which avoids the possible contaminating activities in commercial preparations of fungal α-amylase or malt amylase extracts. Also, the method is rapid, allowing the analysis of 20 samples in duplicate in 2 hr.

The starch damage assay kit provides all of the necessary reagents and a reference flour and employs only one standard solution (glucose), which is readily prepared and stabilized. Both the AACC and Farrand methods determine reducing groups on the degradation products of damaged starch granules by the alkaline ferricyanide method, which requires standardization of two reagents and a titration step, and employs several unstable and corrosive reagents.

CONCLUSIONS

The availability of an assay kit for the determination of starch damage in flour offers a convenient and simple alternative to the current standard methods. Starch damage determinations with the assay kit are highly correlated to those of the standard methods, but the kit procedure is standardized and more rapid. With the kit procedure, 40 samples can be analyzed in 2 hr. The kit is therefore applicable for use in situations where there are large sample numbers, such as in the monitoring of millstream runs and in wheat breeding programs. The starch damage assay kit is now available commercially from MegaZyme (Aust) Pty Ltd.

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Distribution of Polyphenol Oxidase in Flour Millstreams of Canadian Common Wheat Classes Milled to Three Extraction Rates[1]

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ABSTRACT

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Polyphenol oxidase (PPO) levels were determined on individual and pooled millstreams of five cultivars representative of five different classes of Canadian wheat. The wheats were milled on a pilot mill to extraction rates of approximately 75 (conventional), 80, and 85%. Enzyme activity in individual streams ranged widely but increased with increasing bran contamination in the millstreams. With the exception of the soft spring wheat cultivar, PPO levels, as a percentage of total activity, were similar for the different wheats at similar cumulative flour yields. Less than 10% of the total PPO activity was present in cumulative flour streams corresponding to 70% extraction, after which the amount of the enzyme rapidly increased. Slightly more PPO activity appeared in the lower ash streams if the wheats were milled to a higher extraction using this mill flow. PPO activity was linearly correlated with ash content (up to 2.0% ash) and flour grade color figure (up to 5 units).

Polyphenol oxidase (PPO) has been implicated in enzymatic browning reactions in whole wheat flours used for making *chapati* (Abrol et al 1971, Singh and Sheoran 1972). The enzyme is located mainly in the bran of milled wheat and is not readily extractable with common buffers (Marsh and Galliard 1986). A number of studies have elaborated on the chemical and physical properties of the enzyme, as well as its multiplicity (Tikoo et al 1973; Taneja and Sachar 1974; Kruger 1976; Interesse et al 1980, 1981, 1983). Wheat enzyme levels are dependent on wheat class (Lamkin et al 1981).

A number of end products are commonly prepared from flours milled to higher extraction rates in which the presence of PPO

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may play a role in affecting product color. These include Chinese steamed bread (Dexter et al 1984) and Middle East flat breads (Faridi 1988). Color in certain end products, such as Cantonese noodles, is extremely important and may be influenced by this enzyme, even at lower flour milling extraction rates (Miskelly 1984; Kruger et al, in press). The purpose of this study, therefore, was to determine levels of the enzyme that are found in wheat flour millstreams and to ascertain the effects on these levels of increasing flour extraction rate. Five cultivars of Canadian wheat representative of the common wheat classes grown in western Canada were milled to three extraction rates.

MATERIALS AND METHODS

Wheat Samples

The wheats employed in this study (Table I) were obtained as certified seed. The samples were grown within the same year but were harvested from different sites across western Canada. As shown in Table I, the wheats ranged in protein from 9.0 to 15.7%. The Falling Number indicated that all wheats were free of sprout damage.

TABLE I
Properties of Wheats Used in These Studies

Wheat Class	Variety	Falling Number (sec)	Moisture (%)	Protein[a] (%)	Wheat Ash[a] (%)
Canada Western Red Spring	Katepwa	405	11.8	15.7	1.75
Canada Utility	Glenlea	385	11.5	13.1	1.69
Canada Western Red Winter	Norstar	375	11.9	12.0	1.18
Canada Prairie Spring	HY320	335	12.0	11.4	1.54
Canada Western Soft White Spring	Fielder	380	11.8	9.0	1.36

[a] 13.5% moisture basis.

TABLE II
Flour Yield and Ash of Straight-Grade Flours Milled to Extraction Rates of Approximately 75, 80, and 85%[a]

	75%		80%		85%	
Variety	Yield	Ash	Yield	Ash	Yield	Ash
Katepwa	75.7	0.54	79.8	0.66	84.1	0.99
Glenlea	73.9	0.59	79.0	0.70	83.7	1.00
Norstar	77.5	0.42	80.1	0.48	84.2	0.66
HY320	75.0	0.57	78.6	0.66	82.9	0.80
Fielder	71.7	0.43	77.6	0.53	83.2	0.70

[a] All values are percents.

Wheat Milling

Wheats were milled on the Grain Research Laboratory's pilot mill as described by Black (1980). The mill has a capacity in excess of 300 kg/hr. The wheats were cleaned and tempered as described by Black (1980). Temper moisture ranged from 14 to 16.5% depending on wheat hardness and flour extraction rate desired.

Wheats were milled by a typical North American milling that gives a flour extraction rate near 75% (Black 1980) and by two higher extraction milling procedures that give flour extraction rates of about 80 (Dexter et al 1987) and 85% (Dexter et al 1984). Flour yields and resulting ash values of straight-grade flours of the five cultivars at the different extraction rates are shown in Table II.

PPO Assay

Analyses of PPO were carried out using a YSI model 5300 biological oxygen monitor (Yellow Spring Instrument Co., Yellow Spring, OH) per Marsh and Galliard (1986) with slight modifications. Temperature of the assay was raised to 37°C, and the assay medium consisted of 4 ml of air-saturated 0.01*M* McIlvaine's buffer, pH 6.8 (Kruger 1976). Ground wheat or flour was added to the buffer, and the suspension monitored for 5 min to establish endogenous oxygen consumption. Freshly prepared substrate, 0.1 ml of 0.8*M* catechol, was added and the oxygen consumption monitored for 3–5 min. All values were corrected for substrate autooxidation. Activity was linear with added sample in the range of 10–200 mg. Results are expressed as nanomoles of O_2 consumed per minute per gram at 37°C. Analyses were performed in triplicate.

Flour Color

Color was obtained using a Simon series IV (Henry Simon, Stockport, UK) flour color grader, which gives the relative reflectance (with filter no. 58) of a sample. Results are reported as Kent-Jones color units: the lower the number, the less bran contamination in the flour and the brighter the flour color (Kent-Jones et al 1950).

Ash Content

Ash content was determined by using AACC method 08-01

TABLE III
Polyphenol Oxidase Levels[a] in Canadian Wheats Milled to Extraction Rates of 75 (A), 80 (B), and 85% (C)[b]

	Katepwa			Glenlea			Norstar			Fielder			HY320		
Stream[c]	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
B1	125	141	201	299	366	398	52	106	149	174	93	191	93	115	106
B2	76	94	80	189	201	266	33	18	68	114	111	119	56	54	72
B3	60	98	82	133	138	60	41	62	93	77	197	56	63	86	129
B4	277	498	1,410	339	716	116	366	647	796	348	494	987	386	860	1,072
B5			3,239			4,060			1,061			1,274			1,831
S1	13	21	32	25	36	51	15	57	39	20	20	12	40	53	34
S2	0	4	17	12	11	12	7	11	19	12	4	8	16	18	8
M1	12	29	25	22	28	22	13	31	33	16	19	8	39	21	17
M2	24	45	52	45	41	57	14	29	43	21	28	30	46	9	58
M3	39	51	337	55	77	150	32	51	409	35	52	246	53	92	485
M4	63	259	196	66	23	116	55	291	207	52	168	111	101	323	196
M5	133	441	710	127	680	834	141	1,071	573	133	482	544	175	945	515
M6	172	548	585	122	716	627	141	1,014	318	156	479	265	205	907	724
M7			2,754			2,786			1,273			1,963			2,295
M8			4,195			3,900			2,070			3,423			3,237
M9			3,868			4,378			3,502			3,662			4,537
SD	690	915	3,773	1,401	1,328	7,960	445	1,008	1,990	689	1,083	2,896	669	1,305	2,547
BF	697	785	5,143	366	1,130	3,980	323	371	684	737	716	1,393	360	662	1,604

[a] Polyphenol oxidase levels in whole wheat samples were 1,266, 1,769, 1,011, 864, and 1,369 for Katepwa, Glenlea, Norstar, Fielder, and HY320, respectively. All values are in nanomoles of O_2 per gram per minute.
[b] Average coefficient of variation for sample analysis = 4.68%.
[c] B1–B5 = first through fifth breaks, S1–S2 = first and second sizings, M1–M9 = first through ninth middlings, SD = shorts duster, BF = bran flour.

(AACC 1983) on a 4-g sample in a silica dish incinerated overnight at 600° C.

Statistical Analyses

Regression analyses were performed using the SAS version 6.03 (SAS Institute Inc., Cary, NC) software on an IBM-compatible personal computer.

RESULTS AND DISCUSSION

PPO Levels in Individual Millstreams and Pooled Flours

PPO levels on a per gram basis in individual flour streams of Canadian wheat milled to an extraction rate of approximately 75% and to two higher extraction rates of approximately 80 and 85% are shown in Table III. In general, the relative order of increasing enzymic activity in the streams were the sizings, followed by the first through third middling flours interdispersed with break flours, followed by the remaining middling flours, the bran flours, and finally the shorts duster flours. As expected, the bran flours and shorts duster contained notably higher levels than the other flour streams, coincident with the expected anatomical location of the enzyme in the bran (Marsh and Galliard 1986). In general, enzyme activity in a particular flour stream increased on a per gram basis from the 75–80% extraction rate. Direct comparison with the 85% extraction millstreams was not possible because of the separation of additional streams.

The cumulative PPO activity as a percentage of total activity versus the cumulative flour yield on the basis of increasing ash content is shown in Figure 1. It is interesting to note that at a particular extraction rate, all cultivars, with the exception of the soft white spring wheat Fielder, behaved quite similarly in terms of percent PPO activity present at a particular cumulative flour yield. The percent PPO ending up in the flour is fairly low. Thus, at the conventional extraction rate (75%), the percentages of total PPO activity found at 50, 60, and 70% cumulative flour yields were approximately 3, 4, and 5%, respectively, of total wheat PPO activity. Even at the highest extraction rate (approximately 85%), the percentages of total PPO activity found at 50, 60, and 70% cumulative flour yields were only around 4, 6, and 7%, respectively. The results indicate, however, that if wheat is being milled to a higher-than-conventional flour extraction rate for a particular end product, the quality of lower-ash-bulked flour streams, i.e., top patent, may suffer with respect to PPO activity. It is recognized, however, that in a commercial milling operation, the mill flow for an elevated extraction rate can be extended to produce a very fancy patent flour. Under such conditions, PPO levels may be only minimally influenced. Although the increase in total PPO in top patent flours with increased extraction was observed to be minimal compared with the total PPO present in the grain, it is conceivable that products in which color is highly important, i.e., Oriental white salted noodles, could be affected.

Relationship Between PPO Activity and Ash or Color

The anatomical location of the enzyme PPO in the bran suggests that there should be fairly strong relationships between PPO activity and either ash or flour color values. In general, overall relationships were poor when all flour streams were considered. As shown in Figure 2, however, fairly linear correlations were found between PPO activity and ash for the individual flour streams from the wheats milled to different extractions up to an ash value of about 2%, with Pearson correlation coefficients ($P < 0.001$) of 0.98 for Katepwa, 0.96 for Glenlea and Norstar, and 0.94 for Fielder and HY320.

Similarly, the relationship between PPO activity and flour color of individual flour streams for the five cultivars (Fig. 3) were fairly linear up to a Kent-Jones flour color value of approximately 5 units, with correlation coefficients ($P < 0.001$) of 0.93 (Katepwa), 0.88 (HY320), 0.82 (Glenlea and Fielder), and 0.78 (Norstar). McCallum and Walker (1990) have also reported a high correlation ($r = 0.977$) between *o*-diphenol oxidase and flour color with 30 millstreams from New Zealand wheat cultivars.

CONCLUSIONS

The conclusion from a previous study (Marsh and Galliard 1986) and the present study is that PPO is closely associated with the branny layers. As such, its distribution in millstreams closely parallels the effectiveness of the milling procedure, as indicated by the ash content, to remove the bran coat. Milling to a higher flour extraction results in slightly more PPO activity in the low ash millstreams. However, up to a cumulative flour yield of 70%, at least 90% of the PPO enzyme is removed. Whether the remaining PPO activity can bring about enzymatic darkening will depend on the initial PPO activity of the wheat, substrate availability, and the particular end product that is being made.

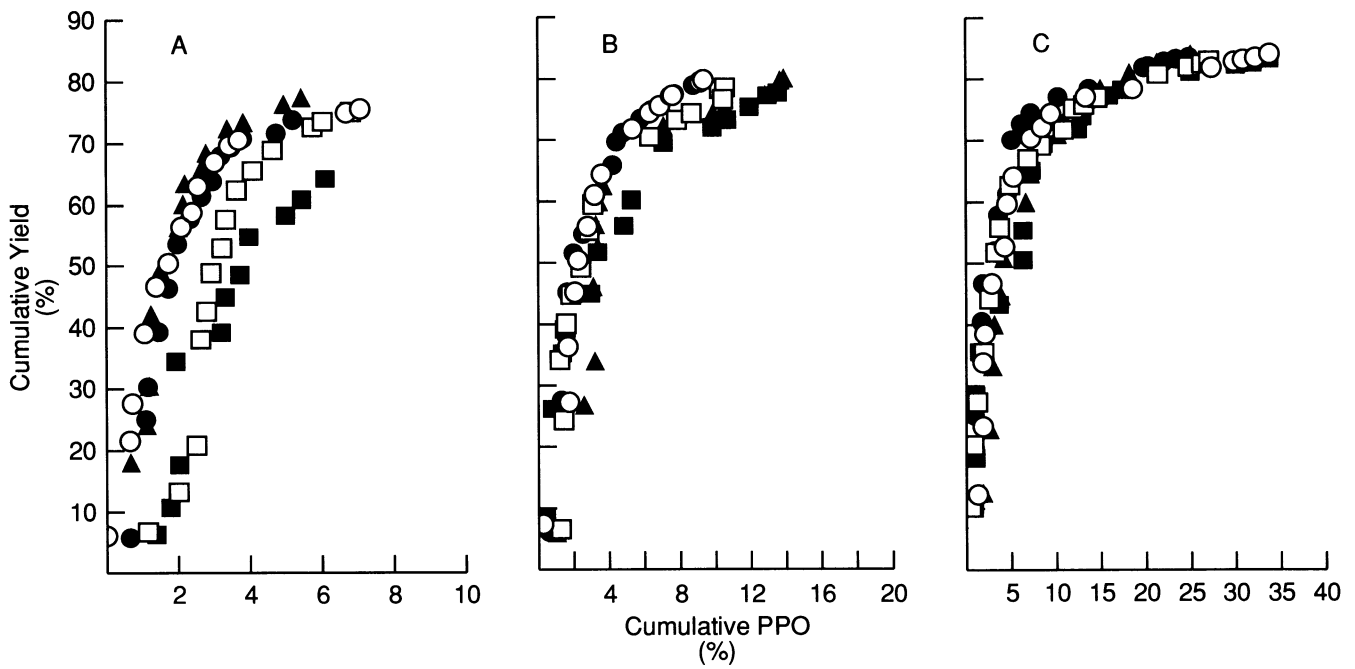

Fig. 1. Cumulative flour yield (%) bulked on the basis of increasing ash versus cumulative polyphenol oxidase (PPO [% of total seed activity]). Flour extraction rates are 75, 80, and 85% (**A–C**, respectively). ○, Katepwa; ●, Glenlea; □, HY320; ■, Fielder; △, Norstar.

Future research is needed to ascertain levels considered deleterious in processing of different products. The results of this study also indicate that wheat flours varying widely in quality parameters but having similar extraction rates have similar PPO levels as a percentage of total PPO. As such, the findings reported here should be roughly extrapolative by others provided that the milling procedure is similar.

The present results apply to wheats that are free of sprout

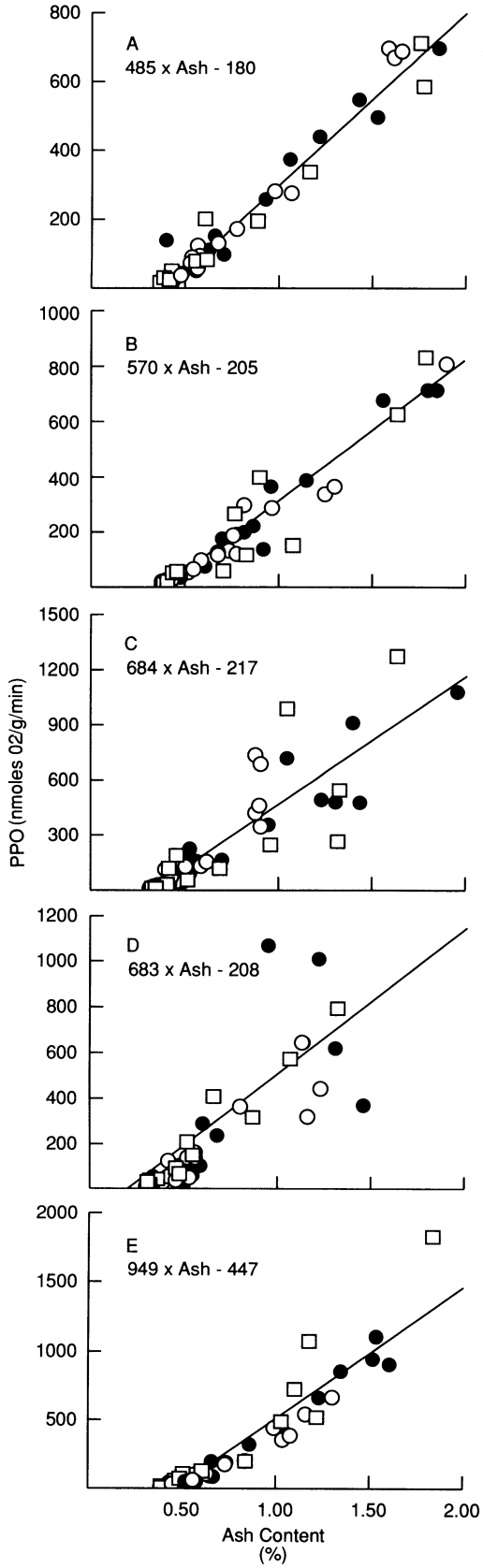

Fig. 2. Polyphenol oxidase activity (PPO, as percent of total grain activity) versus ash content of individual millstreams at flour extraction rates of 75 (○), 80 (●), and 85% (□). **A,** Katepwa; **B,** Glenlea; **C,** Fielder; **D,** Norstar; **E,** HY320.

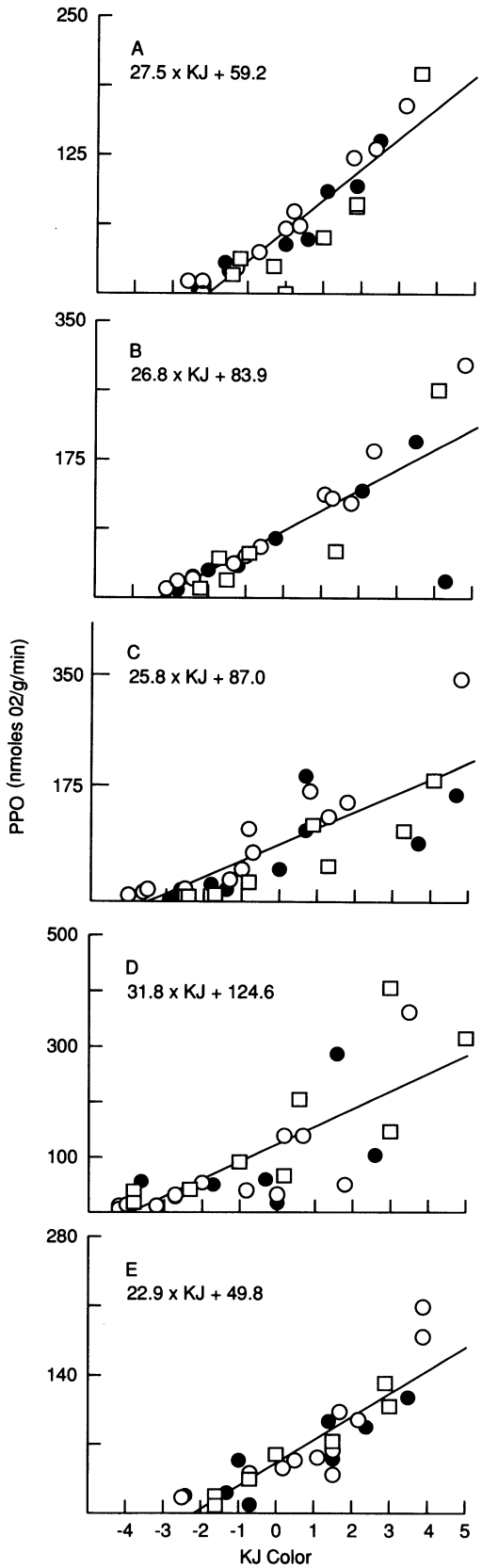

Fig. 3. Polyphenol oxidase activity (PPO) versus Kent-Jones color values (KJ) of individual millstreams at flour extraction rates of 75 (○), 80 (●), and 85% (□). **A,** Katepwa; **B,** Glenlea; **C,** Fielder; **D,** Norstar; **E,** HY320.

damage. PPO can increase up to 33-fold upon germination (Kruger 1976), and the distribution of enzyme in the kernel may be dependent on the severity of sprouting, as has been found for the enzyme α-amylase (Kruger 1981). As such, results on wheats containing sprout damage could be quite different from those reported here.

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Ferulic Acid in Rye and Wheat Grain and Grain Dietary Fiber

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ABSTRACT

Cereal Chem. 70(1):55-59

The aim of the present work was to examine the effect of cross-linking of rye and wheat arabinoxylan by ferulic acid on grain nutritive value, measured in vitro by an enzymatic test. Determination of ferulic acid was based on spectrophotometric measurements of defatted samples at 320 nm. Approximately 80% of the *trans*-ferulic acid, the dominant phenolic acid of rye and wheat grain, was found in the bran of both species. Total content and extractability of free and esterified ferulic acid by water, ethanol, and alimentary enzymes (soluble dietary fiber) from grain meal were significantly higher in rye than in wheat. The activity of peroxidase, the enzyme thought to be responsible for the formation of diferulic bridges, was also significantly higher in rye. Most (85–90%) of the alkaline-soluble ferulic acid in grain was localized in the insoluble dietary fiber, and only about 5% was in the soluble fraction. In spite of the higher solubility of rye arabinoxylans and the higher arabinose-xylose ratio in rye than in wheat grain, the ratio of the number of arabinose residues per ferulic acid molecule was not significantly higher in the soluble fiber of rye. Thus, cross-linking of grain hemicellulose components by ferulic bridges does not appear to contribute to the known differences in the structure, molecular weight, and nutritive properties of soluble fiber of rye and wheat.

Ferulic acid and isoferulic acid (4-hydroxy-3-methoxy and 3-hydroxy-4-methoxy cinnamic acids) are the main phenolic acids of cell walls of monocots (Smith and Hartley 1983). *Trans*-ferulic acid is the dominant isomer and constitutes up to 90% of the total phenolic acids in wheat flour (Sosulski et al 1982). It is esterified to hemicellulosic components of plant cells, primarily to arabinosyl residues at 2-O or 3-O branches of the xylan backbone (Fry 1986, Mueller-Harvey et al 1986). Recently, feruloylated arabinoxylans have been isolated from various plants (Kato and Nevins 1985, Ahluwalia and Fry 1986, Mueller-Harvey et al 1986). Free or esterified ferulic acid could also polymerize with lignin, forming alkali-resistant bonds (Scalbert et al 1985). Cross-linking of pectins in the primary wall by ferulic acid, postulated in dicots, has not been found in monocots (Fry 1983). *N*-feruloylglycine has been detected as the terminal sequence of barley globulin

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