Physicochemical and Functional Properties of Rye Nonstarch Polysaccharides. III. Oxidative Gelation of a Fraction Containing Water-Soluble Pentosans and Proteins

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Cereal Chem. 68(6):617–622

ABSTRACT

Pentosan-protein material from rye is subject to oxidative gelation. Thus, solutions of a fraction (obtained by precipitating water solubles from rye with ethanol) gel when solutions of hydrogen peroxide and horseradish peroxidase are added. The gelation reaction is inhibited by ferulic and vanillic acid but not by fumaric acid. This suggests that the mechanism whereby gelation occurs is comparable to that of wheat pentosan-protein material, in that the ferulic acid aromatic ring and not the propenoic moiety is involved in the gelation. Enzyme treatments showed that although protein probably is not necessary for the oxidative process, the presence of the pentosan moiety is required. Cysteine residues in the protein most likely are not involved in the gelation because *N*-ethylmaleimide has no effect on gelation, and the inhibition of the gelation reaction by cysteine can be rationalized as an effect of competition for the hydrogen peroxide. Ascorbic acid also inhibited the gelation, and its role probably can be ascribed at least partially to the same mechanism. High concentrations of cysteine or ascorbic acid in the presence of peroxidase and hydrogen peroxide reduced the relative viscosity of a pentosan-protein solution to experimental readings below those of the ungelled control solution.

As pointed out earlier (Delcour et al 1989a, 1991), much information is available regarding the physicochemical and functional properties of wheat pentosan-protein material. It has long been known that wheat flour extracts containing water-soluble pentosans gel upon the addition of hydrogen peroxide (Baker et al 1943). Several mechanisms have been proposed to explain the oxidative gelation phenomenon. From all data available, it is clear that a crucial role is to be assigned to ferulic acid.

Over a period of about 25 years, Neukom and coworkers have contributed significantly to our understanding of the phenomenon. In their earlier work (Neukom et al 1962), they suggested that pure protein-free arabinoxylan would not gel, and that glycoprotein is therefore involved in the reactions. Later, Geissmann and Neukom (1973) concluded that oxidative gelation is brought about by oxidative phenolic coupling of ferulic acid residues, causing cross-linking of the polysaccharide molecules. The role of the small amounts of protein present in their pentosan preparations was considered to be rather unclear. Sound evidence that protein-free pentosan extracts gel was presented by Morita et al (1974). However, Meuser et al (1982) confirmed that a wheat glycoprotein fraction has the gelation capacity. They reported that after proteolysis, the gelation capacity of this fraction was lost along with about half of its polyphenol content.

The following explanations of the gelation mechanism have also been proposed:

1. Pentosan ferulic acid could link to tyrosine residues or to ferulic acid linked to the protein (Neukom and Markwalder 1978).
2. Pentosan ferulic acid could be linked to *N*-terminal protein amino groups, thus forming a pseudopeptide linkage much as is the case in barley proteins (Van Sumere et al 1973, Neukom and Markwalder 1978). Upon oxidation, a diferulic acid bridge between protein and pentosan could result.
3. In agreement with the above, Meuser and Suckow (1985) inferred from their own findings that part of the ferulic acid either is linked to the protein of the glycoprotein or serves as a bridge between the protein and the pentosan parts.
4. On the basis of their experimental evidence, Hoseney and Faubion (1981) hypothesized that activated protein thiol groups react with the propenoic double bond of ferulic acid moieties attached to the pentosan to form a gel structure.
5. Lee et al (1968) hypothesized that a weak linkage exists between a hydrochinon-like oxidized form of ferulic acid residue of the arabinoxylan and protein carboxylate moieties with calcium ion bridges.

This last mechanism, however, seems less likely in view of the findings by Geissmann and Neukom (1973). Moore et al (1990) recently found that ferulic acid, vanillic acid, and cysteine (but not fumaric acid) inhibited the oxidative gelation of wheat flour water solubles. The results of this work contradict those of Hoseney and Faubion (1981) in that the lack of inhibitory activity by fumaric acid can be interpreted as evidence that the propenoic moiety of ferulic acid is not directly involved in the gelation reaction. Whether such reactions can be important therefore seems doubtful. In any case, the reaction is not an oxidation phenomenon, and it should therefore not be linked to oxidative gelation.

Moore et al (1990) also found evidence for the presence of some natural unknown inhibiting factor that could be removed by extracting flour with ethanol. Long before that, Baker et al (1943) reported that the viscosity of pentsosans is lowered and their gelation hindered or prevented by solubles from bran, germ, or malt. In agreement with these findings, Neukom and Markwalder (1978) reported that high ash flours contain substances that inhibit gelation.

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Rye meal contains roughly three times more water-soluble, ethanol-precipitable arabinoxylan than does wheat (Meuser et al 1986). In the case of rye hemicelluloses, the occurrence of ferulic and diferulic acid after oxidative gelation was described by Schneider and Pietsch (1985). Delcour et al (1989b) confirmed that ferulic acid and other phenolic acids are present in a saponified water extract of rye.

To the best of our knowledge, however, no data are available with regard to the factors affecting the gelation mechanism of rye pentosan-protein material, whereas much information is available with regard to the corresponding physicochemical property of wheat material.

In view of the above, we investigated the gelation of rye pentosan-protein material and the factors governing the process, as part of a systematic effort aimed at understanding the physicochemical and functional properties of rye nonstarch polysaccharides (Delcour et al 1989a, 1991). More specifically, we were convinced that useful information could result from studies using inhibitors of the oxidative gelation process, much as work by Hoseney and Faubion (1981) or Moore et al (1990).

MATERIALS AND METHODS

Enzymes and Chemicals

Horseradish peroxidase (POD) was from Sigma Chemical Co., St. Louis, MO (Type I, No. P-8125). The commercial pentosanase Veron He was supplied by Röhm (Darmstadt, Germany). Protease was from bovine pancreas (Crude Type I, Sigma P-4630). Bovine serum albumin standard powder, research grade (MW 67,000), was from Serva Feinbiochemica Heidelberg (Cat. No. 11930).

Cereal Sample

The rye (Danko) and wheat (Camp Remy) samples were grown in Belgium in 1989. Rye contained 1.46% Kjeldahl nitrogen and wheat 2.23% (dry basis) when analyzed according to the Analytica procedure (EBC 1987).

Isolation and Characterization of Rye and Wheat Water-Soluble Pentosan-Protein Fractions

Isolation. Kernels were boiled in ethanol (95%) for 60 min. After removal of the solvent and drying at 60°C, the kernels were milled using a Cyclotex 1092 sample mill (Tecator). Whole meal (1,000 g) was extracted with 4,000 ml of deionized water for 4 hr at 6°C with continuous mechanical stirring. After centrifugation (30 min, 2,500 *g*) the extract was filtered (Buchner filtration), and two volumes of ethanol (95%) was added to the extract to precipitate the pentosan material (overnight at 6°C). The precipitate was collected by centrifugation (10 min, 2,500 *g*). The residue was dried by washings with ethanol and diethyl ether and was ball milled.

Moisture content. Moisture content was determined by drying material at 95°C until constant weight.

Protein content and amino acid analysis. Protein content was computed as the sum of amino acid residues determined with an amino acid analyzer (LKB 4150) after acid hydrolysis of the pentosan-protein material. Cysteine was calculated from cystine data.

Polysaccharide content and monosaccharide composition. The procedure described by Delcour et al (1989a) based on gas chromatography of the alditol acetates of the monosaccharides obtained by acid hydrolysis of the sample was applied to determine relative quantities of monosaccharide residues.

The total polysaccharide content was determined by the phenol sulfuric acid method (Dubois et al 1956) as outlined by Eerlingen (1990).

Ferulic acid content. The ferulic acid content was assayed as described by Delcour et al (1989b). The separation by reversed-phase high-performance liquid chromatography of an ethyl acetate extract of saponified pentosan protein was performed on a Rosil C-18 column (Alltech, 250 × 4.6 mm) and monitored by recording the E_{280}-value of the eluate with H_2O/MeOH/HOAc (95/30/5, v/v/v) as elution solvent (1.0 ml/min).

Gel chromatography. An aliquot (1.0 ml) of a solution of pentosan-protein material (1 mg/ml) was separated on a Sephacryl S-500 HR column (47 × 1 cm, Pharmacia) (Hoffmann et al 1991), with 0.3% NaCl (26 ml/hr). Fractions (5 min) were analyzed with the orcinol method (Hashimoto et al 1987). One milliliter of each was hydrolyzed at 100°C (120 min) with 4*N* HCl. One milliliter of the resulting solution was heated at 100°C for 30 min after addition of 3.0 ml of 0.1% $FeCl_3$ in concentrated HCl and 0.3 ml of an orcinol solution (1% in absolute ethanol). Extinction was measured at 670 and 580 nm, and the difference of the two readings was computed. Under the experimental conditons, glucose eluted in fraction 18 (phenol sulfuric acid method, Dubois et al 1956).

Relative Viscosity

Flow times of solutions of the pentosan-protein material were determined with an Ostwald II viscometer (30°C). Flow times were divided by the flow time of deionized water under the experimental conditions and are referred to as relative viscosity readings.

Oxidative Gelation Capacity

A pentosan-protein solution was prepared by mechanical stirring of the material overnight (isolated as outlined above) with distilled water. An aliquot (5.0 ml) was mixed in the viscometer with 0.1 ml of a solution of hydrogen peroxide (0.39 g/L) and 0.1 ml of a solution containing 0.2 Sigma purpurogallin units of POD. Flow times were measured after at least 5 min at 30°C. For the control determination, 0.3 ml of distilled water was added to 5.0 ml of pentosan-protein solution, and the relative viscosity was measured as described above. The difference in relative viscosity between samples that had or had not been submitted to the enzymatic treatment was then taken as a measure for the oxidative gelation capacity. To prepare a rye pentosan-protein material subjected to gelation for amino acid analysis, a rye pentosan-protein solution was subjected to gelation as described above, precipitated with two volumes of ethanol, collected by centrifugation, and further dried by washings with ethanol and ether. An aliquot of this material was then hydrolyzed, and the amino acids formed were analyzed.

Inhibitors, Enzymatic and Alkaline Treatments

The effect of potentially inhibitory chemical compounds on gelation capacity was evaluated by adding 0.1 ml of a solution (neutralized when necessary) to a rye pentosan-protein suspension (5.0 ml, 2.7 mg/ml) before POD/H_2O_2 was added in the viscometer, as described above for measurement of gelation capacity. The molar quantity of the (potential) inhibitor used in these experiments was twice that of H_2O_2 unless otherwise specified.

N-Ethylmaleimide. A solution of 2.7 mg/ml of rye pentosan-protein material was prepared as described above. Relative flow times were determined with and without the addition of 0.1 ml 0.02*M* or 0.2*M* *N*-ethylmaleimide (NEM). The additions of this potential inhibitor were performed 10 min before POD/H_2O_2 was added to the solution in the viscometer. The molar quantity used in these experiments was about one (0.02*M* NEM) or 10 (0.2*M* NEM) times that of the sum of cysteine and lysine present in the material as found by amino acid analysis.

Enzymatic treatments. The pentosanase or protease (1% w/w on pentosan-protein basis) was mixed with a rye pentosan-protein solution in water and stirred at room temperature for 16 hr. The relative flow time of the resulting solution and its gelation capacity were determined as described above.

In the case of protease treatment, the protein content of the solution after treatment was assayed according to the Bradford (1976) method using the Bio-Rad microassay procedure.

Addition of soluble protein. To a rye pentosan-protein solution (2.7 mg/ml), 25% (i.e., 0.675 mg/ml) of soluble protein (bovine serum albumin) was added, and the mixture was stirred overnight. The relative viscosity and gelation capacity were measured as described above.

Effect of treatment with sodium hydroxide. Rye pentosan-protein solution (3.0 mg/ml) was stirred in 0.5*M* NaOH under

nitrogen atmosphere for 16 hr. The solution was neutralized with 2.0*N* HCl, and its relative viscosity was measured. To eliminate the free ferulic acid that potentially could influence the experimental results, an aliquot of the solution was further extracted with diethyl ether after adjusting the pH to 3.0 with 2*N* HCl. The water phase was neutralized, and flow times were determined.

RESULTS AND DISCUSSION

Isolation of Pentosan-Protein Material

Rye and wheat whole meal (1,000 g each) yielded 38.2 and 12.9 g, respectively, of material. The rye water extract was highly viscous and difficult to filter. The ratio of ethanol to water (2/1, v/v) used before for the isolation of wheat pentosan (Abdel-Gawad 1982) resulted in a 66% ethanol solution in water. This proved to be rather low for quantitative removal of the rye arabinoxylan from the solution, because it resulted in a slimy material instead of a clearly defined precipitate. Nevertheless, this extract-to-ethanol ratio was used because Meuser et al (1986) reported that concentrations of ethanol in excess of 60% would also precipitate the water-soluble arabinogalactan peptide.

The composition of the resulting pentosan-protein fractions is given in Tables I and II. The arabinoxylans all had a high degree of branching, as shown by the high ratios of the concentration of arabinose to that of xylose (0.77 and 0.83). The protein and glucose contents were relatively high because neither proteolysis nor amylolysis nor β-glucanolysis were included in the isolation procedure. In this stage of our work we did not want to include such treatments because we were not sure that proteins or glucans would not be involved in the gelation reactions. The results for ferulic acid content in our samples were not in agreement with the data of Meuser et al (1986), who found a higher content in rye than in wheat arabinoxylan-protein complexes (0.33 vs. 0.20%).

TABLE I
Composition of Pentosan-Protein Fractions from Rye and Wheat

Analysis	Rye	Wheat
Moisture, %	8.9	10.2
Protein, %	20	33
Carbohydrates, %	62.5	51.8
X/A/Ga/Gl[a]	1/0.77/0.03/0.7	1/0.83/0.12/1.0
A + X, %	43	32
Ferulic acid, %	0.04	0.08

[a] Ratio of the concentrations of xylose (X), arabinose (A), galactose (Ga), and glucose (Gl).

TABLE II
Amino Acid Content (g of Amino Acid Residues/100 g of Protein) of the Water-Soluble Pentosan-Protein Fractions from Rye and Wheat

Amino Acid	WWSP[a]	RWSP	GRWSP
Aspartic acid	9.8	9.2	9.4
Threonine	4.9	4.6	4.8
Serine	4.8	5.0	5.0
Glutamic acid	16.8	19.8	17.9
Proline	10.7	7.8	10.1
Glycine	4.3	4.4	3.9
Alanine	6.7	6.0	6.4
Half cystine	1.8	0.7	0.5
Valine	6.2	6.0	6.3
Methionine	2.0	1.9	2.0
Isoleucine	3.6	3.8	4.0
Leucine	6.9	7.8	7.8
Tyrosine	3.1	2.5	2.9
Phenylalanine	3.8	4.8	4.7
Histidine	1.9	2.9	2.2
Lysine	6.5	6.3	6.1
Arginine	6.3	6.5	5.9

[a] WWSP = Wheat pentosan-protein material, RWSP = rye pentosan-protein material, GRWSP = rye pentosan-protein material submitted to gelation.

The amino acid analyses of the wheat and rye pentosan material revealed that both protein moieties had similar amino acid compositions. It is noteworthy that in the case of wheat, the content of proline was clearly higher than the content reported by Delcour et al (1991) and Meuser and Suckow (1985), and that both for rye and wheat material, the histidine content is different from that found by the cited authors. However, it is unclear whether these differences are due to differences in composition of the samples.

Relative Viscosity and Oxidative Gelation Capacity

The results in Table II suggest that amino acids are not involved in oxidative gelation, or that their reaction products in the gelation reaction are labile to the conditions of the amino acid analysis. However, one must be aware that in our experimental work we were not able to separate the gelled protein fraction from the ungelled material. In our approach, therefore, any specificity with respect to the constitution of the gelled fraction could not be detected.

Figure 1 shows the relative viscosities before and after gelation of wheat and rye pentosan-protein preparations as a function of their concentration in water. As the chemical composition of the material of rye and wheat is quite similar, the difference in relative flow times are probably due to the higher molecular weight of the rye pentosan, as reflected in the difference in the control relative flow time at equal concentrations. For wheat arabinoxylan, Ciacco and D'Appolonia (1982) observed a direct relation between viscosity and gel strength, and Meuser and Suckow (1985) reported a molecular weight two to three times higher for rye pentosans than for that of wheat. In our work (Figure 2), we were able to confirm that the pentosan material of rye has a higher average molecular weight than that of wheat.

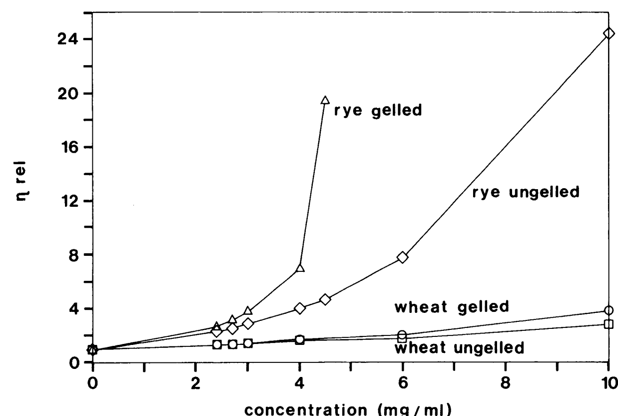

Fig. 1. Relative viscosity (η_{rel}) as a function of concentration (mg/ml) for ungelled and gelled rye and wheat pentosan-protein material.

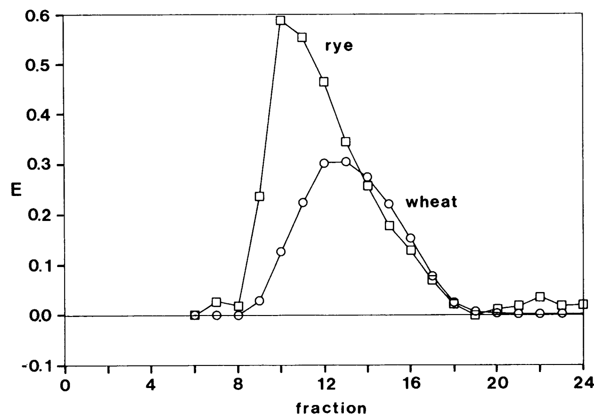

Fig. 2. Gel chromatogram of rye and wheat pentosan-protein material on a Sephacryl S-500 HR column (eluent 0.3% NaCl, 26 ml/hr). Fractions (5 min) were analyzed for pentosans with orcinol (E_{670}–E_{580}).

Inhibitors

Figures 3–7 show the relative flow times upon the addition of several test chemicals in gelation experiments. A decrease relative to the gelled control implies that the gelation is inhibited to a certain degree.

Several phenolic compounds such as ferulic and vanillic acid were shown to be inhibitors of the oxidative gelation. Whereas fumaric and cinnamic acids did not inhibit the reactions, ascorbic acid did. Glycine had no effect on the gelation, but cysteine was an inhibitor. Cysteine and ascorbic acid even reduced the relative viscosity until it was lower than that of the ungelled protein-pentosan solution. Reduction of the control viscosity by cysteine can be explained if the proteins present contain disulfide bridges, because cysteine reduces the molecular weight of such proteins. Patil et al (1975) noted the reduction of the viscosity of solutions and gels of wheat pentosans. In the case of ascorbic acid, we found that the agent has an inhibitory effect on the gelation. This finding is in agreement with that found by Neukom et al (1967) for wheat pentosans. It has also been reported that a gel, once formed, does not liquify on addition of ascorbic acid (Geissmann and Neukom 1973), and that ascorbic (or dehydroascorbic) acid is not a suitable oxidant to increase the viscosity of wheat flour water solubles (Hoseney and Faubion, 1981).

To learn more about the mechanism of inhibition, we changed the ratio of inhibitor to hydrogen peroxide in our experiments from a 2 molar excess of inhibitor to a 1.0, 0.5, or 0.25 molar ratio of inhibitor to hydrogen peroxide. In these experiments we assayed relative viscosity as a function of time after the addition of all reagents. In the case of a 0.5 molar ratio of ascorbic acid to hydrogen peroxide (Fig. 3), the relative viscosity decreased to a value lower than that of the ungelled control (i.e., without the addition of enzyme and hydrogen peroxide) during a certain time. After that time the gelation occurred, and the viscosity was relatively stable. The observed phenomena can be explained if one assumes that ascorbic acid competes for the hydrogen peroxide in the peroxidase catalyzed reaction (Chapon and Chapon 1979). In Figure 3, we observed that the lower the con-

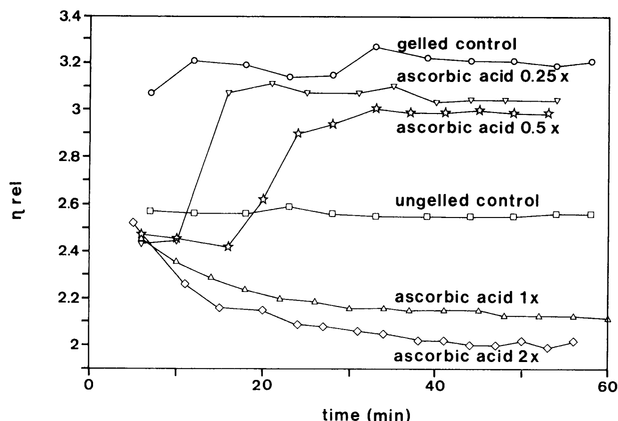

Fig. 3. Relative viscosity (η_{rel}) as a function of time (min) of a rye pentosan-protein solution (ungelled control) to which peroxidase, hydrogen peroxide (gelled control), and ascorbic acid were added in a molar ratio of inhibitor to hydrogen peroxide of 0.25, 0.5, 1.0, or 2.0.

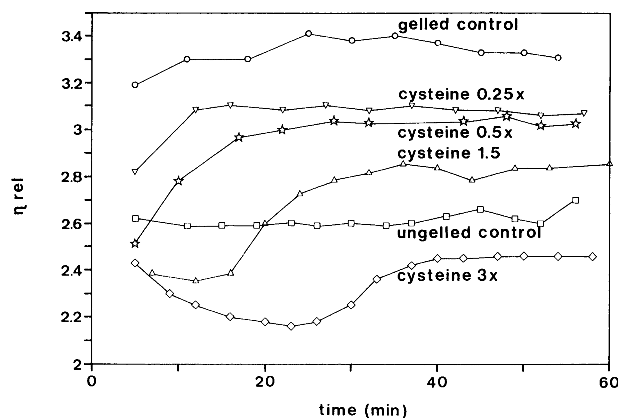

Fig. 4. Relative viscosity (η_{rel}) as a function of time (min) of a rye pentosan-protein solution (ungelled control) to which peroxidase, hydrogen peroxide (gelled control), and cysteine were added in a molar ratio of inhibitor to hydrogen peroxide of 0.25, 0.5, 1.5, or 3.0.

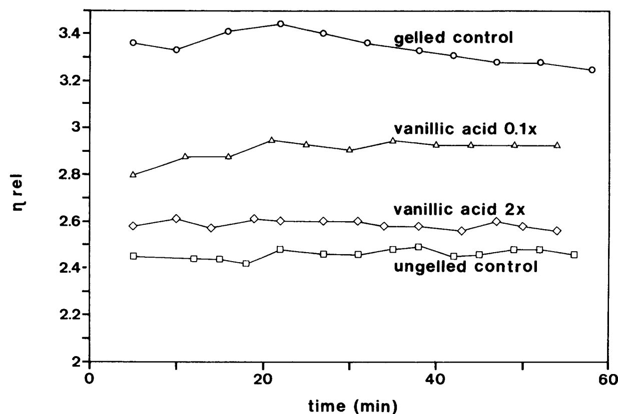

Fig. 6. Relative viscosity (η_{rel}) as a function of time (min) of a rye pentosan-protein solution (ungelled control) to which peroxidase, hydrogen peroxide (gelled control), and vanillic acid were added in a molar ratio of inhibitor to hydrogen peroxide of 0.1 or 2.0.

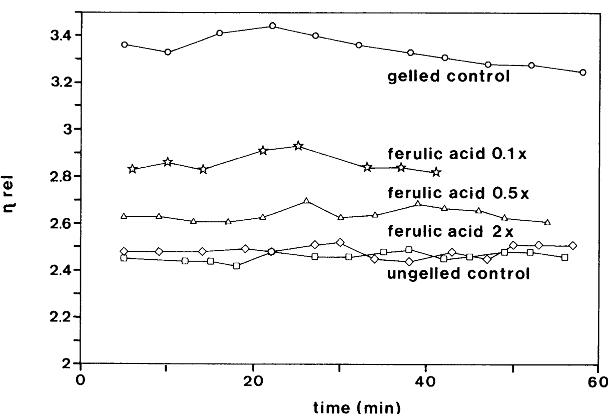

Fig. 5. Relative viscosity (η_{rel}) as a function of time (min) of a rye pentosan-protein solution (ungelled control) to which peroxidase, hydrogen peroxide (gelled control), and ferulic acid were added in a molar ratio of inhibitor to hydrogen peroxide of 0.1, 0.5, or 2.0.

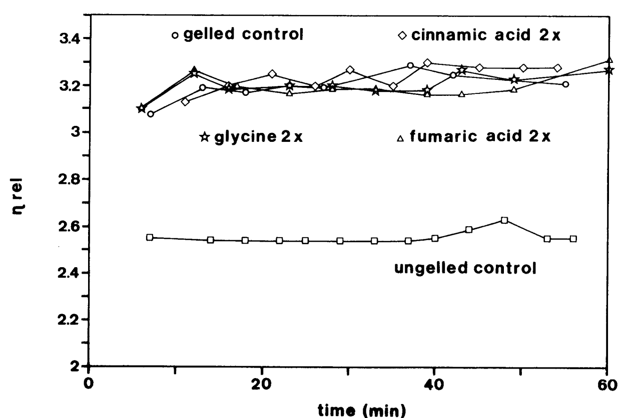

Fig. 7. Relative viscosity (η_{rel}) as a function of time (min) of a rye pentosan-protein solution (ungelled control) to which peroxidase; hydrogen peroxide (gelled control); and cinnamic acid, fumaric acid, or glycine were added in a molar ratio of inhibitor to hydrogen peroxide of 2.0.

centration of ascorbic acid used (molar range of the concentration of ascorbic acid to hydrogen peroxide, 0.25–0.5), the sooner the gelation took place. Furthermore, gelation was no longer observed if at least a 1.0 molar excess of ascorbic acid to hydrogen peroxide was used in the experiments. The decrease in viscosity observed at the higher ascorbic acid levels was not due to the presence of ascorbic acid itself (or dehydroascorbic acid) in the medium. Indeed, when we added ascorbic acid (in a two-molar excess to the regular concentration of hydrogen peroxide), and when the hydrogen peroxide-POD additions were omitted, the viscosity curves matched those of the control solutions without hydrogen peroxide, POD, or inhibitor). Likewise, previous reaction (60 min) of a one-molar ratio of ascorbic acid to hydrogen peroxide in the presence of POD, and subsequent addition of the reacted mixture to the pentosan-protein solution in the viscometer, resulted in viscosity curves matching those of the ungelled control. On the contrary, the two latter experiments with cysteine instead of ascorbic acid showed a decrease in viscosity with time, probably due to rupture of disulfide bridges.

The viscosity-vs.-time experiment with cysteine reveals a much shorter inhibition period than is the case with ascorbic acid. As a result of their work with wheat pentosans, Moore et al (1990) claimed that inhibition by cysteine is not a result of the consumption of hydrogen peroxide, but that it may be due to reaction with the active center of the polymers and prevention of the necessary cross-linking. Under their experimental conditions (characterized by a 1:1 molar ratio of cysteine to hydrogen peroxide), the authors did not observe an increase in viscosity in their gelation experiments. However, we measured relative viscosity as a function of time and observed a delayed increase in viscosity (Figure 4). We therefore concluded that inhibition of the gelation by cysteine was at least partly due to hydrogen peroxide consumption.

With the presence of ferulic or vanillic acid (Figs. 5 and 6, respectively), a time-dependent viscosity change was not observed. It is very likely that the agents inhibit at least partly by reacting with the ferulic acid esterified to the arabinoxylan, thus inhibiting cross-linking between pentosan chains. The amount of inhibitor (in gelation reactions with a 0.5 molar ratio of ferulic acid to hydrogen peroxide) was about 20 times higher than that of the ferulic acid residues present in the material when analyzed according to the procedure outlined.

In agreement with the above, treatment of the pentosan-protein with sodium hydroxide led to a total loss of the gelation capacity, as was to be expected if one assumes that ferulic acid residues on the arabinoxylan are an obligatory condition for oxidative gelation capacity.

The fact that fumaric acid (Fig. 7) did not inhibit the gelation reaction, whereas vanillic and ferulic acids did, suggests that, much as is the case with wheat pentosan-protein material, the reaction is through the aromatic residue of the phenolic compound rather than through the propenoic moiety (Moore et al 1990).

Enzyme Treatments

Enzyme (pentosanase) treatment (Table III) of the rye pentosan-protein material revealed that the arabinoxylan moiety was necessary to impart viscosity and gelation capacity to pentosan-protein solutions in water. Protease also had an effect on viscosity readings, either before or after the oxidative gelation treatment.

TABLE III
Enzyme Treatment of Rye Pentosan-Protein Material and Effect of the Addition of Protein

Sample	Relative Flow Time: Control	Relative Flow Time: with Peroxidase H_2O_2
2.7 mg/ml	2.53	3.22
2.7 mg/ml + Veron He pentosanase	1.61	1.68
2.7 mg/ml + protease	2.20	2.62
2.7 mg/ml + 25% bovine serum albumin	2.64	3.55

Since the results obtained in the Bradford assay showed that the enzyme treatment did not result in a complete hydrolysis of the protein moiety, our data only show that the protein in the pentosan-protein preparation is important for the viscosity of the solutions. We cannot conclude from the experiment whether the protein is involved in the chemical reactions of the oxidative gelation.

The effect of the sulfhydryl blocking agent NEM on the gelation capacity, proved to be negligible, not only with the addition of 0.1 ml 0.02*M* NEM, but also when 0.1 ml 0.2*M* NEM was added. The results are in agreement with those reported for wheat (Kundig et al 1961) and suggest that the protein cysteine residues probably are not involved in the gelation reaction.

CONCLUSIONS

The present work shows that the presence of pentosan material in rye water solubles is necessary for oxidative gelation to occur. The protein moiety is of much less importance, both for the viscous properties of the extracts as well as for the viscosity increase upon gelation. However, the present work was not able to confirm whether the protein has any role in the oxidative gelation process. If the protein is to be active, then it will not react through its activated thiol groups as proposed by Hoseney and Faubion (1981) because the sulfhydryl blocking reagent NEM did not reduce the gelation effect.

A critical role is certainly to be assigned to ferulic acid, much as that observed by numerous authors who studied the oxidative gelation of wheat pentosans. Saponification of the extract or the addition of free ferulic acid inhibit gelation. In agreement with the findings of Moore et al (1990) for wheat pentosans, and contrary to findings reported by Hoseney and Faubion (1981), the aromatic ring and not the propenoic moiety is involved in the reaction of ferulic acid.

The addition of ascorbic acid or cysteine in gelation experiments caused a delayed increase in relative viscosity. We concluded that these components inhibited the gelation through competition for hydrogen peroxide. High levels of ascorbic acid or cysteine reduced the viscosity of a pentosan-protein solution in the presence of peroxidase and hydrogen peroxide. The mechanism of the latter remains unclear, although it is possible that cysteine reduces viscosity by rupture of disulfide bridges.

ACKNOWLEDGMENTS

Author Delcour thanks the Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek for a position as research associate. Helpful discussions with P. Dondeyne and excellent technical assistance by L. Van den Ende are gratefully acknowledged.

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[Received June 20, 1990. Accepted May 24, 1991.]