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Properties of Whey Protein Isolates Extruded under Acidic and Alkaline Conditions[1]

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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 ability 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 Pfleiderer 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