

Factors Affecting the Oxidative Gelation of Wheat Water-Solubles¹

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ABSTRACT

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Viscometry of wheat flour extracts was used to study factors contributing to oxidative gelation. Addition of hydrogen peroxide did not increase viscosity of dilute aqueous extracts of wheat flour but did increase viscosity of more highly concentrated aqueous extracts. Oxidant concentration was a critical factor for gelation. High levels of hydrogen peroxide reduced the increase in viscosity. Addition of ferulic acid, vanillic

acid, and cysteine inhibited increases in viscosity. The mechanism of this oxidative gelation appears to be complicated and influenced greatly by the concentrations of the polysaccharide and the oxidant. Flour extracted with 95% ethanol gelled at a lower concentration than nonextracted flour. This suggests that some unknown inhibiting factor is removed by extracting flour with ethanol.

Concentrated water-soluble pentosan solutions form a gel at room temperature in the presence of oxidants. This unique property of oxidative gelation of flour water-solubles was first described by Durham (1925) and later shown to be caused by the pentosans (Baker et al 1943). Gelation was affected by the pentosan concentration, oxidant concentration, pH, ash, and presence of organic compounds (Baker et al 1943, Neukom et al 1967, Painter and Neukom 1968).

Fincher et al (1974) determined that the water-soluble pentosan fraction consists of three components: an arabinoxylan, a free protein, and a protein covalently linked to an arabinogalactan. Viscometric studies of flour water extracts showed that the polysaccharide accounts for 95% of the intrinsic viscosity, and soluble protein accounts for 5% (Udy 1956). Ferulic acid (4-hydroxy-3-methoxycinnamic acid) was esterified to the largest arabinoxylan fraction (Yeh et al 1980).

Reports conflict regarding the involvement of protein in gel formation. Aqueous flour extracts treated with proteolytic enzymes lose their capacity to gel, and gels treated with proteolytic enzymes liquefy (Baker et al 1943, Painter and Neukom 1968). Additionally, a strong interaction between pentosans and proteins occurs in the presence of oxidizing agents (Udy 1957). However, gel formation with protein-free aqueous extracts has been reported (Morita et al 1974).

Numerous hypotheses concerning the mechanism of oxidative gelation have been developed, but none have been substantiated. Ferulic acid associated with the polysaccharides may be involved in cross-linking pentosans (Fausch et al 1963, Geissmann and Neukom 1973, Neukom 1976, Hosenev and Faubion 1981). The ultraviolet spectrum of water-solubles showed absorption maxima at 320 nm (ferulic acid) and 280 nm (aromatic absorption) (Kuendig et al 1961). Ferulic acid was assumed to be involved in gelation because the absorption at 320 nm decreases during the oxidative gelation.

Detection of diferulic acid by thin-layer chromatography in oxidized crude pentosan solutions, after saponification, prompted Geissmann and Neukom (1973) to suggest that cross-linking occurs through oxidative phenolic coupling of adjacent ferulic acid residues.

Neukom and Markwalder (1978) suggested two ways in which protein may be involved in gelation. First, ferulic acid could be linked to an N-terminal amino group, forming a pseudopeptide linkage. Secondly, the ferulic acid associated with the pentosan could cross-link with the residue of tyrosine. A conflicting hypothesis is that a protein thiyl radical adds to the activated double bond of ferulic acid (Hosenev and Faubion 1981).

Ferulic acid has three potential reactive sites: two on the aro-

matic ring and one at the activated double bond. These reactive sites could serve to cross-link polymers and increase their molecular size. Neukom and Markwalder (1978) suggested involvement of the aromatic nucleus to form diferulic acid bridges. Sidhu et al (1980a) and Hosenev and Faubion (1981) pointed to the activated double bond as the cross-linking site. Hosenev and Faubion (1981) suggested that a protein thiyl radical added to the activated double bond.

Suckow et al (1983) and Martinez-Muñoz (1985) reported that they could not reproduce the results of Hosenev and Faubion (1981). In this study, we sought to clarify the confusion about the mechanism of oxidative gelation.

MATERIALS AND METHODS

Materials

Chemicals. Unless specified otherwise, all chemicals used were reagent grade. Ferulic acid (4-hydroxy-3-methoxycinnamic acid) was from ICN pharmaceuticals, Plainview, NY; L-cysteine HCl from Nutritional Biochemicals Corp., Cleveland, OH; 3% H₂O₂, ethanol (95%) ethyl acetate, and fumaric acid were obtained from Fisher Scientific Co., Fair Lawn, N.J. Vanillic (4-hydroxy-3-methoxybenzoic) acid was from Sigma Chemical Co., St. Louis, MO.

Enzymes. Type I horseradish peroxidase (94 purpurogallin units per milligram) was from Sigma.

Flour. Commercial straight-grade malted flour containing 13.0% protein, 13.4% moisture, and 0.47% ash was used (Ross Mills, Wichita, KS). In certain experiments the flour was supplemented with 0.25% malted barley flour (Malted Ross flour).

Methods

Preparation of flour extracts. Flour and water were combined at specified ratios (w/v). The resultant slurries were stirred for 5 min and then centrifuged for 15 min (360 × g). The supernatant was decanted, and the precipitate discarded. The supernatant was re-centrifuged for 10 min (1,000 × g). The resultant aqueous extracts were used without further purification.

Viscosity measurements. The relative viscosity of aqueous flour extracts was determined using Cannon-Fenske (size 50) capillary viscometers held in a constant-temperature (30 ± 0.1°C) water bath (Moore and Hosenev 1990).

Oxidation of aqueous extracts. Flour and water were combined in ratios of 1:10, 1:5, 1:3, and 1:2 (w/v). Aqueous extracts were treated with various levels of hydrogen peroxide (H₂O₂) for 15 min at room temperature. The optimum oxidant level (ppm) was based on flour weight. Increases in relative viscosity were determined for all flour/water ratios. Analysis of variance was performed, and least significant differences were calculated. The optimum oxidant level for oxidation of aqueous extracts was 15 ppm.

Rheologically active compounds. A number of compounds known to affect the rheology of flour-water doughs (rheologically active compounds) were studied: aqueous solutions of ferulic acid (250 ppm), vanillic acid (250 ppm), and fumaric acid (250 ppm)

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brought to pH 7 with 1.0N NaOH. Cysteine (250 ppm) was dissolved in distilled water.

Rheologically active compounds were added immediately following isolation of aqueous extracts prepared at flour/water ratios of 1:3 and 1:2 (w/v). H₂O₂ (15 ppm) was added after 5 min. The relative viscosity was determined. Aqueous extracts also were treated with various concentrations of H₂O₂ at a constant level of cysteine. Analysis of variance was performed, and least significant differences were calculated.

Flour Extraction

Ethanol (80%) extraction. Flour was mixed with 80% ethanol, 1:3 (w/v), for 30 min and then filtered through a Buchner funnel with Whatman No. 4 filter paper (Martinez-Muñoz 1985). The extraction was repeated three times. The extracted flour was dried overnight at room temperature (22°C) and ground to a uniform particle size in a coffee grinder.

Peroxidase (190 purpurogallin units) was added to extracts from all chemically treated flours to ensure peroxidase activity. Aqueous extracts (from flour/water ratios of 1:10, 1:15, 1:3, and 1:2) were treated with 15 ppm H₂O₂ for 15 min at room temperature. Relative viscosity was determined. Analysis of variance and least significant differences were calculated.

Ethanol (95%) extraction. Flour was extracted with 95% ethanol (1:3, w/v) following the preceding procedure except that a single extraction was performed. The ethanol extract was collected and air-dried. The flour was dried and ground as described above. Aqueous extracts were prepared and treated as described previously.

Ethanol (95%) treatment. Flour and 95% ethanol (1:3, w/v)

were stirred 30 min, poured into a pan, and dried overnight without separation. The resulting dry flour was extracted with water and treated as described previously.

Ethyl acetate extraction. Flour was extracted with ethyl acetate (1:3, w/v), under an inert (N₂) atmosphere. The resulting flour was dried under N₂. Aqueous extracts of that flour were treated with 190 purpurogallin units of peroxidase and 15 ppm H₂O₂. Relative viscosity of aqueous extracts was determined.

Ethanol and ethyl acetate flour extraction. Flour was first extracted with 80% ethanol, then extracted with ethyl acetate, and dried. Aqueous extracts were prepared and treated as described above.

RESULTS AND DISCUSSION

Preliminary attempts to reproduce the results reported by Hosene and Faubion (1981) were unsuccessful. Thus, the water-soluble system was reevaluated to identify factors contributing to oxidative gelation.

Ratio of Flour to Water

The viscosity of aqueous extracts increased as the flour/water ratio increased (Fig. 1). At a 1:1.5 flour/water ratio, the relative viscosity of the flour extract (6.39) was five times greater than the relative viscosity of a 1:10 flour/water extract (1.25). The increased relative viscosity reflects the concentration of water-soluble polymers in the aqueous extract (Neukom et al 1967, Moore and Hosene 1990).

Effect of Oxidant Level and Flour/Water Ratio on Gelation

Hosene and Faubion (1981) reported that addition of an oxidant to aqueous extracts (1:10, flour/water) caused a 10% increase in flow time. However, evaluation of several flours, following their procedure, did not result in an increase in viscosity. In addition, Baker et al (1943) reported that 80 ppm H₂O₂ in aqueous extracts inhibited gelation. The oxidant level reportedly used by Hosene and Faubion was 1.25 ml of 30% H₂O₂ per 100 ml of water-solubles (15,000 ppm based on a flour weight of 25 g). We had difficulty removing the excess bubbles by adding catalase and degassing the solution, as outlined in their procedure. It was not clear whether our inability to reproduce their results was because of the low flour/water ratio, the high concentration of H₂O₂, or the excess bubbles formed.

A critical pentosan concentration must be reached before gelation can occur (Baker et al 1943, Neukom and Markwalder 1978, Martinez-Munoz 1985). Evaluation of the effect of oxidant level with a 1:2 flour/water ratio showed that the increase in relative viscosity passed through a maximum as the oxidant level increased (Fig. 2). The relative viscosities of aqueous extracts treated with 15–40 ppm H₂O₂ (based on flour weight) were not significantly different (Table I). Adding higher levels of oxidant caused a decrease in viscosity.

The optimum oxidant level required did not shift as the ratio of flour to water decreased (Table I). No significant increase in viscosity was found at any H₂O₂ level for the 1:10 flour/water ratio. Using the lower H₂O₂ concentrations eliminated the need to add catalase and degas the solution. The relative viscosities between each flour/water ratio were significantly different (Table II). In dilute systems (1:10 and 1:5), the addition of 15 ppm H₂O₂ has a negligible effect, while in concentrated systems, a large increase in viscosity occurred with addition of 15 ppm H₂O₂. Malted Ross flour formed a gel at a 1:1.5 flour/water ratio. A strong viscoelastic gel formed at a flour/water ratio of 1:1.

Effect of Rheologically Active Compounds

Hosene and Faubion (1981) reported that the addition of certain rheologically active compounds limited the oxidative viscosity increases. They reasoned that if the rheologically active compound was involved in the reaction, then addition of that competition in the process should inhibit increases in viscosity.

Esterified ferulic acid is presumably involved in the oxidative gelation of the water-soluble pentosans (Neukom et al 1967). If

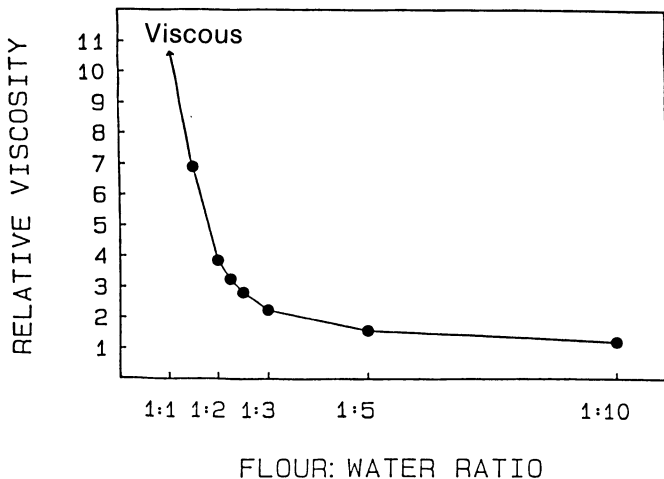


Fig. 1. Effect of flour/water ratio on the relative viscosity of aqueous extracts.

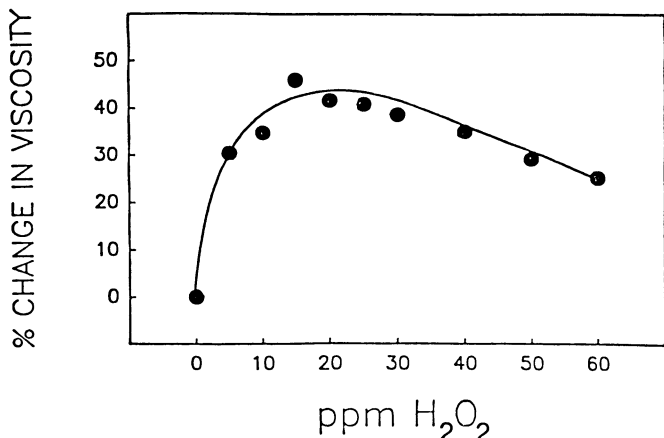


Fig. 2. Effect of oxidant level on the relative viscosity of aqueous extracts prepared at a 1:2 flour/water ratio.

this is true, then addition of free ferulic acid to water-solubles should inhibit increases in viscosity by competing with the less mobile esterified form.

Addition of 250 ppm ferulic acid to the more concentrated extracts (flour/water ratios of 1:3 and 1:2), stopped increases in viscosity (Table III).

To determine whether cross-linking occurs through the aromatic ring or activated double bond, structural analogs of ferulic acid were added to aqueous extracts prior to oxidation. Vanillic acid contains an identical aromatic ring but no activated double bond. Fumaric acid possesses an activated double bond but not an aromatic nucleus.

Addition of 250 ppm vanillic acid inhibited the oxidative increases in viscosity at flour/water ratios of 1:3 and 1:2 (Table II). The increase in viscosity with 250 ppm fumaric acid present was equivalent to that of the H₂O₂ treated control sample. The ability of vanillic acid to inhibit increases in viscosity implicates the aromatic nucleus as the active center. These results contradict those reported by Hoseney and Faubion (1981) but agree with those of Martinez-Muñoz (1985).

TABLE I
Effect of Oxidant Levels on Relative Viscosity at Each Flour/Water Ratio

Hydrogen Peroxide (ppm)	Flour/Water Ratio ^a			
	1:10	1:5	1:3	1:2
0	1.25 a	1.63 b	2.38 c	4.47 g
5	1.63 a	1.72 b	2.70 e	5.84 h
10	1.27 a	1.72 b	2.76 f	6.30 i
15	1.27 a	1.75 b	2.78 f	6.53 j
20	1.27 a	1.75 b	2.75 f	6.34 i
25	1.26 a	1.73 b	2.75 f	6.30 i
30	1.26 a	1.69 b	2.74 f	6.20 i
40	1.24 a	1.67 b	2.71 f	6.05 i
50	1.24 a	1.67 b	2.68 d	5.79 h
60	1.23 a	1.64 b	2.52 f	5.61 h

^a Means followed by different letters are significantly different, both within and between columns (LSD = 0.07, MSE = 0.01).

TABLE II
Effect of Flour/Water Ratio and Oxidation on Relative Viscosity of Aqueous Flour Extracts

Ratio	Control ^a	Hydrogen Peroxide ^a (15 ppm)
1:10	1.25 a	1.27 a
1:5	1.65 b	1.75 b
1:3	2.44 c	2.86 d
1:2.5	2.92 e	3.53 f
1:2.25	3.95 g	4.26 h
1:2	4.33 i	6.25 j
1:1.5	6.39 k	10.84 l
1:1	40.56 m	gel

^a Means followed by different letters are significantly different, both within and between columns (LSD = 0.09, MSE = 0.02).

TABLE III
Effect of Rheologically Active Compounds on Relative Viscosity of Water-Solubles

Ratio	Control	H ₂ O ₂ (15 ppm)	Ferulic Acid (250 ppm)	Fumaric Acid (250 ppm)	Vanillic Acid (250 ppm)	Cysteine (250 ppm)
1:10	1.24 a	1.26 a	1.24 a	1.26 a	1.24 a	1.21 a
1:5	1.58 b	1.71 b	1.62 b	1.69 b	1.56 b	1.62 b
1:3	2.26 c	2.59 d	2.31 c	2.71 d	2.35 c	2.27 c
1:2	3.84 e	5.46 g	4.12 f	5.32 g	4.27 f	3.95 e

^a Means followed by different letters are significantly different, both within and between rows (LSD = 0.17, MSE = 0.04).

Cysteine

Sidhu et al (1980b) showed that ¹⁴C-cysteine became bound to water-soluble pentosans after treatment with ultraviolet light, presumably through reaction with ferulic acid. Addition of 250 ppm cysteine to aqueous extracts stopped gelation (Table III).

From these results, it was not clear whether the cysteine reacted with the ferulic acid in competition with the protein-sulfhydryl or reacted with the H₂O₂ and, thus, inhibited gelation by consuming the oxidant. If cysteine stopped the increase in viscosity by reacting with the H₂O₂, then adding excess H₂O₂ should have resulted in increased viscosity. However, addition of cysteine interfered with increases in viscosity at all H₂O₂ levels (Table IV). This suggests that cysteine might have been interfering with gelation by reacting with the active center of the polymers and preventing the necessary cross-linking.

Effect of Flour Extraction

Martinez-Munoz (1985) found that extracting flour with 80% ethanol lowered the flour/water concentration required to increase viscosity. She concluded that removal of some unidentified interfering substance(s) caused this shift. Free sulfhydryl-containing compounds were implicated as the interfering substance(s).

Flour water-solubles, prepared from flour extracted with 80% ethanol, formed a gel at a flour/water ratio of 1:2 when treated with 15 ppm H₂O₂ (Table V). The ability of the aqueous extract to gel in a less concentrated system suggests that extraction of flour with ethanol removed some inhibitory substance.

If free ferulic acid were naturally present in aqueous extracts, this might explain the required concentrated system for gelation.

TABLE IV
Effect of Increasing Levels of Hydrogen Peroxide at a Constant Cysteine Concentration

Hydrogen Peroxide	Equivalence at 33.8 ppm H ₂ O ₂ per 312 ppm Cysteine	
	Cysteine	Relative Viscosity ^a
1:3 Flour/water ratio ^b		
0	0	2.17 ± 0.11 a
10	0	2.52 ± 0.07 b
10	312	2.25 ± 0.04 a
1:2 Flour/water ratio ^c		
0	0	2.24 ± 0.04 a
30	0	2.50 ± 0.06 b
30	312	2.30 ± 0.04 a
1:1 Flour/water ratio ^c		
0	0	2.17 ± 0.03 a
52	0	2.53 ± 0.03 b
52	312	2.28 ± 0.01 a
1:1 Flour/water ratio ^c		
0	0	2.17 ± 0.02 a
74	0	2.49 ± 0.04 b
74	312	2.27 ± 0.01 a
1:2 Flour/water ratio ^c		
0	0	3.95 ± 0.15 c
7	0	5.64 ± 0.14 d
7	208	4.23 ± 0.20 c
1:1 Flour/water ratio ^c		
0	0	3.83 ± 0.19 c
20	0	5.75 ± 0.31 d
20	208	4.21 ± 0.25 c
1:1 Flour/water ratio ^c		
0	0	3.63 ± 0.34 c
34	0	5.50 ± 0.22 d
34	208	4.03 ± 0.36 c
1:1 Flour/water ratio ^c		
0	0	3.72 ± 0.10 c
49	0	5.34 ± 0.08 d
49	208	4.09 ± 0.19 c

^a Means followed by different letters are significantly different (LSD = 0.39, MSE = 0.05).

^b Equivalence at 33.8 ppm H₂O₂ per 312 ppm cysteine.

^c Equivalence at 22.5 ppm H₂O₂ per 208 ppm cysteine.

TABLE V
Effect of Extracting Flour with 80% Ethanol, Ethyl Acetate,
or a Combination of 80% Ethanol and Ethyl Acetate on Relative Viscosity

Flour/Water	Treatment			
	None	Ethanol	Ethyl Acetate	Ethyl + Ethyl Acetate
1:10				
Control	1.25 a	1.24 a	1.24 a	1.24 a
H ₂ O ₂	1.27 a	1.32 a	1.25 a	1.25 a
1:5				
Control	1.65 b	1.59 b	1.61 b	1.60 b
H ₂ O ₂	1.75 b	1.70 b	1.71 b	1.78 b
1:3				
Control	2.44 c	2.32 c	2.32 c	2.51 c
H ₂ O ₂	2.86 d	3.13 e	2.64 d	3.37 e
1:2				
Control	4.33 f	4.47 f	4.40 f	4.59 f
H ₂ O ₂	6.25 g	gel	6.24 g	gel

^a Means followed by different letters are significantly different, both within and between rows (LSD = 0.40, MSE = 0.14). H₂O₂ = 15 ppm.

TABLE VI
Effect of Different 95% Ethanol Treatments on Relative Viscosity

Treatment	Flour/Water Ratio ^a			
	1:2		1:3	
	Control	H ₂ O ₂ (15 ppm)	Control	H ₂ O ₂ (15 ppm)
Extracted	5.09 a	9.56 c	2.48 d	2.98 e
Stirred	5.11 a	7.49 b	2.50 d	2.90 e
Added extract	5.29 a	7.80 b	2.50 d	3.02 e

^a Means followed by different letters are significantly different, both within and between rows (LSD = 0.41, MSE = 0.22).

Flour was extracted with ethyl acetate to remove any free activated double bond compounds (Jackson 1983). Ethyl acetate extraction of flour did not promote an increase in viscosity (Table V). Flour extracted with both 80% ethanol and ethyl acetate did not show an additional increase in viscosity. Only ethanol extraction achieved removal of the presumed inhibitory substance.

Extraction of flour with 80% ethanol also removed the majority of the gliadin protein fraction. Therefore flour was extracted with 95% ethanol, which does not extract gliadin, to determine whether removal of the gliadin protein fraction removed the inhibitory substance.

Aqueous extracts of flour extracted with 95% ethanol gave (at a 1:2 flour/water ratio) a high viscosity when treated with 15 ppm H₂O₂. Aqueous extracts from flour treated with ethanol, but nothing removed, gave a significantly lower viscosity (Table VI). Thus, the increased viscosity of the 95% ethanol-extracted flour was not a result of exposure to ethanol. Addition of the ethanol extract back to the ethanol-extracted flour, at the same level of extraction, also gave a lower viscosity at a flour/water ratio of 1:2 (Table VI). It appears that addition of the ethanol extract to the aqueous extracts reintroduced some interfering material. The nature of this interfering material was not determined.

SUMMARY

This study clarified some of the contradicting information about the gelation of wheat flour water-solubles. Gelation of aqueous flour extracts requires a concentrated system. Oxidation of dilute aqueous flour extracts did not show a significant increase in

viscosity. The oxidant level used to promote gelation was a critical factor. Excess oxidant appears to cause a decrease in viscosity. Extraction of flour with ethanol decreased the concentration requirement for gelation apparently by removing some interfering substance.

The mechanism of oxidative gelation of wheat flour water-solubles remains unclear. However, the aromatic ring of ferulic acid appears to serve as the cross-linking center for the pentosan polymers. Cysteine appears also to interfere in this reaction. Although the system is better understood, determination of the mechanism of oxidative gelation needs to be explored further.

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