Effect of Processing on Whey Protein Functionality

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ABSTRACT
Whey protein concentrate preparations, prepared by electrodialysis, ultrafiltration, reverse osmosis, gel filtration, and reagent complexation, are highly variable in their composition and functionality. The important functional properties of whey protein concentrates are solubility, whipping and foaming, emulsification, and gelation. Factors affecting these properties include: whey source and composition, cheese or casein manufacturing conditions, heat treatment conditions, fractionation and isolation conditions, storage conditions, overall sanitation conditions, and techniques used for functionality evaluation. Process modifications such as selective heat treatment, selective demineralization or ion exchange, and preteolytic enzyme hydrolysis may be used to alter these functional properties for a desired use application.

INTRODUCTION
The manufacture of protein ingredients from cheese or casein whey has evolved during the last 25 to 30 yr into an established part of the dairy industry of the world. Excellent review articles are available on functional properties of whey protein (i.e., solubility, whipping and foaming, gelation, and emulsification) (2, 48, 49, 50, 52, 68). The major obstacles to overcome in commercial processing of whey protein ingredients are those relating to denaturation during processing and those arising from the high variability of composition and properties of products that are classed as whey protein concentrates (WPC). This latter variability results from many interrelated factors, including source of raw material (whey), cheese manufacturing practices, heat treatment history, protein isolation or fractionation procedures, and storage conditions. The situation is complicated further by general lack of standardization of methods for evaluating protein functionality and lack of close correlation between bench-scale functionality data for individual proteins and predictive behavior in complex food systems.

This review will attempt 1) to provide an overview of the technology of WPC manufacture as it generally relates to protein functionality, 2) to examine those processes that affect specific functional properties, and 3) to discuss potential uses of processing as a means of modifying functionality or in developing WPC products with desired properties.

GENERAL EFFECTS OF PROCESSING ON WHEY PROTEIN CONCENTRATE FUNCTIONALITY
The processing variables and related factors that potentially alter WPC functionality have been summarized in Table 1. Some of these factors have been investigated thoroughly whereas others have not.

Compositional Factors
Composition of whey and of WPC is affected to varying degree by all of the process variables in Table 1. In addition, the relative impact of
TABLE 1. Processing related variables that may affect the functional properties of whey protein concentrates.

<table>
<thead>
<tr>
<th>Processing variable</th>
<th>Direct(^a)</th>
<th>Indirect(^b)</th>
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<tbody>
<tr>
<td>Heat treatment</td>
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<tr>
<td>Heating history</td>
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<tr>
<td>Milk pasteurization</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Whey heat treatment</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Heat during isolation</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Forewarming</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Evaporation and concentration</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Dehydration</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Heating during ingredient applications</td>
<td>x</td>
<td></td>
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<tr>
<td>Fractionation and isolation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Technique used</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Miscellaneous or incidental factors (foaming, pumping, etc.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese or casein manufacturing practices</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>General type of cheese</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rennet (or rennet substitutes)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Starter culture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Process modification (altered cooking, CaCl(_2), direct acid)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Storage factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whey storage conditions</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Whey protein product storage conditions</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Sanitation factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbial load</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Peroxide addition</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Direct protein conformational or denaturation effect.

\(^b\) Indirect protein effect or effect on compositional factors.

many of the process variables on protein functionality is related to compositional factors.

Whey Composition. There are two basic types of whey: 1) acid whey from cottage cheese or casein manufacture, and 2) sweet whey or rennet whey from the manufacture of cheese products involving rennet treatment. The quantity of rennet whey available in the United States and in the world far exceeds the quantity of acid whey available for WPC manufacture (78). However, proportionately larger amounts of acid whey are available in major casein manufacturing countries (New Zealand and the Netherlands).

Components of whey ranked in decreasing order of relative amount are: lactose, nitrogenous compounds (protein, peptides, and amino acids), ash, and lipids. The principal whey proteins are \(\beta\)-lactoglobulin (\(\beta\)-lg) and \(\alpha\)-lactalbumin (\(\alpha\)-la). These two proteins account for approximately 80% of the total whey protein (68). Other proteins include: bovine serum albumin, immune globulins, proteose peptones, and soluble caseins and a variety of minor proteins (enzymes, lactoferrin, etc.). The major mineral components of whey are calcium, phosphorus, sodium, and potassium (74).

Compositional differences and variations between acid and sweet whey have been investigated in detail (1, 37, 42, 44, 63). Generally, rennet whey has a higher pH, more total solids, protein, lactose, and lipids but less calcium and phosphorus than does cottage cheese or lactic casein whey. Although there is variation of nonprotein nitrogen content, sweet whey has generally higher concentrations of peptides and amino acids than acid whey because of proteolysis by rennet (37, 73).

The primary causes of whey compositional variation are cheese or casein manufacturing practices and seasonal variations. Altered cheese making practices generally result in differences in protein components, lactose,
minerals, and low molecular weight nitrogenous compounds (37). Processes in casein manufacture (acidification or lactic fermentation) also affect these major components. Differences in amounts of lactose, ash, calcium, phosphate, and chloride also have been observed between sulphuric acid casein whey and lactic casein whey (42). Protein and lactose content of whey appears to be influenced most by season (63).

Whey Protein Concentrate Composition. Composition of WPC is affected most directly by the fractionation or isolation method used in manufacture. However, WPC composition generally parallels composition of the whey itself (37, 42). A wide range of composition has been reported for WPC prepared by various techniques. The approximate compositional ranges reported are: from 29 to 95% protein, from 1.0 to 80% lactose, from 1.0 to 18% ash, and from 1.0 to 9.0% fat (7, 14, 44, 56). Because economic factors limit the protein purity attainable in WPC manufacture, the typical protein concentration for commercial WPC products ranges from 29 to 60% (7, 45, 56).

Heat Treatment Effects

The factor with the most measurable effect on whey protein functionality is the heat treatment in processing or heating in ingredient applications. Moderate heat treatment (60 to 70°C range) generally results in structural unfolding of the proteins. At higher temperatures, depending on compositional factors, protein aggregation occurs (10, 54). The unfolding step involves molecular interactions such as hydrogen and hydrophobic bonding, whereas the aggregation step involves disulfide linkage and is mediated by calcium ions.

The classical ranking of whey proteins in decreasing order of susceptibility to heat denaturation in milk is: immune globulins, bovine serum albumin, β-lg, and α-la. However, heat denaturation during WPC manufacture is difficult to assess because composition differs from milk and because composition of the system is in a dynamic state changing with each step in the process. The extent of whey protein denaturation is usually determined by solubility measurements (at pH 4.6 or by Harland-Ashworth procedure), electrophoresis, sulfhydryl determination, or available lysine determination (7, 56, 58). These techniques are indirect and relatively insensitive, making interpretation of denaturation data difficult.

Heat denaturation of the major whey proteins (β-lg and α-la) during heating of cheese whey has been shown to follow patterns similar to those in skim milk (29). Differential scanning calorimetry (DSC) curves obtained for heated whey are similar to those for heated β-lg solutions (10). Thus, an important role of β-lg denaturation in the overall physical manifestations of whey protein denaturation has been implicated. However, electrophoretic analyses have shown that α-la was the most highly denatured protein in WPC products (56). These differences may relate to compositional effects on protein denaturation reactions. Compositional factors that alter heat treatment effects on whey protein denaturation include: total solids, pH, lactose concentration, and mineral components (29, 30, 58, 68, 69).

Increased total solids generally has a sparing effect, decreasing the overall rate of protein denaturation during heating of whey (30, 58). This phenomenon more specifically relates to decreases of the rate of β-lg (30, 58) denaturation with increased total solids (30). However, the rate of heat denaturation of α-la is accelerated with increased total solids.

Higher lactose concentration in whey generally decreases rate of protein denaturation by heat (10). There is no published information on the effect of lactose hydrolysis (which is being applied commercially to certain WPC products) on the relative susceptibility of whey proteins to denaturation.

There is disagreement in the literature concerning effects of pH on heat denaturation of whey proteins. Minimal protein denaturation by heating at acid pH near the isoelectric point has been observed by some workers (29, 30), but other researchers have reported lowest protein denaturation at pH from 6 to 7 (10, 58). Further investigations are needed to clarify these discrepancies.

Calcium is the single most critical mineral component of whey that affects heat denaturation and aggregation reactions of proteins. The effect of increased calcium on heat-induced aggregation has been documented (50, 53, 54, 55, 56).

From a practical perspective, protein denaturation in WPC manufacture reflects cu-
cumulative effects of heat treatments applied in the individual processing steps (milk pasteurization, whey pasteurization, forewarming prior to evaporation and concentration, heating during evaporation and concentration, heating during dehydration, etc.). Much of the specific information regarding these heat treatments is of a proprietary nature. It perhaps can be assumed that with the exception of forewarming prior to evaporation (if used), these heat treatments are usually not severe.

Whey Protein Fractionation and Isolation

The technology of WPC manufacture from whey by protein fractionation, isolation, molecular separation, etc., has been in a continual flux with new processes developed and improvements on existing processes. General techniques of WPC manufacture have been summarized in Table 2. The relative technological and economic advantages and disadvantages of these processes has been discussed adequately elsewhere (2, 9, 23, 24, 26, 34, 41, 44, 57, 60, 68, 69, 70, 77). Apparent methods of choice for commercial WPC manufacture are the membrane techniques (primarily ultrafiltration and reverse osmosis). Although higher protein purity may be attained with certain of the methods based on precipitation and complex formation, these methods generally suffer from problems related to residual reagent contamination and may not be readily adaptable to commercialization. Processes for upgrading or further purification by removal of these reagents add steps to the manufacturing process that may not be economically desirable unless unique functionality results.

Membrane processing techniques have a direct effect on WPC composition and indirectly may affect protein denaturation depending upon the process design and operation (i.e., degree of foaming, pumping, mixing, aeration, etc.). There also has been considerable interest in whey pretreatment techniques to standardize composition, maximize processing rates, and prevent membrane fouling (22, 57). Membrane fouling appears to be more of a problem in ultrafiltration than reverse osmosis and is related primarily to complex protein interactions (β-lg with casein components or with bovine serum albumin). However, microbial concentration, protein-calcium interactions, protein-lipid interactions, and calcium-phosphate (apatite) precipitation may be involved. Thus, important compositional factors that could contribute to membrane fouling include protein content, type and physicochemical state of the protein, calcium-phosphate content, pH, and lactate. Whey pretreatment generally involves techniques such as: clarification and centrifugation to remove protein aggregates and lipid components; heating and pH adjustment to induce protein complexation, demineralization, or ion exchange; and altered flow velocity or preconcentration of the whey (22, 57).

Cheese or Casein Manufacturing

Only a paucity of data has been published relating cheese or casein manufacturing factors to WPC functionality. Generally, factors that affect cheese yield (heat treatment, starter culture, cooking conditions, etc.) would be expected to alter composition of the whey and thus affect WPC composition. Other factors related to cheese making may have a more subtle effect on the WPC product.

Rennet (or Coagulant) Effects. Concern has been expressed regarding the possible role of proteolysis caused by residual enzymes (rennet

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TABLE 2. Techniques involved in the manufacture of whey protein concentrates.

<table>
<thead>
<tr>
<th>Membrane fractionation</th>
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<tbody>
<tr>
<td>Ultrafiltration</td>
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<tr>
<td>Reverse osmosis</td>
</tr>
<tr>
<td>Electrodialysis</td>
</tr>
<tr>
<td>Dialysis</td>
</tr>
<tr>
<td>Diafiltration</td>
</tr>
<tr>
<td>Precipitation or complexation with reagents</td>
</tr>
<tr>
<td>Metaphosphates</td>
</tr>
<tr>
<td>Carboxymethylcellulose (CMC)</td>
</tr>
<tr>
<td>Polyacrylic acids</td>
</tr>
<tr>
<td>Iron</td>
</tr>
<tr>
<td>Alcohol</td>
</tr>
<tr>
<td>Physical and chromatographic separation</td>
</tr>
<tr>
<td>Gel filtration</td>
</tr>
<tr>
<td>Ion-exchange</td>
</tr>
<tr>
<td>Inert adsorbents</td>
</tr>
<tr>
<td>Ultracentrifugation</td>
</tr>
<tr>
<td>Foam concentration</td>
</tr>
<tr>
<td>Electro-flocculation</td>
</tr>
<tr>
<td>Heat precipitation</td>
</tr>
</tbody>
</table>
SYMPOSIUM: ASSESSING FUNCTIONALITY OF WHEY PROTEINS

or microbial substitutes) on composition and functionality of whey protein products (17, 32, 39, 71). Residual proteolytic activity could alter directly the concentration of nonprotein nitrogenous compounds and also might have a direct effect on protein conformation and functionality. Whey proteins most susceptible to proteolytic attack by rennet are bovine serum albumin and immune globulins (39). The β-lg and α-la apparently are highly resistant to rennet proteolysis.

The majority of rennet activity in milk during manufacture of Cheddar cheese is recoverable in whey (32). In addition, rennet and microbial proteases used are relatively heat resistant at low pH (4.5 to 6.3) (17, 71). In low-heat processing for WPC manufacture, residual protease activity could present problems. It has been suggested that the pH of whey be adjusted to 6.6 prior to heating to achieve greater inactivation of enzymes in the manufacture of low heat WPC (17). However, further investigations are needed more clearly to assess potential effects of residual proteolytic activity on whey protein processing.

Starter Culture Effects. Little information is available concerning effects of lactic acid bacteria used in cheese or lactic casein manufacture on either composition or functionality of WPC. Therefore, until proven otherwise, the role of starter cultures in whey protein functionality is minimal. Potential effects of starter cultures can be summarized. 1) High metabolic activity (i.e., acid production) of starter organisms or increased starter inoculum can alter whey composition by increasing calcium and phosphate in the whey (73). 2) Lactic acid bacteria vary in their proteolytic activity and, thus, may contribute to residual protease activity in WPC (73). This is probably a minimal effect as these organisms are not highly proteolytic. 3) Recent trends in the use of buffered culture media for propagation of starter cultures may have a subtle effect on ionic composition of whey.

Effects of Added Calcium Chloride. Calcium chloride may be added to milk prior to cheese making to compensate for seasonal variations of milk calcium. Added CaCl₂ has been related to increased viscosity of whey (3). However, effects of added CaCl₂ during cheese making on WPC functionality have not been evaluated sufficiently.

Sanitation Factors

The importance of good sanitation practices in the manufacture of proteinaceous materials cannot be understated. Extraneous microbial or overall bioload has been related to membrane fouling problems (22, 57) and, thus, directly affects WPC processing. Of particular concern are storage conditions for whey (temperature and time) and the practice of pooling wheys prior to processing. Psychrotrophic spoilage organisms are, in general, highly proteolytic, and many of their proteases are heat resistant (73). Large populations of microorganisms in whey could be expected to contribute to altered WPC functionality. Further investigations also are needed in this important area of whey protein processing.

PROCESS EFFECTS ON SPECIFIC FUNCTIONAL PROPERTIES

Solubility

In general, protein solubility of WPC products prepared by membrane or gel filtration techniques is relatively high (14, 19, 25, 45, 49, 50, 56, 66, 68). A range of 88 to 100% protein solubility has been reported for ultrafiltered WPC (14). The WPC products manufactured by complexation or precipitation methods generally have lower solubility and higher solubility dependence upon pH than do WPC from other processes because of residual reagents (56). Removal of protein from hexametaphosphate by ion exchange or gel filtration (24) improves protein solubility.

Heat treatment during processing of WPC preparations generally is expected to have a negative effect on protein solubility. Pasteurization of WPC is the major processing variable in this regard (56).

Heating of WPC dispersions in water at pH near isoelectric points of the proteins (pH 4.3 to 5.0) generally results in measurable reduction of soluble protein (11, 56, 58). Dilute aqueous (1.07 protein) dispersions of WPC at pH 7.0 are relatively stable to heating (19, 66). Considerable reduction of heat stability has been observed at higher protein concentration or with increased ionic strength. Calcium has been implicated especially in lowered soluble protein upon heating of WPC dispersions (25, 50, 66).
Whipping and Foaming Properties

Whipping and foaming properties of whey proteins have been investigated by a large number of researchers (5, 8, 11, 14, 15, 16, 33, 56, 61, 62). The conflicting literature, dating back to 1930, recently has been summarized admirably and reviewed critically by Richert (61). Properties of whipping and foaming depend upon the ability of proteins to unfold and orient at the air-water interface. Thus, it is reasonable that processing or compositional factors that minimize protein aggregation result in more acceptable whipping and foaming properties. A variety of such factors have been investigated: heat treatment, protein solubility and protein denaturation, total solids, pH, calcium content, lipid content, redox potential, and addition of certain reagents such as surfactants, phosphates, reducing and oxidizing agents, or sucrose. From these studies, the following conclusions can be drawn: 1) Laboratory procedures used to induce and thereby measure foam formation differ. Some procedures induce whipping by shearing, whereas others involve foaming by bubbling gases. Such differences themselves cause altered protein structure. 2) Increased total solids (i.e., protein content) generally improve foamability of whey proteins with an apparent optimum near 10% total solids (62, 63). 3) Solution pH has a varied effect on whipping and foaming of WPCs. This variation may relate to interactive effects with other compositional factors (61). 4) The role of calcium in WPC whipping and foaming phenomena is not clearly defined. Generally, calcium addition has a detrimental effect on WPC foamability (61), which may be related to calcium-induced protein aggregation. 5) Foaming is impaired by reducing agents and generally is enhanced by oxidizing reagents (61). This suggests a potential role of sulfhydryl-disulfide mediated protein conformational reactions in the foaming process. 6) Sucrose addition has a varied effect on WPC foams but usually causes decreased overrun (61). Neither effects of lactose content nor lactose hydrolysis on WPC foaming has been investigated sufficiently. 7) Controlled denaturation (protein unfolding) by moderate heating of WPC solutions prior to foam formation results in improved whipping and foaming properties (61, 62).

Emulsification Properties

Perhaps the most conflicting literature related to protein functional properties is in the area of emulsification properties. Much of the confusion results from lack of reliable, standardized methodology for assessing these specific properties.

Like whipping and foaming, emulsification properties depend on the ability of the protein to diffuse to the water-oil interface, unfold, and orient in such a fashion that the hydrophobic groups associate with the oil while hydrophilic groups associate with the water phase. Generally, whey proteins are not considered to be active emulsifiers because they do not possess the appropriate balance of hydrophilic and hydrophobic groups. The hydrophobic and hydrophilic groups of whey protein are distributed uniformly in their primary structure. In contrast, those of casein are concentrated in discrete regions, thus giving rise to the characteristic amphiphilic or "soap-like" structure of caseins (48). Surface adsorption of whey proteins to fat globules is highly dependent on pH, and heat stability of whey protein stabilized emulsions is decreased with added CaCl₂ (76). The individual whey proteins apparently differ in their fat adsorption characteristics. At pH 7.0, β-lg, immune globulin, and lactoferrin are adsorbed selectively. Lowering pH increases α-la adsorption but decreases β-lg adsorption (75).

The following conclusions, adapted from Morr (50, 53), relate to WPC emulsification properties. 1) Processing and compositional factors that alter whipping and foaming properties of WPC would be expected to affect emulsification properties in a similar manner. 2) Emulsion data vary widely depending on conditions used for emulsion formation and evaluation. Of importance are type of equipment and compositional factors (72). Generally, emulsions have been formed in blenders, valve or pressure homogenizers, or ultrasonic generators. Compositional variables include: fat source and properties, ratio of protein to fat, pH, ionic strength, and emulsifier addition. Correlations have been positive among protein solubility, foaming ability, and emulsion stability of ultrafiltered WPC (11). Solubility, foaming, and emulsification properties were affected negatively by heat treatment of WPC at temperatures of approximately 70°C or
above, whereas viscosity and water uptake increased with heat treatment above 70°C.

Gelation Properties

The ability of protein dispersions from WPC products to form gels with heating is a physical manifestation of protein denaturation and aggregation reactions. If conditions are appropriate, a three-dimensional matrix forms with the ability to hold large quantities of water. The gelation properties of whey proteins have been investigated by numerous researchers under a variety of conditions and techniques for evaluation (6, 8, 13, 14, 15, 18, 19, 20, 21, 27, 28, 64, 65, 66, 70). The appearance of whey protein gels varies from translucent and elastic to brittle, aggregated, and curd-like. Generally, translucent gels form at lower protein concentrations (3 to 5%) and at comparatively low heating temperatures (approximately 55 to 70°C). More aggregated, more opaque gels form at higher protein concentration (approximately 10%) and under more severe heating (above 90°C). Translucent gels also form at low ionic strength.

Assessing the processing effects on WPC gelation properties is extremely complex. It generally can be assumed that processing, which results in higher protein purity (reduced lactose, lipid, and ash content) with minimal protein denaturation, produce WPC with gelatin properties appropriate for meat formulations, egg white replacement, or dairy food applications. However, the role of process-related protein denaturation as it influences WPC gelation reactions needs further elucidation.

Aqueous dispersions of commercial WPC adjusted to 10% protein have varied greatly in their gel forming ability when heated under identical conditions (68). Time required for gelation at 100°C ranged from 1 to 17 min for WPC dispersions, which formed gels, whereas some of the products did not gel after 30 min heating. These differences only could be attributed partially to compositional factors such as ratios of protein/ion, protein/fat, or protein/lactose.

Dialysis (or dialfiltration) applied to minimize nonprotein components and improve protein purity alters gel forming ability of WPC. Gels formed by heating dialyzed WPC have been firmer, more cohesive, and more translucent in appearance than those formed by heating nondialyzed WPC (67). Casein WPCs of high protein purity (by diafiltration) had impaired gelation properties when heated at pH 8.5, but their gel properties improved at pH 6.0 as compared to ultrafiltered WPC of lower purity (14). The importance of calcium in the gel formation process at higher pH (where proteins are more negatively charged) was suggested.

Solution pH dramatically affects heat-induced WPC gelation. However, pH effects are interrelated with other compositional factors. Generally, gels formed with heating at low pH (pH 6.0) are more coagulated and less elastic than gels formed at pH 7.0 to 9.0 (28). Gel strength, however, decreases with increased solution pH from 7.0 to 10, and heat-induced gelation of WPC is inhibited by heating at pH 11.0 (64).

A compositional factor of particular significance to WPC gelation is the ionic environment. Calcium has been implicated especially in WPC gelation reactions. Addition of CaCl₂ at 5 to 20 mm or addition of NaCl at 0.1 to 0.3 M typically has resulted in increased gel strength (18, 19, 20, 64, 65, 67). An aggregated appearance of WPC gels formed with heating at higher content of salts has been substantiated by scanning electron microscopy (21).

The general network structure, formed from irreversible heat-induced WPC gelation reactions, is related predominantly to disulfide bonding with some involvement of other nonspecific bondings (hydrogen, hydrophobic, and ionic) that are mediated by calcium (50). The structural dependence on disulfide bonding has been shown by addition of reducing agents, sulphydryl interchange, or sulphydryl modifying reagents prior to heating. A concentration dependence has been noted with added cysteine. Judicious addition of cysteine (approximately 10 mm) increased gel strength of WPC, whereas higher concentrations of added cysteine destroyed gel-forming ability (65). Combined or synergistic effects of added cysteine and calcium on WPC gelation have been examined by multiple regression and response surface methodology (65). Use of sulphydryl blocking agents has resulted in impaired gelation with heating of whey protein products (28). In addition, these workers established a positive correlation between the appearance of whey protein gels and total sulphydryl in WPC.
Generally, WPC of high total sulfhydryl contents produced gels that were more opaque, suggesting more highly crosslinked structures. The free sulfhydryl content in the WPC, however, did not correlate well with gel appearance. After extensive storage at room temperature, whey protein products generally had reduced total sulfhydryl content and also required longer heating for gel formation. A general relationship between total sulfhydryl content in WPC and gel time was established. However, this relationship was not well correlated experimentally (28).

PROCESS MODIFICATION DIRECTED AT ALTERED OR IMPROVED PROTEIN FUNCTIONALITY

A number of process modifications have been applied in attempts to improve protein functionality (12, 35, 36, 38, 40, 46, 47, 59, 76). A general list of these processes and their effects is in Table 3.

Heat Treatment

As discussed, moderate heating just prior to foaming enhances foaming properties of WPC, whereas more severe heating increases viscosity. It is conceivable that, as more knowledge and information is obtained about the relationship between heat denaturation and protein functionality, specific heat processes may be applied at select points during WPC processing to obtain desired functionality effects. However, application of such processing would be complicated by storage factors and by problems associated with subsequent heat treatment and composition in food-use applications.

Heating at Low pH. Relatively severe heating (95°C for 15 min) at low pH has been used experimentally to fractionate and recover whey protein products with unique and interesting functional properties (46, 47). Heating at pH 3.5 resulted in WPC which had low solubility, high water absorption, high viscosity, and low gelation temperature. Soluble WPC was obtained by heating at pH 2.5.

Heating at Alkaline pH. Heating at high pH (or alkaline solubilization) has been suggested as a technique for resolubilizing denatured whey proteins (lactalbumin) (35). However, commercialization of alkaline processing has been hampered by certain questions concerning loss of nutritional quality and occurrence of potentially toxic factors (53).

Demineralization

Use of electrodialysis and ion exchange to remove or alter mineral components is an integral part of some WPC manufacturing processes and is used to a varied degree. Because of the interactive role of the ionic environment with protein functionality, it is conceivable that

TABLE 3. Processing-induced whey protein modifications.

<table>
<thead>
<tr>
<th>Process treatment</th>
<th>Expected functionality effect</th>
</tr>
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<tbody>
<tr>
<td>Heating</td>
<td></td>
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<tr>
<td>Neutral pH</td>
<td>Enhanced emulsification and foaming with moderate heating; enhanced viscosity with high heat</td>
</tr>
<tr>
<td>Acid pH</td>
<td>High protein viscosity, lower gel temperature, high water absorption</td>
</tr>
<tr>
<td>Alkaline pH</td>
<td>Solubilization of heat-denatured protein</td>
</tr>
<tr>
<td>Demineralization or ion exchange</td>
<td>Altered general functionality; improved gelation properties; improved heat stability</td>
</tr>
<tr>
<td>Peroxide treatment</td>
<td>Altered foaming and emulsification</td>
</tr>
<tr>
<td>Enzymatic hydrolysis</td>
<td>Improved solubility at low pH; resolubilization of heat-denatured whey protein products</td>
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</tbody>
</table>

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as improvements are made in these processes, selective dimineralization may be used depending on desired use application.

Investigations in the Netherlands involving the use of dimineralization by ion exchange followed by acidification at pH 4.5 to precipitate immune globulins (insoluble at low ionic strength) and certain lipid components to improve emulsification and foaming properties have been discussed in a recent review (2).

Replacement of calcium with sodium ions during WPC manufacture by ion exchange has resulted in improved gelation properties (36).

Although the time required for gel formation increased as the extent of calcium replacement increased, gels formed from WPC with lowered calcium had improved textural properties (hardness, cohesiveness, and springiness).

**Hydrogen Peroxide Treatment.** Low concentrations of hydrogen peroxide are used in some commercial WPC manufacturing processes as a preservative. Hydrogen peroxide treatment generally alters methionine and cystine/cysteine in the protein and alters foaming properties (4). Although the possible effects of hydrogen peroxide on gelation properties have not been examined, an influence appears likely because of the importance of disulfides to gel formation.

**Proteolytic Enzyme Hydrolysis**

Use of proteolytic enzyme hydrolysis as a means for altering WPC functionality characteristics has not been examined thoroughly. Treatment with proteolytic enzymes improved WPC solubility and has been suggested as a technique for resolubilization of heat denatured whey protein (40, 59).

In summary, processing effects on WPC functionality are both highly complex and variable. Further research is needed particularly: 1) to delineate more clearly the relationship between functional properties and physico-chemical or structural aspects of the proteins, 2) to standardize functionality testing and develop a protocol for such testing that includes functionality assessment in model or utility food systems, and 3) to assess the role of certain processing factors on physicochemical properties of proteins.

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