Polyphenol Oxidase Activities of Hard Red Winter, Soft Red Winter, Hard Red Spring, White Common, Club, and Durum Wheat Cultivars[1]

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ABSTRACT

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Measurement of polyphenol oxidase activity was studied as a possible method for the identification of wheat varieties or classes. A simple, rapid procedure employing a Clark polarographic electrode was developed for determining activity of the enzyme in wheat, and conditions (pH, temperature, etc) were determined for measuring its activity on a variety of substrates. Substrates investigated included phenol, L-tyrosine, catechol, caffeic acid, L-dopa, dopamine, gallic acid, pyrogallol, and *d*-catechin. Thirty wheat cultivars including hard red winter, soft red winter, hard red spring, white common, club, and durum wheats were studied. Different samples of the same cultivar from different growing locations and crop years gave comparable activities. In many cases, differences in polyphenol oxidase activities could be used to distinguish between cultivars. The durum wheats, which had lower activities, could be differentiated from varieties belonging to other classes by polyphenol oxidase activity alone. Lesser differences in activities were noted between the hard red winter and soft red winter wheats, between the hard red winter and hard red spring wheats, and between the white and club wheats.

Monophenol monooxygenase, more commonly called polyphenol oxidase, (EC 1.14.18.1; also called phenol oxidase, phenolase, catechol oxidase, catecholase, tyrosinase, etc) is widely distributed in plants. Isozymes studied include those of mushroom (Bouchilloux et al 1963; Constantinides and Bedford 1967; Jolley et al 1969a, 1969b; Smith and Krueger 1962), broad bean (Robb et al 1965), velvet bean (Zenin and Park 1978), potato (Constantinides and Bedford 1967, Patil and Zucker 1965), *Neurospora crassa* (Fling et al 1963, Fox and Burnett 1962), apple (Harel et al 1965, Harel and Mayer 1968, Stelzig et al 1972), banana (Montgomery and Sgarbieri 1975), pear (Halim and Montgomery 1978, Rivas and Whitaker 1973), peach (Wong et al 1971), and cherry (Benjamin and Montgomery 1973). Since a tyrosinase was reported in bran in 1907 (Bertrand and Muttermilch), presence of the enzyme in wheat has been well documented (Abrol and Uprety 1970, Honold and Stahmann 1968, Kruger 1976, Milner 1951, Milner and Gould 1951, Taneja et al 1974, Tikoo et al 1973). High levels detected in dwarf wheat varieties (Abrol and Uprety 1970, Taneja et al 1974, Tikoo et al 1973) are considered to be responsible for darkening of whole wheat dough and chapaties.

Existence of multiple forms of the enzyme in a species appears to be common, with as many as 11 isozymes reported in potato (Constantinides and Bedford 1967). In wheat, a number of workers have demonstrated the presence of multiple forms with different electrophoretic mobilities. Kruger (1976), for example, was able to separate 12 isozymes in samples of Hercules wheat kernels taken at various stages of maturity. Tikoo et al (1973) detected three to five isozymes in dwarf varieties by acrylamide gel electrophoresis, and Taneja et al (1974) separated 10 isozymes in a dwarf wheat at 36 days after anthesis. By densitometry of an electrophoretically separable isozyme not present in durum wheat, Feillet and Kobrehel (1974) were able to determine common wheat present as an adulterant in semolina and pasta products.

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Multiple forms of polyphenol oxidase are known to be produced by association of monomeric units (Jolley et al 1969b, Taneja et al 1974). Differences observed between isozymes appear, however, to be too great to be accounted for solely by association-dissociation phenomena (Jolley et al 1969a). From the clingstone peach, Wong et al (1971) isolated four isozymes with different pH optima, Michaelis constants, heat stabilities, substrate specificities, and degrees of inhibition by various inhibitors. In mushrooms, some of the isozymes are high in catecholase activity, whereas others are high in cresolase activity (Smith and Krueger 1962). Of nine isozymes with high activity toward DL-dopa, only three were found to have activity toward L-tyrosine (Constantinides and Bedford 1967).

Differences in polyphenol oxidase activities and substrate specificities have been noted between wheat varieties (Kruger 1976, Milner and Gould 1951, Tikoo et al 1973). The present work was undertaken to determine the extent to which these differences might be useful in identifying wheat cultivars or classes. An attempt was made to devise a quantitative procedure allowing more precise characterization than is possible using phenol or catechol color tests (Wrigley 1976, Wrigley and Baxter 1974, Wrigley and Shepherd 1974). Polyphenol oxidase activity was determined by measuring the rate of oxygen consumption with a Clark polarographic electrode.

MATERIALS AND METHODS

Wheat Samples

Wheat samples were obtained from L. C. Bolte, U.S. Grain Marketing Research Laboratory, Manhattan, KS; G. L. Rubenthaler, Western Wheat Quality Laboratory, Pullman, WA; N. D. Williams, North Dakota State University, Fargo; and W. T. Yamazaki, Soft Wheat Quality Laboratory, Wooster, OH. All samples were from the 1977 and 1978 crops except for two (Hyslop and Omar) from the 1975 crop. Thirty-six wheat samples representing 30 cultivars were examined.

Reagents

The substrates employed for measuring polyphenol oxidase activities were of the highest purity obtainable commercially and were used without further purification. "Eastman grade" catechol and L-tyrosine were produced by Eastman Organic Chemicals; caffeic acid (3,4-dihydroxycinnamic acid) and pyrogallol were purchased from Aldrich Chemical Co.; *d*-catechin, L-dopa [3-(3,4-dihydroxyphenyl)-L-alanine], and dopamine (3-hydroxytyramine) were obtained from Sigma Chemical Co.; ACS Reagent grade phenol was purchased from Fisher Scientific Co.; and "Baker

analyzed" gallic acid was obtained from J. T. Baker Chemical Co. "Reagent grade" monosodium phosphate and disodium phosphate from Sigma Chemical Co. and "purified grade" sodium diethylbarbiturate from Fisher Scientific Co. were used to prepare buffers.

pH Optima

Polyphenol oxidase pH optima were determined spectrophotometrically. The initial rate of quinone formation as indicated by the increase in absorbance at 430 nm served as a measure of enzyme activity. With a mortar and pestle, 4.0 g of wheat was ground in the presence of 12.00 ml of distilled water. The resulting slurry was centrifuged at 20,000 × *g* for 15 min at 4°C, and the supernatant, which contained the enzyme, was stored at 4°C until needed. To 1.00 ml of buffer (0.100*M* sodium phosphate containing 0.037*M* diethylbarbiturate with the pH adjusted to the appropriate value) in a 1-cm cuvette was added 2.00 ml of 0.010*M* substrate, freshly dissolved in the same buffer, and 100 μl of enzyme extract. For pyrogallol, because of its greater rate of oxidation by the enzyme, the volume of added substrate, 0.050*M* in this case, was reduced to 100 μl, and volume of the buffer was adjusted to 2.90 ml. Absorbance of the solution was read at 430 nm with a Beckman model 25 spectrophotometer at 15-sec intervals for 3 min after addition of the enzyme extract and at 1-min intervals for the next 12 min. Initial velocities were determined, with polyphenol oxidase activity calculated as change in absorbance per minute.

Polyphenol Oxidase Activity

Polyphenol oxidase activities were measured with a Yellow Springs Instrument model 53 biological oxygen monitor employing a Clark polarographic electrode and equipped with a Linear model 282 recorder calibrated to read 0–100 mV. Temperature of the sample chamber was maintained at 37.0°C with a Thermatron T-50 circulating water bath. Thirty-gram samples were ground in a modified Weber mill (McGinty et al 1977) having a screen with holes 1.0 mm in diameter. Wheat (0.2000 g) was added to 3.00 ml of air-saturated buffer, and the rate of oxygen consumption was measured for 10 min. The rate of oxygen consumption was then measured for 5 min after the addition of substrate. Except for tyrosine, substrates (Table I) were added as 100 μl of aqueous solution with a Hamilton 1710 syringe having a 3½-in. needle, which allowed the addition to be made without removal of the electrode from the sample chamber. Because of its limited solubility, tyrosine was added as 50.0 mg of solid. To avoid problems from air oxidation, substrate solutions were freshly prepared immediately before use. Air-saturated distilled water was the standard used for calibration of the instrument, with correction made for differences in the solubility of oxygen caused by variations in atmospheric pressure.

For all substrates except pyrogallol and gallic acid, measurements were made at 37.0°C in sodium phosphate-diethylbarbiturate buffer, pH 8.3, 0.100*M* with respect to phosphate and 0.037*M* with respect to diethylbarbiturate. Because the rates of oxygen consumption were too high to permit accurate measurements at pH 8.3, activities with pyrogallol and gallic acid as substrates were measured in 0.100*M* sodium phosphate buffer, pH 6.8, also at 37.0°C. Substrate concentrations (Table I) were adjusted to be compatible with the activities encountered in the wheat samples. The increased rate of oxygen consumption after the addition of substrate served as a measure of polyphenol oxidase activity. A typical recording is shown in Fig. 1. The background rate of oxygen consumption was determined graphically at the point of addition, and the rate after addition was also determined graphically after allowing 1 min for equilibration across the electrode membrane. Activity was calculated as microliters of oxygen consumed per milliliter of solution per minute, with all results corrected to a wheat moisture content of 14.00%. Over the range of activities encountered for the samples included in the study, a linear relationship was found between activity and sample size, and thus between activity and enzyme concentration, for each of the nine substrates. The relationship held for sample sizes from 0 to 0.2000 g.

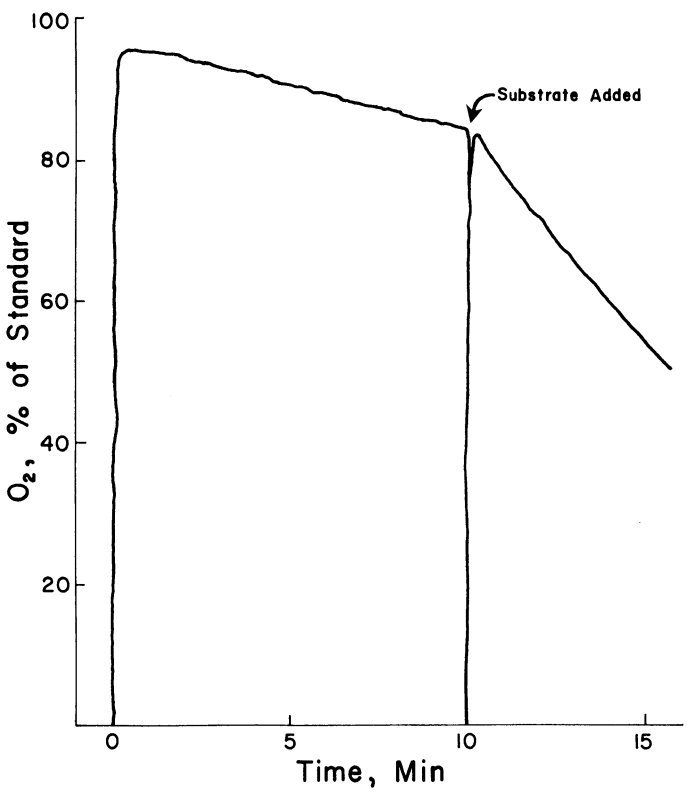

Fig. 1. Oxygen consumption by the hard red spring wheat Waldron before and after the addition of catechol. Wheat (0.2000 g) was added to 3.00 ml of buffer, pH 8.3, and, after sufficient time had been allowed to establish the background rate of oxygen consumption, 100 μl of 2.50 × 10^{-4}*M* catechol was added.

RESULTS AND DISCUSSION

Effect of pH and Temperature

In examining the relationship between wheat polyphenol oxidase activity and pH, a number of buffers were investigated, including borate, Trizma [*N*-tris(hydroxymethyl)aminomethane], glycine, tricine [*N*-tris(hydroxymethyl)methylglycine], phosphate, phosphate/citrate, and phosphate/diethylbarbiturate. In our judgment, best results were obtained with 0.100*M* sodium phosphate containing 0.037*M* diethylbarbiturate. Phosphate was not an effective buffer at the desired pH; activities were lower in some buffers; and, in other cases, anomalous curves were obtained when activity was plotted vs time.

For those substrates that gave peaks in their activity vs pH curves, wheat polyphenol oxidase activity exhibited a pH optimum of about 8.3. Variation with pH of activities toward catechol, pyrogallol, and L-dopa in Parker wheat, expressed as change in absorbance per minute at 430 nm, is shown in Fig. 2. Maximum

TABLE I
Substrate Concentrations Employed for Polarographic Determination of Polyphenol Oxidase Activities in Wheat[a]

Substrate	Final Molar Concentration
Phenol	2.00 × 10^{-3}
L-Tyrosine	Saturated[b]
Catechol	2.50 × 10^{-4}
Caffeic acid	2.50 × 10^{-4}
L-Dopa	2.50 × 10^{-4}
Dopamine	1.00 × 10^{-4}
Gallic acid	1.50 × 10^{-3}
Pyrogallol	1.00 × 10^{-4}
d-Catechin	5.00 × 10^{-4}

[a] Activities were determined with a Yellow Springs Instrument model 53 biological oxygen monitor.
[b] Added as 50.0 mg of solid.

catecholase activity was at about pH 8.3, with activity decreasing above and below this pH and little activity below pH 7.0. Although the shape of the peak was different, activity with pyrogallol as substrate showed an optimum at approximately the same pH (8.4). For L-dopa, no peak was exhibited below pH 9.0, with activity increasing rapidly at the higher pH values examined. Activities toward tyrosine and phenol were too low to allow meaningful readings by the spectrophotometric method, but polarographic measurement of the rate of oxygen consumption indicated that polyphenol oxidase activities for these two substrates varied with pH in a manner similar to that shown in Fig. 2 for L-dopa.

The Yellow Springs Instrument model 53 biological oxygen monitor employed for measuring polyphenol oxidase activity had a temperature range of 25–40° C. Over this range, activity did not show a peak but increased as the temperature was raised. An operating temperature of 37.0° C was selected as representing a satisfactory compromise between sensitivity and convenience. This was the same temperature used by Taneja et al (1974) in their studies on polyphenol oxidase activity during wheat grain development.

Analysis Conditions

Measurement of polyphenol oxidase activity depends on the increased rate of oxygen consumption that occurs on addition of substrate (Fig. 1). About 15 min is required to determine activity for a specific substrate by this procedure. By reducing the time allowed to establish the background rate of oxygen consumption, analysis time can be reduced to less than 10 min with only a slight loss in accuracy. Further reduction may be possible by use of a respiratory inhibitor. Conditions can be adjusted to meet the requirements of a particular analysis.

High activities toward pyrogallol and gallic acid presented experimental difficulty caused by rapid depletion of oxygen from the sample solution. For the purposes of the present study, lowering the activity by reducing the sample weight was not practical because of the small sample sizes that would be required, and taking an aliquot from a sample slurry was considered to be unwise because of the possibility that at least part of the polyphenol oxidase activity in some of the wheat samples might be found in the insoluble fraction. Nor could activity be sufficiently reduced by lowering the reaction temperature. The best option appeared to be to measure activities with pyrogallol and gallic acid as substrates at pH 6.8, where the rate of oxidation by the enzyme was lower than at pH 8.3. Although the rate of change in activity with pH was greater there than at pH 8.3, the validity of making the measurements at pH 6.8 is shown by the precision achieved for activities toward these two substrates (Table II).

Because problems would result from reducing the sample size, activities for the remaining substrates were controlled by adjusting substrate concentrations (Table I). In the case of L-dopa, conditions for measuring activity were somewhat arbitrary. A pH optimum was not found for this substrate below pH 9.0, and no attempt was made to examine pH values above 9.0 because of the rapid oxidation of phenols in alkaline media. At the pH employed, however, the precision obtained for activities toward L-dopa was about the same as for the other substrates (Table II).

Preliminary experiments showed that under the conditions employed for determining wheat polyphenol oxidase activity, accessibility of the enzyme to the substrate was not limited by solubility of the enzyme. Adequate solubilization of the enzyme did occur under the conditions of the assay, demonstrated by the fact that for each of the nine substrates, activity did not change when the time before the addition of substrate was increased from 10 to 60 min. Also, because a deviation from linearity would be expected if solubilization was a problem, the linear relationship found between activity and sample size provides additional evidence that solubilization occurred under the assay conditions.

Activities Toward Different Substrates

Polyphenol oxidase activities of the wheat samples are recorded in Table II. Results were reasonably reproducible. For the cultivar Eagle, standard deviations varied from only 0.5 μl of oxygen per milliliter per minute for gallic acid to 1.8 μl for dopamine.

A unit of activity with respect to one substrate may not be directly comparable to that for another because of differences in substrate concentrations and pH values at which the measurements were made. Each cultivar examined, however, exhibited marked differences in activities toward different substrates. Activities toward phenol were all low despite the fact that the phenol concentrations were the second highest of all the substrates investigated. These low activities are perhaps surprising in view of the preferential use of this substrate in the phenol color test used to distinguish between wheat varieties (Wrigley 1976). Tyrosinase activities were also quite low when the tyrosine concentration was comparable to that used for phenol. At the concentration employed, however, which was greater than that for any other substrate, the numerical values for tyrosinase were the highest activities obtained, and for the hard red spring wheat Coteau (Table II), activity toward tyrosine was almost twice that toward any other substrate for any of the cultivars investigated.

Activities with pyrogallol and gallic acid as substrates were determined at pH 6.8. The low activities for gallic acid reflect chiefly the lower pH. Pyrogallase activities, on the other hand, were quite high even at the lower pH, and at pH 8.3, activities toward pyrogallol were much greater than toward any of the other substrates. Activities were of intermediate value with catechol, caffeic acid, L-dopa, dopamine, and *d*-catechin as substrates.

Differences Among Wheat Cultivars

Polyphenol oxidase activities appear to correlate well with cultivar. This can be seen by reference to the two samples of the soft red winter wheat Monon (Table II), which were from different crop years and growing locations. The excellent agreement between the tyrosinase activities for the two tends to confirm that the unusually high activity was a characteristic of the cultivar, not a result of growing conditions or handling of the grain after harvest.

For each of the six pairs representing different samples of the same cultivar, agreement between polyphenol oxidase activities was good. For paired cultivars, average agreement between activity measurements made using the same substrate was 1.4 μl of oxygen per milliliter per minute, and, of the 108 individual measurements represented, only one pair failed to agree within 3.6 $\mu l \cdot ml^{-1} \cdot min^{-1}$. Activities were in close agreement even for pairs

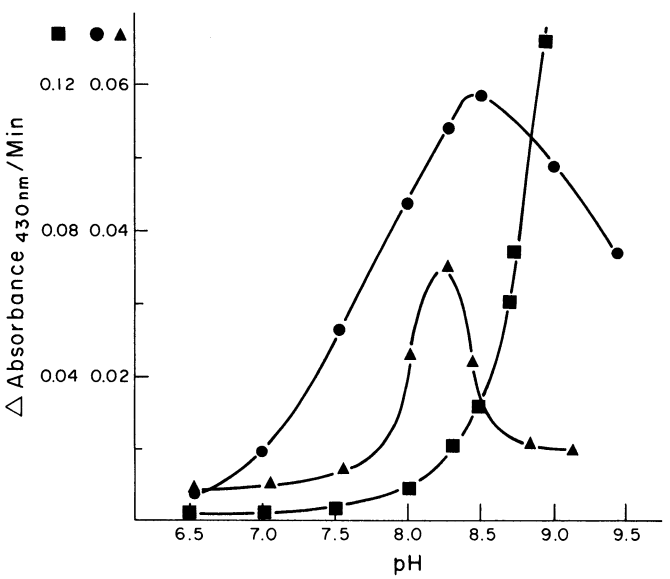

Fig. 2. Polyphenol oxidase activities toward catechol (△), pyrogallol (●), and L-dopa (■) of the hard red winter wheat Parker as a function of pH. For catechol and L-dopa, activity was determined by measuring change in absorbance per minute at 430 nm after 2.00 ml of 0.010 *M* substrate in buffer and 100 μl of enzyme extract had been added to 1.00 ml of buffer at the appropriate pH. For pyrogallol, the volume of buffered substrate, 0.050 *M* in this case, was reduced to 100 μl, and the volume of buffer was adjusted to 2.90 ml.

from different crop years or growing locations. Of the six pairs examined, only the two samples of the hard red winter wheat Parker were from the same growing season. Four pairs, Scout, Centurk, Monon, and Arthur 71, represented both different crop years and different growing locations.

In many cases, differences in polyphenol oxidase activities could distinguish between wheat cultivars. For example, the two white wheats Genesee and Hyslop could easily be differentiated from one another by their activities toward any of the substrates investigated except gallic acid. The high tyrosinase activity of the hard red spring wheat Coteau distinguished it from all the other cultivars examined except the soft red winter wheat Monon, which could be differentiated by its activity toward dopamine.

Differences Among Wheat Classes

Polyphenol oxidase activities for the hard red winter wheats did not differ greatly from those of the soft red winter or hard red spring wheats. The most important finding for the hard red winter wheats was that, at least for the limited number of samples examined, activities agreed with each other within a fairly narrow range. For example, activities with catechol as substrate varied from 25.3 μl of oxygen per milliliter per minute for Scout to only 28.9 $\mu l \cdot ml^{-1} \cdot min^{-1}$ for Eagle. Average range between samples for the nine substrates investigated was only 4.2 $\mu l \cdot ml^{-1} \cdot min^{-1}$.

Activities of the soft red winter wheats showed more variability than did those of the hard red winter cultivars, with tyrosinase activities ranging from 26.4 μl of oxygen per milliliter per minute

TABLE II
Polyphenol Oxidase Activities of Wheat Cultivars

Class and Cultivar	Activity,[a] Oxygen ($\mu l \cdot ml^{-1} \cdot min^{-1}$)								
	Phenol	L-Tyrosine	Catechol	Caffeic Acid	L-Dopa	Dopamine	Gallic Acid	Pyrogallol	*d*-Catechin
Hard Red Winter									
Eagle (1978)[b]	8.8 ± 0.7	27.6 ± 1.3	28.9 ± 1.3	19.9 ± 1.1	13.7 ± 0.7	26.9 ± 1.8	3.4 ± 0.5	24.1 ± 0.7	25.2 ± 1.1
Buckskin (1978)	9.5	26.0	28.2	18.5	14.1	24.8	3.1	23.3	24.6
Scout									
1977	6.2	24.6	25.4	16.5	13.3	20.9	3.4	26.9	20.6
1978	8.3	27.7	25.3	18.6	13.2	21.5	3.7	23.7	23.0
Centurk									
1977	5.7	22.5	25.7	17.8	13.5	24.4	3.0	22.2	20.8
1978	9.3	27.2	25.7	19.6	15.6	27.5	3.0	21.6	22.5
Parker									
1977A	8.3	25.9	26.4	17.0	14.9	24.0	3.0	20.0	20.7
1977B	8.2	27.1	27.4	18.7	14.3	23.2	3.2	23.8	20.2
Soft Red Winter									
Abe (1978)	10.2	26.4	28.5	19.5	15.6	25.4	3.1	26.6	24.0
Arthur (1977)	9.8	27.7	21.4	14.4	9.2	17.4	4.2	23.8	18.5
Monon									
1977	8.6	53.9	18.1	15.4	19.3	15.7	3.6	23.2	16.3
1978	6.3	54.5	17.4	13.6	17.4	16.9	2.7	20.9	17.3
Arthur 71									
1977	12.0	30.2	26.7	17.3	14.8	26.1	3.3	25.0	23.3
1978	8.8	27.6	25.0	18.9	16.6	26.2	3.5	24.2	24.1
Logan									
1977	6.9	43.5	16.1	12.8	12.9	12.8	3.3	24.6	15.0
1978	5.7	40.1	15.3	12.2	14.6	13.7	3.7	24.2	13.2
Hard Red Spring									
Coteau (1978)	8.6	56.9	23.7	20.6	19.6	25.2	3.4	28.6	19.3
Olaf (1978)	4.4	16.7	17.5	11.2	7.4	16.4	3.5	24.5	13.3
Waldron (1977)	7.6	23.5	26.7	16.6	15.0	23.7	4.0	25.0	18.8
Era (1978)	3.8	15.7	15.4	10.2	7.4	15.6	2.4	22.1	12.3
Thatcher (1978)	4.7	20.2	20.4	14.0	10.0	19.0	3.4	25.3	16.1
Kitt (1978)	5.4	21.8	21.0	14.4	9.2	19.7	3.7	22.2	14.3
White Common									
Luke (1977)	3.4	8.6	12.2	8.1	7.4	13.4	3.6	23.8	10.5
Nugaines (1977)	6.8	19.0	22.0	15.9	13.7	22.7	1.9	20.4	22.1
Ionia (1977)	8.1	23.0	21.1	13.7	10.9	20.1	5.4	21.9	14.6
Genesee (1977)	1.5	5.2	6.8	4.3	4.0	7.8	3.8	17.9	5.4
Hyslop (1975)	5.2	44.0	16.1	13.0	18.5	13.4	3.3	28.3	13.8
Club									
Paha (1977)	2.2	5.3	7.5	4.9	3.9	9.2	2.8	14.6	3.6
Moro (1977)	2.7	5.1	8.3	4.8	4.4	9.0	2.9	18.0	5.4
Omar (1975)	2.5	6.0	8.3	4.6	4.0	8.8	3.6	17.5	6.0
Barbee (1977)	3.5	10.8	15.7	10.4	7.7	15.9	3.6	17.1	10.1
Durum									
Rolette (1978)	1.7	3.6	4.5	2.0	2.6	3.8	0.4	7.5	2.5
Rugby (1978)	2.1	5.2	2.8	1.9	2.1	3.8	0.6	12.5	1.7
Ward (1978)	2.5	4.3	3.4	1.2	1.4	3.7	1.1	9.9	2.1
Calvin (1978)	1.8	5.0	3.8	1.5	1.6	3.5	1.4	12.2	2.5
Cando (1978)	2.9	6.0	4.1	2.5	1.4	4.2	0.7	8.4	3.3

[a] Corrected to 14.00% moisture. Each value for each substrate represents the mean of two or more determinations made on 0.2000 g of wheat in 3.10 ml (3.00 ml in the case of tyrosine) of solution. Measurements were made with a Yellow Springs Instrument model 53 biological oxygen monitor.
[b] Standard deviations are shown for Eagle.

for Abe to 54.5 $\mu l \cdot ml^{-1} \cdot min^{-1}$ for Monon. Marketing channels need objective tests to distinguish a soft red winter from a hard red winter wheat, but whether the differences between the activities of the soft red winter and hard red winter wheats would be significant in a more comprehensive study is not clear, due to the limited number of samples. For example, activities toward dopamine of three of the soft red winter cultivars, Arthur, Monon, and Logan, were all appreciably lower than those for any of the hard red winter wheats, but, on the other hand, activities for the remaining two, Abe and Arthur 71, were not. Similar differences can be seen in activities toward catechol and *d*-catechin.

The hard red spring wheats tended to have activities close to those obtained for the hard red winter wheats, but differences did exist. For example, activities toward *d*-catechin were all lower than those exhibited by the hard red winter wheats. Also, with the exception of Waldron, activities with caffeic acid as substrate were outside the range shown by the hard red winter cultivars. Variability in activities between cultivars was about the same as for the soft red winter wheats.

Activities of the white common, club, and durum wheats were on the average lower than activities for the hard red winter, soft red winter, and hard red spring wheats. Activities of the white common wheats tended to be higher than those of the club or durum cultivars with the exception of Genesee, which had activities corresponding more closely to those of the club wheats. The three principal club cultivars, Paha, Moro, and Omar, had activities appreciably lower than those of the white common wheats, but Barbee, a relatively unimportant club cultivar, had activities more like those of the white wheats. Because of inconsistencies between cultivars in both classes, distinguishing club wheats from white common wheats by polyphenol oxidase activities would be difficult.

The durum wheats had significantly lower activities than the other classes. The data indicate that a durum wheat could be distinguished from a cultivar belonging to any other class by polyphenol oxidase activity alone. Activity toward dopamine, for example, would differentiate the durum wheats from any of the other cultivars studied. The highest activity for a durum wheat toward this substrate, 4.2 $\mu l \cdot ml^{-1} \cdot min^{-1}$ exhibited by Cando, was about half the lowest activity for a cultivar in any other class. The exhibition of lower activites by durum wheats is consistent with the fact that they are considered to be a separate species.

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