Factors Affecting the Functional Properties of Whey Protein Products: A Review

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Numerous whey protein products (WPP) have been developed as excellent food ingredients with unique functional properties. However, the functional properties of WPP are affected by several compositional and processing factors. Recently, novel processing technologies such as high hydrostatic pressure, ultrasound, extrusion and tribomechanical activation have been used to modify the functional properties of WPP. Also, WPP have been used as delivery systems for functional ingredients and in edible films. The present paper reviews the latest developments in the role of different factors on the functional properties of WPP with emphasis on novel processing technologies, and interaction with other food ingredients.

Keywords whey protein concentrate, whey protein isolate, modified whey proteins, polysaccharides, functional properties, foaming properties, emulsifying properties, gel formation

Introduction

Whey is a by-product from cheese and casein manufacture; cheese whey constitutes the major part of the global whey production.¹ It has been estimated that world production of cheese reached 16.47 million tons in 2005,¹ which means an output of about 150 million tons of whey. Sweet and acid whey are the two types of whey, which arise from the manufacture of rennet and acid coagulated cheeses and casein, respectively. A third type, namely salted whey, is produced in Egypt from the manufacture of Domiati cheese, the major Egyptian soft cheese.

Utilization and/or disposal of whey have been major concerns to dairy specialists all over the world as it contains valuable constituents that should not be wasted. Furthermore, whey represents an important environmental pollutant.

With the advent of industrial ultrafiltration and chromatographic methods, recovery and fractionation of whey proteins in their native forms has become possible. A wide variety of commercial whey protein concentrates (WPC) and isolates (WPI), whey protein fractions (α-lactalbumin and β-lactoglobulin rich fractions, casein glycomacropeptide, lactoferrin, and lactoperoxidase) and protein hydrolysates (WPH) are available in the market.

Whey protein products are valued as excellent food ingredients because of their unique functional characteristics.² As foodstuffs, they are not used only because of their functional properties, but also because of their high nutritive value and GRAS status.³,⁴,⁵

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However, the utilization of these products as food ingredients has been limited due to the variability in their composition and functional properties.\(^{(6,7,8,9)}\) The need of whey protein products showing consistent functional behavior in food continues to be a major concern to the industry even though the acquired knowledge on factors affecting the functional properties of whey protein during the last two decades has enabled industry to develop whey protein products of more uniform composition and properties. Recently, the potential use of whey protein hydrogels and nanoparticles as a delivery system of functional ingredients has been reported.\(^{(10,11)}\) This trend requires whey protein products of uniform functional properties. Despite recent progress, the factors responsible for the variability of the functional properties of whey protein products are not fully understood.\(^{(7,8,9,12)}\)

Proteins provide various functions in food quality and stability. They form interfacial films that stabilize emulsions and foams, can interact to make gel networks and edible films, and produce stable sols for nutritional drinks. The ability of proteins to provide these properties is called protein functionality. Generally, the functional properties of food proteins can be classified into three main groups\(^{(13)}\) as follows:

1. Hydration properties, which depend upon protein-water interactions that have an important bearing on swelling, adhesion, dispersibility, solubility, viscosity, water absorption and water holding.
2. Interfacial properties, which include emulsification and foaming characteristics.
3. Aggregation and gelling properties, which relate to protein-protein interactions.

Several reviews have been published\(^{(14,15,16,17,18)}\) on the nutritional and functional properties of whey protein products. The aim of the present article is to review recent data on the functional properties of whey protein products with particular emphasis on novel processing technologies, and interactions with other food ingredients.

### Whey Protein Products

Developments in several solid-liquid separation technologies have been utilized by the dairy industry to produce large number of whey products with good functional, nutritional and flavor properties. The main feature of these products is that proteins are largely present in their native form. There are at least two reasons that account for the large number of the developed whey protein products. First, no single product can be utilized in a wide spectrum of food products as each requires a whey protein product of specific properties. Second, production of whey protein products with more protein content utilizes only small portion of whey solids and there will be a need to utilize or discard the remaining effluent stream, which increases the overall cost of production. Generally, whey protein products differ in their composition mainly in their protein, lactose, fat and mineral contents. Also, differences in the relative whey protein concentrations will be found in the different preparations. Table 1 shows the composition of some of the main whey protein products, grouped according to their composition.

#### Whey Protein Concentrates (WPC)

WPCs with protein contents ranging from 35–80% are available. The basic steps in the manufacture of these products are whey pretreatment, ultrafiltration/diafiltration, concentration by evaporation under reduced pressure, and spray drying.
Whey Protein Isolates (WPI)

WPIs, with a minimum protein content of 90%, are either manufactured by ion exchange chromatography or microfiltration. The ion exchange method is based on the retention of major whey proteins by binding to the ion exchanger and subsequent elution by changing pH. In the microfiltration (MF) method, whey is microfiltered using a suitable membrane (pores < 1 μm) to remove lipid and protein aggregates and microbial debris. The MF permeate is ultrafiltered/diafiltered, concentrated and spray dried. WPI prepared by ion exchangers has less casein glycomacropeptide content than that prepared by microfiltration.

β-Lactoglobulin and α-Lactalbumin Rich Fractions

A growing demand for α-lactalbumin for use in infant food formulation encouraged processors to fractionate whey proteins. Several methods have been developed to separate

<table>
<thead>
<tr>
<th>Constituent</th>
<th>WPC</th>
<th>WPI</th>
<th>α-LA rich product</th>
<th>B–LG rich product</th>
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<tbody>
<tr>
<td>Protein</td>
<td>34.0–36.0&lt;sup&gt;a&lt;/sup&gt; 80.0–82.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.0–92.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>76.3–87.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactose</td>
<td>48.0–52.0&lt;sup&gt;a&lt;/sup&gt; 4.0–8.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5–1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.1–13.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat</td>
<td>3.0–4.5&lt;sup&gt;a&lt;/sup&gt; 3.0–4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1–0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.2–10.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash</td>
<td>2.5–4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0–4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.20–0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.2–19.7&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Moisture</td>
<td>3.0–4.5&lt;sup&gt;a&lt;/sup&gt; 3.5–4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.2–3.9&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a</sup>Ref. (19); <sup>b</sup>Ref. (7); <sup>c</sup>Ref. (20); <sup>d</sup>Ref. (21); <sup>e</sup>Ref. (22); <sup>f</sup>Ref. (23); <sup>g</sup>Ref. (24); *α-LA: α-lactalbumin; β-LG: β-lactoglobulin; GMP: glycomacropeptide; BSA: blood serum albumin; and IG: immunoglobulin.
the two major whey proteins, $\beta$-lactoglobulin and $\alpha$-lactalbumin in relatively rich fractions. These methods are based on differential solubility at different pH, temperature and ionic strength.

**Glycomacropeptide (GMP)**

Glycomacropeptide is a peptide arises from the action of chymosin on casein and more specifically $\kappa$-casein during cheese making. It represents the C-terminal part of $\kappa$-casein from residue 106(Met) to 169 (C-terminal Val$^{25}$). This fraction has unique composition, biological activity and nutritional significance$^{25}$ Ultrafiltration and chromatographic methods are used to separate GMP from other whey proteins. Almost pure commercial GMP products are now available.

**Whey Protein Hydrolysates (WPH)**

Enzymatic hydrolysis with a wide range of proteolytic enzymes is generally used to produce WPH of different level of hydrolysis depending on the purpose of its use.

**Modified Whey Proteins (MWP)**

Whey protein polymers have been developed as a cold set ingredient. This product is made by heat polymerization of whey proteins at conditions that do not yield a gel (low ionic strength and high pH). The product forms a gel under cold conditions (20–37°C) by changing the solvent quality (i.e., addition of Ca). Another product is prepared by heat denaturation of WPI in acidic conditions (pH 3.5) and in the presence of Ca.

**Minor Whey Proteins (Lactoferrin, Lactoperoxidase, Basic Milk Proteins)**

These are prepared mainly by chromatographic methods. These products are used mainly as antimicrobial agents or in pharmaceutical preparations. The most widely produced whey protein products as food ingredients are WPC and WPI. Therefore, discussion will be focused mainly on the functional properties of these products.

**Factors Affecting the Functional Properties of Whey Protein Products**

The functional properties of proteins are controlled by their physicochemical properties including their amino acid composition and sequence, conformation, molecular size, shape, net charge, and hydrophobicity. Therefore, changes in the physicochemical properties of proteins influence their functional properties. Whey proteins are a mixture of different proteins of different composition and properties. Therefore, the influence of different factors on the functional of whey protein products is complicated by differences in the effects of these factors on the individual whey proteins and their relative concentration in the product.

**Effects of Whey Protein Composition**

Factors affecting the composition of whey also affect the functionality of whey products.$^{15}$ Most of these factors are interrelated and it is difficult to separate individual effects.
However, factors affecting the composition and functionality of whey protein products can be broadly described. Whey contains a complex mixture of proteins with the most common being β-LG (∼55%), α-LA (∼24%), blood serum albumin (BSA) (∼5%), and immunoglobulins (∼15%)\(^{26}\) and several other minor proteins and protein degradation products. Each of these protein fractions has their characteristic functionality (Table 2).

**Species Differences.** A study\(^{28}\) showed that caprine whey had comparatively high levels of α-lactalbumin (α-LA) and ovine whey high levels of β-lactoglobulin (β-LG) compared to bovine whey. These differences reflected on the functional properties of WPC obtained from caprine and ovine whey,\(^{29}\) where ovine WPC showed significantly better foam overrun, foam stability and gel strength than did bovine and caprine WPC. Caprine WPC showed higher gel strength than bovine WPC and higher emulsifying properties at low pH than bovine and ovine WPC.

**Relative Concentration of Whey Protein Fractions.** Normally, there are inherent and environmental variations in the protein composition of milk. Therefore, it is expected that similar variations will be found in whey obtained from cheese manufacture and in turn in whey protein products. Regester and Smithers\(^{30}\) demonstrated significant variations in β-LG (49.3–59.9%), α-LA (10.8–20.8%), and GMP (23.3–30.9%) contents of WPC from southeast Australia. Changes in the functional properties of that WPCs are related to the relative concentrations of the component whey proteins.\(^{31}\) As each whey protein fraction has its own characteristic functionality, the functional properties of whey protein products can be modified by changing the relative concentration of the different whey proteins through the manufacturing process. For example, using a proprietary membrane system, a commercial modified WPC has been developed that contained higher proportion of high molecular weight proteins (immunoglobulins, lactoferrin, BSA) and phospholipids\(^{32}\). The product had greater emulsifying properties (emulsifying capacity and stability) and smaller initial emulsion droplet than normal WPC.\(^{32}\)

The presence of minor proteins and degradation products plays an important role in modifying the functional properties of the prepared whey protein products. Various types of peptides and nonprotein nitrogenous constituents are found in whey depending on the type of whey (acid/sweet) coagulating enzyme used, plasmin level in milk, storage time and conditions of milk and whey.\(^{15}\) The carry-over of these constituents in the prepared whey products depends on the method used in the preparation of these products. Sweet whey differs from acid whey by the presence of casein glycomacropeptide (GMP), which

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<tr>
<th>Protein</th>
<th>Foaming properties</th>
<th>Emulsifying properties</th>
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<tr>
<td></td>
<td>Capacity</td>
<td>Stability</td>
</tr>
<tr>
<td>B-LG</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>α-LA</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Immunoglobulin</td>
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<td>_</td>
</tr>
<tr>
<td>GMP</td>
<td>+++</td>
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</tr>
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Refs.\(^{14, 21, 26, 27}\)
arises from the action of chymosin on casein. GMP represents 10–20% of proteins in whey,\(^{(30)}\) has low foam stability\(^{(27)}\) and emulsifying properties,\(^{(26)}\) and they do not form gels. The presence of GMP in WPC was found to be detrimental to gel strength and water holding capacity of WPC and its removal improved these functional properties in the product.\(^{(33)}\) Also, whey contains a proteose-peptone fraction, which partially arises from the action of plasmin on \(\beta\)-casein during cold storage of milk before cheese making. Addition of proteose-peptone sharply decreased the foam stability of WPI but did not affect the foam capacity.\(^{(34)}\) Lorenzen and Schrader\(^{(35)}\) suggested that the proteose peptone fraction may account for the differences in the gelling properties of WPI and WPC.

Heat treatment of milk and whey induces a reaction between milk proteins and lactose resulting in lactosylated protein species. Under mild heat treatment (63°C for 20 s) applied to milk before whey separation, an increase in molecular mass of a modified \(\beta\)-LG by 324 is observed in acid and sweet whey.\(^{(36)}\) This specific modification increases with the extent of the heat treatment reaching a maximum of 35% of \(\beta\)-LG after heat treatment at 70°C for 1 h.\(^{(36)}\) A first lactose-binding site is Lys47.\(^{(36)}\) The physicochemical properties of whey proteins (e.g., hydrophobicity of \(\alpha\)-LA and \(\beta\)-LG and conformational alterations in \(\beta\)-LG only) change upon lactosylation.\(^{(37)}\) Holt et al.\(^{(7)}\) found that lactosyl \(\beta\)-LG varied from 7.4 to 31.5% of total \(\beta\)-LG in commercial WPC. Analysis of commercial WPC’s\(^{(38)}\) revealed the presence of different forms of oxidized and/or single or multiple lactosyl whey proteins, which can modify the functional properties of the product due to changes in their hydrophobicity and conformation.

Effects of Minerals. Differences in mineral content is the most important factor that affects the functionality of whey protein products from different types of whey.\(^{(15,39)}\) Acid whey normally contains higher Ca and P than sweet whey. However, WPC prepared from acid whey contains less Ca than that prepared from sweet whey.\(^{(15)}\) Also, WPC prepared from salted whey contains small amounts of NaCl.\(^{(40)}\) Citric acid has been used to improve the flux during the ultrafiltration of sweet whey, since it alters the solubility of Ca and thereby reduces Ca levels.\(^{(15)}\)

The effects of ionic strength and mono- and divalent cations on the functional properties of whey protein products have been demonstrated in several studies. Divalent Ca\(^{++}\) and Mg\(^{++}\) cations significantly improved the foaming properties of WPI,\(^{(41)}\) which has been attributed to time-independent aggregation of the protein in the presence of these ions. This effect was more pronounced when the protein solution was aerated directly after the addition of the cation and decreased gradually with longer incubation time.

Also, the gel properties of whey protein products are affected by mineral content and ionic strength. Generally, the microstructure of whey protein gels can be classified according to the protein aggregate structure within the gel network as fine-stranded, particulate or a mixture of both types.\(^{(42)}\) Several factors are involved in the development of the microstructural and rheological properties of whey gels. Incorporation of protein into the gel network at or after the gel point\(^{(43,44)}\) was found to affect the gel properties. The gel permeability decreased as the concentration of aggregated proteins at the gel point increased. McGuffery and Foegeding\(^{(45)}\) showed that the electrostatic repulsive or attractive interactions are the major factors determining the fracture properties of WPI gels. Essentially, as pH was increased away from the pI, the increased ionic strength caused the protein to unfold more extensively and aggregate more rapidly, producing a more elastic and less permeable network. At neutral pH, the increase of NaCl concentration yielded WPI gels of more open and coarse structure with increased permeability.\(^{(43)}\) Fine-stranded networks are formed when electrostatic repulsion between protein molecules is relatively strong.
i.e., at low ionic strength\(^{(46)}\). At high salt concentrations, protein-protein interactions lead to the formation of a network of aggregated particulates while at intermediate salt concentration the gel network consists of a mixture of particulate and fine-stranded protein aggregates.\(^{(42)}\) The fine-stranded network formed at low ionic strength conditions contained relatively small pores with high water holding capacity and the gels were optically transparent.\(^{(46,47)}\) Particulate networks formed at high ionic strengths contain relatively large pores with low water holding capacity and opaque. Barbut and Drake\(^{(48)}\) reported that a minimum of 75 mM NaCl was required to induce cold gelation in a 10% WPI suspension preheated to 80\(^\circ\)C for 30 min, which was higher than the 25 mM NaCl required for conventional heat-induced gelation. Heating the cold-set gels increased the gel strength by a factor of 2.5 to 8 depending on initial Na. They suggested the use of a two-stage gelation process to enhance characteristics of cold set gels.

Divalent cations, particularly calcium appear to have more significant impact on the gelation of whey protein systems.\(^{(49–52)}\) Gelation time of WPI solution at pH 6.8 decreased six and tenfold in the presence of NaCl and CaCl\(_2\), respectively.\(^{(52)}\) However, the stiffness of the gel obtained after addition of CaCl\(_2\) was lower than in the presence of added NaCl.\(^{(52)}\) It is of interest to note that progressive substitution of \(\beta\)-LG with other whey proteins up to a level of 40% did not alter the behaviour of gelation.\(^{(52)}\)

A modified whey protein concentrate (mWPC) of high water-holding capacity has been prepared by acidification to pH 3.35 followed by extended heat treatment, gelation and spray drying.\(^{(53)}\) In the presence of added Ca\(^{++}\), mWPC showed increased thickening capacity especially under refrigeration temperature as a result of a 2-fold increase in the amount of bound or unfreezable water.\(^{(54)}\) On the other hand, Firebaugh and Daubert\(^{(55)}\) reported that the foam capacity of derivatized whey protein isolate (dWPI) was 50% lower than that of WPI but the foam stability was better.

**Residual Lipids.** Whey normally contains residual lipids, which differ from skim milk lipids in that they have much higher ratio of phospholipids to triglycerides.\(^{(56)}\) The residual lipids remain in whey after centrifugal separation as their density is greater than that of whey cream due to their intimate association with proteinaceous materials as lipoproteins. Although the nature and origin of the residual lipids in whey are not fully elucidated, it has been suggested that they originate from milk fat globule membrane (MFGM) and skim milk membrane (SMM) materials.\(^{(57)}\) Preparation of WPC from whey without pretreatment resulted in product of high lipid content and inferior functional properties.\(^{(58)}\) The MFGM was considered as a strong inhibitor of foaming of WPC and that this effect was related more to the protein portion of the MFGM.\(^{(59)}\) Also, addition of small quantities of MFGM increased the gel strength of \(\beta\)-LG gels while higher levels decreased it.\(^{(59)}\) Residual lipids were reported to inhibit the gelation of WPC by interfering with protein polymerization via formation of intermolecular hydrophobic bonding.\(^{(57)}\) Even at 75% protein content, WPC prepared from whey without pretreatment does not gel but rather forms a white coagulum or curd. Also, residual lipids were implicated in the development of off flavors in stored WPC and WPI, which limit their use as food ingredients.\(^{(60,61)}\)

Two approaches have been developed to minimize the effect of residual lipids on the functional properties of whey protein products:

1. Reduction of residual lipids in whey protein products by different methods including:
   (a) aggregation through calcium binding during heat treatment and subsequent removal of aggregates\(^{(62)}\); (b) thermal aggregation of some whey proteins\(^{(63)}\); (c) direct microfiltration of cheese whey\(^{(57)}\); and (d) electroacidification using bipolar membrane.\(^{(64)}\)
Only 20% of the residual lipids remained in whey after microfiltration using a 0.8 µm membrane and a decrease in lipid content of WPC by six fold from 0.76–0.21% was achieved by electroacidification. The whey protein products prepared from whey treated by these methods were reported to have excellent functional properties.

2. As an alternative to de-fatting, Blecker et al. suggested modifying residual triglycerides in whey products by enzymatic hydrolysis with a specific lipase. This treatment was reported to enhance the emulsifying and foaming properties, and gelling and water holding properties of WPC.

Effects of Processing

De la Fuente et al. suggested that different heat treatments used in cheese making and casein manufacture were probably responsible for differences in WPC composition and functionality rather than changes that occurred during WPC manufacturing. A study on the functional properties of native and cheese whey WPC supports this conclusion. Native WPC, a product prepared by microfiltration of milk, had higher gelling, foaming and emulsifying properties as compared to WPC from cheese whey. Similarly, WPI prepared by microfiltration of milk followed by ultrafiltration and diafiltration of permeate gave a product, which was reported to have superior functional properties as compared to commercial WPI preparations. Drying methods had no significant effect on the functional properties of WPC. On the other hand, Ralkin et al. found that WPC prepared by spray drying at high temperatures (260/138°C) had higher foaming properties than that dried at lower temperature (260/85°C).

The impact of hydrophobic interactions of whey proteins on their functionalities has received much attention. Apparent close relationships between surface hydrophobicity and emulsification, foaming and gelation have been reported. Also, an increase in hydrophobicity of proteins increased their fat binding capacities. The relationship between functionality and hydrophobicity has been shown for both acid and sweet whey protein concentrates. A positive correlation was reported between surface hydrophobicity (SH) and foam formation with a negative correlation between SH and foam stability and emulsion stability.

The level of insoluble (sedimentable) protein at pH 4.6 had a substantial effect on gelling properties of commercial WPC: a high level of this fraction enhanced gel strength. Roufik et al. identified two protein fractions in commercial WPCs using high performance ion exclusion chromatography: the aggregated protein (APF) and the native protein (NPF) fractions. The APF ranged from 10–29% of total proteins and these aggregates are bound by covalent and non-covalent bonds. They suggested that the variations in WPC protein fractions are main factors affecting WPC functional properties.

Effects of Heat Treatments. Heat treatments induce several conformational changes in the structure of whey proteins depending on the heating conditions and these changes are reflected in whey protein functionality. Under moderate heat treatment (60–70°C), structural unfolding of the proteins occurs, while at higher temperatures protein aggregates. Whey proteins differ from each other in their susceptibility to heat denaturation. Schmidt et al. summarized the expected effect of heat treatment as a function of pH as follows:

- Neutral pH: enhanced emulsifying and foaming with moderate heating; enhanced viscosity with high heat;
• Acid pH: High protein viscosity, lower gel temperature, high water absorption; and
• Alkaline pH: Solubilization of heat-denatured protein.

Most heat-induced changes of whey proteins have been carried out on $\beta$-LG since it is the most abundant protein in whey and it affects the overall changes in the functional properties of whey protein products. Several mechanisms have been proposed to explain the heat-induced changes in $\beta$-LG\(^{(12)}\). In almost neutral conditions, heat-induced changes have been regarded as a three-step process\(^{(80)}\) with an overall reaction order of 1.5 for the aggregation step. A three-step model is proposed: (1) initiation, (2) propagation, and (3) termination. The initiation step consists of a reversible reaction (native $\beta$-LG dimer splits into monomer) followed by an irreversible reaction (exposure of the free SH group) resulting in the formation of active monomers. The propagation step corresponds to the buildup of aggregates through SH/disulphide reactions. In the termination step, two active intermediates react to form larger aggregates without an exposed, reactive SH group. Croguennec et al.\(^{(81)}\) found that in the early stages of the denaturation/aggregation of $\beta$-LG, there is formation of native thiol-exposed monomer (Mcys 121) and nonnative thiol-exposed monomer (Mcys 119). The native Mcys 121 monomers form intermediate oligomers (dimer, trimer, tetramer), which rapidly combine to larger aggregates in the presence of CaCl\(_2\) and to a lesser extent in the presence of NaCl. Mcys 119 monomer trapped $\beta$-LG molecules in a conformation that slows down its aggregation behaviour at pH 6.6. Formation of Mcys 119 on heating is related to the rate of condensation of Mcys 121 into intermediate oligomers and/or aggregates independent of the salt conditions. Galani and Apenten\(^{(82)}\) reported that non-covalent interaction had little contribution to the overall aggregation at temperatures below 75°C but became important when $\beta$-LG solution was heated at high temperatures (>90°C).

The aggregation of $\beta$-LG is affected by several factors, de Wit\(^{(83)}\) summarized the thermal behavior of $\beta$-LG as follows: reversible dissociation of native $\beta$-LG dimers and small conformational changes occur up to 55°C, partial unfolding of $\beta$-LG monomers in a “molten globule state” and irreversible modification induced by exposed thiols between 60 and 70°C above 6.8, formation of small oligomers between 65 and 75°C induced mainly through thiol/thiol oxidation reactions, additional unfolding takes place but residual structures remain below 100°C, the oligomers are enlarged by non-covalent associations between 75 and 85°C. Finally, unfolding of residual protein structure is observed between 125 and 150°C, probably induced by cleavage of disulphide bonds. The heat induced changes of $\beta$ = LG are affected by the following:

1. **pH.** At pH higher than 7.0, the sulphhydril groups become more reactive and the rate of conversion of native $\beta$-LG to aggregates increase\(^{(84)}\) but the aggregates have more compact conformation. At pH 2–3, very large aggregates are formed.\(^{(85)}\) Whey proteins, and specifically $\beta$-LG, forms fibrils upon heating at pH 2 and low ionic strength.\(^{(86)}\) Stirring during heat treatment accelerates the kinetics of fibril formation resulting in an increase in the number of fibrils formed.\(^{(85)}\) Heat treatment and pH of whey have marked effects on the solubility and emulsifying properties of WPC.\(^{(88)}\)

2. **Protein concentration.** Larger aggregates of heat denatured $\beta$-LG are formed with the increase of $\beta$-LG concentration.\(^{(89)}\)

3. **Genetic variant.** $\beta$-LG has several genetic variants that differ in their primary structure at one or two amino acid residues along the sequence. $\beta$-LG A and B variants are the most common whereas variant C is only found in small frequency. The $\beta$-LG variants differ in their heat stability and emulsifying properties.\(^{(90,91)}\) The order of thermostability of $\beta$-LG variants is C>A>B.\(^{(90)}\) $\beta$-LG A forms the finest emulsion droplets and $\beta$-LG C
the largest droplets. This has been attributed to high net negative charge of $\beta$-LG A as compared to variant C.\(^{(91)}\) On the other hand, the creaming stability, when compared at the same particle size, is greatest for $\beta$-LG C, with $\beta$-LG A and B having similar and lower stability.\(^{(91)}\) The differences in creaming stability may arise from a higher surface coverage for the $\beta$-LG C droplets.

4. **Presence of other proteins.** Although $\alpha$-LA does not polymerize by itself when heated above 70°C,\(^{(85)}\) it disappears rather more rapidly from heated WPC than $\beta$-LG,\(^{(34)}\) which has been explained by the formation of a complex with $\beta$-LG. The formation of $\beta$-LG/$\alpha$-LA complex is highly dependent on the weight ratio of the two proteins. A mechanism for the formation of this complex has been described\(^{(85)}\). Protein dispersions with different $\alpha$-LA and $\beta$-LG ratios were heated at pH 7.5 and then acidified with glucono-$\delta$-lactone to form gels at room temperature. Increasing the proportion of $\alpha$-LA results in more turbid gels characterized by an open microstructure and reduced elastic and viscous moduli.\(^{(92)}\)

5. **Heating conditions.** Repeated heat treatment has been found to affect the gel properties of WPI dispersions.\(^{(93)}\) Double-heated dispersions gel faster at lower protein and Ca ion concentration and the gels have lower values of shear strain and shear stress at fracture than single-heated dispersion.

The decisive factors for the functional properties of thermally treated whey proteins are their ability to immobilize water in protein structures and to absorb water. The amount of retained water can be increased by thermal denaturation, aggregation and formation of porous structures of the whey proteins. Shearing during heat treatment increases the number of collisions of dissolved and suspended particles, which supports the growth of aggregates. High shear rates break up the flow-induced aggregates. Nuclear magnetic resonance (NMR) showed that denatured and sheared WPC had higher viscosity, shorter relaxation time and the fraction of immobilized phase was about twice that of native WPC.\(^{(94)}\)

**Effects of Novel Processing Technologies.** There is an increased interest in novel nonthermal food processing technologies that focus on preservation and modification of the functional properties of food components while keeping food quality attributes. These methods include irradiation, high hydrostatic pressure, ultrasound and pulsed electric field. Although the main present focus of the use of novel technologies is food preservation, a growing interest has been developed in their use in modifying the functional properties of whey proteins.

**Effects of high hydrostatic (HHP) and dynamic (HDP) pressures.** High hydrostatic pressure (HHP) has several advantages over conventional thermal processing of foods including application at low temperatures, which permits the retention of food quality attributes. HHP affects the functional properties of food biopolymers, mainly proteins, by changing the balance of intramolecular and solvent-protein interactions.\(^{(95)}\) The basic effect of pressure-induced changes in proteins depends on the compression of molecules and on changes of volume during process.\(^{(96,97)}\) The pressure-induced physical modifications of protein structure include disruption and reformation of hydrogen bonds, rupture of hydrophobic interactions, and separation of ion pairs. These changes are dependent on protein structure, pressure, pH, ionic strength, solvent composition, and protein concentration.\(^{(98)}\) At low protein concentration and low pressure (<300 MPa), reversible pressure-induced denaturation occurs, but higher pressures (>300 MPa) induce irreversible and extensive effects on proteins.\(^{(95)}\)
The effects of HHP on the major whey proteins, namely β-LG and α-LA, have received much attention. Comparison between pressure-induced structural changes in β-LG and α-LA confirmed the higher stability of α-LA to HHP. Reversible unfolding of α-LA started at 200 MPa and loss of native structure was irreversible at 400 MPa as compared to 50 and 150 MPa, respectively, for β-LG(99).

High-pressure treatment of β-LG and WPC led to protein unfolding, increase of surface hydrophobicity and formation of aggregates.(100,101) These changes are reflected in their functional properties. Thus, pressurized β-LG has lower emulsifying and foaming capacities than native β-LG due to the reduction of both the diffusibility and concentration of protein available for adsorption at interfaces and the effect of pressure became more pronounced with the increase of pressure and duration used. In contrast, Lee et al(101) reported an increase in emulsifying capacity and stability of HHP-treated WPC. This discrepancy may be explained on the basis of different degrees of aggregation undergone by protein in the two studies since higher concentration of protein (4 folds) was used in the former study.(100) On the other hand, the higher surface hydrophobicity of pressure-treated β-LG is responsible for a greater capacity for protein-protein interactions in the adsorbed layers at interfaces that increase viscosity.

Pressurization of WPI at 150–450 MPa in pH range of 5.0–7.0 was found to affect their foaming properties, i.e., foam volume (overrun) and stability.(102) The foaming properties were decreased as pressure increased and as pH approached the isoelectric point of the proteins.

HHP can induce gel formation in solutions of WPC and WPI. The strength of the formed gels increased with the increase of pressure level and duration of treatment.(101,103,104) Maximum gel strength was obtained in alkaline pH (8.0–9.0), but no gel formation occurred in acidic pH, except in the presence of NaCl. Also, no gel formation occurred when –SH blocking agent was added.(105) Electron microscopy of pressure-induced gels showed a porous structure with few, weak and short-term intermolecular cross-links as compared with heat-induced gels.(106) It has been suggested that pressure promoted incomplete gelation, as compared to heating.

Liu et al.(107) showed that HHP treatment of WPC yields an increase in the number of binding sites for an aromatic hydrophobic probe, whereas aliphatic hydrophobic binding affinity of WPC was enhanced after 10 min of HHP treatment. HHP was found to decrease the binding of several flavour components to WPC.(108) Patel et al.(109) demonstrated time-dependent losses of native whey proteins and a corresponding increase in nonnative proteins and protein aggregates in HHP-treated WPC. They showed that the sensitivity of major whey proteins to pressure was in the order β-LG B > β-LG A > bovine serum albumin > α-LA. They suggested that the intermolecular disulphide bond formation occurred at high pressure and that hydrophobic association became important after HHP treatment.

Photon correlation spectroscopy (PCS) and atomic force microscopy (AFM) reveal protein aggregation in WPC treated with HHP at 250–300 MPa(110) with a main population of aggregates at 7, 26, or 50 nm at 250, 275, or 300 MPa, respectively. It has been suggested(110) that HHP induces aggregation in WPC mainly through hydrophobic interactions. Treatment of WPC and β-lactoglobulin with proteolytic enzymes either after or particularly during HHP treatment considerably enhances their hydrolysis.(111,112) Differences in the degradation pattern are obtained mainly at short hydrolysis time.(113) The hydrolysates of WPC obtained with pepsin at 400 MPa show improved heat stability and superior emulsion activity index than untreated WPC.

Dynamic high pressure (DHP) is different from HHP in that force-induced phenomena of cavitation, shear, turbulence and temperature rise are involved simultaneously.
Bouaouina et al. subjected 3% WPI solution to DHP treatment (up to 300 MPa). They reported that this treatment dissociated large protein aggregates leading to unmasking of the buried hydrophobic groups without affecting protein solubility and improving the foaming and stabilizing properties of WPI.

**Effects of ultrasound.** Ultrasonic treatment is a rapidly growing field of research and development for the food industry. At present, ultrasound is used in food processing for degassing and foam control, mixing and emulsification, and meat tenderization. Its use on whey proteins has been recently studied.

High-intensity ultrasonic processing improved the emulsifying properties of whey protein isolate. Also, the effects of ultrasound on the functional properties of WPC, WPI, and WPH were affected by the applied power level. Low-intensity ultrasound (500 kHz) had no significant effect on solubility, foaming capacity, and stability of tested whey protein products, whereas those properties were increased with increasing power used in ultrasonic treatment, with the most pronounced effect at 20 kHz for 15 min. The increased foaming property was attributed to mechanical homogenization and to partial changing of protein conformation and structure.

**Effects of minimizing particle size.** Particulate size has been reported as one of the most important factors determining the functional properties of WPC. Sieving of whey products within the particle size distribution range of 100–150 microns minimized variations in the functional properties of WPC.

Tribomechanical micronization and activation (TMA) is a patented process where intensive mechanical strains induce friction and collision between particles during a very short time (from 0.0001–0.001s). This treatment leads to a sharp decrease in particle size, an increase in specific area, and increase in the electrical conductivity of water solution of treated material.

Treating whey protein concentrates containing 60 and 80% proteins with tribomechanical micronization and activation decreased their particle size and improved their emulsifying and foaming properties. The most obvious changes were observed when WPC was treated at the maximum rotor speed (22000 rpm) of the TMA equipment.

Compared to other nonthermal treatments, tribomechanical micronization seems to induce the least changes in the properties of WPC and WPI. Kresic et al. compared the effect of HHP (500 MPa/10 min), ultrasound (20kHz/15 min) and tribomechanical activation (40000 rpm) on flow behavior and thermophysical properties of WPI and WPC. Of these treatments, HHP caused the most intensive changes in the rheological behavior of WPC and WPI, which was found to be shear thickening. Also, the decrease of initial freezing point was accompanied with a remarkable increase of specific enthalpy.

**Effects of extrusion.** Extrusion processing texturizes globular proteins by shearing and stretching them into aligned or entangled fibrous bundles with new functionalities. Extrusion of WPI at 35 or 50°C increased their gel strength, but gel strength was lost when extruded at 75 or 100°C. On the other hand, extrusion at these high temperatures had minimal effect on foaming and digestibility of WPI. Texturizing WPI by extrusion under acidic and alkaline conditions had influenced their functional properties. Alkaline conditions increased solubility and pasting properties significantly, whereas acidic conditions increased solubility but decreased pasting properties of WPI. Extruded WPI under alkaline conditions had a stringy texture that could be used in meat applications. The textural parameter of extruded WPC and WPI increases with drying time.
Drying to a moisture contents of 20% and 25% is considered adequate for extruded WPC and WPI, respectively.\(^{(122)}\)

**Effects of Food Ingredients on Whey Protein Functionality**

The effects of protein/food ingredient interactions on the functional behavior of whey proteins in food systems is an important area of study in order to maximize the use of whey proteins in foods.

Interaction of whey proteins and polysaccharides has received much attention as it has direct influence on the macroscopic properties of food products such as flow, stability, texture and mouth feel.\(^{(125)}\) The interactions between proteins and polysaccharides can be classified into two opposing mechanisms,\(^{(3)}\) namely:

1. Attractive forces (i.e., hydrophobic, hydrogen bonds, van der Waals, disulphide bonds), which lead to the formation of complexes.
2. Repulsive forces (i.e., electrostatic, hydration, steric repulsion), which lead to incompatibility and phase separation.

Also, these interactions can be grouped as strong (i.e., hydrophobic, hydration, steric repulsion) or weak (i.e., hydrogen bonds, disulphide bonds) and specific (i.e., disulphide bonds) or nonspecific (i.e., hydrophobic interaction).

Under certain conditions, interaction between whey proteins and polysaccharides may result in the formation of complexes with substantially improved functional properties.\(^{(124–128)}\) A WPC-pectin soluble complex was prepared by dry heat treatment\(^{(124)}\) and was reported to have better emulsifying, foaming and gelling properties as compared to WPC alone. Similar results were obtained by Akhtar and Dickinson\(^{(125)}\) for WPI-dextran conjugate. WPI-dextran conjugate gave much better emulsion stability than WPI or gum arabic alone, which has been attributed to the enhanced steric stabilization provided by the bulky hydrophilic polysaccharide moiety. Also, Neirynck \textit{et al.}\(^{(127)}\) found that conjugation of WPI with low methoxyl pectin led to a substantial improvement in the emulsifying properties of the formed complex. They concluded that the dextrose content should be as low as possible during the dry heat treatment of protein-pectin mixtures. Dry heating of whey protein preparation obtained from whey by complexation with carboxymethyl cellulose (CMC) improved markedly the emulsifying properties of the protein concentrate.\(^{(129)}\)

At pH 4.0, insoluble complexes were formed with a protein-pectin ratio of \(3.84 \pm 0.88\), while at pH 5.5, the protein solubility was independent of the amount of pectin added.\(^{(128)}\) They concluded that pectin has an stabilizing effect on protein-stabilized emulsions both below and above the protein isoelectric point, provided that electrostatic protein precipitation is prevented. Addition of mono- and disaccharides and inulin and starch enhanced foam stability of WPC, WPI, and \(\beta\)-lactoglobulin suspensions.\(^{(126)}\) Also, mono- and disaccharides improved the emulsifying properties of whey protein, while inulin and starch decreased it. The foam and emulsion properties of WPC were enhanced with the addition of mono- and disaccharides.\(^{(130)}\) Addition of \(\iota\)-carrageenan to 4% WPC significantly decreased the interfacial tension while addition of gum Arabic significantly increased it.\(^{(131)}\) However, both additives improved the emulsion activity and stability indexes of WPC.\(^{(131)}\)

The crumbly sensation of whey protein/polysaccharide mixed gels relates strongly to the breakdown behavior of the gels. Gels showing slow, yielding-like breakdown (i.e., high-energy dissipation) are sensed as the least crumbly, and serum release from the gels contributes to large extent to the energy dissipation and thus decreases crumbliness.\(^{(132)}\)
Co-drying alginate, pectin, carrageenan, or konjac flour with WPC improved the physical properties of edible films compared to their dry blends or that made from WPC only.\(^{(133)}\) Films formed from co-dried WPC and different polysaccharides had lower water vapor permeability, higher tensile strength, elastic modulus and elongation than equivalent films formed the dry blended powders. This adds to the conclusion that WPC-polysaccharide complexes exhibit more effective functional properties. Incorporation of increasing amounts of mesquite gum in the formation of WPI films improved flexibility (lower tensile strength and higher elongation at break) but had no effect on water diffusivity nor permeability of the obtained films.\(^{(134)}\)

**Conclusions**

The advent of new applications for whey proteins, such as delivery systems and encapsulation of nutraceuticals, and edible films, requires whey protein products of tailored functional properties. The effectiveness of controlled processing conditions, the use of novel processing technologies such as HHP, ultrasound, micronization and texturization, and formation of complexes with polysaccharides are promising trends that can improve the functional properties of whey protein products. Remarkable progress has been achieved in this respect during the past two decades. However, more research is still needed to open new perspectives in the control of the functional properties of whey protein products and to optimize their processing conditions.

**References**


