

Improved Functional Characteristics of Whey Protein Hydrolysates in Food Industry

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Abstract

This review focuses on the enhanced functional characteristics of enzymatic hydrolysates of whey proteins (WPHs) in food applications compared to intact whey proteins (WPs). WPs are applied in foods as whey protein concentrates (WPCs), whey protein isolates (WPIs), and WPHs. WPs are byproducts of cheese production, used in a wide range of food applications due to their nutritional validity, functional activities, and cost effectiveness. Enzymatic hydrolysis yields improved functional and nutritional benefits in contrast to heat denaturation or native applications. WPHs improve solubility over a wide range of pH, create viscosity through water binding, and promote cohesion, adhesion, and elasticity. WPHs form stronger but more flexible edible films than WPC or WPI. WPHs enhance emulsification, bind fat, and facilitate whipping, compared to intact WPs. Extensive hydrolyzed WPHs with proper heat applications are the best emulsifiers and addition of polysaccharides improves the emulsification ability of WPHs. Also, WPHs improve the sensorial properties like color, flavor, and texture but impart a bitter taste in case where extensive hydrolysis (degree of hydrolysis greater than 8%). It is important to consider the type of enzyme, hydrolysis conditions, and WPHs production method based on the nature of food application.

Keywords: whey protein, whey protein hydrolysate, enzymatic hydrolysis, functionality, food application

Received February 3, 2015; Revised April 6, 2015; Accepted April 27, 2015

Introduction

Whey protein (WP) is now recognized as a value-added ingredient because of its highly functional and nutritional properties. WPs are used widely by dairies, bakeries, confectionaries, meat processing plants, canned goods, and beverage establishments for their various functions in food quality and stability (de Wit, 1998; Foegeding *et al.*, 2002). Because of their nutritional and functional properties, researchers have intensified efforts to expand the utilization of WPs as food ingredients. Technological advances have enabled researchers to enhance the functionality and utilization of WP by producing more concentrated and specialized forms. Pressure-driven membrane processes have been used to concentrate and separate whey protein concentrates (WPCs) or whey protein isolates (WPIs) in order to maintain their functional properties

and make them suitable for other purposes in the food industry (Suarez *et al.*, 1992). Today, WP ingredients are used to replace other proteins or to improve the functional properties of many food products (Spellman *et al.*, 2005).

WPs are comprised mainly of β -lactoglobulin (β -Lg), α -lactalbumin (α -La), bovine serum albumin (BSA), and immunoglobulin, and the effects of WP functionality have been studied extensively (Foegeding *et al.*, 2002; Smithers *et al.*, 2008). β -Lg, a globular and amphiphilic protein with two disulfides and one free cysteine, is the main constituent of WP (around 55% of proteins) and is known to contribute the most to the WP functionality (Bouaouina *et al.*, 2006). Conformation and functionality of WPs are interrelated and dictated by changes in their globular folded structure. Their functional properties are affected by several factors within a food application, including concentration, state of the WPs, pH, ionic environment, (pre-) heat treatment, pressure treatments, and the presence of lipids (Burrington, 1999). The functionality of WPs can be improved by chemical, enzymatic, and physical processes. High pressure treatments are used to enhance the functionality of WPC, but still remain below the expected levels of the industrial food applications (Kresic *et al.*,

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2006). Enzymatic modifications are highly acceptable and applied in the industry not only for functionality improvement but also for bioactive enhancement (Athira *et al.*, 2014; Sharma *et al.*, 2011). These hydrolysates are also being used as protein supplements for infants, senescent, athletes, and bodybuilders (Sousa *et al.*, 2004). The resulting peptides are more easily absorbed but the level of hydrolysis has to be carefully controlled to avoid the formation of bitter peptides (Mann, 2000).

Enzymatic hydrolysis is preferred by food manufacturers due to the availability of a wide range of enzymes that are considered safe and natural. Several enzymes are currently used in the industry as food-grade enzymes and others are being researched for the production of WPHs with tailored functionality and biological activity. The most researched and used enzymes for the production of WPHs are the digestive enzymes trypsin, pepsin, and chymotrypsin (Konrad *et al.*, 2005; Pouliot *et al.*, 2009), plant enzymes mainly papain and bromelain (Nakamura *et al.*, 1993), bacterial proteases mainly those originating from *Bacillus licheniformis* (Creusot *et al.*, 2006; Creusot and Gruppen, 2007a, 2007b; Doucet and Foegeding, 2005) and *Bacillus subtilis* (Madsen *et al.*, 1997), or a mixture of some of these enzymes (Kim *et al.*, 2007). The objective of this review is to emphasize the improvement in functional characteristics of WPHs over those of intact WPs in food applications.

Manufacture of Whey Protein Products

Whey protein concentrates (WPCs) and whey protein isolates (WPIs)

The most valuable component of whey is its protein, which delivers both enhanced functionality and nutritional quality to many formulations. WPs are able to concen-

trate, fracture, and dehydrate efficiently and cost-effectively without damaging the protein structure (Fig. 1). By these methods WPs are produced and introduced to the market as whey protein concentrates (WPCs, 35-80% protein) and whey protein isolates (WPIs, 90-96% protein).

Whey protein hydrolysates (WPHs)

Two main methods of improving WP functionality are modification of proteins through physical treatments, and enzymatic means. Chemical modifications of proteins are possible but due to the necessity to prove the safety of such modifications to humans, they are not generally practiced. Chemical hydrolysis causes loss of some essential amino acids, such as tryptophan, and can result in products with high amounts of free amino acids, which can encumber the body's osmotic balance (Mahmoud, 1994).

For WPs hydrolyzing, enzymatic hydrolysis is the most applicable method in food industry. The functional and biological properties of the WPHs depend to a great extent on the type of enzyme used (specificity and selectivity), hydrolysis conditions (enzyme-to-substrate ratio, incubation temperature, pH, and time) employed, and the source of the protein, native or denatured, WPI vs. WPC, membrane or ion-exchange product, etc. Researchers have shown that limited enzymatic hydrolysis of WPs can result in markedly improved functionality and biological activities (Creusot and Gruppen, 2008; Pouliot *et al.*, 2009; Spellman *et al.*, 2009).

Functional benefits

WPs have typically a globular structure, with high levels of secondary and tertiary structures, in which acidic/basic and hydrophobic/hydrophilic amino acids are distributed in polypeptide chains. The functional benefits depend on (a) hydration, (b) aggregation and gelation, (c)

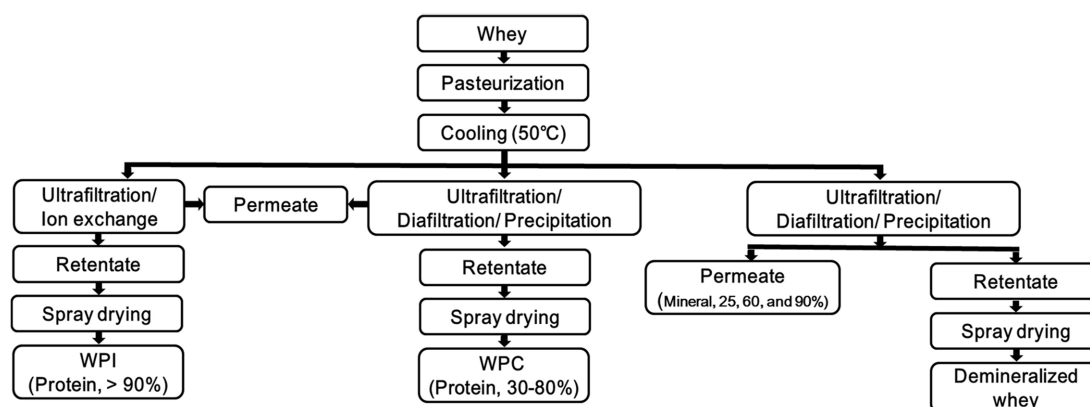


Fig. 1. Production steps of whey products.

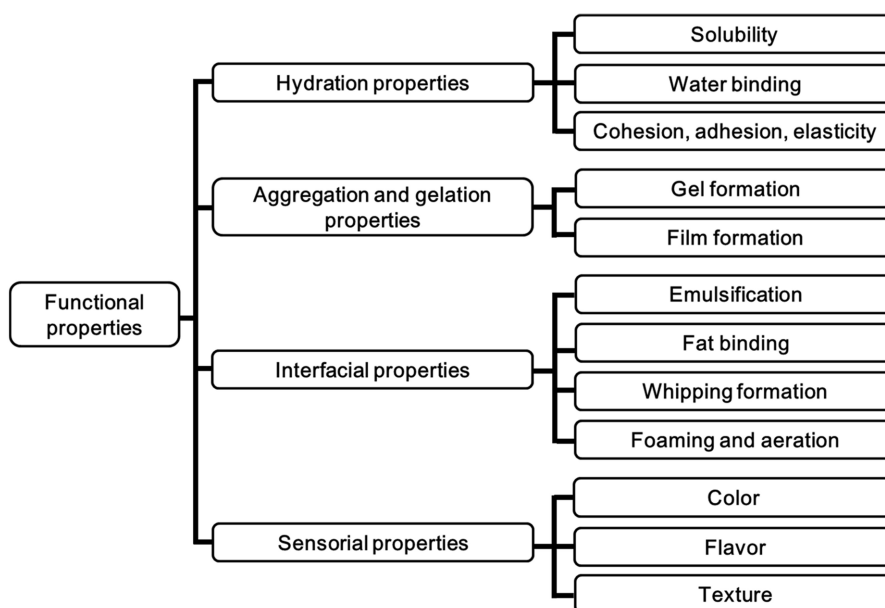


Fig. 2. Classification of functional properties of WPHs in food applications.

interfacial, and (d) sensorial properties of the food protein (Fig. 2) (Kresic *et al.*, 2006). Hydration properties have an important effect on swelling, adhesion, dispersibility, solubility, viscosity, water absorption, and water holding. Aggregation and gelation properties, on the other hand are related to protein-protein interactions while Interfacial properties include emulsification and foaming characteristics. Sensorial properties include flavor, color, and texture properties of the protein.

Hydration properties

Solubility

The solubility of WPCs at a low pH is a unique property that allows it to function in acidic foods and beverages (Kumar *et al.*, 2010; Pelegrine and Gasparetto, 2005). It has a great potential as a food ingredient for the protein enrichment of soft drinks and beverages. Heating WPs can result in a loss of solubility due to the denaturation of the proteins, especially in the pH range of 4.0-6.5 (Burrington, 1999). Most foods are heat processed in some way, and WPs are susceptible to changes during heating. Denaturation at low pH leads to aggregation and insolubility. One challenge for WP is maintaining solubility during heat processing. Solubility of WPCs decreases as temperature increases (Pelegrine and Gomes, 2012). However, the maximum intermolecular aggregation of β -Lg without heat does occur near the iso-electric point due to neutralization of charges and interaction via hydrophobic

forces (Majhi *et al.*, 2006; Mehalebi *et al.*, 2008; Schmitt *et al.*, 2009). Increasing the ionic strength results in salting in of β -Lg (Majhi *et al.*, 2006).

In many cases, limited hydrolysis leads to increased solubility due to the reduced molecular weight and the increased hydrophilicity resulting from the increase in free carboxyl and amine groups. Researchers proposed that hydrolyzing WPs with various proteolytic enzymes under different conditions can be used to improve the solubility over a wide range of pH (Mutilangi *et al.*, 1996; Perea *et al.*, 1993). WPHs had shown less solubility in pH 4 while showing the highest solubility around pH 6-10 (Jeewanthi *et al.*, 2014). More extensive hydrolysis has been shown to increase solubility (Flanagan and Fitzgerald, 2002; Jeewanthi *et al.*, 2014). Also, heat stability was improved upon partial hydrolysis due to loss of the secondary structure, thus contributing to reduced structural changes upon heating (Foegeding *et al.*, 2002).

Adhesion, cohesion, elasticity, and water-binding

The adhesion properties of WPCs help to improve the homogeneous texture of food products. WPCs may be used to bind breadcrumbs or batter to meat, poultry or fish. Effective adhesion between meat pieces is an important quality characteristic in the manufacture of products such as chicken nuggets or restructured ham. The actual meat particle binding occurs during cooking as heat setting of the proteins takes place. WPs aid in this binding by forming strong, irreversible gels that restructure into

an extended three-dimensional network, thereby helping to adhesion (Prabhu, 2006). WPCs decreased the average hardness and chewiness values and increased cohesiveness of the sausages. The adhesion of the WPs is affected by temperature due to denaturation of protein structure (Goode *et al.*, 2013).

Moisture binding is especially important during the cooking stage when the denaturing salt soluble meat proteins experience a progressive decline in water holding capacity (Tsai *et al.*, 1998). Barbut (2007) reported that poultry treated with WPHs had the lowest cooking loss and highest yield with higher water retention capacity compared to those treated with WPI, casinates, β -Lg, and whole milk proteins. The water-binding abilities of WPs can help reduce formula costs as the proteins hold additional water. Viscosity development is closely related to gelation and other protein-protein interactions (Burrington, 1999).

Aggregation and gelation properties

Gel formation

Gelation is an important functionality that is useful in baked goods, processed meats, surimi, desserts, and sour cream. A gel is an intermediate structure between solid and liquid, which carbohydrates or protein strands cross-link to form a network. Gelation is favored by large molecules of proteins since they form extensive networks by cross-linking in three dimensions and by the ability of the denaturing. Rinn *et al.* (1990) reported that WPCs prepared by microfiltration through 0.6 μ m pores exhibited superior gels at 4-5% protein. In restructuring new meat products, WPCs have been utilized for their abilities to exhibit heat-coagulating and heat gelling properties (Morr and Ha, 1993). Cold-set gelation of WPs has also been noted by Bryant and McClements (1998). Hydrolyzed β -Lg also has the ability to form networks associated with gels (Foegeding *et al.*, 2002).

In their un-denatured form, WPs form rigid gels that hold water and fat, and provide structural support. The formation of disulfide bonds and ionic bonding controlled by calcium ions determine gel structure (Burrington, 1999). Hydrophobic interactions can play a major role in partially hydrolyzed proteins and consequently unfolding the protein structure (Pinterits and Arnteld, 2007). This exposes buried hydrophobic groups and other interactive groups which are then free to interact with neighboring polypeptides (Kang *et al.*, 1994). This promotes protein aggregations and subsequent gel setting. The effect of

hydrolysis on the gelation ability of WP is dependent on environmental conditions and on the degree of hydrolysis (DH). On the other hand, extensive hydrolysis of WPI can weaken its gelation properties (Huang *et al.*, 1999). Non heat-set gelation forms strong elastic gels during extensive hydrolysis of WPI at high solid content (20% w/v) with Alcalase 2.4L, a protease from *Bacillus licheniformis* (BLP) than the heat induced gels (Doucet *et al.*, 2001). Among the enzymes investigated for the production of WPH with enhanced gelation properties, BLP is well studied (Creusot *et al.*, 2006; Creusot and Gruppen, 2007a, 2007b; Creusot and Gruppen, 2008; Doucet and Foegeding, 2005; Spellman *et al.*, 2005). Further, Spellman *et al.* (2005) showed, after isolating subtilisin and glutamyl endopeptidase (GE) activities from Alcalase 2.4L, that the GE is the enzyme responsible for the peptide aggregation in WPHs. With enzymatic hydrolysis performing under different conditions, researchers were able to produce gels with different rheological properties (Table 1). The increased net charge on proteins results in increased repulsion between peptides. Interestingly, limited proteolysis with enzymes can also be used to control the gelling ability as well as the gel strength.

Trypsin also showed gelation properties after hydrolysis of WPs. Tryptic hydrolysis of WPs at 6.7 and 2.3% DH prevented gelation at pH 3 and 7, whereas hydrolysis to 2.3% DH with BLP dramatically increased the gelling ability and gel strength at neutral pH (Ju *et al.*, 1995). The aggregates formed upon hydrolysis of β -Lg by BLP consisted of 6-7 major peptides (2-6 kDa) (Otte *et al.*, 1996b). Peptides from β -Lg formed aggregates with other peptides and with intact β -Lg and α -La via hydrophobic interactions leading to gelation. Some researchers have found the key fragments that act as the initiators of aggregation of WPHs (Table 2).

Film formation

In food systems, edible, and permeable film coatings are used to control the transfer of aroma, flavor compounds, moisture, oxygen, and lipid. WP has been shown to make transparent films with good oxygen and aroma barrier properties (Mate and Krochta, 1996; Miller *et al.*, 1997). McHugh *et al.* (1994) produced WPCs films by heating 8-12% (w/w) solutions of WPC at 75-100°C. The suggested optimal condition for producing these films is to heat 10% WPC solutions at 90°C. Exposure of internal S-H and hydrophobic groups after denaturation by heating of WPs support improved film formation. The intramolecular S-S bonds of heat denatured WP films form insoluble

Table 1. Different gel characteristics resulting from whey protein hydrolysates made with BLP under several conditions

Gelation characteristics	Enzyme	Whey product	Hydrolytic condition	Reference
Very high gelation	Subtilisin Carlsberg + Glutamyl endopeptidase (Alcalase 2.4L)	Hydrolysates of WPC 80%	50°C, > 0.4 DH, pH 7	Spellman <i>et al.</i> , 2005
Gelation	BLP(aq) 2% (w/w)	Hydrolysates of WPI	Limited or Extensive	Creusot and Gruppen, 2007a
Gelation	BLP(aq) 2% (w/w)	Hydrolysates of WPI	4°C, Extensive	Creusot <i>et al.</i> , 2006
Strong gelation	BLP	Hydrolysates of WPC	Salt/polysaccharide added	Rocha <i>et al.</i> , 2009
Strong and faster gelation	BLP	Hydrolysates of WPI	Below pH 6.2	Ipsen <i>et al.</i> , 1997
Translucent strong gelation	BLP	Hydrolysates of WPI	Limited	Otte <i>et al.</i> , 1999
Cold gelation	BLP	Hydrolysates of WPI	Salt added/ acidification	Rabiey and Britten, 2009a Rabiey and Britten, 2009b
Soft gelation	BLP	Hydrolysates of WPI	Limited DH 2.2%	Otte <i>et al.</i> , 1996a
White, soft, and thixotropic gelation	BLP	Hydrolysates of WPI	45°C, 1 h hydrolysis	Ju and Kilara, 1998
Stable gelation over wide pH range	Subtilisin Carlsberg	Hydrolysates of WPI	Extensive	Doucet <i>et al.</i> , 2003a Doucet <i>et al.</i> , 2003b
Thin strong gelation	BLP	Hydrolysates of WPI (heat induced)	pH 7	Ju <i>et al.</i> , 1995
Strong elastic gelation	Alcalase 2.4L	Hydrolysates of WPI	Extensive	Doucet <i>et al.</i> , 2001

BLP, protease from *Bacillus licheniformis*; DH, degree of hydrolysis.

Table 2. Key peptides of gelation properties in different types of whey protein hydrolysates

Whey product	Enzyme	Hydrolysis condition	Peptides	Reference
Hydrolysates of WPI	Alcalase	Limited	β -Lg [f135-158]	Doucet and Foegeding, 2005
Hydrolysates of WPI	BLP (aq)	DH 6.8% pH 7	β -Lg [f1-45] β -Lg AB[f90-108]-S-S- α -La [f50-113], α -La[f12-49]-S-S- α -La [f50-113], β -Lg AB[f90-108]-S-S- α -Lg AB[f90-108], β -Lg A[f90-157], β -Lg AB[f135-157/158]	Creusot and Gruppen, 2007b
Hydrolysates of WPI	BLP (glutamyl endopeptidase)	pH 8	Peptides of β -Lg and α -La	Ipsen <i>et al.</i> , 2000
Hydrolysates of β -Lg	Trypsin	5-50°C, pH 4	β -Lg [f1-8], β -Lg [f15-20], β -Lg [f41-60]	Groleau <i>et al.</i> , 2003a Groleau <i>et al.</i> , 2003b
Hydrolysates of WPI	Trypsin	> pH 2	β -Lg [f1-8]	Pouliot <i>et al.</i> , 2009

BLP, protease from *Bacillus licheniformis*; β -Lg, β -lactoglobulin; α -La, α -lactalbumin; DH, degree of hydrolysis.

films while native proteins form soluble films (Perez-Gago and Krochta, 2002). Various measures have been studied to improve barrier capacity and mechanical strength of WPC films. Due to protein chain-to-chain interactions, films with high brittleness are created.

To overcome this and improve the flexibility of the films, hydrolysis of WP provided a much better approach than the addition of plasticizers. Hydrolysates improve the film tensile properties and yield better oxygen barrier

(Sothornvit and Krochta, 2000b). WPHs made good films with oxygen permeability and water vapor transmission rate values similar, but with more flexibility, than WPI films at the same glycerol content (Schmid, 2013; Sothornvit and Krochta, 2000a). Increasing the WPH content leads to a decrease in the molecular weight, in WPI-based films, and also significantly increases film flexibility, compared to films entirely produced from WPHs (Schmid *et al.*, 2013).

Interfacial properties

Emulsification

WPCs are used to improve emulsification in infant formula, meal replacement beverages, soups, gravies, and coffee whiteners (Gauthier *et al.*, 1993). Food emulsions of the oil-in-water type are often stabilized by proteins. Temperature, pH, ionic strength, protein concentration, protein to oil ratio, and oil volume fraction are among the major parameters that affect the physical properties of the emulsion (Guzey and McClements, 2006).

Limited enzymatic hydrolysis was found to be successful in improving interfacial properties (both foaming and emulsification properties) of WPCs (Foegeding *et al.*, 2002; Kilara and Panyam, 2003). Emulsification can be improved through controlled heat denaturation. As the WP unfolds, hydrophobic amino acid residues are exposed, which enhance the ability of the protein to orient at the oil/water interface. Extensive hydrolyzed WPHs can be used as emulsifiers with proper homogenization conditions and careful selection of concentrations (Agboola *et al.*, 1998). The emulsion stability can also be improved by addition of polysaccharides in to the WPHs media (Ye *et al.*, 2004). The interactions between charged polysaccharides and proteins can be used to improve the thickness of the surface layer or to create multi layers of surface (Decher, 1997). The tryptic hydrolysates are reported to be more active on surface and have more emulsification properties than the chymotryptic hydrolysates (Turgeon *et al.*, 1991).

Fat binding and whipping formation

WP also has emulsifying properties allowing fat globules to be structural elements in heat induced WP gels. These emulsification properties have led to the development of new cheese, meat, and confectionary products (de Wit, 1998). Approximately 5-10% of WP is BSA, the smallest protein component of whey and BSA has valuable fat binding properties (Francis and Wiley, 2000). Increased fat binding capacity was associated with an increase in hydrophobicity of the protein (Voutsinas and Nakai, 1983).

Foaming and aeration

Proteins stabilize foams by strongly adsorbing to the air-water interfaces, forming viscoelastic adsorbed layers and leading to a protein network with high viscosity (Rullier *et al.*, 2010). Foaming properties are best when the WPs are undenatured, not competing with other surfactants at the air/water interface, and stabilized by an inc-

crease in viscosity when foam formation occurs. Defatted WPCs can be used as egg substitutes to induce and stabilize foams in aerated food products such as meringues and Madeira-type cakes (de Wit, 1998). Innocente *et al.* (1998) reported that proteose peptone 3, a component that results from the milk fat globule membrane protein, has good foaming properties. At proteose peptone 3 concentrations of 0.05, 0.10, and 0.20 g/100 mL, equilibrium surface tensions at the air-water interface were reported to be 44.75, 36.14, and 32.11 mN/m, respectively. Zhu and Damodaran (1994) suggested that the addition of proteose peptone sharply decreased the foam stability of WPI but did not affect the foam capacity.

Compared to intact WPs, limited enzymatic hydrolysis promotes foaming and aeration through more rapid absorption at the interface by reducing the peptide size (van der Ven *et al.*, 2002). In WPH-35, Alcalase 2.4L resulted in the highest foaming expansion capacity of 287.5% after 5 h hydrolysis with 9.5% DH compared to trypsin, pepsin, protease A, and protease M hydrolysates (Jeevanthi *et al.*, 2014). Davis *et al.* (2005) also reported on the superior forming ability of Alcalase 2.4L and pepsin hydrolysates of β -Lg compared to trypsin. They declared native β -Lg is resistant to hydrolysis by pepsin, which has a broad specificity with a preference for cleaving after hydrophobic residues. In WPH-30, the highest foaming ability has reported by flavourzyme enzyme (Yoon *et al.*, 2010). Excessive hydrolyzed WPHs were showed poor foaming stability due to very short peptides production (Kilara and Panyam, 2003; Ye and Sing, 2006). van der Ven *et al.* (2002) declared that large molecular fractions than 7 kDa has the highest foam stability compared to 44 hydrolysates of WPs. Interfacial films used to stabilize emulsions and foams also have designed from WPHs (Foegeding *et al.*, 2002).

Sensorial properties

Color, flavor, and texture

The flavor profile for whey products ranges from a sweet/dairy (sweet whey) flavor, to virtually no (WPCs, WPIs) perceivable flavor (Keaton, 1999). Acid whey is used in dairy products such as in cheese powders, sauces, and sherbets where a tangy flavor is desired (Kosikowski, 1979). In the bakery industry, acid whey is used in bread, biscuits, and crackers for the gold surface color it provides (Kosikowski, 1979). Color found in whey may result from naturally occurring xanthophylls, Maillard reaction products, and annatto addition (Smith, 2004). Another

added benefit of WPC is that it can improve mouth feel of foods by creating a richer, fuller flavor. This can be especially important when formulating reduced-fat or low-fat meat products where some of the flavor contributed by fat is lost. The incorporation of WPC can be an economic alternative to other high priced flavor enhancing additives (Prabhu, 2006). Baking time and temperatures may require adjustment because crust color might develop more rapidly with whey-based ingredients. WPC 34-80% have been found to improve the color, thickness, and chewiness of full fat and low fat cookie formulations. WPIs has identified as an anti-browning agent that able to suppress enzymatic browning effect which reduce the brown color in apple juice and fresh cut apples (Perez-Gago *et al.*, 2006; Yi and Ding, 2014). Also, some of the benefits recognized by consumers include good crust color developed through the Maillard browning reaction while using high temperature in food. WPCs are also bland tasting and contribute no foreign or off-flavors when used as ingredients (Burrington, 1999). Aldehydes including hexanal, have been suggested as the compounds responsible for off-flavors in liquid and dried whey products (Tomaino *et al.*, 2004; Wright *et al.*, 2009). Concentrations increased with storage time concurrent with increased off-flavors (Wright *et al.*, 2009). Off-flavors in whey products can carry into ingredient applications and negatively affect consumer acceptance (Drake *et al.*, 2009; Wright *et al.*, 2009).

A major disadvantage of protein hydrolysis is the release of bitter-tasting peptides that limits the use of WPHs to low concentrations at which bitterness is not detected (Sinha *et al.*, 2007). Rios *et al.* (2004) have mentioned that limiting the hydrolysis to less than 8% is a better option to minimize the bitter peptides. Small hydrophobic peptides and non-protein nitrogen released during the enzymatic hydrolysis are the major contributors to the bitter taste (Adler-Nissen, 1986; Matoba and Hata, 1972). Hydrophobic amino acids were bitterer when both the α -amino and carboxyl groups were involved in peptide bond formation than when the bond formation occurred at the N- or C-terminus of peptides (Matoba and Hata, 1972). WPHs produced with Alcalase 2.4L were bitterer than hydrolysates generated with Prolyve or Corolase, under similar reaction parameters (Spellman *et al.*, 2009). On the other hand, free amino acids are also converted to flavorful compounds by heat and chemical interaction with other compounds. When WPs are enzymatically hydrolyzed, they develop flavor enhancing properties. WPHs can refine, brighten, accentuate, and naturally enhance key

flavor notes in soups, sauces, dips, and meat products (Prabhu, 2006). WPHs have the tendency to bind a variety of flavor chemicals. As a flavor enhancer, WPH addition to liquid whey and further production to spray dried WPC successfully led to a decrease in cardboard flavor and short chain aldehyde concentration compared to the control liquid whey and WPCs. WPH has its own distinct flavor and aroma as well (Drake *et al.*, 2009).

Conclusion

WP products are used in food applications considering their functional benefits over the other proteins in the industry. The major functional activities of the protein depend on their hydration, gelation, interfacial, aggregation, and sensorial properties. These properties are better enhanced via enzymatic hydrolysis than via chemical or technical means. WPHs enhance functionality due to their ability to expose the globular protein structure, reduce the average molecular weight and, increase the ionic strength, molecular charges, and protein-to-protein interactions, properties which are lacking in non-hydrolyzed WPCs and WPIs. Moreover, the enzymatic hydrolysis avoids the heat denaturation of proteins and maintains the functionality through the food production process. The optimum functional ability of WPHs would be achieved by using proper homogenization conditions, hydrolysis conditions (enzyme-to-substrate ratio, temperature, pH, and time), the type of enzyme, and environmental conditions in food industry.

Acknowledgements

This work was supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093824).

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