongoing dairy ingredient research, visit InnovateWithDairy.com, USDairy.com/DairyResearchInstitute or USDEC.org. For assistance with new or improved products using dairy ingredients, contact Dairy Technical Support at techsupport@InnovateWithDairy.com.

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