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/STUDIES ON THE OXIDATIVE GELATION MECHANISM/  
EFFECT OF INHIBITORS, TIME, AND CONCENTRATION OF  
WATER SOLUBLES ON THE RELATIVE VISCOSITY OF  
WHEAT FLOUR WATER SOLUBLE PENTOSANS

by

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INTRODUCTION

Water soluble pentosans are important constituents of wheat flour. They are polysaccharides of pentose sugars. They can bind large amounts of water forming clear viscous solutions. Gums or hydrocolloids disperse in water to give a thickening and sometimes a gelling effect.

The wheat flour water soluble pentosans are composed primarily of two fractions, an arabinoxylan and a peptide bound arabinogalactan. Ferulic acid is known to be associated with the largest molecular weight arabinoxylan fraction. Part of the arabinoxylan fraction react with oxidizing agents to form viscous solutions and gels. The mechanism of this reaction is generally considered to involve ferulic acid. The mechanism, however, is not completely understood.

The water soluble pentosans have been shown (Jelaca and Hlynka (1972), Patil and Finney (1976)) to contribute to bread loaf volume. Bread volume is also improved by oxidation of the dough system. This mechanism of oxidative improvement in dough systems and the mechanism of oxidative gelation of the water solubles could be related. However, this has not been clearly demonstrated. Therefore, it is desirable to have a better understanding of the oxidative gelation mechanism in the hope that it may give information on this complex system.

LITERATURE REVIEW

The oxidative gelation phenomena of wheat flour solubles has been of interest and the subject of many studies throughout the years. This phenomena was initially reported by Durhum (1925). He reported that absorption of flour could be increased when flour and water suspensions were treated with hydrogen peroxide. He found the finer the middlings were ground the higher the viscosity. He also reported a relation between the hardness of wheat and the increase in viscosity, where the harder the wheat the higher the viscosity obtained. He did not identify what water suspension constituents were responsible for the oxidative gelation.

Baker et al (1943) reported that the wash water from a strong gluten as well as the concentrate of flour extracts form gels upon the addition of oxidants. This gel resembled an undisturbed egg white and could be whipped to a stiff foam resembling beaten egg white. However, after resting overnight at room temperature the gel turned into a liquid which would not gel upon subsequent oxidation again. Baker et al (1943) showed that by washing the gluten the properties of the gluten could be altered, indicating that the dough properties were affected by soluble constituents. They isolated the gel forming substance. The substance was

found to be a nonprotein that had an abundance of pentose sugars, indicating that the water soluble pentosans were the gelling ingredient. In the course of their experiments they found that some flour extracts would not gel by any of the methods used. They suggested that certain flours contain an interfering substance to gel formation and the presence of enzymatic activity that broke down the gel.

Ferulic acid, a phenol constituent of the higher plant cell walls, was later found to be involved in the gelation process (Kuendig and Neukom 1961, Faush et al 1963, Geissmann and Neukom 1973). In 1968, Painter and Neukom reported the gel form with a protein-polysaccharide fraction that contained ferulic acid. Morita et al (1974) showed that the gelation reaction could occur without the protein. After a DEAE cellulose chromatography refractionation of a gel forming polysaccharide they found no protein in the gel forming fraction. However, absorption at 280 nm indicated the presence of ferulic acid. Thus Morita demonstrated that protein was not necessarily involved in the gelation reaction. Hosney and Faubion (1981) reported that cysteine stopped the formation of a gel when added to the water solubles prior to the addition of  $H_2O_2$ , indicating that protein was involved in the reaction.

Fincher and Stone (1974) defined more exactly the nature of the water solubles compounds of wheat flour. They found an arabinoxylan and a galactose-containing polysaccharide. Using two different methods they found arabinose, xylose and

galactose to be present in the same preparation in the polysaccharide portion of each preparation on the different methods. They found a large molecular weight arabinoxylan fraction of the polysaccharide associated with a free protein which was insoluble in  $(\text{NH}_4)_2\text{SO}_4$ . They also suggest the association of the low molecular weight polysaccharides and protein, and that this arabinogalactan peptide was soluble in a solution of saturated ammonium sulphate.

Yeh et al (1980) showed by absorption at 290 nm and 320 nm that ferulic acid was associated only with the largest molecular weight arabinoxylan fraction of the polysaccharide. Painter and Neukom (1968) had shown the esterification of ferulic acid to the arabinoxylan. They also showed earlier that the disappearance of the ferulic acid peak after the gel was formed which suggested that ferulic acid was involved in the mechanism of oxidative gelation.

The mechanism of the water soluble gelation phenomena is not yet understood, but a number of theories have been developed. Painter and Neukom (1968) suggested that gelation was brought about by a metal ion chelated by oxidized ferulic acid residues and a polypeptide causing a crosslinkage of the macromolecules. They assumed that there had to be a minimum number of crosslinkages to maintain a gel structure. The divalent metal ion is held in an accessible position so that another chain could attach itself to the other side of the metal ion. This theory was not

substantiated and a new model was developed. In the framework of this new theory Geissmann and Neukom (1971) suggested that gelation was caused by an oxidative coupling of the ferulic acid chains, causing a crosslinkage of polysaccharide chains. Analysis of polyphenols revealed the presence of ferulic acid and also of diferulic acid not present before oxidation. Geissmann and Neukom (1971) explained the presence of diferulic acid as product of the oxidative phenolic coupling of ferulic acid residues adjacent to the arabinoxylan fraction. The diferulic acid crosslink is assumed to be responsible for the formation of the three dimensional gel network. Hosoney and Faubion (1981) proposed a different mechanism to explain the oxidative phenomena. It is a mechanism involving the addition of a protein radical to the activated double bond of ferulic acid. The thiol radical of the protein is bound to the activated double bond of ferulic acid. The ferulic acid is esterified to the arabinoxylan fraction of pentosans and with the resultant crosslinking responsible for the oxidative gelation.

The present study was performed trying to extend and confirm this last theory, and trying to contribute to and extend the knowledge about this phenomena.

## MATERIALS AND METHODS

### Viscometry

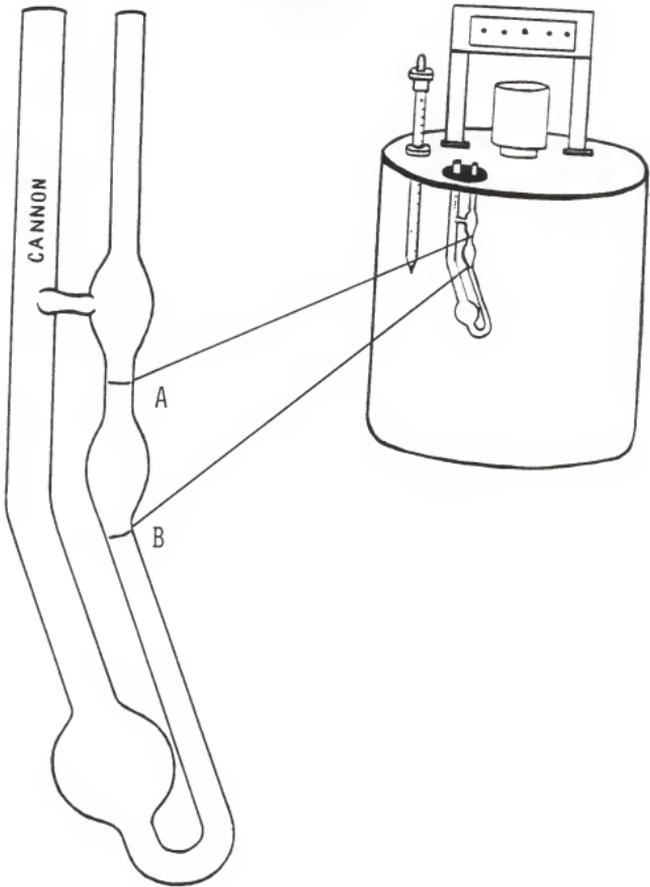
Viscosity measurements of the water solubles (WS) fractions were made using capillary viscometric tubes (Cannon-Fenske) of size 50. The tubes were held in a water bath at  $30 \pm 0.1$  °C.

Each capillary tube was identified by a number and calibrated using water. The tubes were allowed to equilibrate in the water bath for 5 min before any measurements were taken. A sample (5 ml) was pipetted into the tubes and pumped above line "A" (fig 1). The time for the meniscus of the sample to travel from point "A" to point "B" was then determined.

### Flours

4 straight grade flour, patent flour, and 2nd middlings from Kansas State University pilot flour mill as well as a commercial straight grade flour (Ross malted), were used. This gave a broad spectrum of flours.

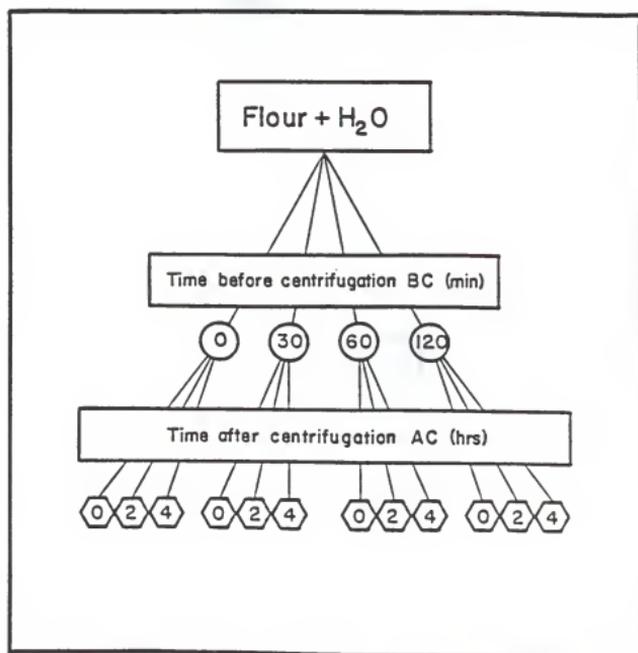
Figure 1. Viscometric tube and viscometer



Extraction of Water Solubles

Water soluble fractions were produced from various water to flour ratios. The ratios used ranged from a dilute 10:1 water to flour to a concentrated 2:1 water to flour. The resulting slurry was allowed to rest and then was centrifuged to recover the WS. The resting periods were classified as before centrifugation (BC) when the flour-water slurry was at rest, and after centrifugation (AC) when the supernatant was at rest, and they consisted of four periods for each classification (fig 2). For the BC the first period labeled "0 time" indicates no waiting interval, the second period labeled "30 time" indicating an interval of 30 min etc. These intervals started from the moment the flour and water were mixed together to the time when the flour slurry was to be centrifuged. The WS fractions were recovered after a 15 min centrifugation at 420xG at which moment the precipitate (ppt) was discarded. These WS fractions were then centrifuged again at 750xG for 10 min. The resulting WS fractions were then used for the measurements. The measurements obtained immediately after this process were followed by measurements from the classification AC where the supernatant was allowed to rest for two hrs in the capillary tube. Measurements were taken thereafter every two hrs for a total of six hrs.

Figure 2. Diagram representing the process of sample preparation. During time before centrifugation the flour slurry was at rest, during time after centrifugation the supernatant was at rest.



### Sample Preparation

The water solubles (WS) fractions from the various water-flour ratios were used as 25 ml samples. Always catalase was used in a mixture of 0.06  $\mu$ l per 25 ml WS after  $H_2O_2$  was added to the prepared sample.

### Preparation of Concentrated Samples

#### Using Baker's Procedure

To prepare concentrated samples 100 g of flour was mixed with 200 ml of cold distilled water for five min in a waring blender at high speed. The batter temperature was kept below 40.5 °C. Air and foam were minimized by placing the Waring blender under a strong flexible plastic bag fastened to a domestic vacuum. The batter thus obtained was centrifuged for 30 min at 420xG to obtain the water solubles.

#### Baker's Procedure to

#### Fractionate Water Solubles

Samples were prepared at a 2:1 water-flour ratio using 100 g flour samples, giving about 125 ml. To those WS fractions 70 g of dry  $(NH_4)_2 SO_4$  was added with stirring. Then the mixture was centrifuged for 15 min at 420xG. After centrifugation a cream-like precipitate was recovered. The supernatant was discarded. A cream-like precipitate, which

was the soluble part, was recovered, sometimes in two phases as shown in fig 3. This creamy material was dissolved in water to give 100 ml total volume.

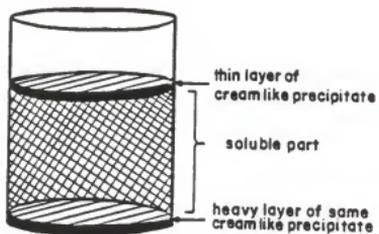
#### Extraction of Flour

Flours were extracted with ethanol (80% V:V) at a 3:1 V:W ethanol-to-flour ratio. The slurry was mixed for 15 min using a magnetic stirrer, after which it was separated using a Buchner funnel. This procedure was repeated for a total of three cycles. The recovered flour was air dried overnight and ground with a Waring blender. This extracted flour was used in the same way as nonextracted flour. To the WS of the extracted flour 50 ppm of peroxidase was added to remove the possibility that ethanol denatured the native peroxidase.

Figure 3. Sample of WS after extraction with  $(\text{NH}_4)_2\text{SO}_4$   
The recovered precipitate manifested itself  
sometimes in two phases

## Water Solubles

extracted with  $(\text{NH}_4)_2\text{SO}_4$



## RESULTS AND DISCUSSION

To confirm the hypothesis of Hoseney and Faubion (1981) concerning the mechanism of oxidative gelation of wheat flour water solubles, their procedure was used. However, we did not obtain results similar to those reported. In general, no significant increase in viscosity was obtained using their procedures. Therefore, a search of the earlier literature on the oxidation phenomena was undertaken.

### Effect of Hydrogen Peroxide on Various Water-Flour Ratios

Baker (1943) reported that no gels were obtained with flours milled from sprouted wheat. This led us to suspect that the inability to reproduce the earlier data may have been the use of malted flour. Therefore, three new flours were selected for use. The chosen flours were a 2nd middlings, a patent flour, and a straight grade flour, all from the KSU pilot flour mill. None of the flours were malted.

Baker's observations were on a water soluble fraction obtained with a 2:1 water-flour ratio. This was much more concentrated than the 10:1 ratio we had used and raised the question of how concentration affected the gelation process.

To answer that question, a series of measurements was taken on extracts from the different flours, and over a series of concentrations. The concentration effect was found to be dramatic (fig 4 and 5). Gelation occurs at a high (1:1) water-flour ratio with the Ross malted flour. No gelation was found with the 2nd middlings flour at the 2:1 ratio. At lower concentrations the different flours behave to give different results. The viscosity depended strongly on the flour-water ratio. If as suggested in Baker's report (1943) sprouted flour extracts did not gel, then the results obtained in this experiment would be unlikely, because the malted flour (Ross malted) did give a gel. With the 2nd middlings flour, at a 10:1 water-flour ratio,  $H_2O_2$  had a small effect. However, the Ross malted flour, at the same water-flour ratio,  $H_2O_2$  had no effect, showing that different flours give different effects.

The 2nd middlings flour was studied further at a concentration of 6:1 (water:flour) where it showed an increase in viscosity as a result of  $H_2O_2$  treatment. Ferulic acid at 250 ppm, 500 ppm and 1000 ppm were used with the assumption that ferulic acid would stop the increase in viscosity. However, the results showed no changes in viscosity (tables 1 and 2). The conclusion drawn from those results was that ferulic acid did not interfere with the oxidation at a concentration of 6:1. Thus, the observed increase in viscosity was due to a different process than the one reported by Hoseney and Faubion (1981) and further

Figure 4. Effect of flour concentration on the relative viscosity of WS of nonextracted Ross flour treated with  $H_2O_2$

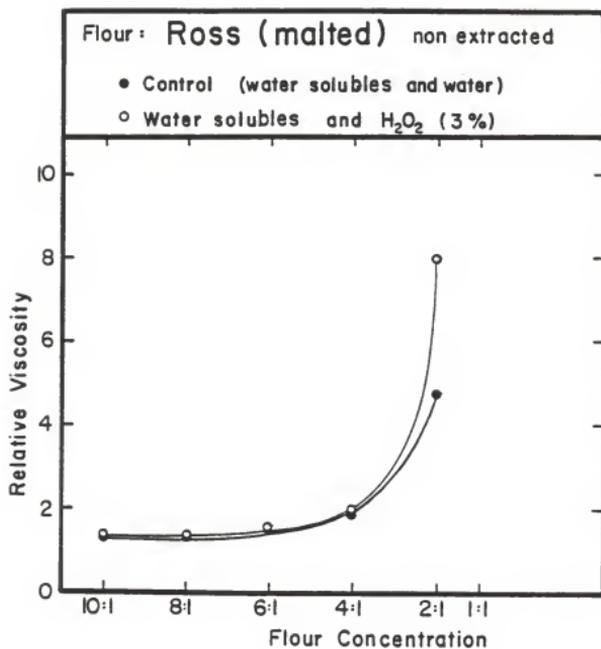


Figure 5. Effect of flour concentration on the relative viscosity of WS of nonextracted 2nd middlings flour treated with  $H_2O_2$

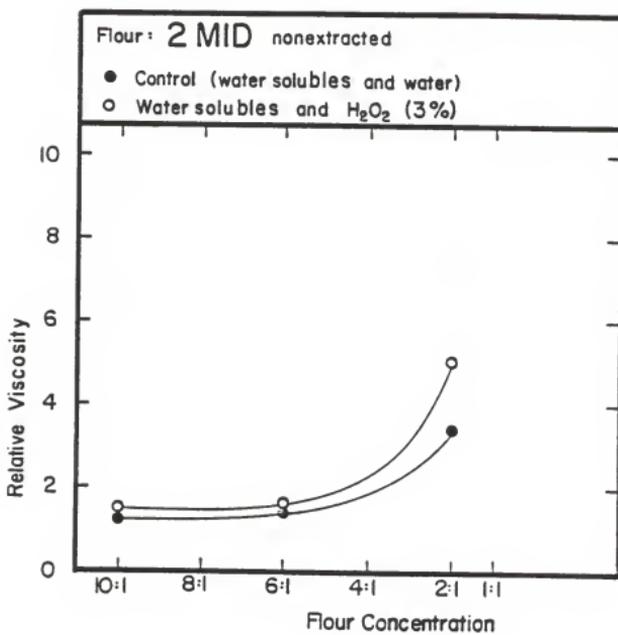


Table 1 : Effect of different concentrations of ferulic acid (FA in ppm) on the water solubles of nonextracted 2nd middlings flour treated and nontreated with  $H_2O_2$   
(x=mean value of relative viscosity,  
s=standard deviation)

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<u>Water Solubles</u>	<u>Relative Viscosity</u>	<u>Standard Deviation</u>
+ $H_2O$	2.10	0.02
+ $H_2O_2$ (3%)	2.42	0.11
+ 250 ppm FA + $H_2O_2$	2.39	0.11
+ $H_2O$	2.28	0.28
+ $H_2O_2$ (3%)	2.40	0.20
+ 500 ppm FA + $H_2O_2$	2.48	0.19
+ $H_2O$	2.06	0.03
+ $H_2O_2$ (3%)	2.28	0.04
+ 1000 ppm FA + $H_2O_2$	2.29	0.05

---

Table 2 : Effect of variuos reagents on the  
relative viscosity of WS of  
nonextracted 2nd middlings flour

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<u>Treatment</u>	<u>Relative Viscosity</u>
WS + Water	1.47
WS + H <sub>2</sub> O <sub>2</sub> (3%)	1.57
Fumaric Acid (250 ppm)	1.56
Vanillic Acid (250 ppm)	1.57
Ferulic Acid (250 ppm)	1.57
L-Cysteine (50 ppm)	1.56

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the results imply that the ferulic acid is not involved in the increase in viscosity.

The effect of added cysteine on the viscosity of extracted WS was also studied. Cysteine (50 ppm) did not interfere with the increase in viscosity as a result of  $H_2O_2$  oxidation at a WS concentration of 6:1. This also is inconsistent with the results of Hoseney and Faubion (1981).

Baker (1943) observed a egg white like gel after treating the WS extract with certain oxidative reagents. To confirm those results his procedure was used. The WS fractions (2:1) collected by his process were used. Three to six drops of  $I_2$  of 0.0520 N were added to a beaker which contained 25 ml of WS and 0.4 ml or 0.6 ml of starch at 1%.  $I_2$  did not cause the WS to gel. Adding excess  $I_2$  did not cause the formation of a gel either.

Between 0.01 g and 0.05 g of  $NaClO_3$  were added to 50 ml of WS fractions in a beaker. None of those concentrations caused the WS fractions to gel. Water was boiled to remove oxygen and then a flour-water slurry was mixed under nitrogen. The resultant WS fraction was then treated with  $I_2$ ,  $H_2O_2$  (3%) and  $NaClO_3$  (20 ppm). None of the samples gave a gel.

D-isoascorbic acid (200 ppm), a reducing agent, was added to water and the water was stirred with flour and mixed by hand for five min, then centrifuged as mentioned above. The WS fraction was treated again with  $I_2$ , 1 ml  $H_2O_2$  (3%) and  $NaClO_3$  (20 ppm). No gelation was observed.

Baker (1943) showed that WS from certain flours did not gel, however, those WS could be caused to gel after they were purified. He used  $(\text{NH}_4)_2\text{SO}_4$  to fractionate the WS fractions and eliminate an interference. Using his procedure we obtained samples that when treated with  $\text{I}_2$  did not form a gel, however, when treated with  $\text{H}_2\text{O}_2$  formed a strong gel. The  $\text{H}_2\text{O}_2$ -treated samples gave egg-white-like gels.

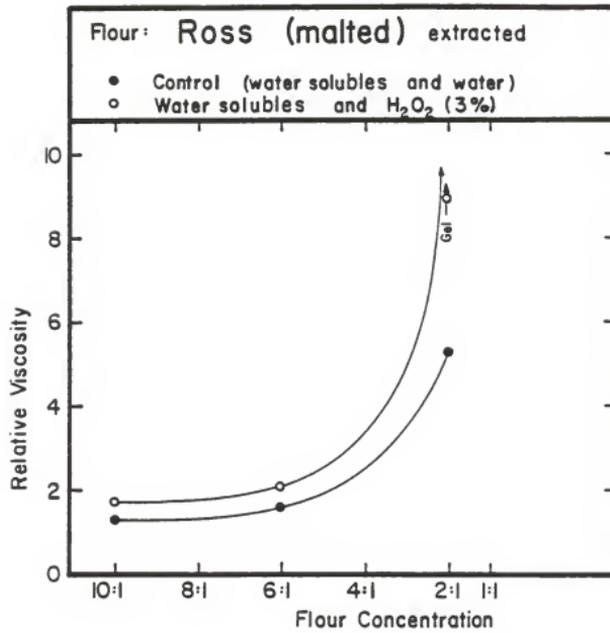
However, the results were based on visual observation. From those results we concluded that there was an interfering substance in flour and that the interference could be eliminated by precipitating protein and pentosans from a soluble material.

#### Influence of Residual Gas Bubbles and Enzymes on the Gelation Process

It was found that extracting flour with 80% ethanol (Fincher and Stone (1974)) was also effective in removing the inhibitor. Ethanol extracted flour was then used to study the effect of WS concentration (fig 5). The water solubles from the extracted flour were similar to those from the nonextracted flour (fig 4). Gelation occurred at a lower concentration of water solubles (2:1) and an increase in viscosity occurred at the lower flour:water ratio upon treatment with  $\text{H}_2\text{O}_2$ .

Those results were evidence for the efficiency of ethanol

Figure 6. Effect of flour concentration on the relative viscosity of WS of with ethanol extracted Ross flour treated with  $H_2O_2$



extraction in removing the interfering materials. The interfering material could now be removed with two different procedures, but we still did not know what it was. These results also indicated the relation between the increase in viscosity and the formation of a gel. Although these results showed that a high viscosity could be achieved with ethanol extracted flour, it was still difficult to obtain reproducible viscosity results. The relatively large error associated with the viscosity readings suggested that other factors were interfering with reproducibility. Most probably this factor was not related to the inhibitor extracted with ethanol. The data given later suggests that the nonreproducible results were because of cleavage by enzymes. A second possibility was that the variability was caused by bubbles resulting from the use of  $H_2O_2$  could not be ruled out.

An experiment was conducted using a WS fraction from a 10:1 water-flour ratio of Ross extracted flour. The WS were boiled with evaporation minimized. After cooling, peroxidase,  $H_2O_2$  (3%), and, after some time, catalase were added to the WS fraction. Viscometric measurements were taken each 5 min for a total of 25 min.

The data (table 3) showed that  $H_2O_2$  increases the relative viscosity of the samples even when the WS were boiled. In the boiled samples treated with  $H_2O_2$  the relative viscosity gradually decreased with time. The large difference between the samples at 5 and 10 min suggests that

Table 3 : Effect of boiling on the relative viscosity of extracted Ross flour, treated and nontreated with  $H_2O_2$ . For the treated sample two measurements were taken with time intervals of 5 min  
( $x$ =mean value of the relative viscosity  
 $s$ =standard deviation)

WS + Water (boiled)

$x = 1.25$

$s = 0.02$

WS + Water (nonboiled)

$x = 1.26$

$s = 0.08$

WS (boiled) +  $H_2O_2$  (3%)

Relative  
Viscosity

Measurement 1

Relative  
Viscosity

Measurement 2

	1st	1.44	1.85
T	2nd	1.38	1.31
I	3rd	1.34	1.28
M	4th	1.31	1.26
E	5th	1.29	1.31

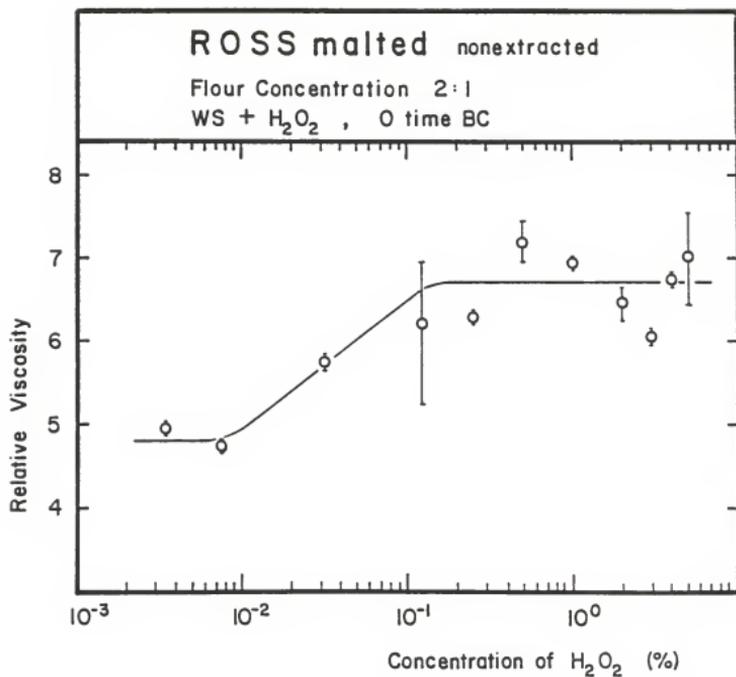
the high viscosity in the 5 min samples could be due to bubbles produced from  $H_2O_2$ . If the increase in viscosity was caused by bubbles from the  $H_2O_2$  breaking down, then with time the bubbles should disappear and the viscosity should be the same as the control. However, the results here were higher than the control even after 25 min. This suggests that there may also be some oxidative increase in viscosity.

To minimize the variability described above, the samples were carefully prepared following a pattern of timed measurements. The procedure was timed using the wetting of the flour as a starting point. The first measurement was after 80 min which gave enough time for the reagents to react and to avoid disturbance by bubbles. The measurements then were performed at 30 min intervals.

Influence of Hydrogen Peroxide  
on Extracted Flours

The effect of  $H_2O_2$  on concentrated WS fractions of extracted flours at a 2:1 water-flour ratio was investigated. As shown in fig 7, the relative viscosity was dependent on the  $H_2O_2$  concentration at concentrations between 0.0035% and 0.125% of  $H_2O_2$ . With  $H_2O_2$  concentration greater than 0.125% the relation between  $H_2O_2$  concentration and viscosity had a zero slope.

Figure 7. Effect of  $H_2O_2$  concentration on the relative viscosity of nonextracted Ross flour, treated with  $H_2O_2$ , at a flour concentration of 2:1



Stopping of the Formation of Gels in Extracted  
and Non-Extracted Flours

A WS extract (2:1 water to flour) from flour was treated with saturated  $(\text{NH}_4)_2\text{S}_2\text{O}_8$ . The precipitate was dissolved in water and treated with 50 ppm L-cysteine. The cysteine appeared to interrupt the formation of a gel.

Viscometric measurements of WS fractions obtained from ethanol extracted straight grade flour, ethanol extracted Ross malted flour, and nonextracted 2nd middlings flour all at concentration of 3:1 showed a small decrease in viscosity, when treated with 50 ppm of L-cysteine, and hydrogen peroxide. The samples were compared with the control samples treated with  $\text{H}_2\text{O}_2$ . This small decrease in viscosity seems to indicate that thiol groups in proteins were involved in the reaction, but that it was not the only mechanism.

Ferulic acid contains an aromatic ring and an activated double bond, the latter was thought to be the bond involved in the oxidation reaction. Hosoney and Faubion (1981) reported that fumaric acid, a reagent containing an activated double bond like ferulic acid, would stop the oxidative reaction, whereas vanillic acid, a reagent containing an aromatic ring like ferulic acid, did not stop the oxidative reaction. To confirm those data the same experiment was performed using a more highly concentrated WS fraction from ethanol extracted flour. The data (table 4)

Table 4 : Effect of various reagents on the relative viscosity of WS of different flours

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	<u>Straight Grade</u>	<u>Ross Malted</u>	<u>2nd Middlings</u>
	<u>Extracted</u>	<u>Extracted</u>	<u>Nonextracted</u>
WS + Water	2.21	2.25	2.12
WS + H <sub>2</sub> O <sub>2</sub> (3%)	2.26	2.94	2.12
Fumaric Acid	2.75	3.40	2.35
Vanillic Acid	2.43	2.68	2.29
Ferulic Acid	2.47	2.65	2.32
L-Cysteine	2.68	2.79	2.25

---

obtained from this experiment seem to indicate that at higher concentration of WS both vanillic and ferulic acid interfered with the oxidative gelation reaction. This suggests that the aromatic ring was involved in the reaction.

If a protein in the oxidative reaction binds at the site of the activated double bond of ferulic acid, then by blocking the thiol groups this reaction would be stopped. The data (table 5) show that NEMI (N-ethylmaleimide) did not interfere with the oxidative reaction. This also suggests that thiol groups are not involved in the oxidative reaction.

The Action of Various Agents with the  
Water Solubles of Extracted Flours at  
High Concentrations

Some oxidative reagents such as  $KBrO_3$  and  $KIO_3$  were also monitored for their influence in highly concentrated WS fractions. Ethanol extracted Ross malted flour at a concentration of 3:1 was used and it was found that  $KBrO_3$  and  $KIO_3$  did not increase the viscosity (table 6).

Table 5 : Effect of NEMI on the relative viscosity of wS of extracted Ross flour. The measurements were taken at intervals of 5 min

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		<u>WS+water</u>	<u>WS+H<sub>2</sub>O<sub>2</sub> (3%)</u>	<u>NEMI+H<sub>2</sub>O<sub>2</sub> (3%)</u>	
		Relative	Relative	Relative	
		Viscosity	Viscosity	Viscosity	
T	↓				
I		1st	2.24	3.18	3.21
M		2nd	2.21	3.02	3.06
E	3rd	2.20	2.94	3.02	

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Table 6 : Effect of  $KIO_3$  on the relative viscosity of WS of extracted Ross flour as compared to samples treated with  $H_2O_2$ . The measurements were taken at intervals of 15 min

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		<u>WS + <math>H_2O_2</math> (3%)</u>	<u>WS + <math>KIO_3</math></u>
T			
I	1st	2.76	2.79
M	2nd	2.66	2.70
E	3rd	2.60	2.61

		<u>WS + <math>H_2O_2</math> (3%)</u>	<u>WS + <math>KBrO_3</math></u>
T			
I	1st	3.05	3.15
M	2nd	2.74	2.73
E	3rd	2.67	2.66

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Influence of Resting Time on  
Water Solubles Gelation

An experiment was set up using both nonextracted and extracted flours at a water-flour ratio of 2:1. The flour used was patent flour from the KSU pilot mill. The reagent used was  $H_2O_2$  (3%). Both time intervals BC and AC were allowed for the samples.

As shown in fig 8a and 8b, at 0 time BC with a nonextracted flour the increase in viscosity as a result of treatment with  $H_2O_2$  was obvious (compare fig 8a and 8b) at a concentration of 2:1, but it was lower than the increase in viscosity for the extracted flour (fig 8c and 8d). The viscosity decreased with increasing time AC. The viscosity increased with time before centrifugation and reached a maximum approximately one hour BC. When comparing both extracted and nonextracted flours a higher viscosity was found with the nonextracted flours.

These results suggest the presence of enzymatic activity that degrades the oxidized viscous solution. Also the increase in viscosity as a result of time before centrifugation suggests that more water soluble pentosans are generated. The insoluble pentosans are broken into smaller molecular weight fractions, which are soluble. This process continues until the insoluble pentosans are solubilized, and a further break down of the molecules results in a decrease of the relative viscosity of the water

solubles. To support this observation fig 9 shows a linear regression of the relative viscosity versus the time AC with samples measured allowing zero time BC.

The results shown in fig. 8c and 8d suggest that the extraction of the flour with 80% ethanol seems to denature the enzymes responsible for the increase in relative viscosity.

Figure 8a. Effect of time before centrifugation (BC) and time after centrifugation (AC) on the relative viscosity of WS of nonextracted patent flour treated with  $H_2O_2$ .

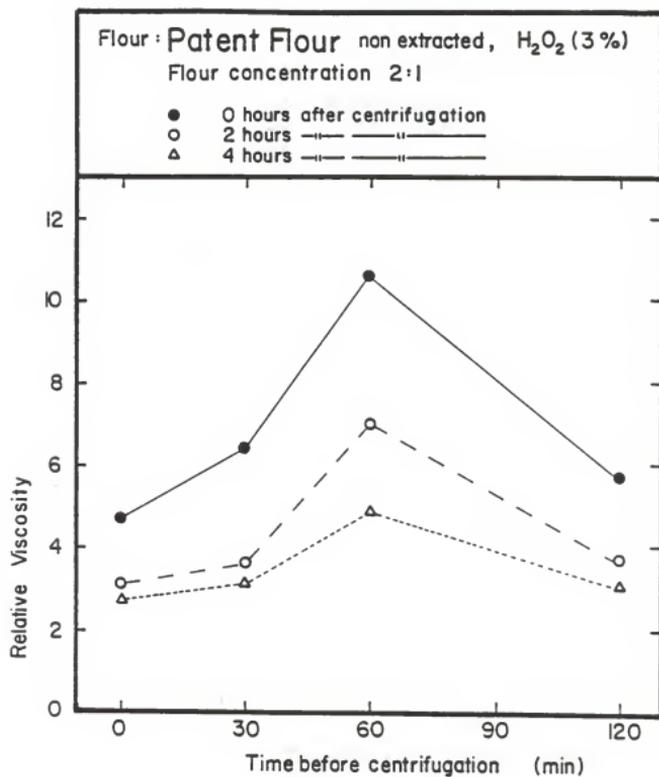


Figure 8b. Effect of time before centrifugation (BC) and time after centrifugation (AC) on the relative viscosity of WS of nonextracted patent flour control

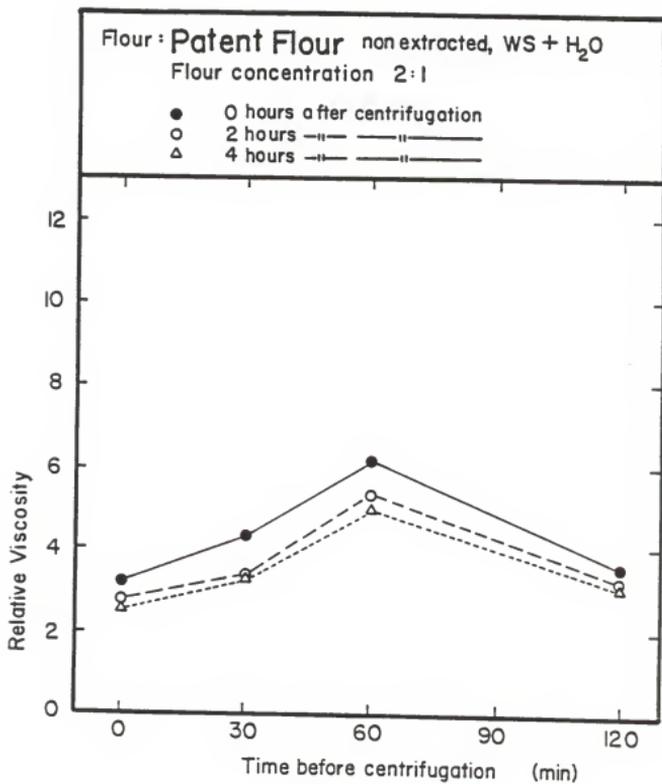


Figure 8c. Effect of time before centrifugation (BC) and time after centrifugation (AC) on the relative viscosity of WS of extracted patent flour treated with  $H_2O_2$

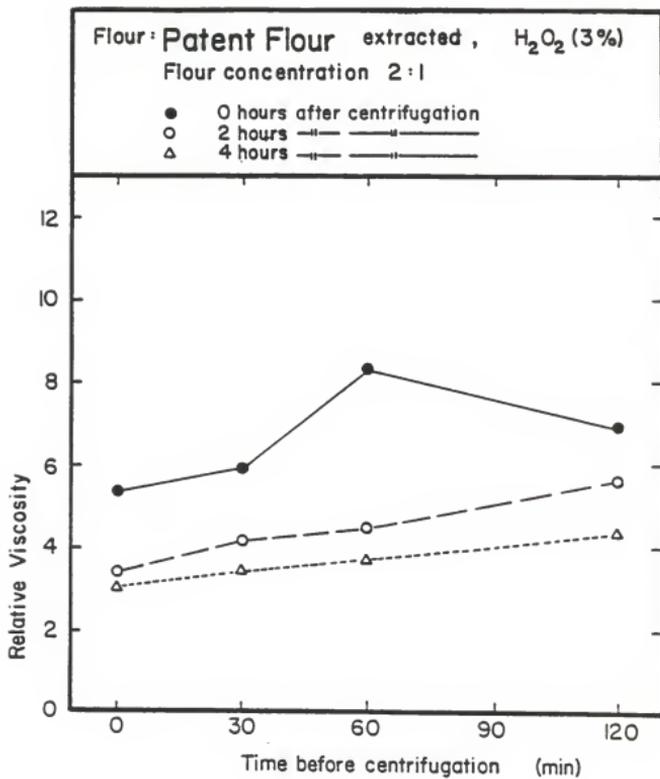


Figure 8d. Effect of time before centrifugation (BC) and time after centrifugation (AC) on the relative viscosity of WS of extracted patent flour control

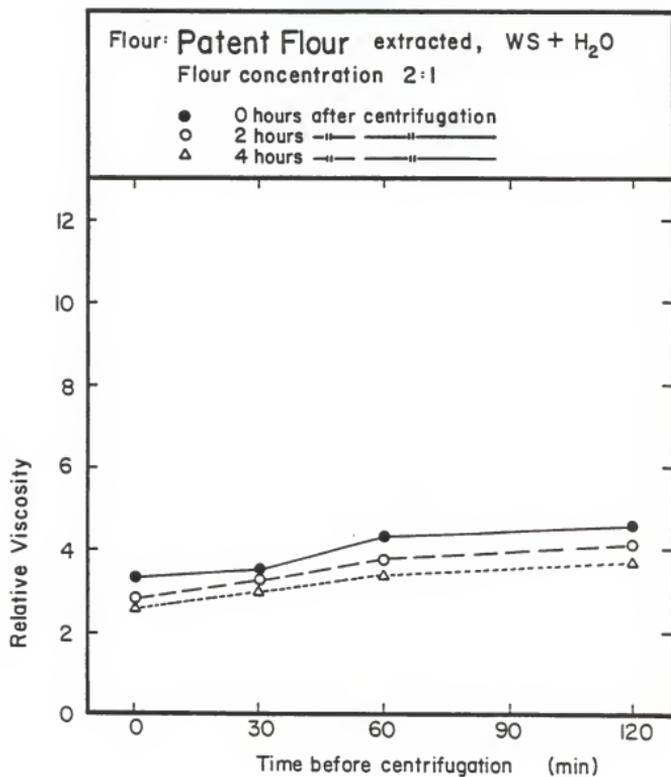
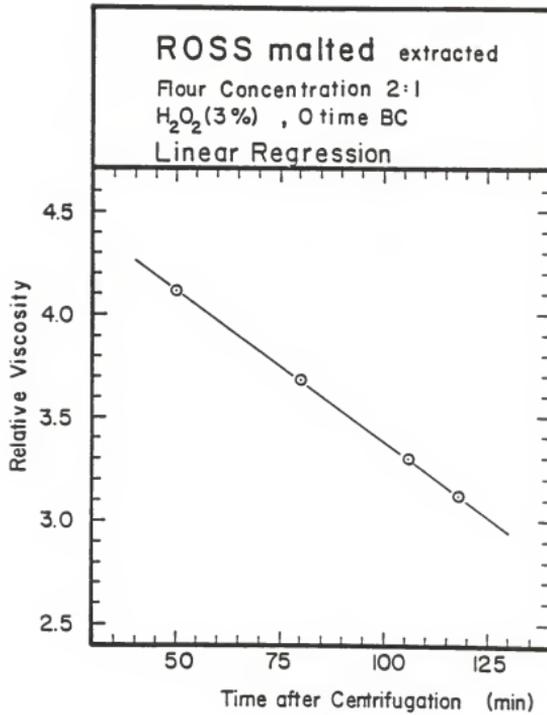


Figure 9. Linear regression of the relative viscosity of WS of extracted Ross flour treated with  $H_2O_2$ , versus time after centrifugation (AC)



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STUDIES ON THE OXIDATIVE GELATION MECHANISM :  
EFFECT OF INHIBITORS, TIME, AND CONCENTRATION OF  
WATER SOLUBLES ON THE RELATIVE VISCOSITY OF  
WHEAT FLOUR WATER SOLUBLE PENTOSANS

by

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AN ABSTRACT OF A MASTER'S THESIS

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Water soluble pentosans have the capability of gelling upon addition of certain oxidants. The gelation and change in viscosity were found to be related to concentration of the water solubles and to be affected by time. This study was based on viscometric measurements. The increase in viscosity and the gelation were found to be related to highly concentrated water solubles. The viscosity of the water solubles changed with the length of time the flour suspension was allowed to rest. Various flours gave slightly different results. At least some flours contained a unidentified substance that interfered with the gelation process. Extraction procedures were used to remove that substance. Ferulic and vanillic acid stop the formation of a gel at high concentration of water solubles. Fumaric acid and cysteine did not have any effect on the gel formation. These results suggest that the aromatic ring and not the activated double bond of ferulic acid are involved in the gelation mechanism. This phenomena is influenced by the flour concentration. The effect of time on the viscosity suggests the possibility of enzymatic activity working on the pentosans.