Recovery of Soybean Whey Protein with Edible Gums and Detergents

ALLAN K. SMITH, ARLO M. NASH, A. C. ELDRIDGE and W. J. WOLF
Northern Regional Research Laboratory, Peoria, Ill.

Soybean whey, the filtrate obtained from acid-precipitated curd in isolating soybean protein from defatted meal, contains approximately one third of the original soybean meal. Because of its high biological oxygen demand (BOD) it presents a serious waste disposal problem. Whey proteins, which constitute about 12% of whey solids, can be precipitated in varying quantities, up to 100%, as insoluble complexes with negatively charged colloids such as alginic acid, gum karaya, Irish moss extractives, and anionic detergents. The complexes with edible gums retain the enzymatic activity of the original proteins and the properties of the original gums. The detergent complexes have no enzymatic activity. Removing protein from whey lowers the BOD by 8 to 18%.

In processing defatted soybean meal for isolated protein (*2*), two by-product fractions are obtained—an insoluble residue and soybean whey. Although yields vary, each fraction represents about one third of the meal. The residue, used in mixed animal feeds, contains 33 to 52% protein (*2, 9, 15*). Soybean whey contains salts, carbohydrates, nonprotein nitrogen, proteins not precipitated by inorganic acids, and unidentified constituents.

The protein concentration of the laboratory-prepared whey is approximately 0.3 to 0.4%; dry whey solids are about 8 to 12% protein (*18*). The BOD (*1*) of soybean whey from a plant with a 5-ton daily capacity of isolated protein is estimated to be equivalent to that needed by a city of 25,000 to 30,000 population using 80 to 100 grams of oxygen requirement per day per capita. As an economical recovery method has not yet been developed, whey presents a serious waste disposal problem.

Smith, Schubert, and Belter (*18*) showed that whey solids content and composition depend on origin of the meal and method of whey preparation. Rackis and associates (*16*) reported that soybean whey contains at least 13 proteins including two trypsin inhibitors, hemagglutinin (*7*), and amylases. Also present is the enzyme lipoxidase (*5*).

The proteins of whey are the lower molecular weight proteins of soybeans (*20*) and have a good balance of essential amino acids (*15*).

This paper describes the recovery of proteins in soybean whey by forming insoluble complexes with anionic materials, and the decrease in BOD of whey by removing protein.

Materials and Methods

All precipitants were commercial samples (listed below) except sulfated wheat starch and flour (*19*), carboxymethyl amylose (*17*), 5% cross-linked dicarboxyl starch (*4*), and dialdehyde starch (*11*). These were experimental samples prepared in this laboratory by procedures given in the Literature Cited.

Protein Precipitant	Source
Carrageenan (Sea Kem Type 9 Irish moss extractives)	Food Technology, Inc.
Kelgin (medium viscosity sodium alginate)	Kelco Co.
Kelgin LV (low viscosity sodium alginate)	Kelco Co.
Gum karaya	Baker's Franklin Div., General Foods Corp.
Agar (Bacto-Agar)	Difco Laboratories
Locust bean gum	Stein Hall & Co., Inc.
Duponol C (sodium lauryl sulfate)	E. I. duPont de Nemours & Co.
Nacconol FSNO (sodium alkyl aryl sulfonate)	National Aniline Div., Allied Chemical & Dye Corp.
Calgon (sodium hexametaphosphate)	Calgon Co.
Saponified rosin (Dresinate XX)	Hercules Powder Co.
Gum guaiac	S. B. Penick and Co.
Gum tragacanth	E. H. Sargent Co.

Preparation of Whey and Isolation of Protein Complexes. Whey solutions were prepared by two methods. In one, undenatured oil-free meal is extracted twice with water (pH 7.5), first at a solvent to meal ratio of 10 to 1 and a second time at 5 to 1. After centrifugation the supernatants of the two extracts are combined and adjusted to pH 4.5 with hydrochloric acid; the precipitated curd is then removed to obtain the whey. In the other method oil-free meal is suspended in water (pH 7.5), stirred, adjusted to pH 4.5, and centrifuged. After re-extraction of the insolubles with water at pH 4.5, the two extracts are combined. Since the residue and acid-precipitated protein are collected together, one centrifugation is eliminated.

Whey solutions contained about 4 mg. of protein per ml. as determined by Kjeldahl analysis. Correction for nonprotein nitrogen was made by precipitating the whey proteins with 0.8*N* trichloroacetic acid and then determining nitrogen in the filtrate. Protein content was calculated using a conversion factor of 6.35 based on a nitrogen content of 15.75% for the proteins precipitated by adjusting a whey solution to 80% saturation with respect to ammonium sulfate, dispersing the precipitated proteins in water, dialyzing until salt-free, and and freeze-drying.

Preliminary precipitation experiments were made at pH 2.0 and 4.5 at varying weight ratios of precipitants to protein. Protein concentrations were determined by the phenol reagent of Lowry and coworkers (*8*) or by Kjeldahl analysis.

Under optimum conditions, the whey proteins were precipitated at room temperature by adding a concentrated solution of the selected reagent. The reagents and whey were at pH 2.0 or 4.5. The resulting suspension was stirred for 1 hour at room temperature, and the precipitate was separated in a centrifuge before drying by lyophilization; the nitrogen in the complex was determined by Kjeldahl analysis.

Lipoxidase. Lipoxidase activity was determined using Holman's procedure (*5*).

Amylase. Amylase activity was determined by measuring the maltose hydrate (*12*) liberated from a 1% purified glycogen solution in 0.02*M* acetate buffer, pH 6.0, for 5 minutes at 30° C. Amylase units are defined as milligrams of maltose hydrate liberated per milligram of protein under these conditions.

Phosphatase. Phosphatase was determined by measuring phosphate liberated from a 0.5% solution of β-glycerylphosphate in 0.05*M* phthalate buffer at pH 6.0 in 30 minutes at 30° C. (*10*). The phosphatase unit is the amount of enzyme required to produce one gamma of phosphorus under the conditions of the assay.

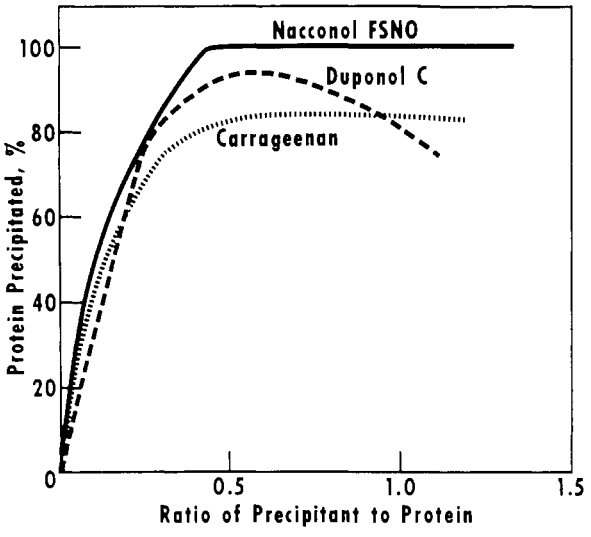

Figure 1. Precipitation of soybean whey protein at pH 4.5 at various weight ratios of precipitant to protein

Table I. Isolation Conditions, Yields, and Composition of Soybean Whey Protein Complexes

Complexing agents	pH	Precipitant to Protein Ratio: Used	Precipitant to Protein Ratio: In complex	Complex: Protein, %	Complex: Yield[a], grams	Recovery: Precipitant[b], %	Recovery: Protein, %
Carrageenan	4.5	0.57	0.6	62.8	6.9	101	96
Kelgin (low viscosity)	4.5	0.32	0.3	75.9	3.5	58	58
Kelgin (medium viscosity)	4.5	0.53	0.9	52.4	5.5	111	64
Gum karaya	4.5	1.7	2.0	33.4	8.4	73	62
Agar	4.5	0.63	2.5	28.5	3.1	79	20
Locust bean gum	4.5	0.71	0.9	51.5	1.4	21	16
Duponol C	4.5	0.74	0.3	67.8	6.5	62	97
Nacconol FSNO	4.5	0.41	0.3	78.7	4.8	55	84
Sodium polyphosphate	2.0	1.6	0.2	85.6	4.1	8	78
Calgon	2.0	1.2	0.2	77.2	6.3	27	108
Saponified rosin	2.0	1.9	3.9	20.5	9.7	90	44
Gum guaiac	4.5	1.3	5.2	16.1	6.9	97	25
Dialdehyde starch	2.0	0.24	0.9	64	2.5	85	36
Gum tragacanth	4.5	0.52	2.0	33.8	1.4	38	10

[a] Based on whey obtained from 100 grams of soybean meal.
[b] Calculated by difference from protein content of complex.

Table II. Lipoxidase Activity of Gum-Protein Complexes

Complex	Activity Units[a]/ Mg. of Complex	Equiv. to 100 G. Meal Units[a] $\times 10^{-4}$	Recovery, %
Whey solids (for comparison)	~5[b]	10.3	..
Carrageenan complex	13.3	9.2	89
Kelgin complex (low viscosity)	22.3	7.8	76
Kelgin complex (medium viscosity)	9	5.8	56
Karaya complex	10.8	9.1	88
Agar complex	1.7	0.6	6

[a] Theorell units.
[b] Calculated from activity of whey solution.

BOD. BOD of the whey solutions was determined by standard methods (*1*) using a 5-day incubation period.

Results and Discussion

Figure 1 shows typical protein precipitation curves at pH 4.5 for Nacconol FSNO, Duponol C, and carrageenan. The character of the precipitate formed with gums varied. For example, locust bean gum and gum tragacanth gave gelatinous precipitates, whereas carrageenan and Kelgin gave flocculent precipitates.

Preliminary precipitation tests were also made with impure synthetic products such as sulfated wheat starch and flour, carboxymethyl amylose, and 5% cross-linked dicarboxyl starch. When small-scale precipitation tests and the Folin reagent assay procedure were used, yields of 83% and 87%, respectively, were obtained with sulfated wheat starch and flour at pH 2.0 and at a precipitant-to-protein ratio of 4.5. Carboxymethylcellulose and cross-linked starch precipitated 50% of the protein at a precipitant-to-protein ratio of 0.3 and 4.5, respectively.

Table I shows the weight ratio of precipitant to protein used, the weight ratio of precipitant to protein in the isolated product, yield of complex, protein in the product, and percentage of protein and precipitant recovered. More than 50 precipitating reagents were tested, but only 14 of the more promising are listed in Table I.

Although detergent-protein interaction is not fully understood, it is known that insoluble protein-detergent complexes are formed at pH values where detergents and proteins are oppositely charged (*14*). Insolubilization is probably caused by charge neutralization. Isoelectric points are known for two whey proteins: trypsin inhibitor, pH 4.5 (*6*), and soybean hemagglutinin, pH 6.1 (*13*). However, the average isoelectric point of whey proteins is 5.1 (*3*). Thus, the formation of insoluble detergent- and gum-whey protein complexes at pH 2.0 and pH 4.5 may be due to charge neutralization between the anionic precipitants and the positively charged proteins.

Although insoluble as prepared, the complexes were soluble in aqueous systems in the pH range of 7.0 to 9.0, indicating dissociation of the complexes or conversion to soluble complexes. Viscosity of such solutions of gum-protein complexes was close to that of the original gum, indicating that the complexes can function in food products like the original gum while contributing a nutritious protein to the food system.

Because the whey solution is known to contain a number of enzymes, the complexes were assayed for lipoxidase, amylases, and phosphatases. As expected, these tests were negative for complexes formed with Duponal C and Nacconol FSNO, two protein denaturants. However, enzyme activity was found in complexes with such gums as carrageenan, Kelgin, and karaya. Assay of these complexes for lipoxidase is given in Table II. Variations in activities of the complexes reflect efficiency of lipoxidase recovery and precipitant content of the complexes. Tests on bleaching of carotene and unbleached flour with the carrageenan complex showed that it will be necessary to incorporate an unsaturated fat, for example, sodium linoleate, in the system for good bleaching results. Only 15% of the amylase and phospha-

Table III. Biological Oxygen Demand of Treated Soybean Whey

Treatment	Protein (N_2^1 X 6.35), Mg./Ml.	Protein Precipitated, %	BOD, P.P.M.	Decrease of BOD, %
None	4.26	..	13,730	..
Kelgin (1.87 g./l.)	0.32	93	12,590	8.3
Nacconol FSNO (1.7 g./l.)	0.88	79	11,230	18.2
Duponol C (2.55 g./l.)	0.88	79	11,500	16.2
Heat, 80° C., 15 min.	2.39	44	12,320	10.3

tase activities of whey were found in the gum-protein complexes. These low activities are attributed partly to the insolubility of the complexes at the assay pH of 6.0.

Table III compares BOD of the original whey with that of whey preparations treated in various ways to remove proteins. Although Kelgin precipitated more protein, the decrease in BOD was less than with Nacconol. This may result from incomplete precipitation of Kelgin in the complex at this ratio of Kelgin to protein. Apparently protein removal does not solve the BOD problem, but recovery of valuable products, such as lipoxidase or high-quality edible protein, should contribute to solving the waste disposal problem.

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WHEAT MALTING

Effects of Gibberellins and Certain Synergists on Enzyme Production during the Malting of Wheat

JAMES R. FLEMING, JOHN A. JOHNSON, and BYRON S. MILLER

Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station, Manhattan, Kan.

NUMEROUS WORKERS have studied the response of cereal grains to treatment with gibberellins during the malting process (*9*). Enzyme activities, extract yield, degree of modification, and other criteria of malt quality were improved by the proper use of gibberellic acid (GA). Dickson, Shands, and Burkhart (*7*), Bawden, Dahlstrom, and Sfat (*2*), Kringstad, Busengdal, and Rasch (*15*), and Linko and Enari (*16*) have investigated the effects of various other plant regulators on the malting process and malt quality. These substances, however, did not stimulate growth processes, but reduced growth. The results suggested that such materials might be useful in curtailing malting losses. Coumarin (*14*) and potassium bromate (*19*) have also been used for this purpose.

Moffatt and Radley (*24*) found that copper, silver, and manganese salts of GA were more effective than those of the alkaline earth elements or GA when applied to pea plants.

Investigators employing excised plant parts and tissue cultures (*5, 11, 31*) have demonstrated synergistic responses when GA and other growth substances were present at proper levels. Comparatively few attempts have been made, however, to use other substances with GA during malting. Macey (*18*) reported that combinations of GA and potassium bromate were effective in increasing enzyme activities, while curtailing malt loss. Similar results were obtained when 2,4-dichlorophenoxyacetic acid and 2(3)-benzoxazolone were used in combination with GA (*16, 17*). Pollock (*26*) found that the effect of GA on the germination of some dormant barleys could be enhanced by the use of hydrogen sulfide. Mastovsky, Karel, and Kahler (*21*) reported that combinations of glucose and GA were more effective than GA alone.

This study investigated the possibility of further increasing the effect of gibberellins on the production of α-amylase and protease during the malting of wheat through the use of GA in combination with other chemicals.

Experimental

Malts were prepared on a laboratory scale, using 50-gram samples of Triumph (Hard Red Winter) wheat. Grain was steeped at 50° F. to 42 % moisture con-