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Improving Functionality of Whey Protein Concentrates from Different Sources

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Abstract: The nutritional appeal and physical functional properties of whey proteins have increased their level of use in various food products. Fractionation technologies based on ultrafiltration have created Whey Protein Concentrates (WPC) ranging from 35 to 80% protein contents leading to their increased use in different food products; but their functional properties can vary widely and may depend on the processing history, particularly drying. Because of this variability in functional properties the amount of WPC used is kept to a minimum to reduce the effects of their variable properties on overall quality of formulated foods. This study was carried out with six commercial WPC with 80% protein content (WPC80) to compare sieving and blending as methods of reducing variations in solubility, gel strength, foam volume and stability of solutions of WPC80. There was a significant difference ($p<0.05$) in functional properties of sieved or blended WPC80 samples. Sieving to reduce particle size of WPC80 below 150 microns increased solubility up to 50% for the least soluble samples ($p<0.05$). Blending all six WPC80 from different manufacturers averaged the solubility values, but sieving was better for improving individual functional properties for below average WPC80.

Key words: Sieving, blending, whey protein concentrates, functionality

Introduction

The use of whey protein concentrates world-wide in formulating many products is increasing due to their reported nutritional and health benefits. But variability in whey proteins and their functionality in products is a significant problem in formulating products. Particularly as whey protein concentrates continue to vary in functionality and flavor after manufacturing and during storage. Storage below 20°C has been recommended to retain initial physical functionality (Hsu and Fennema, 1989) and flavor (Whetstine *et al*., 2003) but this will present a significant challenge for most international users.

Whey protein concentrates widely used for product formulation range from 35 to 80% protein. The proteins are denatured to varying degrees by the manufacturing processes, affecting their solubility. The quality and functionality of whey protein concentrates vary depending on the source of cheese and process history (Huffman and Harper, 1999; Hurley *et al*., 1990; Schmidt *et al*., 1984). Also, other factors such as extent of heat treatment that contribute to variability have been enumerated (Hurley *et al*., 1990; Regester *et al*., 1992; Mehra *et al*., 1999; Walstra *et al*., 1999).

One method of reducing variability, by size classification, indicates that factors that contribute to poor functionality such as loss of solubility can be mitigated by this process (Onwulata *et al*., 2004). The process, sieving of Whey Protein Concentrates (WPC) from different manufacturers to a particle size in the range of 100 to 150 microns, minimizes functionality variation, forcing the different WPC within a narrow functionality range. As the U.S. whey protein concentrate market continues to increase, simple methods must be developed to maintain functional consistency

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as food manufacturers rely on uniformity of raw materials in providing dependable quality in their products. Therefore the goal of this study was to compare the relative effectiveness of sieving or blending WPC80 powders as methods of improving functionality in whey protein concentrates from six commercial suppliers.

Materials and Methods

Whey Protein Concentrate (WPC) was purchased from the following suppliers: The Milky Whey Inc. (Missoula, MT); Foremost Farms USA (Baraboo, WI); Arla Foods, Inc. (Union, NJ); Kerry Foods (Beloit, WI); and Proliant Inc (Ames, IA). All whey protein concentrates were low heat processed, contained approximately 80% protein (WPC80) and was intended for use in extruded snack food applications. The samples were randomly assigned letters A through F. Proximate composition of the WPC80 products as purchased was determined as follows:

A: Moisture 4.9%, protein 75.8%, fat 2.7%, Ash 2.8%, carbohydrate 13.8% by difference;
B: Moisture 3.9%, protein 77.0%, fat 4.2%, Ash 3.1%, carbohydrate 11.8% by difference;
C: Moisture 4.0%, protein 77.5%, fat 4.0%, Ash 2.6%, carbohydrate 11.9% by difference;
D: Moisture 3.4%, protein 76.8%, fat 1.9%, Ash 3.2%, carbohydrate 14.7% by difference;
E: Moisture 3.6%, protein 76.0%, fat 3.6%, Ash 4.5%, carbohydrate 12.3% by difference;
F: Moisture 3.9%, protein 74.3%, fat 3.1%, Ash 4.8%, carbohydrate 13.9% by difference (Onwaulata, 2004).

The WPC80 products were sieved through a standard 100 mesh (150 micron opening), making two classes of WPC80 products, as purchased and sieved. The mean particle sizes of the as purchased WPC80 was determined (Table 1) and the powders were mixed together to create blended samples as follows: hundred grams of A through F was blended to form ATF sample; the three samples with the highest mean particle sizes, A, B and E were mixed to form ABE sample; then the three samples with the smallest mean particle sizes, C, D and F were mixed to form CDF sample. The experiment was replicated twice and analyses were done in triplicate. Analysis of SAS Co-variance, Estimate and Contrast were used to identify differences in physical properties among the six as purchased, sieved and blended products. Bonferroni's Multiple Range Test was used for mean separation. The Statistical Analysis System (SAS) package was used (SAS Institute Inc, Cary, NC) in all cases. Significance of differences was defined as $p \leq 0.05$.

Moisture content was determined by the AOAC Method 925.10 (AACC, 2000). Approximately 1.5 g of WPC80 product were dried in a vacuum oven at 100°C overnight (AACC, 2000).

Ash content was determined by AOAC 923.03 (AACC, 2004). Ash was determined from 3 g samples combusted in a muffler furnace at 550°C for 16 h (AACC, 2004).

Fat content was determined using AACC Method 30-25 (1995). A one gram sample of WPC80 product was placed in an Erlenmeyer flask. One milliliter sulfuric acid and 4 mL of water were added to the flask and mixed gently. After 60 min, the contents of the flask were transferred to a 60 mL separatory funnel using 25 mL of dichloromethane: methanol solution (1:1). After 15 min, the bottom layer was drained into a weighing pan and then evaporated. The amount of fat was calculated according to AACC (1995).

Protein content was determined using the LECO Protein Analyzer Model FP2000 (LECO Corporation, St. Joseph, MI). A 0.2 g sample was placed the sample holder and analyzed. Percent protein was calculated with the nitrogen conversion factor 6.38 for whey protein.

Particle size distribution was determined for the original product and the 2 sieved samples purchased from the manufacturers. Each sample was analyzed using the Accusizer Optical Particle Sizer model 770 (Particle Sizing Systems Santa Barbara, CA, USA). The particle size distribution of the samples was determined.

Particle density of the WPC80 samples was determined with an air pycnomter Horiba Model VM-100 (Horiba Inc. Irvine, CA).

Gel strength as described by Ju and Kilara (1998), was measured by Bloom determinations using a TA-XT2 Texture Analyzer. An 11% protein solution was made (3.2 g of dried sample mixed with 26.7 mL deionized water and 3.3 mL (0.03 M $CaCl_2$) and allowed to sit for 15 min. To initiate gelation, the sample was heated to 80°C for 30 min in a water bath, cooled in an ice bath for 15 min and then stored overnight at 4°C. Gel strength was determined using a 0.5 inch analytical probe to a depth of 6 mm at the rate of 1 mm/sec.

As described by Kilara (1984), 1.0 g product was mixed with 90 mL deionized water. The protein suspension was adjusted to pH 7 and then stirred at 125 rpm for 2 h. The suspension was then centrifuged for 20 min and decanted. The supernatant was freeze dried overnight. The LECO Protein Analyzer Model FP2000 (LECO Corporation, St. Joseph, MI) was used to analyze the solids from the freeze-dried supernatant for protein content. Protein solubility was calculated as described by Kilara (1984). Percent protein denatured is the inverse of percent solubility.

Foam volume and stability of the WPC80 products were determined using the method described in Phillips *et al.* (1990). 2.3 g samples of WPC80 product was mixed with 35 mL deionized water and then heated to 60°C for 15 min. The slurry was whipped for 15 sec a in Waring Lab Micronizer FPC70 (Waring Products Division, New Hartford, CT) and then transferred to a 100 mL graduated cylinder where the foam volume was read initially and for every 5 min for 1 h. Foam stability (foam capacity at specific time) over the 1 h period was calculated as described by Phillips *et al.* (1990).

Viscosity analysis of the pasting behavior of the WPC80 products 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 WPC80 paste viscosity, were determined by RVA Application Method No. 48, using 28 g specimen, 13.5% wet basis. Specimens were stirred initially at 1000 rpm for 60 sec followed by constant stirring at 320 rpm. At equilibrium, the specimens were heated from 50 to 80°C in 3 min, held at 80°C for 5 min, then cooled to 30°C in 4 min. Cold (initial), maximum (peak), trough, final and breakdown viscosities were recorded (1998).

For scanning electron microscopy, WPC80 products (1 to 2 mg) were injected into 10 mm dia. Spectrapor dialysis tubing (Spectrum Medical Industries, Inc., Los Angeles, CA) and equilibrated with a fixative solution containing 2% glutaraldehyde and 0.1 M imidazole HCl (pH 7.0) for 24 h. Samples were washed in imidazole buffer and dehydrated by exchange with 50% absolute ethanol for 24 h. The samples in the tubing were frozen in liquid nitrogen and fractured manually with the cooled blade of a surgical scalpel. Fractured fragments were thawed into absolute ethanol and critical point dried in liquid carbon dioxide. Dry fragments were glued to aluminum specimen stubs with colloidal silver paste (Electron Microscopy Sciences, Ft. Washington, PA) and coated by DC sputtering with a thin layer of gold for imaging in a model JSM 840A scanning electron microscope (JEOL USA, Peabody, MA), operated in the secondary electron imaging mode. Digital images were collected with an Imix workstation (Princeton Gamma-tech, Princeton, NJ). Image analysis of digital images (Fast Fourier Transformation) was done as described earlier (Cooke *et al.,* 1995) to resolve possible differences in topographical features of the different whey samples.

Results

The WPC80 samples contained between 75 to 80% protein as purchased. Their functional properties such as protein solubility, foaming and foam stability, gel strength and amount of denatured protein, were reported previously (Onwulata *et al*., 2004). The proximate compositions were similar for the sieved and blended WPC80 in moisture, protein and carbohydrate Table 1. Fat and ash content were significantly different ($p<0.05$) among samples A through F and between sieved and blended samples. Also, particle sizes and all functional properties varied significantly ($p<0.05$). We observed previously, that smaller particle size correlated with lower fat content and with higher solubility (Onwulata *et al*., 2004). This time, no definitive association of particle size with composition was observed. Average mean particle size was skewed due to the presence of aggregated or fused WPC80.

Table 1: Proximate composition and physical properties of the sieved and blended WPC80

Product	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)	Particle size
A*	5.3	74.8	2.3^{abc}	2.7^{d}	15.1	273^{b}
B*	4.2	78.0	3.6^{b}	3.1^{c}	11.3	297^{ab}
C*	4.3	77.6	3.0^{ab}	2.5^{d}	11.6	258^{bc}
D*	3.3	78.5	1.4^{bc}	3.2^{c}	15.2	53^{e}
E*	3.9	77.3	3.0^{ab}	4.5^{b}	11.9	305^{a}
F*	4.2	74.2	2.80^{abc}	4.8^{b}	14.0	199^{cd}
ATF	5.1	79.2	1.5^{abc}	3.2^{c}	11.6	145^{d}
ABE	5.6	80.0	2.9^{ab}	5.9^{a}	5.7	183^{d}
CDF	5.5	79.1	0.6^{c}	3.4^{c}	11.6	161^{d}

Table 2: Physical properties of the sieved and blended WPC80*

Product	Solubility (%)	Gel strength (N)	Foam volume (%)
A	62.5	22.7^{ab}	60.0^{ab}
B	63.4	11.6^{cd}	80.5^{a}
C	60.4	6.5^{d}	65.5^{ab}
D	65.6	27.3^{a}	46.8^{b}
E	60.5	9.5^{cd}	75.0^{a}
F	58.0	11.2^{cd}	57.5^{ab}
ATF	61.9	15.7^{bcd}	53.5^{ab}
ABE	58.2	11.9^{bcd}	79.5^{a}
CDF	60.8	18.9^{abc}	69.5^{ab}

Table 3: RVA pasting properties of the sieved and blended WPC80

Product	Peak viscosity (cP)	Final viscosity (cP)	Setback viscosity (cP)
A*	161^{abc}	445^{bcd}	322
B*	178^{ab}	563^{b}	425
C*	140^{abc}	445^{bcd}	331
D*	267^{a}	988^{a}	738
E*	41^{c}	109^{d}	63
F*	128^{bc}	521^{bc}	654
ATF	116^{bc}	199^{bcd}	114
ABE	106^{bc}	177^{cd}	84
CDF	117^{bc}	215^{bcd}	111

Means within a column with the same letter(s) are not significantly different. *: Samples passed through a standard 100 mesh sieve. Density for all powders was 1.3 ± 0.04 (g cm^{-3})

Table 2 show significant differences in gel strength and foam volume, but none in solubility. As we reported before, sieving WPC80 through a 100 mesh sieve narrows the solubility to within a uniform range, here by 7%; as purchased, the solubility of the six WPC80 varied by over 50% (Onwulata *et al*., 2004). Blending the six WPC80 samples produced the same results as sieving; the three largest particle-sized samples, ABE and the three smallest, CDF were similar in solubility. The reason is that the blending action reduced the particle sizes, effectively making them uniformly small and soluble (Table 1). The large spread in gel strength from 6.5 to 27.3 N for the sieved samples contrasts with the narrower spread for the blended samples 11.9 to 18.9 N. The same is true for the foam volume spread from 46.8 to 80.5% for the sieved samples and from 53.5 to 79.5% for the blended. The Contrasts and Estimates of true statistical differences in functional properties between the sieved and blended are discussed later.

The Scanning Electron Micrographs (SEM) of the surface of the sieved WPC80 products A through F reveal different levels of aggregation, sizes and surface morphology, depending on the method of manufacture (Fig. 1). All products showed characteristic shape of spray-dried powders (Caric, 1994). WPC80 samples, A, B and E show many more fused particles than others; C and F show large non-fused particles (>120 microns) and product D shows mostly cracked particles. It was suggested that product D was first spray dried with a high capacity nozzle and then milled (Onwulata *et al*., 2004). The SEM of the blended products, ATF, ABE and CDF, labeled A, B and C, respectively (Fig. 2), show both aggregation and wide distribution of particle sizes for all three. ATF and CDF show some cracked particles mostly from product D, while product ABE are large and smooth.

The pasting viscosity profiles of the sieved and blended WPC80 products (Table 3) show differences in peak and final, but none in setback viscosity. There were significant differences ($p<0.05$)

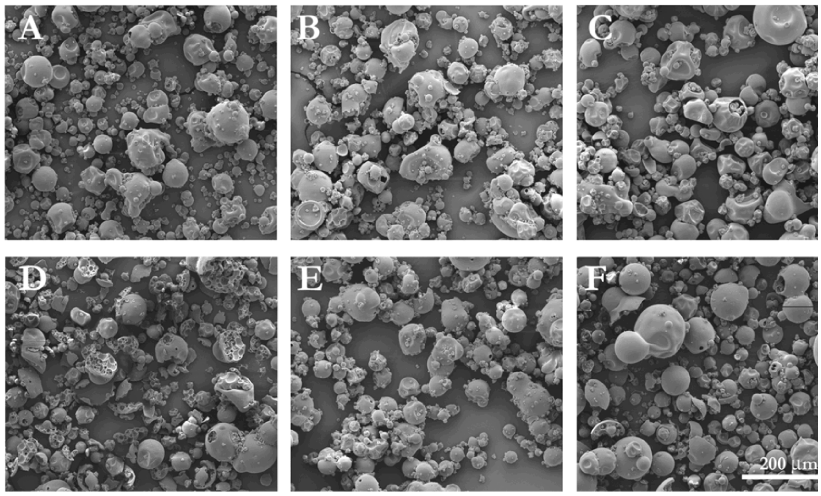

Fig. 1: Scanning Electron Micrographs of the sieved WPC80 products (A-F)

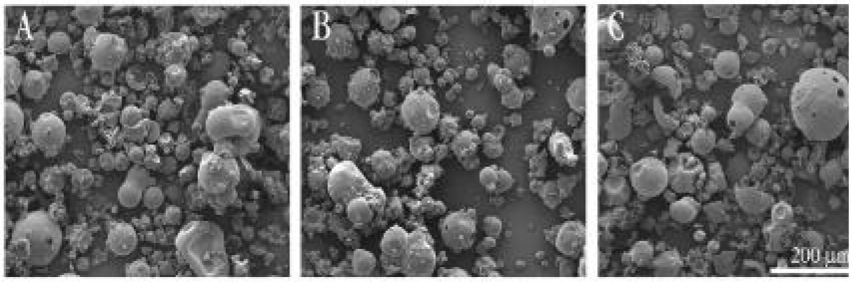

Fig. 2: Scanning Electron Micrographs of blended WPC80 products, A: ATF (all six products); B: ABE (three largest); C: CDF (three smallest)

differences in peak and final viscosities; peak viscosity spread was from 41 to 267 cP and final was from 106 to 988 cP. This contrasts with the narrower spread for the blended samples, peak viscosity spread from 106 to 117 cP and final from 177 to 199 cP. The Contrasts and Estimates of true statistical differences in functional properties follow:

The SAS Contrast and Estimate Procedures were used to compare the means of the six sieved and three blended samples of WPC80 for all properties reported (Table 4). The F-tests and probability

Table 4: SAS contrast and estimate values of sieved versus blended samples

	Contrast of sieving vs. blending		Estimate of variance: Sieving vs. blending		
WPC physical property	F-value	Pr > F	Estimate	t-value	Standard error
Moisture	21.09	0.001	-1.20	-4.59	1.54
Protein	3.27	0.100	-2.80	-1.81	9.18
Fat	17.87	0.000	1.00	4.23	1.42
Ash	517.56	0.000	-0.86	-22.75	0.20
Carbohydrates	4.01	0.080	3.62	2.00	10.92
Particle size	123.27	0.000	84.67	11.10	45.75
Solubility	0.86	0.370	1.44	0.93	9.35
Gel strength	0.35	0.570	-0.71	-0.59	7.21
Foam volume	1.18	0.310	-3.29	-1.09	18.19
peak viscosity	7.56	0.020	39.58	2.75	86.39
Final viscosity	57.37	0.000	314.80	7.57	249.40
Setback viscosity	17.90	0.000	319.30	4.23	452.80

*SAS: The Statistical Analysis System

estimates indicate differences between the two methods, sieved versus blending. The pooled means of the sieved WPC80 samples were at least ($p<0.05$) significantly different in composition and pasting properties from the blended. But were similar to the blended samples in functionality attributes solubility, gel strength and foam volume. By SAS Estimates of variance, sieving was better (positive SAS Estimate values) than blending, in solubility and pasting properties, while blending was better (negative SAS Estimate values), in gel strength and foam volume. Overall, comparing the functionality differences, sieving WPC80 samples is significantly better than blending. Generally, reducing WPC80 powders to particle sizes that passes through a 100-mesh sieve, by sieving or blending, produces uniform functionality, their obviously different manufacturing and processing history, notwithstanding.

Discussion

Recombined milk powder is used widely in places where fresh milk supply is limited such as in countries where no milk is produced (Caric, 1994). These countries without adequate refrigeration and storage networks are importing increasing amounts of whey proteins (Anonymous, 2000). The same issues of milk powder stability in hot humid environment, its effects on aggregation and agglomeration resulting in reduced or no functionality long after manufacture, are the same for whey proteins in general and WPC in particular, as it is the biggest whey protein product exported out of the U.S. (Anonymous, 2000). For example, freshly made whey proteins have mild sweet bland flavors; but when used in large amounts in flavored products, their off-flavors comes through, therefore producers limit their use (Whetstine *et al*., 2003). There appears to be very little information on physical and functional changes in whey proteins after drying, in storage. One study describes changes in functional properties, solubility, foam stability, emulsifying capability and development of browning color for whey concentrate containing 52% protein when stored above 20°C and also for products with water activity values greater than 0.2 (Hsu *et al*., 1989). So, changes due to agglomeration and aggregation in WPC80 products, as seen with increasing particle size (Table 1), may point to increasing variability. This variability can be ameliorated by sieving the particles or blending them to reduce particle sizes.

Variability in physical and functional properties of WPC80 was documented earlier (Onwulata *et al*., 2004). Others have reported considerable variability in chemical composition, ash, pH, solubility and digestibility attributed mostly to product source and processing (Schmidt *et al*., 1984; Regester *et al*., 1992; Hawks *et al*., 1993). Processing affects functionality through extent of protein denaturation or insolubility. The level of insoluble denatured proteins in WPC products determines functionality (Huffman and Harper, 1999; Schmidt *et al*., 1984; De-la-Fuente *et al*., 2002; Puyol *et al*., 1999; deWit and Klarenbeek, 1984). Sieving and blending alike, improved solubility (Table 2) sieving mostly by increasing solubility for very insoluble powders and blending by averaging. Though gel strength and foam volume still varied, significantly, blending narrowed the values. Same trend was seen also for pasting properties, peak and final viscosities.

The properties of any food particulate system are primarily dependent on its particle size and distribution (Yan and Barbosa-Canovas, 1997). The control of mean particle size and size distribution is used for granulation; the reduction of particle size by shear, is used to narrow particle and increase viscosity in binary powders (Bardin *et al*., 2004). To reduce variability in powders with wide particle size ranges, intense mixing by rotating impeller blades at high speeds are recommended (Bardin *et al*., 2004). However, for the WPC80 products, simple minimal shear V-blender tumbling was sufficient to reduce their particle sizes (Table 1). We had earlier observed that removing large particles by sieving in the WPC80 products improved foam volume, particularly in the smallest particle size fractions (Onwulata *et al*., 2004).

Any method used to reduce the size of the whey protein concentrates will improve their functionality. Sieving to remove the large particles improved solubility and functionality; also, blending products with minimal shear reduces particle sizes as well and improves functionality. But sieving to reduce particles in the range of 100 to 150 microns alone, improves functionality for the least performing powders.

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