Polysaccharide Interactions With Wheat Proteins and Flour Doughs

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Cereal Chem. 56(2):68-73

ABSTRACT

Because of the generally lower bread baking quality of soft wheat flours, polysaccharide additives that might improve their performance were explored. Extracellular microbial polysaccharides, carrageenans, and an alginate were added to soft wheat flour doughs before mixing in the farinograph. When added in concentrations lower than 0.6%, two microbial polysaccharides increased peak time and dough stability as measured by the farinograph. Furthermore, by adding certain microbial polysaccharides and small amounts of a commercial carrageenan gum, the mixing characteristic of the dough could be varied extensively, even approximating those of strong flours. To determine polysaccharide-protein interactions, the polysaccharides were added to partially purified gluten solutions and the turbidity and viscosity were determined. The reactions, which varied from no apparent interaction to strong association and precipitation, suggest possible use of the gums as scavengers for proteins in dilute wastewater solutions or in texturized protein foods.

Previous studies have shown that dextrans (2-D-glucans produced from sugar by *Leuconostoc mesenteroides* and related bacteria) interact with wheat proteins to varying degrees and also alter mixing characteristics of flour to which they are added (Jones and Erlander 1967, Wilham et al 1959). Other microbial polysaccharides (PS) have been isolated and partially characterized (Jeanes 1973; Seymour et al 1976; Slodki 1962; Slodki et al 1961, 1972), but only limited studies have been conducted on their interactions with wheat proteins of flour. Of these PS, xanthan gum from *Xanthomonas campestris* NRRL B-1459 is produced commercially and its industrial and food uses have multiplied rapidly (Jeanes 1974). Because there is such diversity among the available microbial and plant PS, many uses conceivably can also be found for some of these. Aqueous solutions of certain of these PS have extremely high viscosity that may be from 10 to possibly 300–400 times as great as those of dextrans at concentrations of 1–3% (Jeanes 1973).

Soft red winter (SRW) wheat flour costs less than hard red winter (HRW) wheat flour, since it is not as desirable for baking bread and some other bakery products. The different baking qualities of SRW flours may be due to lower protein content and probably different protein composition (Huebner and Wall 1976). Because xanthan gum lengthens the mixing time of SRW flour dough on the farinograph (Christianson 1976), other PS may exhibit similar behavior and possibly improve the baking quality of poor quality wheat flours. Because the gluten is weak in the SRW flour, we studied the interaction between the protein and the PS to see if the PS might be influencing gluten properties in modifying mixing characteristics. This article describes results of studies to determine the effects of PS added to wheat flour or to SRW gluten proteins in solution.

[1]Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

MATERIALS AND METHODS

The SRW wheat flour was a mixture of SRW wheats, designated standard baking flour for 1975, and was obtained from W. T. Yamazaki, Wooster, OH. The HRW wheat flour was a mixture of HRW wheats, designated regional baking standard for 1975, obtained from K. F. Finney, Manhattan, KS. The flour was stored at −20°.

The PS were obtained from A. Jeanes and M. Slodki of this Center. All of the samples, except those specifically noted, were prepared in the laboratory by the methods described by Jeanes (1973) and, if anionic, as the potassium salts. The refined polytetran was from the Pillsbury Company and the carrageenans were from the Marine Colloid Company. The xanthan (Keltrol) and sodium alginate (Kelcosol) were from the Kelco Company. Table I summarizes the sources and compositions of the PS.

Dough Mixing Studies

Dough consistency was determined in the Brabender mixer on a mixture of 50 g of SRW wheat flour, a designated amount of PS, and sufficient water to bring the peak of the curve to 500 Brabender units (BU) according to the official AACC method (1962). No attempt was made to reduce the amount of flour by the small amount of PS added or to add an equivalent amount of starch to the control standard run. This method necessitated changing the amount of water for each determination in order to have the peak at 500 BU. If too little water was used, the curve would extend above 500 BU and yield a sharper peak, thereby incorrectly indicating a lower stability reading.

Almost all of the PS were freeze-dried previously, which gave them a light, fluffy, tough consistency and made them difficult to dissolve in water. Consequently, these preparations had to be dispersed in water 1–2 hr before use. The dispersion was added within 25–30 sec to the wheat flour in the farinograph mixing bowl, while mixing according to the official AACC method.

Interactions in Solution

Gluten was prepared by the method of Jones et al (1959) by preparing a dough ball, washing out the starch with 0.1*N* NaCl, dissolving the gluten in 0.1*N* acetic acid, and centrifuging at 35,000× *g* for 30 min. The supernatant gluten solution was heated to 95° to inactivate any enzymes and then lyophilized. Approximately 0.5% stock solution was made up in 0.01*N* acetic acid. The percent protein in the solution was determined by Kjeldahl nitrogen analysis; from this stock solution, solutions for addition to the PS solutions were prepared by dilution with 0.01*N* acetic acid.

The PS were dispersed in 0.01*N* acetic acid and dialyzed overnight against that solution. Samples giving cloudy solutions were centrifuged at 25,000× *g* for 1 hr. At that speed, some of the higher molecular weight PS tended to separate from the liquid.

The PS concentrations in the solutions were determined by the phenol-sulfuric acid method of Dubois et al (1956). Almost all the solutions were made to approximately 0.4%; those that were too viscous were diluted to about 0.2%. The dispersions were diluted further as needed for interactions with the protein solutions.

For the interaction studies, 2.5 ml of the PS solution was placed in matched tubes to which 2.5 ml of the protein solution was added. The tube contents were stirred with a slight circular motion of the tube by wrist action during addition of the protein solutions. After 10 min, the absorbance of the solutions was read at 545 nm in a Beckman Model B spectrophotometer to determine the turbidity. A PS solution diluted with an equal volume of 0.01*N* acetic acid was used to obtain a blank absorbance reading. After about 10 min, the absorbance generally decreased steadily, especially at higher concentrations, due to coagulation and settling out of the precipitate.

Viscosity

The viscosities of the solutions were determined with a Cannon-Fenske viscometer No. 100 at 25.0°. Only a relative viscosity was determined at one concentration. The concentration of the PS used to react with the protein varied from 0.005% to 0.05% depending on the viscosity of the solutions. The protein solution was either 0.02 or 0.04%.

RESULTS

Dough Mixing Studies

Our main objective was to find a PS that would increase the stability of SRW wheat doughs with short mixing and stability times. Because the cost of these PS would be considerably more per pound than the flour, they also would have to be effective at very

TABLE I
Microbial and Algal Polysaccharide Preparations

Organism	NRRL Strain[a]	Description	Reference
Arthrobacter viscosus	B-1973	Heteropolysaccharide (mannuronic acid)	Jeanes, 1973
Xanthomonas campestris	B-1459	Heteropolysaccharide (glucuronic acid, pyruvic acid)	Jeanes, 1973
Bacillus polymyxa	B-1828	Heteropolysaccharide (glucuronic acid)	Jeanes, 1973
Cryptococcus laurentii var. *flavescens*	Y-1401	Heteropolysaccharide (glucuronic acid)	Jeanes, 1973
Hansenula capsulata	Y-1842	Neutral mannan	Seymour et al, 1976
Torulopsis pinus	Y-2023	Neutral mannan	Seymour et al, 1976
Hansenula holstii	Y-2448, Y-2154[b]	Phosphomannans (diesterified phosphoric acid)	Slodki et al, 1961
Pichia pinus	Y-2579	Phosphoglucomannan	Slodki et al, 1972
Polysaccharides from Other Sources			
Polytetran (*Sclerotium glucanicum*)		Neutral glucan	
Carrageenan		Heteropolysaccharide (monoesterified sulfuric acid)	
Sodium alginate (Kelcosol)		Polyuronic acid	

[a]B and Y designate bacteria and yeast, respectively.
[b]Related strains that produce similar phosphomannans.

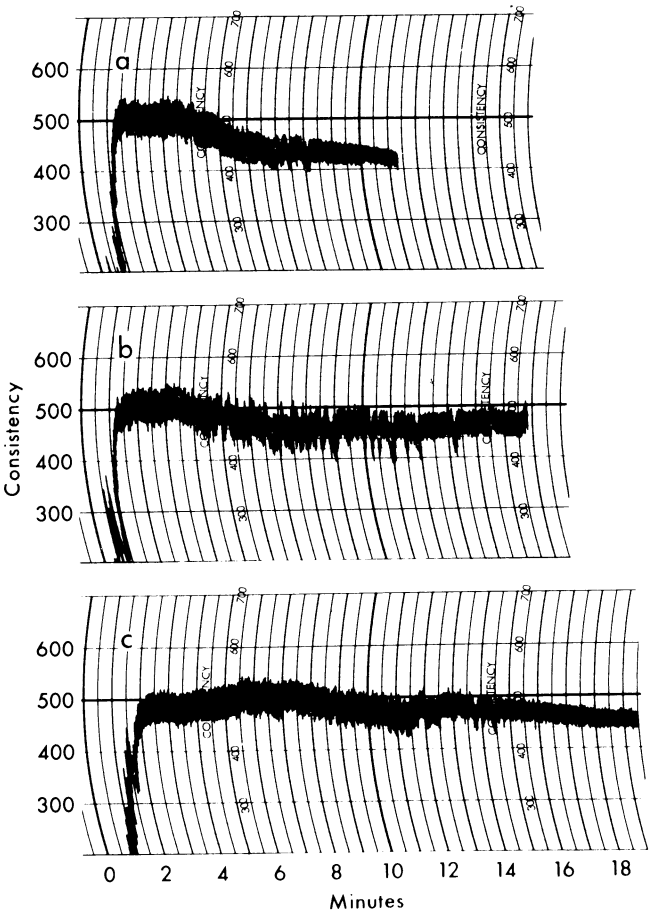

Fig. 1. Farinograph curves of 50 g of standard mix soft red winter wheat flours. **a,** no additives, 27.5 ml of H_2O; **b,** added 0.3 g of PS B-1459, 29.5 ml of H_2O; **c,** added 75 mg of calcium carrageenan, 27.8 ml of H_2O.

low concentrations. Some initial tests with xanthan gum and two commercial algal gums showed a good increase in stability at gum concentrations of 0.6% or less. Therefore, we used 0.6% or less in our tests. Farinograms (Fig. 1) demonstrate the increased stability of doughs containing xanthan gum and carrageenans compared with doughs of the SRW wheat flour alone. Jones and Erlander's studies (1967) of flour mixed with dextrans showed only a decrease in stability; in those studies, however, 1% of dextran was added relative to the amount of flour, and the addition of water was kept constant so that an increase in consistency due to higher water absorption gave an apparent decrease in stability. By increasing the water proportional to the increase in absorption, the consistency would have remained more stable. We compared three dextrans (from *L. mesenteroides* B-512F, B-742 and *Streptobacterium dextranicum* B-1254) at the 1% level as was done with the PS, and all three showed a slight increase in peak and stability times.

Christianson (1976) found that the xanthan gum gave an increase in peak time due to the slower absorption time of the PS; however, for the PS that we added, both dry and previously dispersed in water, no differences in peak times were noted. Evidently, the small amount used had little effect on the absorption time.

Table II lists the results of the farinograph studies. PS B-1973 increased the stability but not the peak time of SRW doughs, whereas PS B-1973 (old) increased both. Carrageenans gave the greatest increases in the peak and stability times and had the unusual characteristic of giving two peaks (Fig. 1). The double peaks could be eliminated by keeping the carrageenan addition low and adding a small amount of another PS such as Y-2023 (Fig. 1c). PS Y-2154 and PS Y-2448, which are similar phosphorylated mannans, only slightly improved peak and stability times (Table II). Addition of other gums either had little effect on the mixing curve or resulted in a slight decrease in stability.

Addition to dough of PS B-1973, which has a high content (25%) of O-acetyl (Jeanes 1973), increased stability and slightly increased peak time, whereas introduction of the deacetylated sample increased the peak time but slightly decreased dough stability (Fig. 2a, c; Table II). A sample of old PS B-1973 that had lost some O-acetyl groups only slightly increased the peak and stability times

TABLE II
Changes in Farinograms After Addition of Polysaccharides to Soft Red Winter Flour[a] Doughs

Polysaccharide NRRL Strain	Quantity Added (g)	Peak (min)	Stability (min)	H_2O Needed (ml)
None		3	4	27.5
B-1973	0.1	3.5	6.5	28.6
B-1973 (old)	0.2	4	6.5	29.0
	0.1	4	5	28.8
B-1973 (deacetylated)	0.1	4.5	3	28.7
B-1828	0.15	3.5	3	28.6
B-1459	0.1	3	5	29.1
	0.3	3	9–11	29.5
Y-1401	0.3	3.5	4	28.7
Y-1842	0.3	3.5	4.3	28.5
Y-2023	0.3	4.7	3.5	28.8
Y-2154	0.3	4	4.5	28.5
	0.15	3	4	28.2
Y-2448	0.25	3.5	5	28
Y-2579	0.3	4.3	3.5	28.7
	0.15	3	3.5	27.8
Sodium alginate[b]	0.15	4	5	31.0
Lambda carrageenan[c]	0.15	1.2 and 10	>10	27.8
Kappa carrageenan[c]	0.15	1.2 and 6	11–12	27.8
Calcium carrageenan[c]	0.1	2 and 8	12–14	27.8
Calcium carrageenan[c] Y-2023	0.075 0.075	6	>10	27.8

[a]50 g.
[b]From the Kelco Company.
[c]From Marine Colloid Company.

(Fig. 2b). Although the stability times increased little for most doughs with added PS, there was considerably less drop off in the curve (Fig. 1 and 2) than is normally obtained with straight flour. Consequently, the stability of some doughs, such as that containing PS B-1973, may be better than indicated by the stability times in Table II.

As demonstrated in Fig. 1c, mixing curves can be obtained from a SRW wheat flour with peak times and stabilities comparable to curves for a hard wheat or even for a long mixing hard wheat, provided the right amounts or mixtures of suitable PS such as calcium carrageenans plus PS Y-2023 or PS Y-2448 (Table II) are added. Carrageenan and xanthan gum were the most effective in increasing the stability of the soft wheat flour doughs.

Interaction in Solutions

The interactions of different PS with soft wheat gluten varied considerably (Fig. 3). Some PS produced no turbidity (Table III). Some produced considerable turbidity with no immediate settling of precipitate, but others (ie, PS B-1973 and PS B-1459) formed flocculent or stringy precipitates that prevented turbidity measurements. However, the interaction with deacetylated PS B-1973 (Fig. 3b) was easily readable to over 1.0 absorbance unit before the reactant products aggregated. PS Y-1401 (Fig. 3a) caused the highest amount of readable turbidity at any given protein concentration, but its absorbance, like that of the others, could not be accurately determined above 1.0. As shown in Fig. 3a, lowering the concentration of the PS Y-1401 to 0.025% achieved the maximum interaction at about 0.07–0.09% protein. Unlike the results with dextrans (Jones and Erlander 1967), the turbidity did not decrease when more protein was added. This was generally true for the other PS also.

The phosphoglucomannan PS Y-2579 exhibited unusual behavior (Fig. 3a), producing a maximum absorbance when the gum concentration was one-half the protein concentration. With further increase in protein concentration, a fine precipitate formed. Upon settling, it reduced the absorbance until a protein/PS dispersion of nearly 4:1 was attained, whereupon the turbidity increased again. The interaction appears to depend on a relative percent of each component; doubling the percent of PS required doubling the protein in order to obtain nearly twice the absorbance. Tests with crude gliadin and glutenin samples gave similar but slightly different results, which suggest that the biphasic change in absorbance may result from slight differences in reactivity among specific proteins in the gliadin and glutenin fractions.

Another difference between PS was that some, such as PS Y-1401 and PS B-1973, reacted immediately at low concentrations of protein whereas other (ie, PS Y-2448) gave no noticeable reaction until a specific higher concentration was reached (Fig. 3a, b). Also, unexpectedly, some of the PS at a higher concentration did not form turbidity at as low a concentration of protein as the PS at a lower concentration (Fig. 3a) (PS Y-2579). This also was essentially true for other PS. For almost all of the PS, a certain ratio of the two polymers apparently is necessary before the interaction takes place.

In contrast, PS B-1459 and PS B-1973 reacted with the proteins

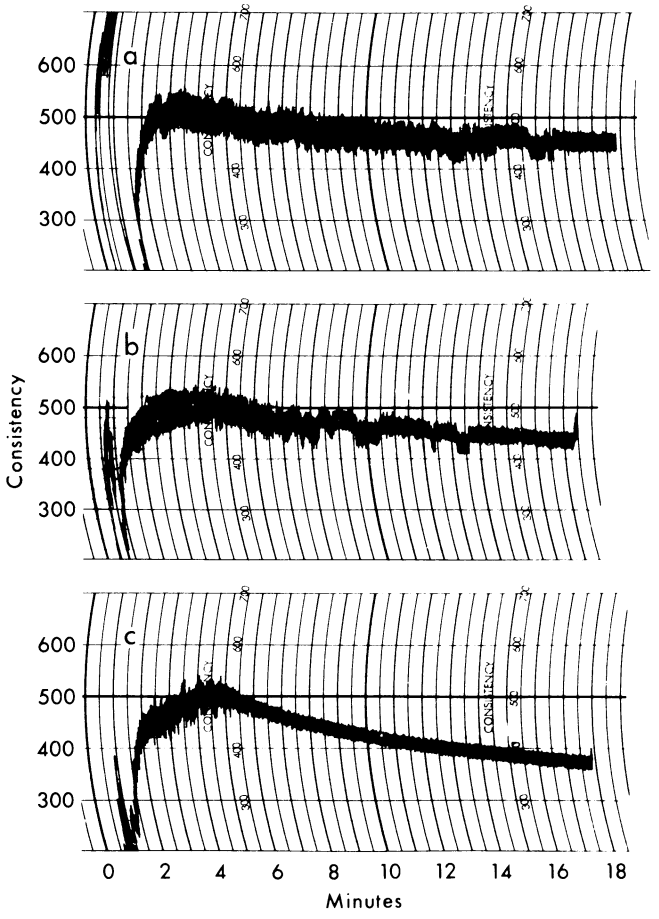

Fig. 2. Farinograph curves of 50 g of standard mix soft red winter wheat flours. **a,** added 0.15 g of PS B-1973, 28.7 ml of H_2O; **b,** added 0.1 g of PS B-1973 (old sample), 28.8 ml of H_2O; **c,** added 0.1 g of PS B-1973 (deacetylated), 28.2 ml of H_2O.

TABLE III
Gluten-Polysaccharide Interactions

	Turbidity				Viscosity	
	None	Precipitate				
Sample		Yes	Stringy	None	Increase	Decrease	No Change
B-1828				X[a]		X	
B-1973			X			[b]	
B-1973 (deacetylated)				X		X	
Y-1401		X				X	
Y-1842	X					X	
Y-2023	X					X	
Y-2154				X[a]		X	
Y-2448				X		X	
Y-2579		X[c]				X	
Refined polytetan	X				X		
Keltrol (B-1459)			X			[b]	
Calcium carrageenan				X[a]		X	
Kappa carrageenan				X			X
Kelcosol (sodium alginate)				X		X	

[a]Precipitate formed only after increased concentration, absorbance >1.0–1.5.
[b]Not measurable due to stringy precipitate.
[c]Fine precipitate formed only at a specific ratio.

to form stringy precipitates at low concentrations. When dilute solutions containing as little as 0.002% of these PS and protein were mixed, precipitates formed immediately that became stringy with slight mixing and generally floated near the top of the solution.

The interactions of a number of PS with gluten from a standard mix of HRW wheat also were tested, and the turbidity measurements were essentially the same as those of the SRW wheat.

Trace amounts of divalent cations may have been present that could crosslink negatively charged carboxylate, phosphate, or sulfate groups. Therefore, interactions also were done with EDTA or $MgCl_2$ present. The interactions with EDTA present did not differ noticeably, which indicates that divalent cations were not present. On the other hand, lack of difference with the $MgCl_2$ added might suggest that divalent cations play no part in these interactions. One exception was PS Y-2154; at a protein concentration of 0.06%, a slightly increased concentration of $MgCl_2$ brought about an increase in the absorbance in that solution over that with or without EDTA present. At protein concentrations lower than 0.05% or higher than 0.07%, the absorbance with $MgCl_2$ present was the same or less than that with EDTA present.

Viscosity

Because of the heavy turbidity or precipitate at higher concentrations, only one viscosity measurement was made with a low concentration of protein. The concentrations of the various PS had to be adjusted according to their relative viscosities so that they gave a reasonable measurement time for the particular flow viscometer used. After mixing PS with proteins, nearly all gave a decrease in relative viscosity except for the refined polytetran. Interactions involving PS B-1459 and PS B-1973 could not be read because they formed a stringy precipitate. For most PS, the decrease in viscosity was small and, because the amount of precipitate formed at different concentrations changed the viscosity, little if any significance can be attached to these results. The only interaction with a large viscosity decrease was given by deacetylated PS B-1973, which like the PS B-1973 has a high viscosity but does not form a stringy precipitate with the protein.

DISCUSSION

The differences in behavior in doughs and with gluten to which various types of PS were added indicates associative interactions between these polymers. The most pronounced effects on dough rheology were shown by heteropolysaccharides with numerous charged groups. For example, B-1459 (xanthan gum) and carrageenan most effectively increased the peak time and the dough stability. These PS also interacted with gluten at lowest concentrations. Probably the most effective interaction between these PS and wheat gluten is ionic. Electrostatic effects of the PS do not explain all the interactions, however, as Y-2154 and Y-2448 should have similar ionic charges but give different reactions. Also, changes in PS concentrations do not have results on solubilities similar to those observed for Y-1401 and Y-2579. Other effects, such as the configuration of the molecule and the availability of charged groups, also may be important.

Hydrogen bonding also may play an important role. In acid solutions the carboxylate groups on the acidic heteropolymers, such as xanthan, are mainly associated and could not combine extensively with the NH_4^+ group on the proteins. However, through hydrogen bonds, the carboxyl groups could combine with amide and other groups on the protein. The acetyl groups make B-1973 more hydrophobic and can associate with nonpolar residues of protein. Removing the acetyl groups exposes hydroxyl groups, rendering the PS more hydrophilic with more groups to react by hydrogen bonding with the protein. Hydrogen bonding also may account for the action of dextrans and neutral mannans in slightly increasing mixing peak time and dough stability. These neutral PS offer extensive hydroxyl sites to form noncovalent links to the numerous amide groups on gluten proteins.

Although two of the PS improved dough stability in the farinograph, they were not as effective as the carrageenans, especially at low concentrations. We do not yet know whether any of these PS would be useful for baking. Although PS B-1459 was used to make a product like bread without starch (Christianson 1976), adding it to wheat flour decreases the loaf volume (D. D. Christianson, personal communication). To date, extensive tests with varying percentages of PS added to wheat flour have not been

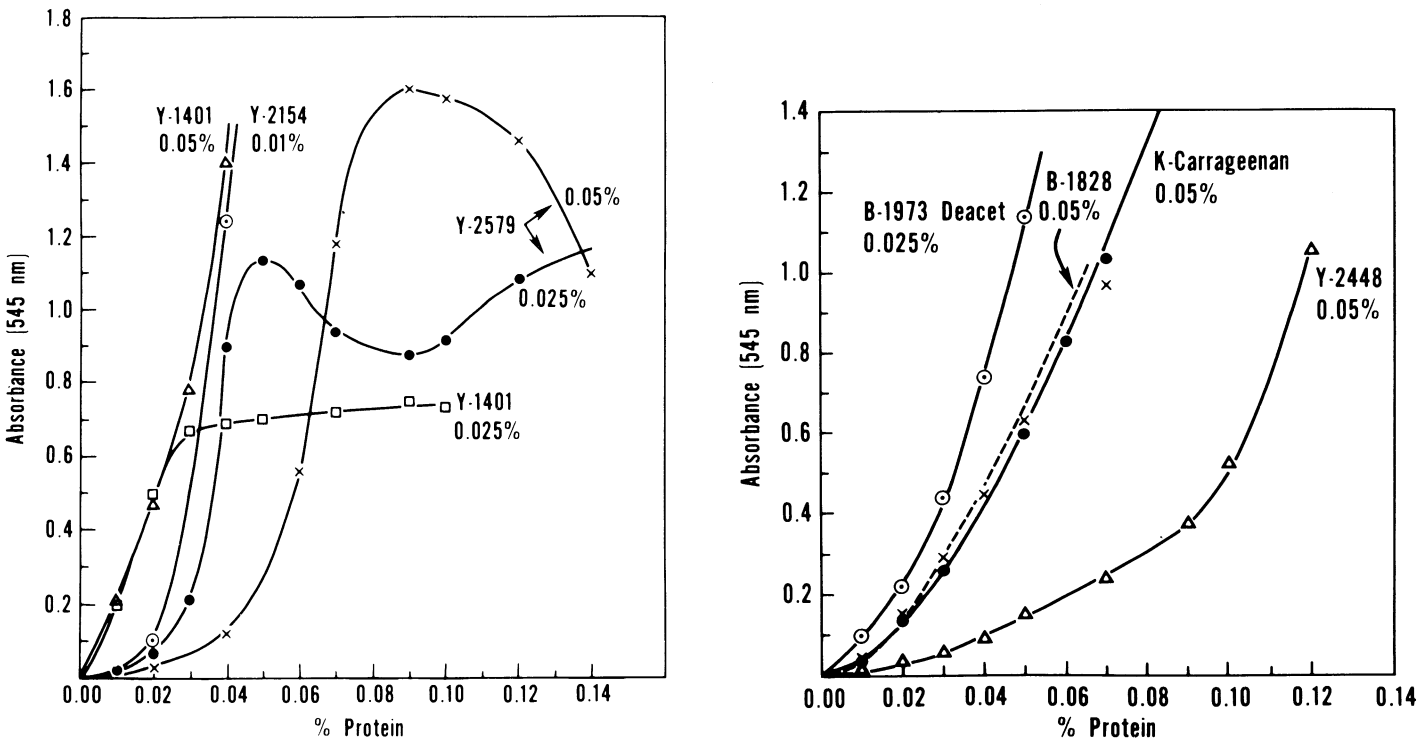

Fig. 3. Soft red winter wheat gluten-polysaccharide turbidity curves in 0.01 *M* acetic acid. Percentage of polysaccharides in final solution given next to each curve, percentage protein at base of graph.

made. However, this work shows that PS react differently with wheat proteins, and some of the interactions may be useful.

Baking is only one potential area of use of PS. Uses of PS B-1459 (xanthan gum) are many and diversified (Jeanes 1974). Many applications could be developed for some other PS. Since the PS B-1973 reacts well with protein at low concentrations, it might be useful as a scavenger to remove protein from dilute solutions now being discarded as waste and causing pollution problems. Conceivably a number of these PS could have other uses in the food industry to modify the functionality of proteins used as supplements in meat and cereal products.

ACKNOWLEDGMENTS

We thank Mrs. Felicula Porcuna for technical assistance on this project.

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[Received December 12, 1977. Accepted June 26, 1978]