

Arabinoxylanases and Cellulases of Wheat¹

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ABSTRACT

Enzymes of six varieties of wheat and their mill fractions were assayed by viscosity reduction (endoenzyme) and sugar analysis (exoenzymes) with flour pentosans and carboxymethyl cellulose as substrates, and by *o*-nitrophenyl- β -D-xyloside hydrolysis. Endoactivity on pentosans was mainly in the bran and shorts and absent in all but three of the flours. Arabinose from side chains and xylose from the main chain were produced by enzymes from grain and mill fractions. Both endo- and exocellulases were present in grain and mill fractions. Grain and mill fractions were active on nitrophenyl- β -D-xyloside. Steeping of wheat, which caused a significant decrease in falling number value, did not cause a marked activation of endopentosanase, endocellulase, or xylosidase activity. Hydrolysis of pentosans by enzymes of Selkirk grain first rapidly reduced viscosity and released arabinose sugar, then released xylose sugar.

In wheat flour, water-soluble and water-insoluble pentosans are reported to increase farinograph absorption (1,2) and mixing time (2). Water-soluble pentosans that contained protein are reported to increase loaf volume (3) and tailings that contained water-insoluble pentosans decreased loaf volume (1). Cawley (4) reported that the endogenous enzymes of flour and pentosanase of snail juice eliminated loaf volume improvement of water-solubles of flour. Kulp (1) reported that enzymolysis of the tailings fraction with commercial fungal pentosanases, which also had carboxymethyl cellulase and protease activity, decreased farinograph absorption and the loaf volume decreasing effect of the

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tailings fraction. Kulp also suggested that a synergistic attack of both pentosanases and cellulases may be required to degrade the tailings fraction because pentosans and cellulose of this fraction may be chemically or physically entangled.

Recently Lee and Ronalds (5) reported that enzymes of an Australian flour reduced the viscosity of crude flour gum, carboxymethyl cellulose, and guaran (galactomannan) solutions but caused only a small drop in viscosity of a solution of purified flour arabinoxylan. Also the extract hydrolyzed a number of nitrophenyl glycosides including nitrophenyl- β -D-xyloside used in our study to measure β -xylosidase activity.

This paper reports on the exo- and endo-arabinoxylanase and carboxymethyl cellulase activity, and the β -D-xylosidase activity of wheat grain and mill fractions of three hard red spring, one hard red winter, and two durum wheat cultivars. The distribution of the different enzyme activities in the wheat kernel and the possible importance of the different enzymes in doughs are indicated by the results.

MATERIALS AND METHODS

The hard red spring cultivars Justin, ND-33, and Selkirk and the durum cultivars Leeds and Wells were grown in 1967 in North Dakota. A hard red winter cultivar Cheyenne was grown in 1967 in Wyoming. Samples were milled on a Buhler experimental mill to flour, bran, and shorts (6). Bran was ground on a Udy cyclone hammer mill (Udy Analyzer Co., Boulder, Colo.) using a 0.040-in. screen. Whole wheat was ground on a Labconco mill (Laboratory Construction Co., Kansas City, Mo.) at a No. 15 setting. Protein content of the samples is given in Table I.

The carboxymethyl cellulose used as substrate was 0.42 degree of substitution from Hercules Inc., Wilmington, Dela. *o*-Nitrophenyl- β -D-xylopyranoside was from Pierce Chemical Co., Rockford, Ill.

Arabinoxylan was isolated from wheat flour by a modification (7) of the method of Simpson (8). Substrate prepared by this method was used for the whole wheat and mill fraction studies. A substrate prepared by a method published by Kulp (1) at about the end of this study was used in a hydrolysis-time study. This substrate produced a more viscous solution but contained more protein (2.0% compared to 1% in the earlier method of preparation). Arabinoxylan substrate prepared by the Simpson method is reported to contain an arabinose:xylose ratio of 1:1.63 (9) and substrate by Kulp a ratio of 1:1.72 (1).

For thin-layer analysis adsorbosil No. 3 adsorbent, a silica gel with 10% MgSiO₃ binder, was purchased from Applied Science Laboratories, State College, Pa. Standard sugars were obtained from Mann Research Lab., New York, N.Y.

Analytical Methods

Protein in extracts was determined by a biuret method (10). The standard curve was prepared from a lyophilized water-soluble extract of flour of known protein content (Kjeldahl analysis). Protein on wheat and mill fractions was determined by a standard micro- or macro-Kjeldahl procedure (6) using a factor of 5.7 to convert nitrogen to protein.

TABLE I. PROTEIN CONTENT OF SAMPLES

Cultivar	Mill Fraction	Protein % d.b.	Cultivar	Mill Fraction	Protein % d.b.
Selkirk	Grain	17.9	Cheyenne	Grain	15.9
	Flour	15.8		Flour	14.6
	Bran	20.7		Bran	20.0
	Shorts	19.4		Shorts	19.1
Justin	Grain	16.3	Leeds	Grain	17.4
	Flour	15.1		Flour	16.8
	Bran	18.4		Bran	18.4
	Shorts	19.2		Shorts	20.1
ND-33	Grain	20.1	Wells	Grain	16.7
	Flour	17.0		Flour	15.8
	Bran	25.3		Bran	18.0
	Shorts	23.6		Shorts	20.7

Moisture on wheat samples was determined on a model 919 Motomco moisture meter, and on mill fractions the 130° C. air oven method was used (6).

Enzyme Activity

Whole wheat or flour (125 g.) and bran or shorts (75 g.) and three ice cubes containing 0.6% NaCl were blended with 250 ml. of 0.6% NaCl for 3 min. in a Waring Blendor. After centrifugation at $8,500 \times g$ for 30 min. at 5° C., the resulting supernatant was dialyzed at 5° C. for 24 hr. at pH 5.0 against 4 liters of 0.02M sodium acetate buffer containing 0.6% NaCl.

Enzyme assays were done in duplicate. For activity on flour arabinoxylan, 1 ml. dialyzed extract was incubated for 10 hr. at 37° C. with 5 ml. of arabinoxylan (10 mg. per ml. in 0.02M sodium acetate buffer, pH 5.0, containing 0.6% NaCl). Controls containing 1 ml. of heated extract (20 min. boiling water) and 5 ml. substrate were also incubated 10 hr. Two drops of toluene was used to prevent growth of microorganisms. After incubation the sample and control tubes were heated for 20 min. in a boiling water bath.

For cellulase activity, 1 ml. of dialyzed extract was incubated at 37° C. with 5 ml. carboxymethyl cellulose (0.9 mg. per ml. in 0.02M sodium acetate buffer, pH 5.0, containing 0.6% NaCl) for 1 hr. to measure viscosity reduction and for 10 hr. to measure sugar liberation. One-hour incubation was used for viscosity reduction to obtain zero-order kinetic. Controls and heat treatment at the end of incubation were as described for the arabinoxylan substrate.

Prior to viscosity measurements, insoluble foreign material was removed by filtration through fiber-glass discs (Udy filter assembly). Viscosities were determined on 4-ml. aliquots of hydrolysates and controls at $25^\circ \pm 0.2^\circ \text{C.}$ in Ostwald flow-type viscometers of 2 ml. (min.) sample size. Flow time of buffer alone was 80 to 100 sec. The decrease in specific viscosity per hour was used as an estimate of endoenzyme activity and this change in viscosity was predominantly of zero-order kinetics for both arabinoxylanase and cellulase activity. The standard error of the mean was calculated to be ± 0.04 decrease in η sp. per hr. per g. enzyme source $\times 10^{-2}$.

For estimating exoenzyme activity, analysis for sugars liberated by the enzyme extract was done by a new thin-layer method. A 4-ml. aliquot of the hydrolysate

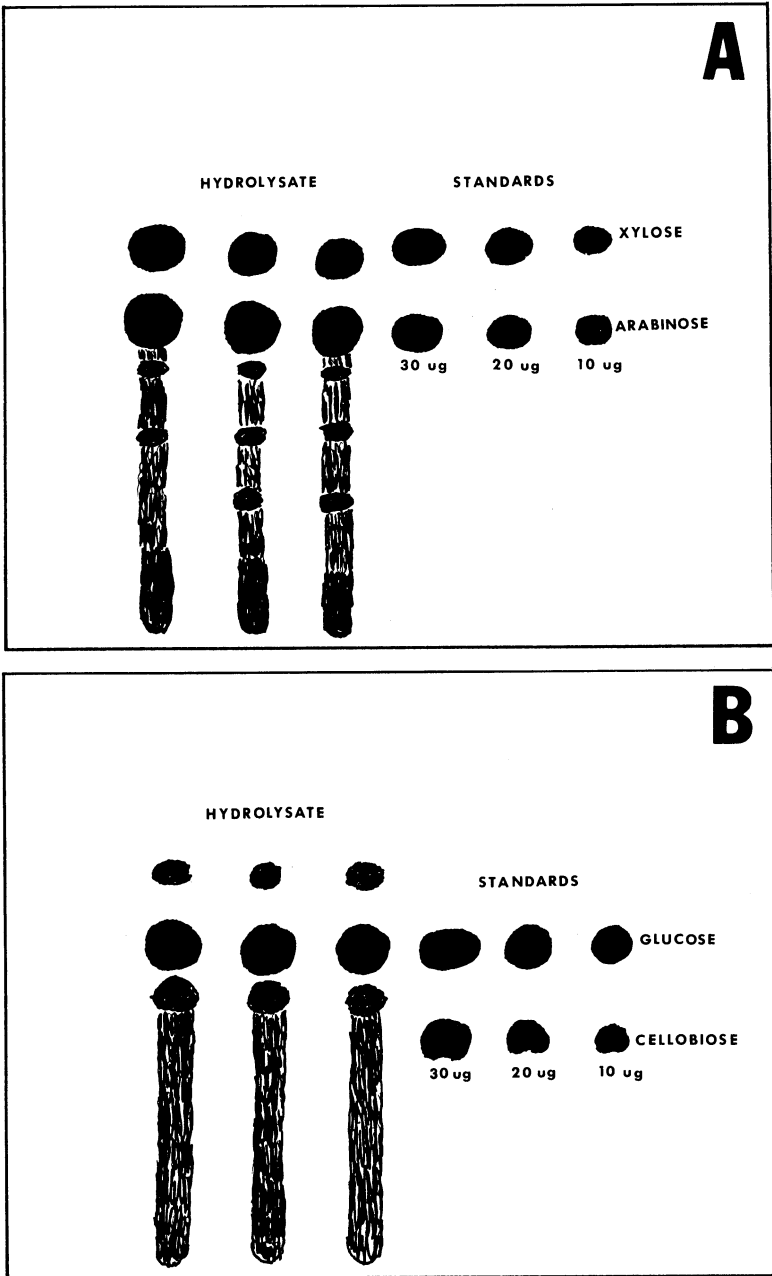


Fig. 1. Sugar standards and replicate sugar analysis on hydrolysates produced by: A, bran enzymes from Wells variety of wheat acting on arabinoxylan substrate, and B, shorts enzyme from ND-33 variety of wheat acting on carboxymethyl cellulose substrate. Chromatograms were developed with n-propanol:H₂O 7:1.5 (v./v.) and the sugars visualized with diphenylamine-aniline-phosphoric acid spray.

was evaporated to dryness under vacuum on a rotary evaporator while heating with hot water. Several drops of 1-octanol was added to prevent foaming. A small amount of distilled water was added to dissolve the solids and then 15 ml. of 1:1 (v./v.) chloroform:methanol solution was added to precipitate unhydrolyzed pentosan and protein. After removing and washing the precipitate on a glass disc filter assembly (Udy Analyzer Co., Boulder, Colo.), the chloroform:methanol solution was evaporated to dryness. To deionize the hydrolysate, the solids were dissolved in 15 ml. 70% (v./v.) ethanol and stirred 15 min. with an ion-exchange resin mixture of approximately 500 mg. of both Amberlite IR-120 and IR-45 resin. When this step was omitted, a white area that interferes with separation of sugars appeared on the thin-layer chromatograms. The resin was removed by filtration, washed with 70% ethanol, and the combined filtrate and washings were evaporated to dryness. The solid material containing the sugars was dissolved in 0.3 ml. 70% ethanol for application on the thin-layer plates.

A number of adsorbents and solvents were tested for separation of arabinose

TABLE II. ENDOZYME ACTIVITY OF WHEAT GRAIN

Cultivar	Viscosity Decrease			
	Arabinoxylan η sp./hr./g. $\times 10^{-2}$		Carboxymethyl cellulose η sp./hr./g.	
	Sample basis	Protein basis	Sample basis	Protein basis
Selkirk	4.8	27	2.5	14
Justin	0.6	4	2.2	13
ND-33	0.3	1	2.3	11
Cheyenne	2.4	15	2.4	15
Leeds	1.5	9	2.5	14
Wells	1.4	8	2.4	14

TABLE III. EXOZYME ACTIVITY OF WHEAT GRAIN

Cultivar	Arabinoxylanase		Carboxymethyl Cellulase	
	Arabinose	Xylose	Glucose	Cellulose
	μ mole/min./g. sample $\times 10^{-2}$		μ mole/min./g. sample $\times 10^{-2}$	
Selkirk	4.7	2.8	6.2	trace
Justin	2.1	1.3	4.6	trace
ND-33	3.8	2.2	3.2	trace
Cheyenne	5.0	1.4	1.7	0.4
Leeds	3.2	1.8	4.0	trace
Wells	5.7	1.3	1.7	trace
	μ mole/min./g. protein $\times 10^{-2}$		μ mole/min./g. protein $\times 10^{-2}$	
Selkirk	26	16	35	trace
Justin	13	8	28	trace
ND-33	19	11	16	trace
Cheyenne	31	9	11	3
Leeds	18	10	23	trace
Wells	34	8	10	trace

from xylose and glucose from cellobiose. The best results were obtained with a silica gel adsorbent containing 10% MgSiO₃ binder (adsorbisol No. 3, Applied Science Lab., State College, Pa.) and a solvent system of ethyl acetate:ethanol:water 50:40:13 (v./v./v.) or 1-propanol:water 7:1.5 (v./v.). The adsorbent was applied as a 0.25-mm. slurry in 0.02M acetate buffer (pH 5); after air-drying the adsorbent was activated at 125°C. for 1 hr. The first solvent, ethyl acetate:ethanol:water, required 5 hr. for two developments on 20×20-cm. plates while the latter solvent of propanol:water required 10 hr. for two developments. During hot, humid summer days, the propanol:water solvent had to be used because of erratic results with the other solvent.

TABLE IV. ACTIVITY OF WHEAT GRAIN ENZYME ON *o*-NITROPHENYL- β -D-XYLOPYRANOSE

Cultivar	Activity, $\mu\text{mole xylose/min./g.} \times 10^{-2}$	
	Sample basis	Protein basis
Selkirk	3.7	21
Justin	4.0	25
ND-33	4.4	22
Cheyenne	4.4	28
Leeds	4.1	24
Wells	6.9	41

TABLE V. ENDOENZYME ACTIVITIES OF WHEAT MILL FRACTIONS

Cultivar	Mill Fraction	Viscosity Decrease			
		Arabinoxylan $\eta \text{ sp./hr./g.} \times 10^{-2}$		Carboxymethyl cellulose, $\eta \text{ sp./hr./g.}$	
		Sample basis	Protein basis	Sample basis	Protein basis
Selkirk	Flour	0.0	0	2.4	15
	Bran	6.0	29	3.3	16
	Shorts	6.1	31	4.2	22
Justin	Flour	0.0	0	2.5	16
	Bran	7.3	40	3.4	18
	Shorts	4.3	22	3.9	20
ND-33	Flour	0.0	0	2.3	14
	Bran	3.7	15	3.7	15
	Shorts	3.8	16	4.2	18
Cheyenne	Flour	0.3	2	2.4	16
	Bran	0.5	3	3.2	16
	Shorts	2.7	14	3.9	20
Leeds	Flour	0.3	2	2.6	15
	Bran	5.3	29	4.1	22
	Shorts	3.0	15	4.5	22
Wells	Flour	0.0	0	2.4	15
	Bran	3.4	19	3.3	18
	Shorts	2.9	14	4.4	21

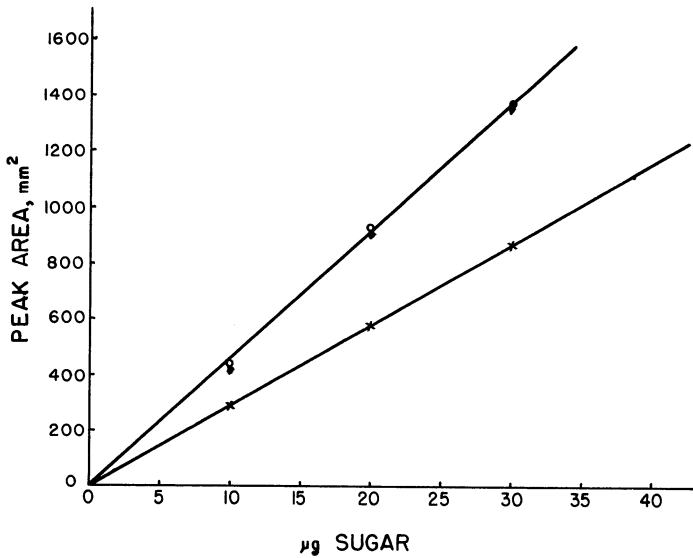


Fig. 2. Standard curves for thin-layer sugar analysis. Top curve= arabinose and xylose; bottom curve = glucose.

TABLE VI. EXOARABINOXYLANASE ACTIVITY OF WHEAT MILL FRACTIONS

Cultivar	Mill Fraction	$\mu\text{moles}/\text{min.}/\text{g. sample} \times 10^{-2}$		$\mu\text{moles}/\text{min.}/\text{g. protein} \times 10^{-2}$	
		Sample Bases		Protein Bases	
		Arabinose	Xylose	Arabinose	Xylose
Selkirk	Flour	1.6	2.9	10	18
	Bran	4.1	4.7	20	23
	Shorts	5.1	7.2	26	37
Justin	Flour	3.9	1.6	26	11
	Bran	10.0	6.2	54	34
	Shorts	7.9	4.5	41	23
ND-33	Flour	4.7	1.6	28	9
	Bran	10.3	4.6	41	18
	Shorts	5.6	7.5	24	32
Cheyenne	Flour	2.8	1.9	19	13
	Bran	16.0	6.4	80	32
	Shorts	19.0	8.2	99	43
Leeds	Flour	2.3	3.0	14	18
	Bran	6.1	2.4	33	13
	Shorts	7.7	2.4	38	12
Wells	Flour	4.5	2.5	28	16
	Bran	14.0	7.0	78	39
	Shorts	7.0	5.9	36	28

To visualize the sugars in early work, the thin-layer plate was placed in iodine vapors for 2.5 min., allowed to set in air for 2.5 min., and then sprayed with 1% soluble starch (11). The sugars yielded geometrically uniform spots on the thin-layer plates and the area under curves obtained with a densitometer were linear with sugar concentration. Fading of the dark blue spots with time and variations among plates was corrected by a standard curve run on each plate. In the latter part of our study, the diphenylamine-aniline-phosphoric acid spray was used that was found by DeStefanis and Ponte (12) to give good results. This spray gave uniform spots (Fig. 1A and 1B), did not fade with time, and gave linear standard curves (Fig. 2). The liberation of arabinose from arabinoxylan and glucose from carboxymethyl cellulose was predominantly linear with the 10-hr. incubation time. The liberation of xylose from arabinoxylan substrate was not linear because of an initial delay before liberation began, whereas the amount of cellobiose from carboxymethyl cellulose substrate with 10-hr. incubation was usually only a trace, presumably because of hydrolysis of this sugar by cellobiase (13). The standard error of the mean for this analysis was calculated to be ± 0.56 μ moles sugar per min. per g. enzyme source $\times 10^{-2}$.

The β -D-xylosidase activity was determined by incubating 1 ml. of dialyzed extract with 1 μ mole *o*-nitrophenyl- β -D-xylopyranose (in 4.0 ml. 0.02M sodium acetate buffer, pH 5.0) at 37°C. and the light absorption of liberated *o*-nitrophenol was determined at 10- or 20-min. intervals in a 0.75-in. tube at 380 nm. on a Spectronic 20 spectrophotometer. After an initially rapid burst of activity, the reaction followed zero-order kinetics, and the rate under the latter condition was determined graphically. Standard error of the mean was calculated to be 0.25 μ moles nitrophenol per min. per g. enzyme source $\times 10^{-2}$.

All enzyme activities are expressed on a dry sample weight basis and sample protein basis. Results are an average of duplicate analysis, except for β -xylosidase activity which are an average of triplicate analysis.

RESULTS AND DISCUSSION

Activity of Wheat Grain

Reduction of viscosity of a polysaccharide solution was considered in this study to be due mainly to endoenzymes that are fragmenting the large substrate molecules. Endoenzyme activity of grain is indicated by reductions in viscosity of arabinoxylan and carboxymethyl cellulose (CMC) solutions (Table II). A marked difference in endoarabinoxylanase activity was found between cultivars on both a sample and protein basis, while the activity on CMC was rather constant between cultivars. Kulp (1) has reported low levels of endoarabinoxylanase and endocarboxymethyl cellulase activity in a sample of wheat.

From arabinoxylan, the extent of hydrolysis of the arabinose side chains and xylose units from the main xylose chains is indicated by the results in Table III. The arabinose side chains are hydrolyzed more extensively than the xylose units from the chain. Higher rate of release of arabinose is not, however, always accompanied by large xylose hydrolysis.

Enzymes of wheat, rye, oats, maize, and barley grain are reported by Preece and MacDougall (14) to hydrolyze rye arabinoxylan by first linearly releasing arabinose side-chains and then after 15 hr. simultaneously releasing linearly

TABLE VII. EXOCARBOXYMETHYL CELLULASE ACTIVITY OF MILL FRACTIONS

Cultivar	Mill Fraction	$\mu\text{mole}/\text{min.}/\text{g. sample} \times 10^{-2}$		$\mu\text{mole}/\text{min.}/\text{g. protein} \times 10^{-2}$	
		Sample Basis		Protein Basis	
		Glucose	Cellobiose	Glucose	Cellobiose
Selkirk	Flour	4.4	0.7	28	4
	Bran	1.5	trace	7	trace
	Shorts	2.2	trace	11	trace
Justin	Flour	1.2	0.7	8	5
	Bran	3.6	trace	20	trace
	Shorts	1.8	trace	9	trace
ND-33	Flour	7.5	2.5	44	15
	Bran	4.6	trace	18	trace
	Shorts	4.5	trace	19	trace
Cheyenne	Flour	1.0	0.9	7	6
	Bran	15.0	trace	75	trace
	Shorts	18.0	trace	94	trace
Leeds	Flour	2.9	0.5	17	3
	Bran	3.9	trace	21	trace
	Shorts	2.8	trace	14	trace
Wells	Flour	8.9	2.1	56	13
	Bran	2.5	trace	14	trace
	Shorts	2.0	trace	10	trace

xylose from the main chain. The acetone precipitate of wheat used by them as an enzyme preparation was calculated to be four- to tenfold lower in arabinase activity ($0.6 \mu\text{moles}$ arabinose per min. per g. of wheat $\times 10^{-2}$) than observed here (Table III) for an extract. Xylobiose and other xylose oligosaccharides in our hydrolysates were indicated by unidentified sugars that migrate slower than xylose and arabinose on thin-layer plates.

The exocarboxymethyl cellulase activity (Table III) varied considerably between cultivars and produced mainly glucose. Cellobiase enzyme in the digest may have hydrolyzed most of the cellobiose, if produced. Preece et al. (13) report abundant cellobiase activity in wheat grain.

The activity on *o*-nitrophenyl- β -D-xylopyranose (Table IV, β -xylosidase activity) was rather constant between varieties, except in Wells variety where the activity was significantly higher. This activity is usually considered to be exoxylanase.

The above wheat enzyme activities on flour arabinoxylan are low in comparison to a commercial fungal enzyme (Rhozyme HP-150, Rohm and Haas Co.) which was on a weight basis over 1,000 times more active in reducing the viscosity of arabinoxylan, and about 100 times more active in hydrolyzing *o*-nitrophenyl- β -D-xylopyranose. The low activity in wheat, however, may well be enough to cause significant changes in the properties of pentosans of flour and affect dough and baking results.

Activity of Mill Fractions

Endoarabinoxylanase activity was absent or low in wheat flour (Table V). Low

TABLE VIII. ACTIVITY MILL FRACTIONS ON
o-NITROPHENYL- β -XYLOPYRANOSE

Cultivar	Mill Fraction	$\mu\text{mole xylose}/\text{min.}/\text{g.} \times 10^{-2}$	
		Sample Basis	Protein Basis
Justin	Flour	1.1	7
ND-33	Flour	1.8	9
Cheyenne	Flour	3.3	23
	Bran	4.0	20
Leeds	Shorts	7.9	41
	Flour	3.0	18
	Bran	6.0	33
Wells	Shorts	7.8	39
	Flour	4.6	29
	Bran	7.2	40
	Shorts	9.4	45

TABLE IX. ENDOENZYME AND β -D-XYLOSIDASE ACTIVITY
OF STEEPED WHEAT

Cultivar	Steep Time hr.	Falling Number (units)	Decrease in $\eta_{sp.}/\text{hr.}/\text{g.} \times 10^{-2}$		$\mu\text{mole xylose}/\text{min.}/\text{g.} \times 10^{-2}$
			Arabinoxylan	Carboxymethyl cellulose	<i>o</i> -Nitrophenyl- β -D-xylopyranose
Selkirk	0	394	4.3	290	...
	5.0	334	0.0	265	7.6
	10.7	212	0.0	259	8.7
	20.0	184	0.0	302	5.9
	30.0	153	0.0	291	5.7
Leeds	0	489	3.6	289	...
	5.0	482	1.3	294	6.1
	10.7	453	1.5	291	7.8
	20.0	438	1.4	291	5.8
	30.0	259	1.6	259	5.9

activity in flour has also been reported by other workers (1,5). In contrast to our results, however, Kulp (1) reports about threefold higher activity in flour than in whole wheat. In all cases, flour was much lower in activity than the corresponding whole wheat (Table II). The bran and shorts mill fractions contained almost all the endoarabinoxylanase activity.

Our results indicate that the large pentosan molecules in white flour would be slowly fragmented by natural flour enzymes during baking, but more of a breakdown of pentosans would be expected in whole-wheat dough with a possible decrease in water absorption and increase in dough stickiness.

Exoarabinoxylanase activity (Table VI) was found in all mill fractions but this activity was significantly higher in the bran and/or shorts. Generally, more arabinose than xylose was liberated by enzymes of mill fractions. Removal of arabinose side chains of pentosans can cause a decrease in solubility (15).

Endocellulase (Table V) was most active in the bran and shorts but flour was also active. Exocellulase (Table VII) produced mainly glucose but flour also produced a significant amount of cellobiose. Enzymes producing glucose were not concentrated in any particular mill fraction. As noted earlier, the role of

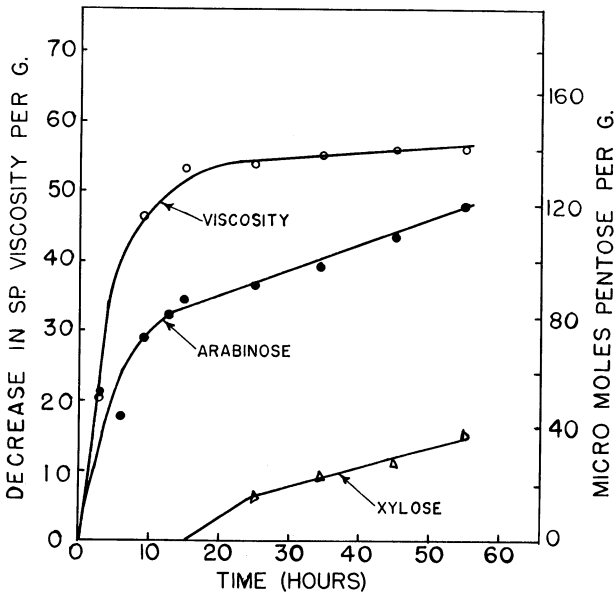


Fig. 3. Hydrolysis of flour arabinoxylan by extract of Selkirk whole wheat. Open circles = viscosity, solid circles = arabinose, triangles = xylose.

cellulase enzymes may be the digestion of cellulose entangled with pentosans (1).

Bran and shorts mill fractions were highest in β -D-xylosidase activity (Table VIII), but flour was also quite active. The lower activity found in this study for flour was near that reported by Lee and Ronalds(5) for a single Australian flour.

Grain Steeping Endoenzyme and Xylosidase Activity

The effect of wet conditions on certain enzyme activities was determined on wheat steeped at 16°C. for 0 to 30 hr. (Table IX). Grain grade was reduced from No. 1 to sample grade in 5 to 10 hr. steeping and some of the α -amylase was activated, as indicated by lower falling number values. The endoarabinoxylanase activity decreased markedly with steep time while endocellulase activity decreased slightly. Xylosidase activity increased and later decreased. Thus, there was no large activation of arabinoxylanases and cellulases on wetting wheat under the conditions used here.

Time Study of Arabinoxylanase Hydrolysis

The hydrolysis of flour arabinoxylan by enzymes of Selkirk wheat grain was followed in a time study. The rapid drop in viscosity and liberation of arabinose indicate internal chain bonds and arabinose side chains of flour pentosan were rapidly hydrolyzed in the initial stages of hydrolysis (Fig. 3). The substrate used here was prepared by the method of Kulp (1) (without acid treatment), and it is hydrolyzed more rapidly by Selkirk wheat than the pentosan prepared by the Simpson method (8) with pH 1 treatment⁴ and used in the rest of our

⁴Pancreatin and acid treatment is reported to cause some hydrolysis of arabinose side chain from pentosan (9).

experiments. Xylose was not released to any extent for 15 hr. until about 10% of the arabinose side chains had been removed. Thereafter, xylose was released at a moderate linear rate. Preece and MacDougall (14) report similar release of arabinose and xylose with the hydrolysis of rye arabinoxylan by grain enzymes of barley, oats, and rye. The rapid initial decrease in viscosity suggests that certain internal bonds in the xylose chain are at first rapidly hydrolyzed by wheat enzymes, and the late release of xylose suggests that some arabinose side-chains must be removed before hydrolysis of xylose from the main chain can occur.

This study verifies in wheat grain and its mill fractions the presence of enzymes that can hydrolyze the external and internal bonds of pentosans and cellulose. The very low amount of endoarabinoxylase activity found in flour indicates that in white bread production very few of the internal bonds would be hydrolyzed. Thus, the water-absorbing ability of the natural flour pentosans should not be altered because of fragmentation of the large pentosan molecules. Pentosans in production of whole-wheat bread may, on the other hand, be altered because of the moderate endoarabinoxylase activity in the bran and shorts. In white bread production, the greatest effect on pentosans would probably occur because of hydrolysis of the arabinose side-chains of the pentosans which may affect its solubility properties. The role of the celluloses is rather difficult to see. However, as suggested by Kulp (1) these enzymes may liberate cellulose-entangled pentosans and make them more available for solubilization and enzymatic hydrolyses.

Acknowledgment

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Literature Cited

1. KULP, K. Enzymolysis of pentosans of wheat flour. *Cereal Chem.* 45: 339 (1968).
2. JELACA, S. L., and HLYNKA, I. Water-binding capacity of wheat flour crude pentosans and their relation to mixing characteristics of dough. *Cereal Chem.* 48: 211 (1971).
3. D'APPOLONIA, B. L., GILLES, K. A., and MEDCALF, D. G. Effect of water-soluble pentosans on gluten-starch loaves. *Cereal Chem.* 47: 194 (1970).
4. CAWLEY, R. W. The role of wheat flour pentosans in baking. II. Effect of added flour pentosan and other gums on gluten starch loaves. *J. Sci. Food Agr.* 15: 834 (1964).
5. LEE, J. W., and RONALDS, J. A. The glycosidases and glycanases of wheat-flour dough. *J. Sci. Food Agr.* 23: 199 (1972).
6. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Approved methods of the AACC (7th ed.). The Association: St. Paul, Minn. (1962).
7. MEDCALF, D. G., D'APPOLONIA, B. L., and GILLES, K. A. Comparison of chemical composition and properties between hard red spring and durum wheat endosperm pentosans. *Cereal Chem.* 45: 539 (1968).
8. SIMPSON, F. J. Microbial pentosanases. I. A survey of microorganisms for the production of enzymes that attack the pentosans of wheat flour. *Can. J. Microbiol.* 1: 131 (1954).
9. LINTAS, C., and D'APPOLONIA, B. L. Note on the effect of purification treatment on water-soluble pentosan. *Cereal Chem.* 49: 731 (1972).
10. ROSENTHAL, H., and CUNDIFF, H. Preparation of a new biuret reagent. *Clin. Chem.* 2: 394 (1956).
11. KREBS, K. G., HEUSSER, D., and WIMMER, H. In: *Thin-layer chromatography, a laboratory handbook* (2nd ed.), Egon Stahl, ed., and M. R. F. Ashworth, translator, p. 882. Springer-Verlag: New York (1969).
12. DeSTEFANIS, V. A., and PONTE, J. G., Jr. Separation of sugars by thin-layer chromatography. *J. Chromatog.* 34: 116 (1968).

13. PREECE, I. A., AITKEN, R. A., and DICK, J. A. Non-starchy polysaccharides of cereal grains. VI. Preliminary study of the enzymolysis of barley β -glucosan. *J. Inst. Brew.* 60: 497 (1954).
14. PREECE, I. A., and MacDOUGALL, M. Enzymic degradation of cereal hemicelluloses. II. Pattern of pentosan degradation. *J. Inst. Brew.* 64: 489 (1958).
15. PERLIN, A. S. Structure of the soluble pentosans of wheat flours. *Cereal Chem.* 28: 382 (1951).

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