Composition and Properties of Whey Protein Concentrates from Ultrafiltration

F. E. McDONOUGH, R. E. HARGROVE, W. A. MATTINGLY, L. P. POSATI, and J. A. ALFORD
USDA, ARS, Agricultural Research Center
Beltsville, MD 20705

Abstract

Chemical and nutrient composition and functional properties of whey protein concentrates from ultrafiltration of sweet and acid wheys were studied for potential food uses. Vitamins passed readily through the membrane; thus, vitamin content was slightly higher than in whey. Amino acid values were considerably higher, increasing in direct proportion to increases in protein. Lysine availability was not significantly affected by fractionation or by subsequent heat treatment. Since this process results in substantial removal of minerals along with the permeate, the protein to ash ratio of the protein concentrate increased. Unlike most other methods of recovering protein from whey, solubility was not adversely affected by ultrafiltration. However, protein concentrates were susceptible to heat; normal pasteurization temperatures resulted in approximately 20% denaturation. Whey protein exhibited excellent water retention. Addition of 1.5% protein to skim milk followed by heating formed a custard-like gel with sufficient body to stand alone without leakage. Approximately twice as much egg albumin was required to achieve comparable results. Whipping properties were very good when butterfat content was less than 2%. Excellent stable whips could be produced by a combination of heat and pH adjustment.

Introduction

The high cost of disposal and the need to reduce environmental pollution have prompted considerable efforts to increase use of cheese whey. Because of the substantial economic and nutritional potential, much of this effort has been aimed at recovering the protein from whey. The nutritional superiority of whey protein has been established (3, 8, 13). Unfortunately, use of whey protein has been limited because of poor physical properties of commercial products. Until recently whey protein has been available only as a heat denatured, insoluble, gritty powder.

Rapidly developing processes for recovering protein from whey include electrodialysis, formation of complexes, ethanol precipitation, Sephadex gel filtration, and ultrafiltration (UF). Products from some of these processes are now commercially available. Although claims have been for unique functional properties, they have been general, and more specific data are needed. Morr et al. (10) surveyed functionality of whey-protein concentrates (WPC) produced by several methods but indicated a need for more detailed studies. This paper reports composition and useful properties of WPC prepared by UF.

Materials and Methods

Whey. Cheddar cheese whey was manufactured by the Dairy Foods Nutrition Laboratory at the Agricultural Research Center, Beltsville, MD. Cottage cheese whey was obtained from a local manufacturer. The Cheddar whey was pasteurized at 72.8 C for 15 s to prevent further acid development. All whey was centrifugally clarified with a desludging separator and held at 1.7 to 4.4 C prior to use.

UF procedure. A model UF-44S pilot-plant ultrafiltrator (Abcor, Inc., Cambridge, MA.)[1] was used to prepare WPC. The unit contained twenty 2.5 cm tubes each 3 m long, totaling 4.1 m^2 of HFA-180 membranes with a normal retention of 98.2% for whey protein. Operation was by batch procedure with 1,100 to 1,800 liter lots. A temperature of 48 C and inlet-outlet pressures of 3.1-1 kg/cm^2 were utilized. Whey was continuously circulated through the tubes and back to the holding tank until permeate was removed to the desired protein concentration. This required from 3 to 20 h.

Analytical. Total solids were determined ac-

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[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products that may be suitable.

cording to the Mojonnier procedure, lactose by the copper reduction method as presented by AOAC (1), ash by combustion at 550 C, and total nitrogen by the standard micro-Kjeldahl procedure. Protein was calculated by multiplying total nitrogen by 6.38. Amino acid analyses were with a Beckman Model 120 C Amino Acid Analyzer in accordance with the method of Spackman et al. (14). Aliquots of each sample were sealed under vacuum in ampules containing 6 N HCl and were hydrolyzed at 110 C for 22, 46, and 70 h. Values for threonine, serine, and ammonia were extrapolated to zero time. Available lysine with its epsilon amino group free for chemical reaction was measured as the difference between total and blocked lysine as described by Blom et al. (4). Cystine was determined as cysteic acid after performic acid oxidation, followed by acid hydrolysis according to the method of Moore (9). Tryptophan was measured by the procedure of Spies (15). Vitamin analysis was by an independent laboratory using standard AOAC methods.

Concentration and drying. Commercially acceptable methods of concentrating and drying WPC were utilized. After preheating through a Mallory tube heater at 60 C for 15 s, WPC was further concentrated to 25 to 50% solids in a Weigand Falling Film Evaporator with an inlet tube temperature of 82.2 C. Spray drying was in a Grey Jensen Cyclone dryer with a pressure nozzle using 132 C drying air.

Protein solubility. The general procedure for determining solubility was to acidify and remove insoluble material by centrifugation. Each sample was diluted 10:1 with distilled water, adjusted to pH 4.6 with 10% acetic acid, and maintained at 40 C in a water bath. After 10 min, 1 ml of a N-sodium acetate solution was added and the sample cooled to room temperature. Insoluble material was then removed by centrifuging for 40 min at 65,000 $\times$ g. The clear supernatant was analyzed for nitrogen by micro-Kjeldahl and compared with similar analysis of the original sample.

Whippability. In whipping trials a Hamilton Beach electric mixer was set at full speed for 10 min. Overrun was calculated by comparing weight of a given volume of WPC to weight of an equal volume of foam. Immediately after being whipped, foam was transferred to a 200-ml Buchner funnel; stability was indicated by the length of time required for the first drop of fluid to drip from the funnel.

Water affinity. Skim milk was fortified with WPC to give various amounts of whey protein. Fresh egg white was used for comparative purposes. The mixtures were heated to 85 C for 5 min to denature the protein and to trap the water. Degree of entrapment was indicated by an increase in viscosity or strength of gel as measured with a Brookfield LVT viscometer at 3 rpm with the spindle having a crosspiece length of 1.1 cm. With gel structures, the rotating spindle would normally create a channel and exert a negligible and meaningless torque. To overcome this effect, the viscometer was mounted on a Helipath Stand which lowered the spindle in a helical path through the test material during measurement to insure that the rotor always measured undisturbed material.

Results and Discussion

Composition. Since UF membranes are almost completely permeable to lactose, minerals, and short chain polypeptides, removal of permeate from whey by UF removes almost the same percentage of these components as water. Thus, to remove 90% of the lactose from whey, one would have to remove 90% of the original volume through the membranes. Theoretical data on composition of products resulting from various degrees of volume reduction (VR) have been presented by Fenton-May et al. (5). The data assume 100% retention of protein and zero retention of all other components. Our results are in Table 1. Whereas theoretical values for 80, 90, and 95% VR predict concentrates containing 36, 53, and 69% protein, corresponding experimental values were 30, 42, and 55% protein. Differences between theoretical and actual values arise from imperfect membranes; neither 100% retention of protein nor zero retention of other solids was achieved.

By varying VR practically any concentration of protein can be produced. However, the high lactose:protein ratio in whey makes it difficult to prepare WPC with more than 50 to 60% protein. One could wash additional non-

TABLE 1. Typical composition of whey protein concentrates from ultrafiltration[a].

	% Water removed (volume reduction)			
Fraction	0	80	90	95
Total solids	6.60	9.50	13.00	18.00
Lactose	4.80	5.50	5.60	5.80
Protein	.67	2.85	5.50	9.54
Ash	.74	.79	.77	.75

[a] Representative results from both cottage and Cheddar whey.

TABLE 2. Comparison of vitamins in fluid whey and whey protein concentrate[a].

Vitamin	Whey mg/kg	WPC[b] mg/kg
Thiamine	.31	.32
Folic acid	.07	.11
Niacin	1.18	1.28
Riboflavin	.16	.20
Choline	108.00	136.00
Pantothenic acid	3.94	4.43

[a] Representative results from both cottage and Cheddar whey.
[b] 92% volume reduction; 53% protein (dry weight).

protein solids from the concentrate by diluting with water and following with a second UF cycle. Concentrates containing up to 80% protein have been prepared by this method, but the expense increases considerably. Holsinger prepared concentrates of up to 90% protein with a combination of UF and Sephadex gel filtration (6).

Ash. Low minerals are essential for some human food requirements such as infant foods and low sodium foods. Results in Table 1 highlight the advantage of UF as a partial demineralizer. The ash values represent 11.2% of the dry weight in the original whey, and 8.3, 5.9, and 4.0% of the dry weight in the 80, 90, and 95% VR concentrates. The WPC by 95% VR compares favorably in ash content with electrodialyzed products and is vastly superior to those produced by polyphosphate extraction procedures which result in 10 to 16% mineral (10). The overall low ash of WPC should enhance its value for dietetic and health food applications.

Vitamins. Table 2 compares vitamin composition of fluid whey and WPC. Since HFA-180 membranes are designed for a molecular weight cut-off of 15,000 to 20,000, one would expect a high percentage of the relatively small vitamins to pass through the membrane. This is shown by the small increase of vitamins in the concentrate. Mass balance calculations indicate that from 11 to 14% of each vitamin remained in the concentrate. This is not surprising since the WPC represents 10% of the original volume of whey. Apparently, most of the water-soluble vitamins in whey are in free form.

Amino acids. Amino acid quantities are enhanced considerably by UF (Table 3). The five-fold increase correlates well with the protein which increased from approximately 11% of the solids in the whey to 53% of the solids in the WPC. Comparing ratios of amino acids in whey and WPC indicates no significant shift in the amino acid profile. Thus, nearly all of the amino acids must be bound to or are part of the protein. Mavropoulou and Kosikowski (7) reported this recently when they gave an average of .59% for free amino acids in four commercial whey powders. Of the total lysine, 98.6% was chemically available in the whey, 97.7% in the WPC, and 95.5% in the powder indicating little heat damage to the amino acids during processing. The amino acid compositions of WPC from sweet and acid wheys were not significantly different.

TABLE 3. Amino acid composition of Cheddar cheese whey and whey protein concentrate (dry weight).

	Whey	WPC[a]
	%	
Lysine	1.12	5.47
Histidine	.24	1.14
Arginine	.29	1.72
Threonine	.63	3.09
Valine	.67	3.25
Methionine	.23	1.12
Isoleucine	.60	3.05
Leucine	1.32	6.53
Phenylalanine	.42	2.04
Tryptophan	.24	1.37
Aspartic acid	1.24	6.19
Serine	.59	2.73
Glutamic acid	2.16	9.16
Proline	.64	2.54
Glycine	.25	1.20
Alanine	.56	2.73
Cystine	.24	1.25
Tyrosine	.36	1.71

[a] 92% volume reduction; 53% protein.

TABLE 4. Effect of processing on solubility of whey protein concentrate[a].

Treatment	Solubility (%)
Whey	
Clarified	98.7
WPC	
91% VR, 48 C for 20 h	98.4
Pasteurized, 72.7 C for 15 s	81.0
Pasteurized, 62.7 C for 30 min	80.0
Preheated, 60 C for 15 s	98.3
Evaporated, 45% total solids	98.0
Spray dried	97.8

[a] From cottage whey: 91% volume reduction (VR); 50% protein.

Solubility. In Table 4 neither UF nor the additional treatments essential to production of a powder significantly altered protein solubility. These treatments included preheating, evaporation and spray drying as outlined earlier. Heat lability of the protein was demonstrated, however, when attempts were made to pasteurize the WPC; both 78.2 C for 15 s and 62.4 C for 30 min resulted in approximately 20% denaturation. Thus, pasteurization appears to be the only processing problem although this may be resolved by further studies on time-temperature relationships. If acceptable procedures cannot be found, alternatives such as peroxide treatment can be used, or, with proper sanitary control, pasteurization can be limited to raw whey.

Water affinity. One of the more interesting properties of whey protein is its ability to hold water after being heat-denatured. Berlin et al. found the amount of water bound by denatured and undenatured protein was essentially the same (2). Thus, an explanation for the excellent water holding properties of WPC in our study appears to be formation of a classical gel structure rather than a true binding of unfreezable water. Entrapment of water is by a network of cellular protein filaments. Upon heating WPC-skim milk mixtures, formation of a whey protein-casein complex is likely. Addition to milk is not necessary for gelaton, however; heating of a 10% solution of WPC with 50% of the solids as protein gave a firm gel with no leakage. The excellent water affinity of WPC is demonstrated in Fig. 1. Various amounts of WPC (10% solids, 50% protein) were added to skim milk to give from .5 to 2% whey protein based on total volume of milk. The mixtures were heated to 85 C for 5 min to denature the protein and entrap the water. Viscosity of the skim milk first increased upon addition of as little as .6% added whey protein and accelerated rapidly from that point. When the skim milk contained 1.5% whey protein, a custard like gel was formed with sufficient body to stand alone without leakage when it was removed from the container. In contrast, approximately twice as much egg albumin was required to achieve comparable results. Water affinity of WPC from Cheddar (sweet) whey was slightly lower than from cottage (acid) whey but was still superior to egg albumin.

Whippability. Whipping properties of WPC are shown in Table 5. Richert et al. (12) reported effects of heat and other factors upon foaming properties of WPC. They found WPC prepared by UF had poor whippability. However, they used 4% dispersions. We found solids percent was critical. WPC with solids lower than 25% developed foams of high overrun which were extremely coarse and unstable with insufficient strength to resist rupturing. Those with 35% solids were too viscous for foam development. This was surprising; Holsinger (6) was able to obtain stable foams from 35% solids with 80% protein produced by combining UF and Sephadex gel filtration. With 25% solids as a base, whippability could be improved in several ways. Richert et al. (12) reported that heating temperature and redox potential significantly affected overrun of WPC dispersions and that foam stability was affected by pH. While temperatures of 65 to 70 C greatly improved foaming properties, higher temperatures impaired foaming properties. Our results agree; heat treatment and pH adjustment were effective in producing good foams. Peter and Bell (11) have shown

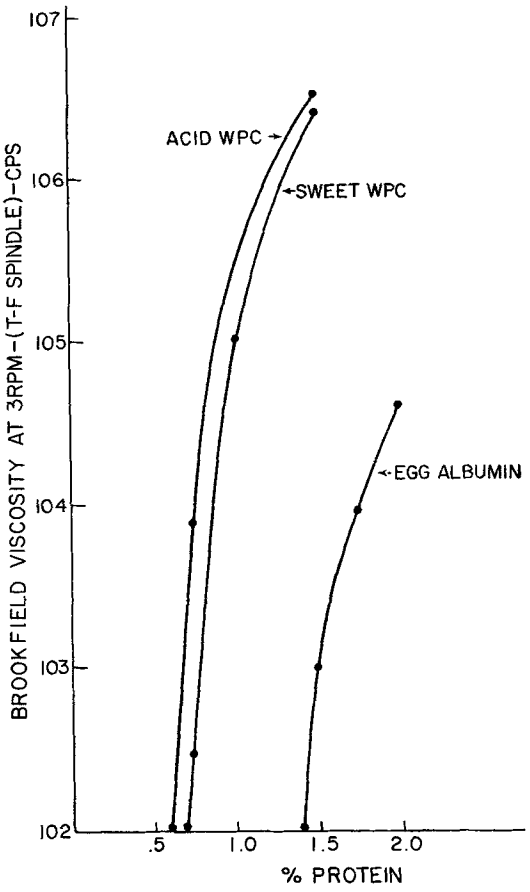

FIG. 1. Water affinity of whey protein as indicated by strength of gels formed by heating whey protein concentrate or egg white added to skim milk.

TABLE 5. Whippability of whey protein concentrate[a].

Treatment	Overrun	Stability
Cottage WPC	%	
5% total solids	700	10 s
15% total solids	600	30 s
25% total solids	400	8 min
35% total solids	0	0
65.5 C for 15 min[b]	260	50 min
71.1 C for 15 min[b]	260	42 min
71.1 C for 60 min[b]	200	30 min
pH: 5.0[b]	425	10 min
pH: 6.0[b]	450	22 min
pH: 7.0[b]	490	30 min
pH: 8.0[b]	550	48 min
71.1 C for 15 min + pH 6.0[b]	300	6-7 h
Cheddar WPC[b]		
pH: 6.0	500	16 min
pH: 7.0	470	20 min
pH: 8.0	450	22 min
1% fat	520	38 min
2% fat	490	19 min
3% fat	325	1 min

[a] 91% volume reduction; 50% protein.
[b] 25% total solids.

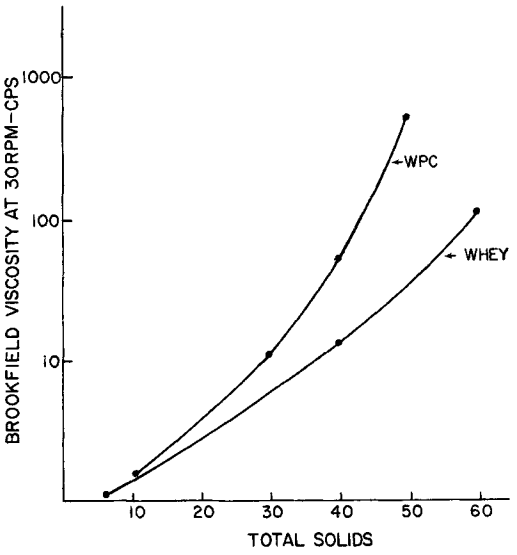

FIG. 2. Comparison of viscosity of whey and whey protein concentrate (50% protein) at various concentrations.

that adding surface tension depressants to whey protein did not increase foaming properties. Improvement by heat treatment is apparently a result of partial denaturation which increases affinity for water and influences permanency of foam.

Adjustment of pH of cottage WPC with CaOH had a beneficial effect on whippability. Excellent, brilliant white, fine-grained whips with good stability were formed at pH 6.0 and above. Stability and overrun continued to improve when cottage WPC was adjusted beyond pH 6.0, but adjusting the pH of Cheddar WPC had little effect. A combination of heat and pH adjustments produced an excellent whip with a stability of several hours. However, the surface of this foam did dry out and become slightly more coarse after 2 to 3 h. Butterfat is an excellent foam depressant. Concentrates having 3% fat produced coarse whips which lacked stability. Concentrates with 2% fat were good but did not match the excellent stability when fat was 1% or less. Concentrates from cottage cheese whey were sufficiently low in fat to be whippable, but those from Cheddar cheese whey had to be run through a cream separator.

Viscosity. Specific viscosity, as affected by solids and protein content, is in Fig. 2. Although protein content contributed to viscosity, no problems should be encountered in pumping and processing of WPC since significant increases in viscosity were not observed until concentration reached 45% solids. Pilot plant trials have shown that because of water affinity, WPC with higher than 45% solids could not be dried with our equipment. Although other equipment may be available which can dry WPC at this concentration, we can visualize no real need for evaporating to higher solids.

Protein ingredients are often added to foods for technical function. As an example, retention of water improves freshness of baked goods. Our data help to establish both the nutrient amounts and some of the functional properties. It is probable that WPC has many applications in the food industry, and it will find extensive use as specific applications are developed.

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