# A Mechanism for the Oxidative Gelation of Wheat Flour Water-Soluble Pentosans[1]

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ABSTRACT

The oxidative gelation of wheat flour water-soluble pentosans was studied with viscometry. Such common oxidants as potassium bromate, potassium iodate, and ascorbic acid did not increase the viscosity of wheat flour water solubles, but hydrogen peroxide, ammonium persulfate, and formamidine disulfide did. Hydrogen peroxide was effective only in the presence of the enzyme peroxidase, which flour contains. The enzyme tyrosinase was also effective in increasing viscosity. Ferulic acid, fumaric acid, and cysteine stopped formation of the gel when hydrogen peroxide was added. Vanillic acid had no effect on gel formation. These results suggest a mechanism involving the addition of a protein radical to the activated double bond of ferulic acid. The ferulic acid is esterified to the arabinoxylan fraction of pentosan. The resultant cross-linking is responsible for the oxidative gelation of wheat flour water solubles. The gelation appears to be responsible for some, but not all, oxidative changes in bread doughs.

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Durham (1925) reported that adding hydrogen peroxide to a flour-water slurry increased the slurry's viscosity. Baker et al (1943) showed that the water-soluble pentosans were responsible for the increases in viscosity. Udy (1956) studied the viscometry of flour-water extracts and concluded that 95% of the intrinsic viscosity was due to the polysaccharides and 5% to the soluble protein, with essentially no viscosity coming from the other soluble components.

Neukom and coworkers (Fausch et al 1963; Geissmann and Neukom 1971, 1973a, 1973b; Markwalder and Neukom 1976; Neukom 1976; Neukom et al 1967) studied water-soluble pentosans and the oxidative gelation phenomena. Their work has been recently reviewed (Neukom and Markwalder 1978), and several possible structures for the gel were suggested.

The work of Fincher et al (1974) and Fincher and Stone (1974) showed that wheat flour water-soluble pentosans are composed primarily of two fractions, an arabinoxylan and a peptide-bound arabinogalactan. Their work clarified the confusing literature on water-soluble pentosans.

Yeh et al (1980) showed ferulic acid to be associated only with the largest molecular weight part of the arabinoxylan fraction and the level of ferulic acid to decrease as dough is overmixed. Sidhu et al (1980b) showed that when a mixture of wheat flour water solubles and $^{14}$C-cysteine was irradiated with ultraviolet light, the cysteine was bound to the water-soluble pentosans.

We investigated the gel formed by wheat flour water-soluble pentosans when hydrogen peroxide was added and looked at effects of certain compounds rheologically active in dough on formation of that gel. From these data, we hoped to learn more about the mechanism of the oxidative gelation reaction.

## MATERIALS AND METHODS

### Materials

*Enzymes.* Bovine liver catalase, E.C. 1.11.1.6 (9,200 units per milligram of protein), type I horseradish peroxidase (94 purpurogallin units per milligram), and mushroom tyrosinase, E.C. 1.14.18.11 (2,230 units per milligram) were obtained from Sigma Chemical Co., St. Louis, MO.

*Chemicals.* Ferulic acid (4-hydroxy-3-methoxycinnamic acid), phenylthiocarbamide (1-phenyl-2-thiourea), formamidine disulfide HCl, and L-cysteine HCl were obtained from Sigma Chemical Co.; trans-cinnamic acid, 3-(*p*-hydroxyphenyl) propionic acid (98%), and vanillic (4-hydroxy,3-methoxybenzoic) acid (97%) were obtained from Aldrich Chemical Co., Milwaukee, WI; reagent grade $H_2O_2$ (30%) and fumaric acid were from Fisher Scientific Co., Fair Lawn, NJ. All other chemicals were reagent grade.

*Flour.* The flour was experimentally milled from a composite of many varieties of hard red winter wheat harvested at a number of locations. The flour, which was not treated, contained 11.5% protein and 0.42% ash.

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## Methods

*Isolation of Wheat Flour Water Solubles.* Flour was slurried with distilled water (1:10, w/v) for 30 min at room temperature. The suspension was stirred every 5 min. The resulting slurry was centrifuged for 15 min at 1,200 rpm (300 × *g*) and the supernate decanted and recentrifuged 10 min at 2,000 rpm (1,000 × *g*). The supernate from this spin was defined as control water solubles and used for subsequent studies.

*Viscosity Measurement.* Viscosities of water-soluble solutions were measured by a Cannon-Fenske type of capillary flow viscometer (ASTM size 50). For each measurement, 5.0 ml of water-soluble solution was placed in a viscometer previously equilibrated to 30 ± 1°C in a constant temperature bath. Solutions to be tested were brought to temperature by a further 5-min equilibration of the loaded viscometer. At the end of this incubation, readings were taken and data recorded as seconds required for a constant volume of test solution to flow through the capillary. At a flow time of 377 sec, the standard deviation was 1.6 sec or 0.4%.

*Gelling Reaction by Hydrogen Peroxide.* Control water solubles (100 ml) were mixed with 1.25 ml of 30% $H_2O_2$ and allowed to react 15 min at room temperature. Then 0.30 ml of catalase (~150,000 units) was added and the solution was stirred and allowed to react 5 min. The numerous small bubbles present after the first two reactions were removed by briefly degassing the test solution under reduced pressure. Oxidized and degassed solutions were tested for viscosity as described above.

*Incubation of Yeast Plus Water Solubles.* Control water solubles (500 ml) were mixed with baker's yeast (Anheuser-Busch, St. Louis, MO) to create a 2% (w/v) mixture and swirled gently to disperse any small clumps. The resulting suspension was allowed to incubate for various times with gentle swirling at 20-min intervals.

For the final 30 min of each incubation, the yeast was allowed to settle from suspension, and then the upper, clear layer was decanted. After this solution was centrifuged 3 min at 400 rpm (40 × *g*), the viscosity of the supernatant was tested. Microscopic examination of the solution after centrifugation revealed no residual yeast cells or cell fragments.

*Boiled Water Solubles.* Aliquots (10 ml) of control water solubles were placed in 20-ml test tubes, loosely stoppered, and placed in a boiling water bath for 10 min. Tubes were cooled to room temperature and adjusted to their original volume before viscosity was measured.

**TABLE I**
**Effect of Hydrogen Peroxide and the Flour-Water Ratio on the Flow Time (sec) of Flour Water Solubles**

| Hydrogen Peroxide (ml/100 ml) | Flour-Water Ratio | |
|---|---|---|
| | 1:10 | 1:5 |
| 0 (control) | 377.9 | 505.0 |
| 0.50 | 389.4 | 524.9 |
| 1.00 | 400.6 | 541.0 |
| 1.25 | 403.0 | 536.1 |
| 1.50 | 413.7 | 541.0 |
| 2.00 | 400.5 | 534.3 |

**TABLE II**
**Effect of Potassium Bromate, Potassium Iodate, Ammonium Persulfate, and Formamidine Disulfide on the Flow Time of Flour Water Solubles**

| Treatment[a] | Flow Time (sec) |
|---|---|
| Control | 377.0 |
| Potassium bromate (40 ppm) | 376.4 |
| Potassium iodate (20 ppm) | 377.7 |
| Ammonium persulfate (50 ppm) | 411.3 |
| Formamidine disulfide | |
| 50 ppm | 382.4 |
| 100 ppm | 408.8 |

[a] Calculation of treatment levels was based on the weight of flour extracted.

**TABLE III**
**Effects of Boiling and of Adding Peroxidase on the Flow Time of Flour Water Solubles**

| Treatment | Flow Time (sec) |
|---|---|
| Control | 364.0 |
| $H_2O_2$ | 398.2 |
| Boiling + $H_2O_2$ | 364.2 |
| Boiling + Peroxidase[a] + $H_2O_2$ | 389.4 |

[a] 1 ml of a 1:50 solution.

**TABLE IV**
**Effects of Certain Rheologically Active Compounds on the Flow Time of Flour Water Solubles**

| Treatment[a] | Flow Time (sec) |
|---|---|
| Control | 371.1 |
| $H_2O_2$ | 395.2 |
| $H_2O_2$ plus | |
| Ferulic acid (250 ppm) | 368.0 |
| Fumaric acid (250 ppm) | 371.2 |
| Vanillic acid (250 ppm) | 386.5 |
| Cysteine (50 ppm) | 361.5 |

[a] Calculation of treatment level was based on the weight of the flour extracted.

## RESULTS AND DISCUSSION

The water-soluble material was extracted from flour and used for viscosity studies with no further purification. Adding $H_2O_2$ to the water solubles increased the viscosity of the solution (flow time increased from 378 to 417 sec), which demonstrates the well-known oxidative gelation phenomenon. Next, the effects of flour-water ratio and of $H_2O_2$ concentration were investigated (Table I). As might be expected, increasing the flour-water ratio increased the flow time at each level of $H_2O_2$. With both flour-water ratios studied, 1.5 ml/100 ml of $H_2O_2$ gave the maximum response.

When we studied the effects of oxidizing agents other than $H_2O_2$ by adding various levels of $KBrO_3$ and $KIO_3$ (Table II), viscosity

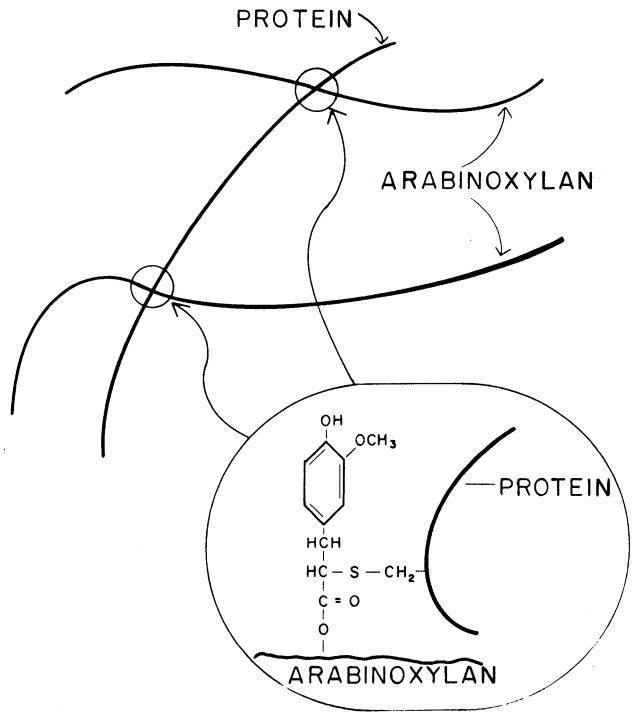


**Fig. 1.** Mechanism of oxidative gelation.

TABLE V
Effect of Tyrosinase on the Flow Time of Flour Water Solubles

| Treatment | Flow Time (sec) |
|---|---|
| Control | 242.8 |
| Tyrosinase (0.5 mg) | 270.8 |
| Tyrosinase (0.5 mg) plus | |
| Ferulic acid | 241.5 |
| Fumaric acid | 232.1 |
| Vanillic acid | 270.5 |
| PTC ($10^{-4}M$) | 247.8 |

TABLE VI
Effect of 2% Yeast on the Flow Time of Flour Water Solubles

| Fermentation Time (hr) | Flow Time (sec) |
|---|---|
| 0 (control) | 224.4 |
| 1 | 224.0 |
| 2 | 233.9 |
| 3 | 234.8 |
| 4 | 240.1 |

did not increase. Nor did it when we added dehydroascorbic acid (data not shown). But two other oxidizing agents, ammonium persulfate and formamidine disulfide, effectively promoted the gelation (Table II). Why did certain oxidants promote the gelation whereas others did not? Ammonium persulfate and formamidine disulfide are known to create free radicals (Sullivan and Dahle 1966), whereas $KBrO_3$, $KIO_3$, and dehydroascorbic acid are not considered to be strong generators of radicals. Hydrogen peroxide is a special case; by itself it does not generate a radical, but in the presence of the enzyme peroxidase it does (Yamazaki 1977). We therefore boiled the system to see whether the enzyme peroxidase was required for hydrogen peroxide to be effective (Table III). Clearly, peroxidase was required. This suggested that a free radical may be required for the gel to form.

Neukom and coworkers had shown that the ferulic acid esterified to the water-soluble pentosan was involved in oxidative gelation of the water-soluble pentosans. Thus, we reasoned that adding ferulic acid that was not esterified to the water-soluble pentosans should stop the increase in viscosity on addition of $H_2O_2$; 250 ppm ferulic acid completely stopped the increase in viscosity (Table IV). Ferulic acid appears to have two active centers that could affect the cross-linking that is presumably involved in the increase in viscosity. One is the aromatic nucleous that Neukom and Markwalder (1978) suggested and the other the activated double bond suggested by the work of Sidhu et al (1980a, 1980b).

To distinguish between those two possibilities, we added 250 ppm of fumaric acid, containing the activated double bond but no aromatic nucleous, and of vanillic acid, containing an aromatic nucleous but no activated double bond. Fumaric acid stopped the increase in viscosity, but the vanillic acid did not (Table IV). Thus, the activated double bond, not the aromatic nucleous, appears to be involved in the gelation reaction.

The next question was: What is added to the activated double bond during the gelation reaction? Sidhu et al (1980b) had shown that $^{14}C$-cysteine was added to the water-soluble pentosan under ultraviolet light, presumably by reacting with ferulic acid. So we first added 50 ppm cysteine to the water solubles and then hydrogen peroxide. The flow time did not increase, indicating that the sulfhydryl group was involved in the cross-linking. This suggests a mechanism of oxidative gelation, as shown in Fig. 1.

Because Japanese workers (Kunivori et al 1976) have shown that tyrosinase (mushroom extract) effectively creates or causes an oxidative effect on dough, we used tyrosinase to see if gelation of the water-soluble pentosans resulted. Adding 0.5 mg of tyrosinase (without hydrogen peroxide) sharply increased viscosity (Table V). Addition of ferulic, fumaric, and vanillic acids separately to the water solubles with tyrosinase gave the same results as each did with hydrogen peroxide. Thus, the reaction appears to be the same. Adding the tyrosinase inhibitor phenylthiocarbamide also stopped the reaction (Table V). The literature suggests that tyrosinase also acts through a free-radical mechanism (Yamazaki 1977).

The enzyme lipoxygenase creates free radicals and also has an oxidizing effect on wheat flour doughs (Frazier et al 1977, Hoseney et al 1980). In the presence of yeast, lipoxygenase loses its rheological (oxidative) effect, whereas yeast alone has an oxidative effect on dough (Hoseney et al 1979). In an effort to determine whether yeast's oxidative effects in dough were related to the gelation phenomenon, we added yeast to the water-soluble fraction and determined viscosity with time (Table VI). Clearly, yeast caused the water solubles to increase in viscosity. The same mechanism may possibly cause the rheological effect of yeast on dough and the oxidative gelation of the water-soluble fraction. Such similarity of mechanism would explain why lipoxygenase in the presence of yeast has no additional rheological effect.

From these results, the oxidative gelation of pentosans appears to involve the addition of a protein thiyl radical to the activated double bond of ferulic acid (or related compounds) esterified to the arabinoxylan fraction of the pentosan. Such covalent grafting of polypeptide and polysaccharide chains would create a multiple cross-linked entity of high molecular weight, which, in the water-solubles system, would be reflected by viscosity increases. In dough, the postulated cross-linking would be reflected in changes in dough's rheological properties.

In this model, the thiyl radical is formed by reagents in native flour systems that create free radicals; ie, $H_2O_2$, peroxidase, ammonium persulfate, formamidine disulfide, tyrosinase, yeast, etc. The necessary radicals are not created by many of the common oxidants used in breadmaking, ie, potassium bromate, potassium iodate, or ascorbic acid. Thus, even if the oxidative gelation explains the rheological effect of some oxidative reagents, it does not explain them all.


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