

WHEAT PRODUCTS

Gel-Forming Phosphorylated Derivative of Wheat Gluten

ALI MOHAMMAD, D. K. MECHAM, and H. S. OLCOTT
Western Regional Research Laboratory, Albany, Calif.

A derivative of wheat gluten was prepared by treatment with phosphoric acid and urea at 140° C., an adaptation of a procedure used for phosphorylation of cellulose. The phosphorylated product was capable of absorbing about 200 times its weight of water to form a firm gel. This preparative method has several advantages over those previously described for making the gel-forming derivative by sulfation or phosphorylation.

AN INTERESTING GEL-FORMING MATERIAL, called gluten sulfate, was described by Reitz, Ferrel, and Olcott (6) in 1944. It absorbs 100 to 300 times its weight of water almost instantly and forms a firm, odorless, tasteless, and non-toxic gel. The suitability of gluten sulfate for use in therapeutic jellies, ointments, and other pharmaceutical preparations, and as a thickening or emulsifying agent in ice cream and other food products, seemed worthy of consideration. However, the estimated cost of its production (\$8 to \$10 per pound based on reaction of undenatured gluten with cold concentrated sulfuric acid) ordinarily would prohibit incorporation in such products. Various other sulfation procedures were investigated; none gave a suitable product at less cost.

Subsequently Ferrel, Olcott, and Fraenkel-Conrat (2) showed that gluten can be phosphorylated to yield a product with properties very similar to those of gluten sulfate; but the method used (treatment with phosphoric acid containing excess phosphorus pentoxide for 3 days at room temperature) was at least as costly as the sulfation methods.

The authors have now found that gluten phosphate can be made by an adaptation of the method described by Coppick and Hall (7) for phosphorylation of cellulose. Essentially this process consists of treating the material to be phosphorylated with a mixture of phosphoric acid and urea, drying, and heating at temperatures near 140° C. (3). As applied to wheat gluten, the principal advantage over other phosphorylation and sulfation procedures is that much smaller quantities of acid are needed, eliminating the cost of use and disposal of much ex-

cess acid. Estimates have placed the cost of production of gluten phosphate by this method at about \$2.00 per pound.

Method of Preparation

In the most convenient method for laboratory use, 300 grams of wet gluten (approximately 100 grams dry weight), 18.2 ml. of 85% orthophosphoric acid, and 30 grams of urea were mixed with a mechanical mixer, such as a small kitchen mixer, using a dough paddle. The soft sticky mixture was spread on a tray in a layer approximately 1/4 inch deep, and dried in an air-draft oven at 40° to 55° C. After 24 hours, the partially dried material was cut into small pieces and drying was continued until the mixture became brittle rather than gummy; with some preparations this required up to 4 days. The dried mixture was ground in a small hammer mill; and the ground dry mixture was heated at 140° C. for 30 minutes in vacuum (an Abderhalden dryer was used with xylene as the refluxing jacketing liquid). One hundred grams of heated mixture were suspended in water and 0.1 *N* sodium hydroxide was added to pH 7.6 (pH 7.5 to 7.8 permissible). Additional water was then added to a total volume of 7 liters, and the mixture was allowed to stand for 10 to 20 minutes. The supernatant was decanted; the residue was washed three times with 7-liter portions of 70% acetone and once with 100% acetone; and the washed product was dried in an air stream or in vacuum.

Gluten phosphate prepared by this method consistently had a hydration capacity near 200 (grams of water held as a firm gel by 1 gram of product). The

total phosphorus content was about 1.6%.

Yields varied from 27 to 31% of the weight of heated ground mixture, or approximately from 39 to 48% on the basis of the dry weight of gluten taken. With some preparations, the product settled incompletely during the step of washing.

Variations in Procedure

A satisfactory product was obtained from commercial dry "gum" gluten (undenatured) but the mixing procedure was modified slightly. One hundred grams of dry gluten were mixed with a solution of 18.2 ml. of 85% phosphoric acid and 30 grams of urea made to 100 ml. with water; as this mixture contained less water than that prepared from wet gluten, a mixer giving a better kneading action, such as a double-arm Z-mixer, was more suitable than a paddle mixer. Preparation A, Table I, was prepared from dry gum gluten; properties as given in the table are similar to those of wet gluten preparations. No gel could be obtained from heat-denatured (commercial "devitalized") gluten.

Drying. This is probably the least commercially feasible step of the process as described. During drying, a horny layer forms on the surface of the mixture and removal of remaining moisture is very slow.

Grinding. On small preparations, a Wiley mill with 80-mesh screen was used. It was observed that material not ground so finely gave less homogeneous and opaque rather than transparent gels.

Heating. With lower heating temperatures, down to 130° C., gel-forming

materials were obtained (preparations B, C, and D, Table I), but were of relatively poor hydration capacity. Heating in air gave variable results and a somewhat darkened product.

Washing. No organic solvent is required in the washing process in order to obtain a satisfactory product, as illustrated by preparation E, Table I. However, with water washing the volume of gel becomes so large that large quantities of wash liquid must be handled, and the final drying of the product requires the removal of much water. Of the organic solvents tried (acetone, ethyl alcohol, isopropyl alcohol), acetone gave the product of highest hydration capacity and reduced the gel volume sufficiently to make drying the product in an air stream easy. The effect of acetone concentration on gel volume of a washed preparation of gluten phosphate is shown by the following data:

Acetone, % by Volume	Gel Volume, Ml./G.
0	232
50	142
70	68
80	8

The ratio of wash liquid to solid given in the method of preparation probably is appreciably higher than necessary. Preparation F, Table I, was given one wash with water and one with 70% acetone, and then was filtered and washed twice on the filter with a small portion of anhydrous acetone. Hydration capacity was satisfactory, even though the total phosphorus content is somewhat high, suggesting that all inorganic phosphate was not removed.

The use of ammonium bicarbonate solution for washing gluten sulfate has been suggested by Ramage (5). Attempts to adapt this technique to the preparation of gluten phosphate were unsuccessful.

Influence of Phosphoric Acid And Urea and of Heating

A considerable number of combinations of acid and urea level and heating time were tried; these trials were made with dry gum gluten and with water-washing of the heated dried mixture. With 3 grams of urea per 10 grams of gluten, less than 1.8 ml. of phosphoric acid did not give maximum gel volume, while larger amounts lengthened the drying time appreciably. The level of urea was not found to be critical over a fairly wide range (1.5 to 4.5 grams per 10 grams gluten), but 3.0 grams per 10 grams of gluten gave as good or better gels than other quantities at several acid concentrations.

Variation in time at 140° C. with several combinations of acid and urea levels showed that shorter times than 30 minutes were not sufficient to develop maximum gel formation; in some cases decreases occurred beyond 45 minutes.

Properties

The properties of gluten phosphate, in so far as they have been determined, are very similar to those of gluten sulfate. The ammonium, potassium, and lithium salts are similar to the sodium salt in gel-forming properties; but the calcium,

barium, aluminum, and ferric salts exhibit no tendency to form gels. The effects of sodium, aluminum, barium, and ferric chlorides at different concentrations on gel volume obtained with a sodium salt preparation are given in Table II.

The chemical nature of gluten phosphate prepared by the acid-urea process has not been investigated. Because the method was adapted from that used for cellulose, and because of observations on the phosphorylation of proteins with phosphoric acid plus phosphorus pentoxide (2), it has been assumed that phosphorylation of protein aliphatic hydroxyl groups occurs.

On a 17.5% nitrogen basis, wheat gluten as an average contains 4.5 moles of serine and 2.1 moles of threonine per 10⁴ grams (4); complete phosphorylation of the serine and threonine to *O*-orthophosphate esters would introduce about 2% phosphorus. These calculations are only approximations, inasmuch as the low yields suggest that fractionation of the gluten components may have occurred. Some attempts to sulfate gluten by using sulfuric instead of phosphoric acid in the acid-urea-gluten mixture were unsuccessful, no gel being obtained. Also, because little free acidity remains in the heated dry acid-urea-gluten mixture, it seemed likely that most of the urea is hydrolyzed during the various stages of processing. Assuming ammonium phosphate is formed, some trials were made with ammonium phosphate-urea mixtures as a phosphorylating agent. Only small yields of very poor gels were obtained, however.

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Table I. Properties of Gluten Phosphate Prepared by Various Procedures^a

Preparation Description ^b	Yield ^c , %	Hydration Capacity ^d	Total P, %	Total N, %
A. From dry gum gluten	49	164	1.59	15.5
B. Heated 40 min., 130° C.	47	126	1.44	15.6
C. Heated 30 min., 133° C.	53	146	1.45	15.6
D. Heated 30 min., 139° C.	47	204	1.57	15.4
E. Water washing	47	200	1.53	15.4
F. One 70% acetone wash, then anhydrous acetone	41	188	2.81	14.4

^a Analytical and yield figures uncorrected for moisture, which varied from 7.5 to 9.0%.

^b Unless otherwise indicated, preparations were from wet gluten, heated 30 minutes at 140° C., and washed once with water, followed by two washes with 70% acetone.

^c As % of gluten.

^d Grams water held as a firm gel by 1 gram of product.

Table II. Effects of Salts on Gel Volume

Salt Concn., %	Hydration Capacity ^a , Ml. per Gram			
	NaCl	CaCl ₂	AlCl ₃	FeCl ₃
0.0	164	185	185	185
0.005	147	132	130	130
0.010	136	108	110	120
0.030	103	64	10	40
0.070	74	30	8	20
0.100	59	20	8	10
0.200	43			
0.600	22			
1.00	14			

^a Grams water held as a firm gel by 1 gram of product.