Properties of Whey Protein Isolates Extruded under Acidic and Alkaline Conditions¹

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ABSTRACT

Whey proteins have wide acceptance and use in many products due to their beneficial nutritional properties. To further increase the amount of whey protein isolates (WPI) that may be added to products such as extruded snacks and meats, texturization of WPI is necessary. Texturization changes the folding of globular proteins to improve interaction with other ingredients and create new functional ingredients. In this study, WPI pastes (60% solids) were extruded in a twin-screw extruder at 100°C with 4 pH-adjusted water streams: acidic (pH 2.0 ± 0.2) and alkaline (pH 12.4 ± 0.4) streams from 2 *N* HCl and 2 *N* NaOH, respectively, and acidic (pH 2.5 ± 0.2) and alkaline (pH 11.5 ± 0.4) electrolyzed water streams; these were compared with WPI extruded with deionized water. The effects of water acidity on WPI solubility at pH 7, color, microstructure, Rapid Visco Analyzer pasting properties, and physical structure were determined. Alkaline conditions increased insolubility caused yellowing and increased pasting properties significantly. Acidic conditions increased solubility and decreased WPI pasting properties. Subtle structural changes occurred under acidic conditions, but were more pronounced under alkaline conditions. Overall, alkaline conditions increased denaturation in the extruded WPI resulting in stringy texturized WPI products, which could be used in meat applications.

Key words: pH, extrusion, whey protein isolates, electrolyzed water

INTRODUCTION

Extruders provide mechanical and thermal energy for mixing, cooking, melting, and forming biomaterials. A demonstrated benefit of extrusion processing is the abil-

ity to change the molecular structure of food ingredients (Batterman-Azcona and Hamaker, 1998). Because extrusion processing offers versatility in forming materials for various functional uses, there has been a steady increase in food products created by this technology. These products include breakfast cereals, snacks, meal replacement bars, and confectionery candies (Breitenbach, 2002).

The use of extrusion to impart fibrous texture to plant proteins for use as meat extenders has been practiced for many years (Atkinson, 1970). Rhee et al. (1981) reviewed the processes for texturization of soy proteins. High moisture (≥60 weight %) and temperatures above 150°C are needed for texturizing and forming fibrous structures by extrusion from soy isolates (Kitabatake et al., 1985). Extrusion shear-induced fibrous networks are formed through formation of disulfide bonds, and cross-linking of protein chains through amide bonds between free-carboxyl and amino side groups (Harper, 1981). Adjustment of pH through acid or alkali treatment influences conformation, molecular interactions of proteins, and development of structure in soy proteins (Dahl and Villota, 1991). Whey proteins can be modified using chemicals, heat, or shear in extrusion processes. Chemical treatment alone alters the reactive groups of the amino acids, resulting in changes in the noncovalent forces that influence conformation, such as van der Waals forces, electrostatic interactions, hydrophobic interactions, and hydrogen bonding (Kester and Richardson, 1984).

For whey proteins, texturization is more difficult to accomplish. Whey proteins are degraded by prolonged heat treatment above 140°C (Walstra et al., 1999). Thermal denaturation of the 2 major protein fractions in whey protein isolates, β -lactoglobulin (50%) and α -lactalbumin (22%), takes place between 50 and 75°C and is accompanied by unfolding and unmasking of the SH groups (Linden and Lorient, 1999). When the whey proteins are denatured, they become insoluble, and aggregate (Walstra et al., 1999). The extent of denaturation is determined by proportion of protein insoluble at pH 7 and depends on heating temperature, time, and the pH of whey at heating (Ennis and Mulvihill, 2000). Moreover, adjusting the acidity (H⁺) or alkalinity (OH⁻) of whey

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proteins while heating increases loss of solubility, and denaturation (Harwalker, 1979). However, during texturization in the extruder, insoluble cross-linked protein aggregates are aligned by flow and complex shearing action of the extruder screws (Queguiner et al., 1992).

Heating and shear alter the conformational structure of the whey protein through partial denaturation of the protein, thereby exposing groups that are normally concealed in the native protein (Kim and Maga, 1987). By varying extrusion temperatures and denaturing whey proteins at temperatures below 100°C, various textured protein products were created that retained their physical functionality such as foaming and digestibility (Hale et al., 2002; Onwulata et al., 2003; Onwulata and Tomasula, 2004). Differences in the interaction of denatured whey depending on pH have been reported in milk (Anema et al., 2004). Anema et al. (2004) showed, for instance, that at pH 6.5, about 70% of the denatured β -LG and α -LA were associated with casein, whereas at pH 6.7, only about 30% were associated with casein.

Electrolyzed water has been used as an adjunct for enhanced food quality and safety in Japan, and its use has been reported to improve kneading quality of wheat flour and dough alone without using other food additives (Kato et al., 2001). Improvement in the textural properties of Japanese rice cooked with electrolyzed water (pH 9 to 10) was also reported by the same authors. Kobayashi et al. (1996) reported increased volume and texture, as measured by the adhesiveness and hardness ratio, signifying the effect of pH on protein structure and function. Therefore, the use of electrolyzed water in affecting structural changes in proteins was worth investigating. Because small shifts in pH affect milk protein structure, this study was conducted to determine the effect of pH with extrusion shear on texturization of whey protein isolates and the resulting effects on physical properties of the extrudates.

MATERIALS AND METHODS

Materials

Whey protein isolate (**WPI**) Provon 190 purchased from Glanbia Ingredients (Glanbia Foods Inc., Richfield, ID) was used for this work. Electrolyzed water for the acid or alkaline extrudates was generated with an Amano 800 electrolyzer (Amano USA Inc., Roseland, NJ) at pH of 2.0 ± 0.2 and 11.5 ± 0.2 , respectively, for comparison studies. Nonelectrolyzed pH-adjusted water was also prepared. For acid, water was adjusted to pH of 1.4 ± 0.2 with 2 N HCl, and alkali to pH 12.5 ± 0.2 with 2 N NaOH. Electrolyzed water was generated by electrolysis of sodium chloride solution producing low pH water at the anode (HCl) and hypochlorous (OCl^-) at the cathode. Acidic electrolyzed water (**AcEW**) was generated using

a flow type electrolysis apparatus (ROX-20TA, Hoshizaki Electric Co. Ltd., Toyoake, Aichi, Japan). The current passing through the electrolysis apparatus was set at 16 A, and the voltage between the electrodes was set at 18 V. Acidic electrolyzed water (40 ppm free available chlorine) was prepared within the anode compartment of an electrolytic cell and alkaline electrolyzed water (**AlEW**) was prepared within the cathode compartment. The properties of each solution were determined, including pH and free available chlorine concentration. The pH of the tested solution was measured with a pH meter (HM-11P, Toa Electronics Ltd., Tokyo, Japan). Within 1 h, the initial concentration of the free available chlorine was determined with EPA-approved chlorine test kits (Hach Co., Loveland, CO). Estimated pH values were 2.6 ± 0.1 , 11.4 ± 0.1 , and 6.8 ± 0.1 for AcEW, AlEW, and deionized water (**DW**), respectively. Free available chlorine concentration of AcEW and chlorinated water was 40.3 ± 1.5 ppm.

Extrusion Processing

The screw profile of the Krupf Werner Pfeiderer ZSK30 twin-screw extruder used for this study was reported earlier (Onwulata et al., 2001). The screw speed of the extruder was maintained at 200 rpm. The screw elements were selected to provide low shear at 200 rpm. The barrel temperature profile for extrusion was 35, 35, 35, 50, 100, 100, 100, 100, and 100°C, from the feed section to the die. Water input to the extruder was at the rate of 23 g/min. The pH-adjusted water was injected with an electromagnetic dosing pump (Milton Roy, Acton, MA) to bring the moisture content of the feed to approximately 20 g of H_2O /100 g of mixture (wet basis). Feed rate of WPI was 35 g/min and WPI extrudates assayed were coded as follows: **NEXT** = nonextruded WPI (control); **HACI** = WPI extruded at pH 2.0 ± 0.2 (pH was adjusted with 2 N HCl); **NEUT** (extrudate control) = WPI extruded at pH 6.8 ± 0.2 with DW; **EACI** = WPI extruded at pH 2.5 ± 0.4 with AcEW; **EALK** = WPI extruded at pH 11.5 ± 0.2 with AlEW; and **NALK** = WPI extruded at pH 11.5 ± 0.4 with 2 N NaOH. Extruded WPI samples were collected, freeze-dried in a laboratory freeze dryer for 5 min, and stored at 4.4°C until analyzed. Moisture content was measured using a vacuum oven (method #925.09; AOAC, 1998).

Buffering

To understand the buffering effect of the WPI, nonextruded pastes of WPI and pH-adjusted water streams were made by adding a specified acid or alkali to deionized water, and the pastes were made using a Hobart commercial mixer (The Hobart Mfg. Co., Troy, OH). Five

different pH water streams were prepared as follows: pH 2.6 adjusted with 1 *N* HCl; pH 1.4 adjusted with 2 *N* HCl; pH 6.8, deionized water; pH 11.6 adjusted with 1 *N* NaOH; and pH 13 adjusted with 2 *N* NaOH. Whey protein isolate pastes were made at the ratio of 60 g of WPI/40 mL of the appropriate pH-adjusted water stream, and pH was measured every 15 s for 2 min. The maximum residence time of pH-adjusted pastes in the extruder was recorded.

Solubility

Solubility of extruded WPI was determined with a 1.0-g sample of extrudate stirred with 90 mL of deionized water at 125 rpm and pH 7.0 for 2 h. Samples were then centrifuged at $5,000 \times g$ for 20 min. The supernatant was freeze-dried overnight. The nitrogen/protein conversion factor was 6.38 and percentage protein solubility was calculated as described by Kilara (1984). Insolubility or denaturation was calculated as $(100 - \text{solubility})$.

Color

Tristimulus color measurements of WPI extrudates were measured instrumentally with a Hunter Laboratory (ColorQuest XE model) infrared spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA). Extrudates were scanned through a small-angle port (2°). Hunter L^* , a^* , b^* values were used to calculate total color difference (ΔE), defined as the square root of $(L^2 + a^2 + b^2)$. Standard reference tile values were $L = 93.5$; $a = -0.89$; and $b = 1.01$, where L = lightness, a = redness, and b = yellowness.

Rapid Visco Analyzer Pasting Viscosity

Post-extrusion, pastes of texturized extrudates were made as described, to determine their viscosity. Analysis of the pasting behavior was conducted with a Rapid Visco Analyzer (RVA; model RVA-3D, Foss North America, Eden Prairie, MN) equipped with Thermocline for Windows software. Pasting properties, a measure of WPI paste viscosity, were determined by RVA Application Method No. 48, using a 28-g specimen, 13.5% wet basis (Parkes et al., 1998).

Stereo Fluorescence and Confocal Microscopy

Short (1 cm) segments of extruded ribbons were cut perpendicular to the axis of extrusion with a stainless steel razor blade by hand and sectioned surfaces were illuminated with either white light from a fiber-optic illuminator (model 190, Dolan-Jenner Industries, Inc., Woburn, MA) or violet light (425 to 440 nm) from a high-

pressure Hg lamp. Images were obtained with a stereo fluorescence microscope, equipped with a color CCD and digital imaging hardware and software (model MZ FLIII, Leica Microsystems, Inc., Bannockburn, IL). For confocal microscopy, the cut faces of ribbon segments were applied to the glass bottom of microwell dishes (MatTek Corp., Ashland, MA) and illuminated with blue light (488 nm) from an argon laser. Maximum projection images of green autofluorescence (510 to 550 nm) within approximately 30 μm -thick stacks of optical sections were collected using a $10\times$ lens mounted in an optical microscope integrated with a spectrophotometric confocal microscope system (model IRBE, Leica Microsystems, Inc., Exton, PA). The distribution of intensity in digital images was analyzed using Fovea Pro3.0 plug-ins (Reindeer Graphics, Asheville, NC) with Photoshop v7.0 (Adobe Systems Inc., San Jose, CA).

Transmission Electron Microscopy

Small segments of extruded ribbons in glutaraldehyde fixative solution were trimmed into small blocks ($\sim 1 \times 2$ mm) and washed in 0.1 *M* imidazole buffer. Then, the blocks were immersed in 2% osmium tetroxide solution buffered with imidazole buffer for 2 h, washed in distilled water, and gradually dehydrated in a series of ethanol solutions. After several changes of absolute ethanol, blocks were immersed in propylene oxide and transferred to a 1:1 mixture of propylene oxide and epoxy resin mixture and slowly agitated to promote infiltration of the plastic into the sample blocks. Following infiltration, the blocks were transferred to epoxy resin mixture and cured for 2 d at 55°C . Thin sections of the cured blocks were stained with solutions of uranyl acetate and lead citrate, and then examined in the bright-field imaging mode using a electron microscope (model CM12, FEI Co., Inc., Hillsboro, OR). Images were recorded on photographic film at an instrumental magnification of $45,000\times$.

RESULTS AND DISCUSSION

The buffering capacities of WPI pastes under different pH conditions are shown in Figure 1. Using the same amount of WPI (60 g) and pH-adjusted water (40 mL), a paste with the same consistency as the paste that leaves the extruder was created and the buffering time depended on the initial pH. Comparatively, 1 *M* HCl of lower molarity weaker acids or bases took a shorter time, 15 to 30 s, to buffer whereas high molarity acids and bases took a little longer, 30 to 45 s. This time is within the range of the average residence time of materials in the barrels of the extruder (30 to 75 s). The buffering effect is seen with both acid and alkali; although the

Figure 1. The buffering properties of extruded whey protein isolates adjusted with 5 different pH-adjusted waters as follows: 1 N HCl (pH 2.62), 2 N HCl (pH 1.4), deionized water (pH 7.2), 1 N NaOH (pH 11.6), and 2 N NaOH (pH 13.2).

materials start with different pH conditions, they end up close to the normal pH of WPI, $\text{pH } 6.2 \pm 0.2$. The short time (<30 s) to complete buffering means that all reactions are carried out within the extruder, allowing for maximum short-term effect with minimal long-term alteration of the proteins. Buffering capacity of milk is due to the presence of protein and salts (Walstra et al. 1999). In general, whey proteins have maximum buffering capacity between pH 3 and 4 for proteins with acidic residues and $\text{pH} > 8$ for those with alkaline residues (Salaun et al., 2005). For this experiment, we used acid-precipitated whey proteins, which reportedly have higher buffering capacity due to higher inorganic mineral content, particularly phosphates. Heat treatment of whey proteins has been shown to increase buffering capacity (Lucey et al., 1993). The combination of shear and heat treatment explains the short buffering times observed in this study.

There was a significant ($P < 0.05$) difference in solubility between nonextruded pastes (NEXT) and the pH-adjusted extrudates. The solubility of extruded WPI was affected by pH of the water (Table 1). There was a slight increase (~18 to 20%) in solubility associated with acid extrudates over the extruded control (NEUT). The difference between EACI and HACI extrudates was not significant, but both were higher in solubility than NEUT. The electrolyzed alkali extrudates were similar to the control, but significantly more denatured than the other pH-adjusted products.

The effect of pH on yellowness values and total color difference (ΔE) of WPI extrudates is shown in Table 1.

On the color scale, the b coordinate is a measure of yellowness. All pH-adjusted extruded samples were significantly ($P < 0.05$) more yellow and darker than the NEXT paste. The yellowness order was NEXT (pH 6.8) < HACI (pH 1.4), NEUT (pH 6.8) < EACI (pH 2.5) < EALK (pH 11.5) < NALK (pH 13.2). Examination of the b coordinates of WPI extrudates show that the most yellow were NALK extrudates (Figure 2). The 2 acid WPI extrudates were similarly bright, and were brighter than NEUT, but slightly darker than NEXT. The NALK extrudates were significantly darker ($P < 0.05$), indicating very strong effect of alkaline conditions. The total

Table 1. The effect of pH of extruded whey protein isolates (WPI) on the solubility and color of WPI

Extrusion condition ¹	Solubility (%)	Color (b)	Color difference ² (ΔE)
EACI (pH 2.5)	22.3 ^b	10.9 ^b	89.9 ^b
HACI (pH 1.4)	21.8 ^b	9.1 ^{bc}	89.8 ^b
NEUT (pH 6.8)	18.4 ^c	9.1 ^{bc}	86.9 ^{bc}
EALK (pH 11.5)	18.6 ^c	12.1 ^b	90.3 ^b
NALK (pH 13.2)	15.3 ^d	15.7 ^a	73.8 ^c
NEXT (pH 6.8)	73.5 ^a	6.7 ^c	94.7 ^a

^{a-d}Means within a column with the same letter are not significantly different ($P < 0.05$).

¹EACI = WPI Extruded at pH 2.5 with electrolyzed acid water; HACI = WPI extruded at pH 1.4 with 2 N HCl-adjusted water; NEUT = WPI extruded at pH 6.8 with deionized water; EALK = WPI extruded at pH 11.5 with electrolyzed alkali water; NALK = WPI extruded at pH 13.2 with 2 M NaOH-adjusted water; NEXT = nonextruded WPI paste.

² ΔE is the total color difference.

Figure 2. Photograph showing the effect of pH on extruded whey protein isolates. A) HAlC (extruded at pH 1.4 with electrolyzed acid water); B) NEUT (extruded at pH 6.8 with deionized water); and C) NALK (extruded at pH 13.2 with 2 N NaOH-adjusted water).

color difference of EALK extrudate was brighter than NALK, indicating less effect. Yellowness may be the effect of ammonium sulfide, olfactorally perceptible from NALK extrudates.

Rapid Visco analysis is a time-temperature profile or pasting viscogram of a material that show the points of

Table 2. The effect of pH of whey protein isolates (WPI) on the Rapid Visco Analyzer pasting properties of extruded WPI

Extrusion condition ¹	Peak viscosity (cP)	Final viscosity (cP)	Setback viscosity (cP)
EACI (pH 2.5)	34 ^d	23 ^b	14 ^b
HAlC (pH 1.4)	108 ^b	268 ^a	171 ^a
NEUT (pH 6.8)	96 ^{bc}	263 ^a	175 ^a
EALK (pH 11.5)	128 ^b	320 ^a	202 ^a
NALK (pH 13.2)	208 ^a	381 ^a	186 ^a

^{a-d}Means within a column with the same letter are not significantly different ($P < 0.05$).

¹EACI = WPI Extruded at pH 2.5 with electrolyzed acid water; HAlC = WPI extruded at pH 1.4 with 2 N HCl-adjusted water; NEUT = WPI extruded at pH 6.8 with deionized water; EALK = WPI extruded at pH 11.5 with electrolyzed alkali water; NALK = WPI extruded at pH 13.2 with 2 M NaOH-adjusted water; NEXT = nonextruded WPI paste.

peak, final, and setback viscosities (Almeida-Dominguez et al., 1994). Peak viscosity is the first peak in the swelling region that characterizes viscous properties. Pasting properties reveal important information about a product's degree of denaturation, and the effect of processing conditions on degree of denaturation (DesRochers, 1998). The RVA peak viscosities for gels of pH-adjusted texturized WPI (Table 2) show the NALK extrudate to be significantly more viscous than the rest of the samples. The rest of the samples were similar to the neutral extrudate, but EACI was less viscous than its control (NEUT). Final and setback viscosities were similar for all extrudates except EACI, which was significantly lower ($P < 0.05$). The effect of pH-adjustment on protein pasting properties depended on the molar strength of the solution used to adjust pH.

Differences in structure of the WPI extrudates produced under different pH conditions were probed by macroscopic and microscopic imaging. Cross-sections of extruded ribbons illustrate several differences, examined by stereomicroscopy (Figure 3, panels A, B and C). At pH 1.4, the ribbons were narrow and dry, and frequently split horizontally along the midline, while the outer edges were smooth. At neutral pH 6.8, the ribbons were slightly thicker and moist with rough or fluted edges; and at alkaline pH, the ribbons were thickest, moist, and the edges were irregular on a submillimeter scale. When the edges of the ribbons were examined by fluorescence confocal microscopy, the differences in structure were apparent, especially at the edges. Following extrusion, the ribbons were immersed in a glutaraldehyde solution; the reaction of glutaraldehyde with protein typically produces autofluorescence in the visible light spectrum, which can be imaged with a confocal laser scanning microscope or other epifluorescence imaging system. When excited with blue light (488 nm) from an argon laser, the protein-glutaraldehyde com-

Figure 3. Two sets of images illustrating the effects of pH on extruded whey protein isolate ribbons. A (HACI; extruded at pH 1.4 with electrolyzed acid water), B (NEUT; extruded at pH 6.8 with deionized water), and C (NALK; extruded at pH 13.2 with 2 *N* NaOH-adjusted water) are cross-sections of extruded ribbons imaged by reflected light illustrating general features and variations in color following fixation in glutaraldehyde solution; D (HACI), E (NEUT), and F (NALK) are confocal microscope images including edges of the cross sections demonstrating the differences in breadth of the dense bands and granularities that correspond to intense autofluorescence.

plexes in the WPI extrudates emitted visible fluorescence extending from 500 to >600 nm. When maximum projection images at the cross-sectioned edges of the ribbons were made, distinct differences in the distribution of fluorescence were apparent, depending on the pH of

the extrusion process. At pH 1.4, a narrow region of the edge (<250 μm) was most intense, but the whole edge area was composed of numerous irregular, fluorescent granules, with dimensions up to about 50 μm in diameter (Figure 3D). Similarly, ribbons extruded at neutral pH

Figure 4. Plots of the horizontally averaged relative intensities in Figure 3 D, E and F, showing the edge effects at the different pH values.

also had a thin bright edge, including the superficial irregularities ($<250\text{ }\mu\text{m}$) but the matrix of the ribbon contained nearly uniform fluorescence that was not resolved in the form of granules compared with the acid pH ribbons (Figure 3E). Notably, at alkaline pH, the increased fluorescence intensity at the edge was much deeper or broader than that of the others, extending more than $500\text{ }\mu\text{m}$ from the edges, and the intensity decreased gradually, rather than abruptly as in the acid and neutral pH samples (Figure 3F). For comparison, profiles of the fluorescence intensities from each type of ribbon were made by averaging the intensity along the horizontal axis of each image, and the computed line profiles for the maximum projection images are illustrated in Figure 4. The comparison reveals that at acid and neutral pH, the intensities of fluorescence are maximal at short distances ($<250\text{ }\mu\text{m}$) from the edge, and they drop sharply to a baseline level. The intensity peak at alkaline pH also occurs at a depth of less than $250\text{ }\mu\text{m}$, but the intensity does not drop rapidly to low levels; instead, fluorescence extends at more than one-half the peak height to depths of more than $500\text{ }\mu\text{m}$ into the matrix of the ribbon. These results suggest that the pH conditions of extrusion affect the aggregation and conformation of the WPI proteins in the ribbons, as expressed by a differential reaction with glutaraldehyde, producing different patterns of autofluorescence. Averaged intensities along the horizontal axes of the images are compared in Figure 4. A maximum projection image of the fluorescent surface of the 3 extrudates shows different degrees of aggregation, and that HACI extrudates were most aggregated (see Figure 3D). The HACI extrudates were aggregated into the most discrete, granular-shaped fluorescent material, delineated by narrow spaces with no

fluorescence. The NEUT extrudates had the most continuous, and least well-defined fluorescence (see Figure 3E) and in the alkaline extrudates, the fluorescent material was aggregated into particles or clumps (Figure 3F).

Compared with NEXT (pH 6.8; Figure 5A) and NEUT (Figure 5B) pastes, the folding of the acid and alkali samples are more extensive. The fluorescence images of the surfaces of HACI and NALK extrudates (Figures 5C and 5D) show different folding patterns or aggregation from the control extrudates. The NALK extrudate has large folds, whereas the HACI folds are smaller with spaces in the matrix. The increased folding with HACI and NALK extrudates suggests greater insolubility or denaturation. This was confirmed by the solubility analysis (Table 1). Under acid conditions, the autofluorescence was clustered into the form of small, well-defined particles ranging from less than 1 to $2\text{ }\mu\text{m}$ in diameter and under neutral conditions the distribution of intensity was similar. Alkaline conditions produced broader distribution of sizes and less well-defined shapes of fluorescent aggregates.

The pH of the electrolyzed water created the visible differences in the organization of the fine structures of products extruded with electrolyzed water and may have been the result of electrolysis products. The autofluorescence from the 2 electrolyzed water extrudates, EACI and EALK, showed different degrees of aggregation, with acid extrudates being more aggregated (Figure 6A) than the alkali extrudates (Figure 6B). These images show the fluorescence variation due to degree of aggregation and differences in clumping. Extruded samples contain dense aggregates of material with irregular shapes and sizes, interspersed with electron-lucent areas with smaller aggregates (Figure 7). Ultrastructure in the dense clumps resembles the fine granularity (5 nm) found in the fully denatured materials examined previously (Onwulata et al., 2003). Control samples (NEXT and NEUT) did not contain the irregular dense clumps of material; instead the ultrastructure is a uniform dispersion of loosely packed small clumps, containing very fine granularity (5 nm) unlike the long periodicity (200 to 300 nm) found in the fully hydrated "gel". When examined by transmission electron microscopy of stained thin sections, nonextruded WPI paste was homogeneous, consisting of fine granules around 5 nm in diameter and short threads about 15 to 25 nm long (Figure 7A). At neutral pH (Figure 7B), the ultrastructure of the extrudate contained regular, circular or ellipsoidal electron-dense aggregates ranging from under 100 to over 300 nm in diameter, and the aggregates were separated by less electron-dense areas containing randomly oriented threads, around 5 nm wide and 100 nm long in the interstices that merge with the surfaces of the close-packed aggregates. At acid and alkaline pH (Figure 7C

Figure 5. Confocal fluorescence images illustrating the effects of pH on the microstructure of whey protein isolate (WPI) ribbons: A) diffuse fluorescence in NEXT (nonextruded WPI paste); B) diffuse clusters of fluorescence in NEUT (WPI extruded at pH 6.8 with deionized water); C) and D) well defined, cumulative clusters of fluorescence in HACL (WPI extruded at pH 1.4 with 2 *N* HCl-adjusted water) and NALK (WPI extruded at pH 13.2 with 2 *N* NaOH-adjusted water), respectively.

and D), the ultrastructure of extrudates contained very large, irregular electron-dense aggregates, separated by equally large and irregular areas of low electron density filled with short threads that were confluent with the surfaces of the aggregates, similar to those found at neutral pH.

Although it is known that pH buffering (the resistance to change in pH on addition of acid or base) retards any physical modification of WPI (Fox and McSweeney, 1998), we observed in this study that buffering of WPI was more effective with comparatively weaker acids and bases, but not with strong acids and bases (Figure 1). Buffering capacity can be altered depending on the severity of heat treatment of various milk protein fractions (Salaun et al., 2005). Heat treatment lower than 100°C

for 10 min induced a slight increase in buffering capacity at pH 5 of milk, whereas intense heat treatment at 120°C for 10 min increased its buffering capacity intensely and shifted maximum peak pH (Lucey et al., 1993). Gezimati et al. (1998) showed that combining heating and shear alters the conformational structure of the protein by exposing groups that are normally hidden away in the native protein.

We have reported here that extrusion induces shear-generated structural changes leading to loss of solubility with increasing pH in a short time (<40 s). Previously, Kavanagh et al. (2000) showed that mixtures of α -LA and β -LG formed coupled network gels at pH 3 and 7, and fine-stranded fiber-like aggregates at pH 2, in the presence of 0.1 *M* NaCl, and that aggregation of globules

Figure 6. Confocal fluorescence images illustrating the effects of pH on the microstructure of whey protein isolate (WPI) ribbons showing well defined, cumulative clusters of fluorescence: A) diffuse fluorescence in EACI (WPI extruded at pH 2.5 with electrolyzed acid water); and B) diffuse clusters of fluorescence in EALK (WPI extruded at pH 13.2 with 2 N NaOH-adjusted water).

occurred at pH 7. They showed also that aggregation occurs more with acid because of modest increases in intermolecular β -sheet with shoulder, which develops in the carbonyl-stretching amide I region. Our results

(Figures 5 and 6) confirm the formation of aggregates, and shows further varying degrees of aggregation induced by shear and acidic conditions, and demonstrated that NALK samples produced with water adjusted with 2 N NaOH at pH 13 were most aggregated.

The network structure in a heat-induced globular gel depends strongly on the balance between the attractive and repulsive forces during aggregation. If the pH is far from the isoelectric point, and the ionic strength is sufficiently low, then intermolecular electrostatic repulsion is dominant. Structural transitions are reflected by the macroscopic properties—fine-stranded gels are translucent, whereas particulate gels are opaque and synergetic. A fine-stranded gel formed at neutral pH is rubbery and deformable to a large strain without fracturing (Ikeda and Morris, 2002). At acidic pH, intramolecular disulfide bonding is unlikely to occur; the structures are brittle. Our results show brittleness of HACI extrudate pH (1.4) with fractures (Figure 3A). Bilayer fluorescent patterns of protein and glutaraldehyde complexes observed through confocal scanning laser microscopy (Figure 4) agree with the observation that heat induces gelation of globular proteins at low pH (2 to 2.5), and at the molecular level, rod-like structures are formed (Onwulata et al., 2003), whereas aggregation of globules occurs at pH 7. Gosal et al. (2004a) showed that β -LG formed aggregates with worm-like fibrils when heated in various alcohol-water mixtures at pH 2, and that thermally induced aggregation occurred more cooperatively in a nucleation and growth process. At higher concentrations, heated β -LG self-assembled in a uniform fibrillar pattern (Gosal et al., 2004b). It is reasonable to consider that whey protein aggregates generated under extrusion at high temperature would also contain more aligned fibrillar structures. Finally, Pelegri and Gasparetto (2005) have shown that strong links between pH and temperature affect solubility of whey proteins in the pH range 3.5 to 7.8 and temperature range of 40 to 60°C and that denaturation occurs. We show that extrusion texturization occurs in the short time frame of 15 to 60 s within the extruder; therefore, pH-adjusted extrusion-texturized WPI will maintain their characteristic quality after treatment, and provide added functionality due to newly induced conformation.

CONCLUSIONS

Denaturation and aggregation of whey protein isolates are pH dependent, with strong alkalis producing the more textural effect. Denaturation at the molecular level produces rod-like microstructures and forms the basis for fine-stranded fiber-like structures in texturized products. We have demonstrated that whey protein can be texturized by high shear at different pH values to form

Figure 7. Thin sections of embedded whey protein isolate (WPI) ribbons illustrating the ultrastructure of the protein aggregates: A) uniform, fine granularity of EACI (WPI extruded at pH 2.5 with electrolyzed acid water); B) micrometer- and submicrometer-diameter circular dense aggregates surrounded by fine filaments in the interstices in NEUT (WPI extruded at pH 6.8 with deionized water); C) and D) irregular dense aggregates and interstitial filaments in EALK (WPI extruded at pH 11.5 with electrolyzed alkali water) and NALK (WPI extruded at pH 13.2 with 2 *N* NaOH-adjusted water), respectively.

stringy products. Whey proteins denatured under acidic and alkaline conditions form small primary aggregates that combine to form large clusters and are aligned by extrusion shear to form fibrous structures. Fibrous protein extrudates may be spun into meat alternatives similar to textured soy products.

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