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Activation of Polyphenol Oxidase in Extracts of Bran from Several Wheat (*Triticum aestivum*) Cultivars Using Organic Solvents, Detergents, and Chaotropes

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Polyphenol oxidase (PPO), known to induce browning in wheat-based products, has been shown to be activatable in wheat *(Triticum aestivum)* bran extracts by chemical compounds. The activity in the extracts could be increased to varying degrees with acetone, methanol, ethanol, 2-propanol, and *n*-butanol as additives in the extraction buffer. The most potent alcoholic activator was *n*-butanol (about a 3-fold increase), followed by 2-propanol and ethanol, whereas methanol had the least effect. Ionic detergents in the extraction buffer were also good activators, with sodium dodecyl sulfate (SDS) being more potent (3-fold increase) than cetyltrimethylammonium bromide (CTAB) that had only half as much effect, whereas the nonionic detergent, Triton X-114, was ineffective. The chaotropes, urea and guanidine·HCl (GND), were the most potent activators of all, increasing the activity over 4-fold. Of the two chaotropes, GND was more effective at lower concentrations (<6 M) than urea. However, the enzyme activity lessened at a higher concentration of GND (6 M), while there was a further increase in the activity with 6 M urea treatment. The activity lessened with higher concentration of GND presumably as a result of extensive denaturation of the enzyme, as GND is known to be a more potent denaturant than urea. It is hypothesized that in wheat PPO exists in an inactive form which may be activated by the presence of activators, hitherto unknown, similar in effect to that elicited by the chemical denaturants in this study.

KEYWORDS: Wheat; *Triticum aestivum*; bran; polyphenol oxidase; enzyme activation; denaturants

INTRODUCTION

Polyphenol oxidase, sometimes referred to as phenol oxidase, catecholase, phenolase, catechol oxidase, or even tyrosinase, is considered to be an *o*-diphenol: oxygen-oxidoreductase (EC 1.14.18.1). This array of names for apparently the same enzyme is due to the broad substrate specificity it displays. The enzyme uses copper ions as a prosthetic group which participates in several redox reactions. Oxygen is required for the catalytic processes that lead to hydroxylation of monophenols to *o*-diphenols (monophenolase activity) and oxidation of *o*-diphenols to highly reactive *o*-quinones (diphenolase activity) (*1, 2*). The resulting *o*-quinones may participate in the formation of melanoid colored products (melanins) in some food systems (*3, 4*). Because of generating undesirable browning with negative esthetic attributes, PPO becomes a liability in some segments of the food industries, notably in fruits and vegetables (*5*) and some cereal foods, such as Chinese noodles (*6, 7*).

Polyphenol oxidase is widely distributed in microorganisms, plants, and animals. This enzyme has been shown to be present in wheat *(Triticum aestivum)* (*8*−*13*) as well. It is known to play a role in the physiology of plant photosynthesis and in the protection of plants from invasion by pathogens and insects. Several studies have investigated variations in its activity not only with quality characteristics for wheat products (*14, 15*), but also with cultivars, growing locations (*13, 15*−*17*), and milling fractions (*13, 14, 18*−*20*).

Some studies have demonstrated an array of PPO variants in wheat anatomical parts (*12*) or from partially purified fractions (*21, 22*), but it is not known whether some of these isoforms exist in latent forms. However, it has been reported in a variety of other crops that PPO (tyrosinase) exists in both latent and active forms (*4*). It was interesting, therefore, to determine whether there are active and latent forms of this enzyme in wheat, and if so, what would be the activation mechanism. The more likely mechanism would be through chemical activation reported in the literature in such instances as activation by different physicochemical treatments such as with acids (*23*) or exposure to detergents (*24*−*26*), fatty acids (*27*), and alcohols (*28, 29*). The results of these studies pointed to the potential mechanism of PPO activation in various living organisms apparently resulting from a conformational change in its structure (*26*). We presume, therefore, that the activity of polyphenol oxidase associated with unwanted browning encountered in wheat-based products is a consequence of activation. However, reports to that effect are scarce in the literature. Therefore, in

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an attempt to shed light on potential PPO activation mechanism(s), we employed the known protein denaturants to elicit a conformational change and correlate this with the level of activity induced. We report here the effect of extraction buffer additives on the activity of PPO from various wheat cultivars. The enzyme was extracted using buffer systems containing different classes of chemical compounds including alcohols, a ketone, detergents, or chaotropes. In addition, we demonstrated that PPO extracted from bran with an aqueous buffer alone could be further activated by adding to the extract known amounts of some of the chemical compounds listed above for various durations.

MATERIALS AND METHODS

Wheat Samples. Samples of hard red (Jagger and Prowers) and hard white (Betty, Heyne, and Lakin) winter wheats were obtained from 1998 and 1999 harvests. These included experimental lines and check varieties submitted by public breeding programs in participating midwest states (Colorado, Kansas, Nebraska, South Dakota, and Oklahoma) for quality testing under the auspices of the Wheat Quality Council. The bagged samples of wheat were transported to Manhattan, Kansas, for milling by the Hard Winter Wheat Quality Laboratory, Department of Grain Science and Industry, Kansas State University, and the Wheat Quality Laboratory, USDA Wheat Research Center, also in Manhattan. Milling was accomplished on the Miag Multomat Mill to produce bran and flour. Samples of bran were collected, immediately put into polyethylene bags, and stored at 8 °C until use.

Reagents. L-Dihydroxyphenylalanine was purchased from Sigma (St Louis, MO). Other reagents were of the highest grade and were purchased from Sigma, Fisher (Pittsburgh, PA), or BioRad (Hercules, CA).

Extraction of Wheat Bran with Phosphate Buffer Alone. A 0.5-g sample of each bran was weighed and soaked overnight in a refrigerated cabinet (8 °C) in 100 mM phosphate buffer, pH 6.5 (1:10, w/v). Homogenization was accomplished employing a Power Gen 700 homogenizer at a speed setting of 5 for 1 min with the sample tube immersed in ice. The samples were then gently shaken on a wrist-arm shaker at 8 °C for 1.5 h followed by centrifugation at 4800*g* in a swinging bucket rotor for 30 min. The clear supernatants were collected, and aliquots with known amounts of protein were used for the determination of PPO activity.

Extraction of Wheat Bran with Phosphate Buffer Containing Chemical Additive. Solutions of various concentrations of the organics were prepared in the 100 mM phosphate buffer pH 6.5. The chaotropic agents, urea and GND, were each prepared in concentrations ranging from 0.5 to 6 M. The detergents SDS and CTAB were in concentrations from 0.1−5%, and Triton X-114 concentrations ranged from 0.1 to 0.5%. Phosphate extraction buffers containing 1−40% (V/V) of either methanol, ethanol, 2-propanol, *n*-butanol, or acetone were also prepared. These buffer preparations were then individually used for the extraction of PPO to determine their effects on the level of the enzyme activity compared to that attained under similar extraction conditions but with phosphate buffer alone. The extracts were similarly centrifuged as described above and supernatant aliquots with known protein concentrations were used to estimate the activity as described below.

Estimation of Protein Concentration. Protein concentrations in the extracts used for the assays were determined by the Coomassie Blue dye method of Bradford (*30*). Bovine serum albumin supplied by Pierce Chemical Co. (Rockford, IL) was used as the standard.

Quantitation of PPO Activity. Polyphenol oxidase activity was spectrophotometrically determined as described by Kruger (*12*) using DOPA as the substrate with minor modifications. Essentially, the reaction was carried out in a 1-cm light path cuvette containing a volume of an extract equivalent to 500 μg of protein. The temperature was kept constant at 30 °C using a Beckman Peltier temperature controller attached to the cell-holder of the spectrophotometer. The reaction was started by addition of 1 mL of 10 mM DOPA to a final reaction volume of approximately 1.3 mL and rapidly mixing, blanking, and then starting the recording of the increase in absorbance. The activity was monitored employing a diode array spectrophotometer (Beckman DU 4700) equipped with molecular biology/enzyme kinetics software. An increase in absorbance at 480 nm as a result of DOPA being converted into dopachrome by PPO in the extract was monitored. The assay was carried out over a 3 min period during which the initial rate could easily be determined. The spectrophotometer kinetics software package was then utilized to estimate the enzyme activity by measuring the slope of the reaction as the initial rate. One unit of PPO activity was defined as the amount of the enzyme giving a change in absorbance of 0.001 per min under the assay conditions described above.

Direct Activation of PPO in Buffer Extracts Prior to Assay. Bran samples from 3 wheat cultivars were used in this experiment: Lakin, Prowers, and Betty. Appropriate aliquots of phosphate-buffer-extracted samples containing 500 μg of protein were each put into a cuvette. A predetermined concentration of the most potent PPO chemical activators was adjusted in each of the cuvettes to give the following concentrations in the mixtures: 5% SDS, 6 M urea, 2 M guanidine·HCl, or 5% *n*-butanol. The extracts containing the additives were then carefully mixed and incubated for various time intervals before assaying for PPO activity. Preincubation duration with each of the activators ranged from 0 to 60 min prior to addition of substrate for PPO assay. The spectrophotometric assay was carried out as described above.

Statistics. Each experiment was repeated at least 3 times, and each assay was performed in triplicates. The results are expressed as means of the values obtained, and the standard deviations of the means were calculated.

RESULTS

Effects of Acetone in Phosphate Extraction Buffer on PPO Activity. Acetone concentrations ranging from 1 to 20% in the extraction buffer had little effect on PPO specific activity in extracts of bran from the five wheat varieties tested (**Figure 1**) with only a slight increase at 40% in the extracts.

Effects of Alcohols in Phosphate Extraction Buffer on PPO Activity. The results of PPO activities in the alcohol extraction series are presented in **Figure 2**. Methanol had the least effect on the activity. In fact, there was a slight decrease in the activity as the concentration of methanol increased, but at 40% a moderate increase was observed. There was no noticeable effect of ethanol when used in concentrations up to 20% (**Figure 2B**). Ethanol and 2-propanol extracts behaved essentially the same way, with moderate increases in the activity at 40% concentration (**Figure 2B** and **C**). The most significant effect of alcoholic extracts of bran was observed with *n*-butanol. The specific activities of PPO were increased in all concentrations of *n*-butanol (**Figure 2D**). At 1% of the alcohol, there was already an appreciable increase in the extractable PPO activity (up to 2-fold). A plateau was reached at approximately10% *n*-butanol, and activity remained high even at 40% concentration, amounting to approximately a 3-fold increase in the activity in most of the variety extracts compared with that of the controls.

Effects of Detergents in Phosphate Extraction Buffer on PPO Activity. Extractions with SDS and CTAB had positive effects on PPO activity, but Triton X-114 had no apparent effect on the activity (**Figure 3**). In the case of an anionic detergent, SDS, a concentration of 0.1% already had a visible effect on the activity. The effect was most pronounced at the 1 and 2% concentration levels, with a peak at 2% for most of the cultivars tested (**Figure 3A**), where the specific activity went up about 3-fold. At 5% SDS concentration, the activities decreased, but still were approximately 2-fold higher than those in the controls. The extract of Lakin wheat bran was least affected by any of the concentrations of SDS used in the extraction buffers (**Figure 3A**).

The cationic detergent CTAB also had a positive effect on the PPO specific activity (**Figure 3B**), but to a much lesser extent than SDS. Less than a 1.5-fold increase in the activity in most extracts was observed. Again, the cultivar with lowest PPO activity in the controls, Lakin, was least affected by the presence

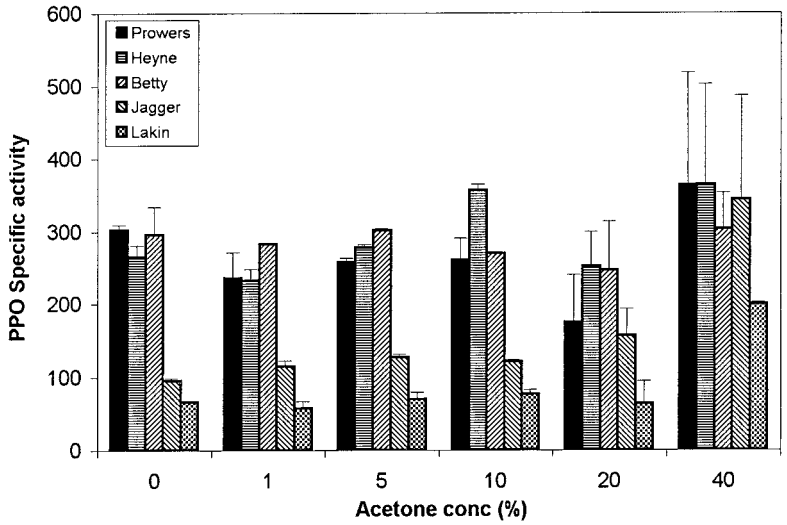


Figure 1. Influence of acetone in the extraction buffer on PPO specific activities in extracts of bran from 5 cultivars of hard winter wheat. The bran extracts were from Prowers, Heyne, Betty, Jagger, and Lakin cultivars as designated in the figure. Samples were extracted with either 0.1 M phosphate buffer, pH 6.5, alone or with the buffer containing varying concentrations of acetone (1, 5, 10, 20, or 40%). Aliquots containing 500 μg of protein were assayed for PPO activity using 10 mM DOPA substrate as described in Materials and Methods. Each sample was replicated at least 3 times. Vertical bars represent ± STD.

of the detergent in the extraction buffer showing no difference from the control value (**Figure 3B**).

Extraction with buffers containing a nonionic detergent, Triton X-114, in the concentration range from 0.1 to 0.5% had, overall, no influence on the PPO activity (**Figure 3C**). In the cultivars that have high PPO activities in the controls (Prowers, Heyne, and Betty) Triton X-114 even had a slight lowering effect on the specific activity. The detergent had virtually no effect on the enzyme activities in the Jagger and Lakin bran extracts (**Figure 3C**).

Effects of Chaotropes in Phosphate Extraction Buffer on PPO Activity. Extraction of bran with phosphate buffer containing either urea or guanidine·HCl had a profound influence on PPO specific activity (**Figure 4A** and **B**). It is clear that concentrations above 0.5 M had a strong effect on the activities. In the case of urea, a greater than 4-fold increase in the PPO specific activities was observed in the cultivars with higher control values such as Prowers, Heyne, Betty, and Jagger (**Figure 4A**). Polyphenol oxidase in the extract from the cultivar with lower activity in the control, such as Lakin, was less affected by urea in the concentration range of 0.5−3.0 M, as only approximately a 2-fold increase was observed. However, there was a much stronger enhancement in the activities in the extractions with 6 M urea that approached a 4-fold increase.

Extraction in the presence of guanidine·HCl yielded a pattern of activities similar to that observed for urea, but 0.5 M guanidine·HCl concentration had a more profound effect on PPO activity than urea at this concentration (**Figure 4A** and **B**). Comparable levels of activation was observed at 3 M concentration (**Figure 4A** and **B**) where more than a 4-fold increase in activity was found. At 6 M guanidine·HCl, the activation level was lower than that at 3 M concentration, as it amounted to only a 3-fold enhancement compared with that of the controls (**Figure 4B**).

Direct Activation of PPO in Bran Extracts with SDS, Urea, Guanidine, and n-Butanol. Phosphate buffer extracts of bran from 3 cultivars, Lakin (white, low PPO), Prowers (red, high PPO), and Betty (white, high PPO), were tested for potential PPO activation by direct exposure to the most potent activating chemical agents demonstrated in the extraction experiments above: SDS, urea, guanidine, and *n*-butanol. The results of exposure of the phosphate buffer extracts to these chemicals for various durations are shown in **Figure 5A**, **B**,- and **C**. It is evident that 2 M guanidine·HCl had the highest ability to activate PPO extracted from the 3 cultivars. At peak activation, about a 5-fold increase in the PPO activity was observed in Lakin extract, from approximately 60 to 300 PPO units. These increases were approximately 3-fold those in the Prowers and Betty extracts. In Lakin and Betty extracts, the peaks were attained within the first 5 min of exposure, and the levels were maintained throughout the 60 min of study in the case of Lakin, but a slight decline from about 700 units to 550 units was observed with Betty extracts. On the other hand, in Prowers the peak activity was attained between 10 and 15 min followed by a decline from approximately 650 PPO units to 450 units.

The second-best direct activator of PPO was 6 M urea with approximately 1.5−2-fold increases in the activity in all of the cultivars studied (**Figure 5A**,**B**, and **C**). Activation with urea peaked within 5−15 min of exposure, and the levels were maintained for the rest of the study period.

n-Butanol was a rather poor direct activator, as there was hardly a change in the activity throughout the study period in all of the cultivars (**Figure 5A**, **B**, and **C**).

A negative effect on PPO activity was induced by SDS in the Lakin bran extract, and SDS had very little effect on the Prowers sample, as the levels of PPO activity remained essentially unchanged (**Figure 5A** and **B**). However, there was a 1.25-fold increase in the activity of the Betty bran extract caused by addition of SDS (**Figure 5C**). The peak was attained within 5−15 min of exposure, and a level of about 300 units was maintained throughout the study period.

In all cases of direct activation of PPO in the bran extracts with the denaturants as outlined above, the peak activation was attained within the first 15 min of exposure. Longer exposures had no accumulative effect; in fact, in some instances such as in the case of activation with guanidine·HCl, there was a slight drop in the activity after the first 15 min. The plateau was maintained for the rest of the period (**Figure 5A**, **B**, and **C**). Even increasing contact time for 24 h did not yield any higher PPO activity in the extracts.

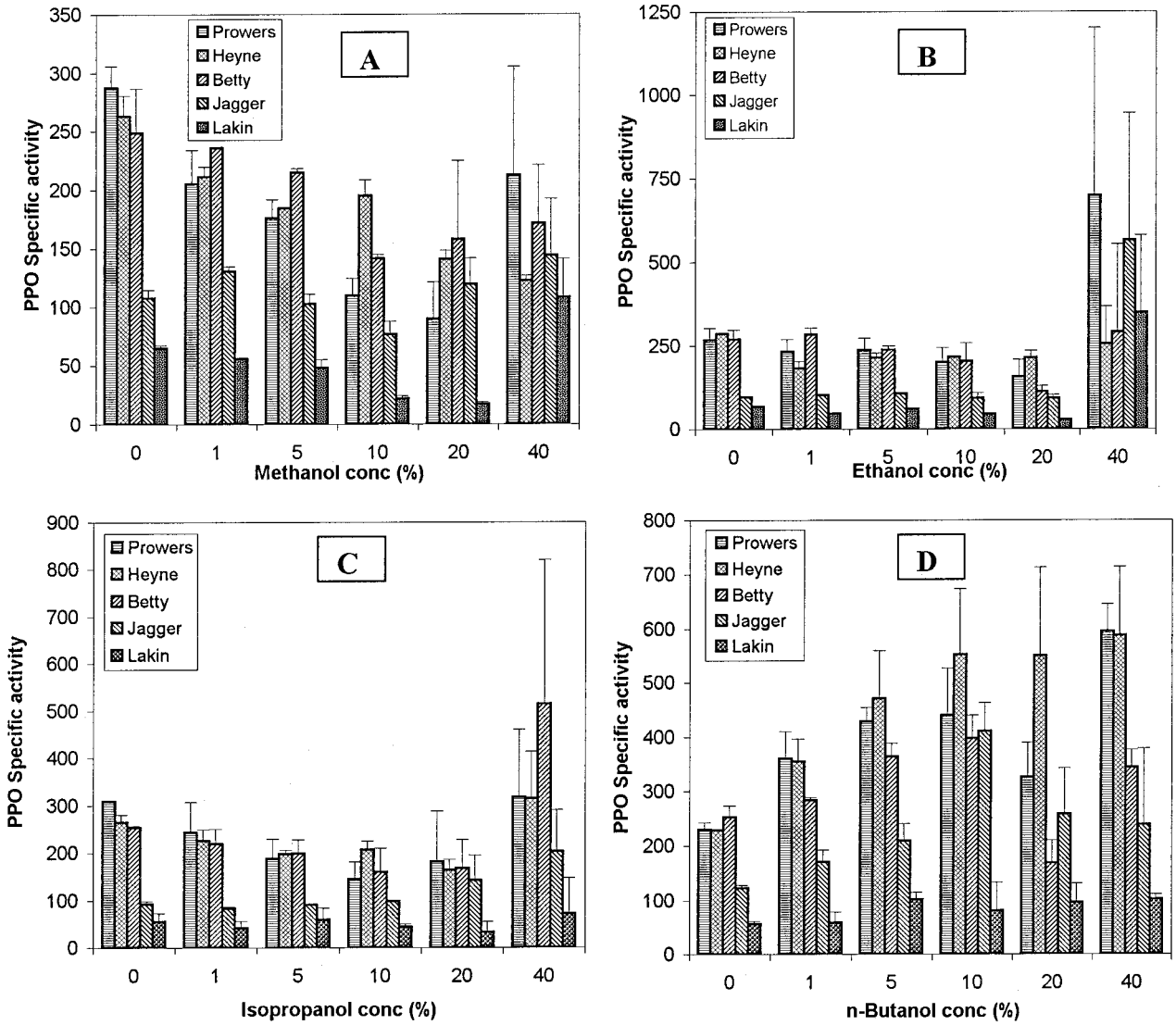


Figure 2. Effect of alcohols added to extraction buffer on PPO specific activities in extracts of bran from 5 cultivars of hard winter wheat: Prowers, Heyne, Betty, Jagger, and Lakin, as designated in the figure. Each sample was extracted with either 0.1 M phosphate buffer, pH 6.5, alone or with the buffer containing varying concentrations of methanol (A), ethanol (B), 2-propanol (C), or *n*-butanol (D) (1, 5, 10, 20, or 40%). Aliquots containing 500 μg of protein were assayed for PPO activity using 10 mM DOPA substrate as described in Materials and Methods. Each sample was replicated at least 3 times. Vertical bars represent ± STD.

Polyphenol oxidase activity was also observed in the extracts made with phosphate buffer alone (**Figure 5A**,**B**, and **C**) though at lower levels than in those that had been chemically activated. It appears that some degree of latent PPO activation must have occurred in these fractions either prior to or during the extraction processes.

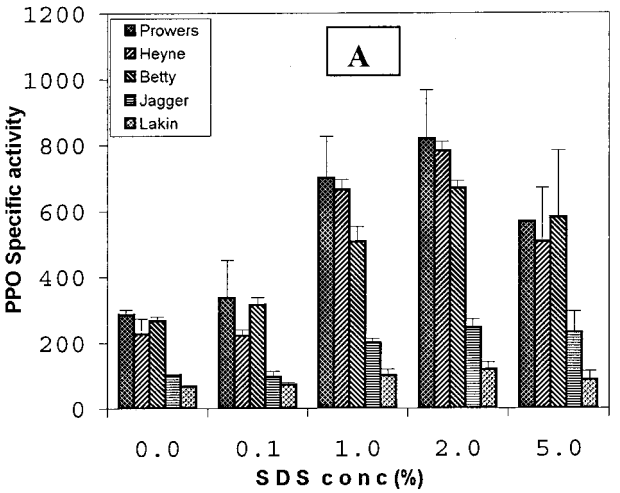
DISCUSSION

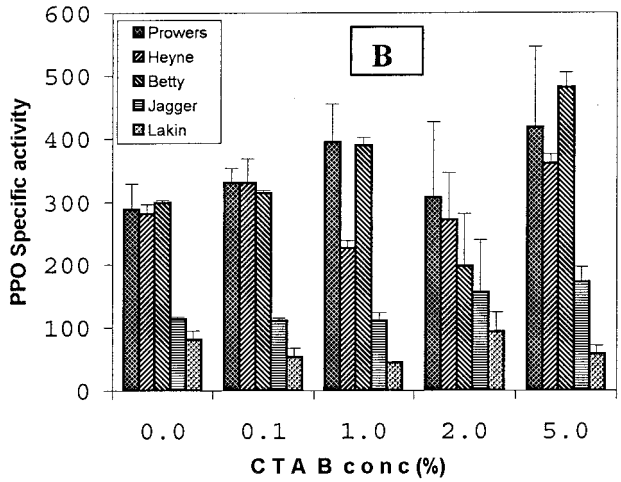
Polyphenol oxidase (tyrosinase) has been reported to exist in both latent and active forms in a variety of crops (*4*). No study, however, has shown this to be the case in wheat kernels. PPO activity has been correlated with unwanted browning in some cereal foods such as Chinese noodles (*6*, *7*). This would suggest that PPO has to be activated by some mechanism to elicit its effect. Some studies have indicated that the potential mechanism of PPO activation in various living organisms is induction of a conformational change in its structure (*26*). It is conceivable, therefore, that the activity of PPO associated with unwanted browning encountered in wheat-based products is brought about by PPO activation. We have, therefore, in this study attempted to elucidate the potential PPO activation mechanism by employing the known protein denaturants to elicit a conformational change and correlate this with the level of activity induced.

In our recent studies, it was confirmed that the bran fraction had the highest level of PPO activity and that the flour fraction had the least (*13*). We, however, did not resolve whether the PPO activity observed was due to the whole extractable enzyme or whether some of the extracted enzyme was in its latent form and therefore could not be measured.

To resolve this question, we conducted PPO activation studies with extracts from bran, as that fraction was the richest source of the enzyme in the wheat kernel. The ability of several classes of chemical compounds to activate the enzyme was tested, including a ketone, alcohols, detergents, and chaotropes. The results showed that acetone had little effect on PPO activity in the hexaploid hard winter wheats studied.

Of the 4 alcohols tested, methanol, ethanol, and 2-propanol had about the same effects on PPO activity. An apparent decrease in activity was noted at lower concentrations compared with that of controls, but these decreases were minimal. However, at higher concentrations, increases in PPO activity





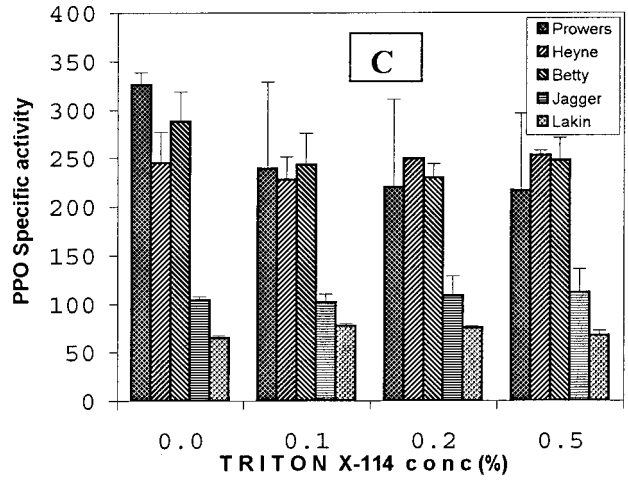
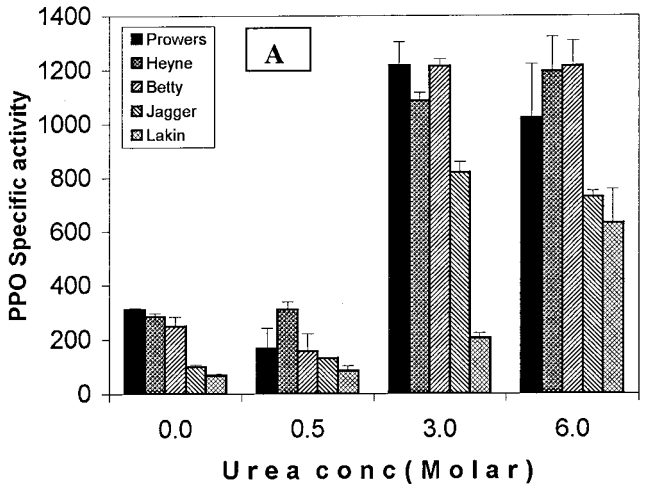


Figure 3. Comparison of PPO specific activities in extracts of bran from 5 cultivars of hard winter wheat made with buffers containing detergents. The bran extracts were from Prowers, Heyne, Betty, Jagger, and Lakin cultivars as designated in the figure. Each sample was extracted with either 0.1 M phosphate buffer, pH 6.5, alone or with the buffer containing varying concentrations of SDS (A), CTAB (B) (0.5, 1, 2, or 5%), or Triton X-114 (C) (0.1, 0.2, or 0.5%). Aliquots containing 500 μg of protein were assayed for PPO activity using 10 mM DOPA substrate as described in Materials and Methods. Each sample was replicated at least 3 times. Vertical bars represent ± STD.

were evident, particularly those caused by ethanol and 2-propanol. The most dramatic effects were found when *n*-butanol was used in the extraction buffer. A 2-fold increase in the PPO



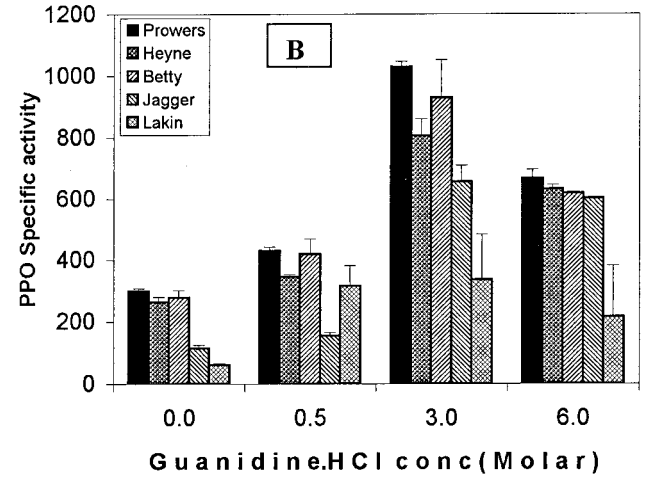


Figure 4. Specific activities of PPO in extracts of bran from 5 cultivars of hard winter wheat extracted with buffers containing chaotropes. The bran extracts were from Prowers, Heyne, Betty, Jagger, and Lakin cultivars as designated in the figure. Each sample was extracted with either 0.1 M phosphate buffer, pH 6.5, alone or with the buffer containing varying concentrations of urea (A) or guanidine·HCl (B) (0.5, 3.0, 6.0 M). Aliquots containing 500 μg of protein were assayed for PPO activity using 10 mM DOPA substrate as described under Materials and Methods. Each sample was replicated at least 3 times. Vertical bars represent ± STD.

activity compared with that of the control was observed even at 1% *n*-butanol. A 3-fold increase was noted with increasing concentrations of *n*-butanol, peaking at a 10% concentration, and maintained even at higher concentrations (up to 40%).

Asada et al. (*28*), working with fruitfly prophenoloxidase found that the best activator of hemolymph proenzyme was 2-propanol, followed by *n*-butanol, then methanol, and the least was ethanol. In the present study, the activation of wheat PPO by extracting with phosphate buffer containing these alcohols was most effective with *n*-butanol followed by 2-propanol, then ethanol, and the least being methanol. Although phenoloxidase in animals may be related to plant PPO, the way in which these enzymes respond to activators may vary according to the evolutionary hierarchy of the source. It is worth noting that relatively high levels of activation were observed with *n*-butanol in both systems. *n*-Butanol is the most hydrophobic of the 3 alcohols tested. There appears, therefore, to be a correlation between the hydrocarbon chain length of the alcohol used and the level of PPO activation. Methanol has the shortest chain length and it is the weakest activator, whereas *n*-butanol has the longest chain length and it is the strongest activator. It is not clear, however, whether the activation mechanism is related

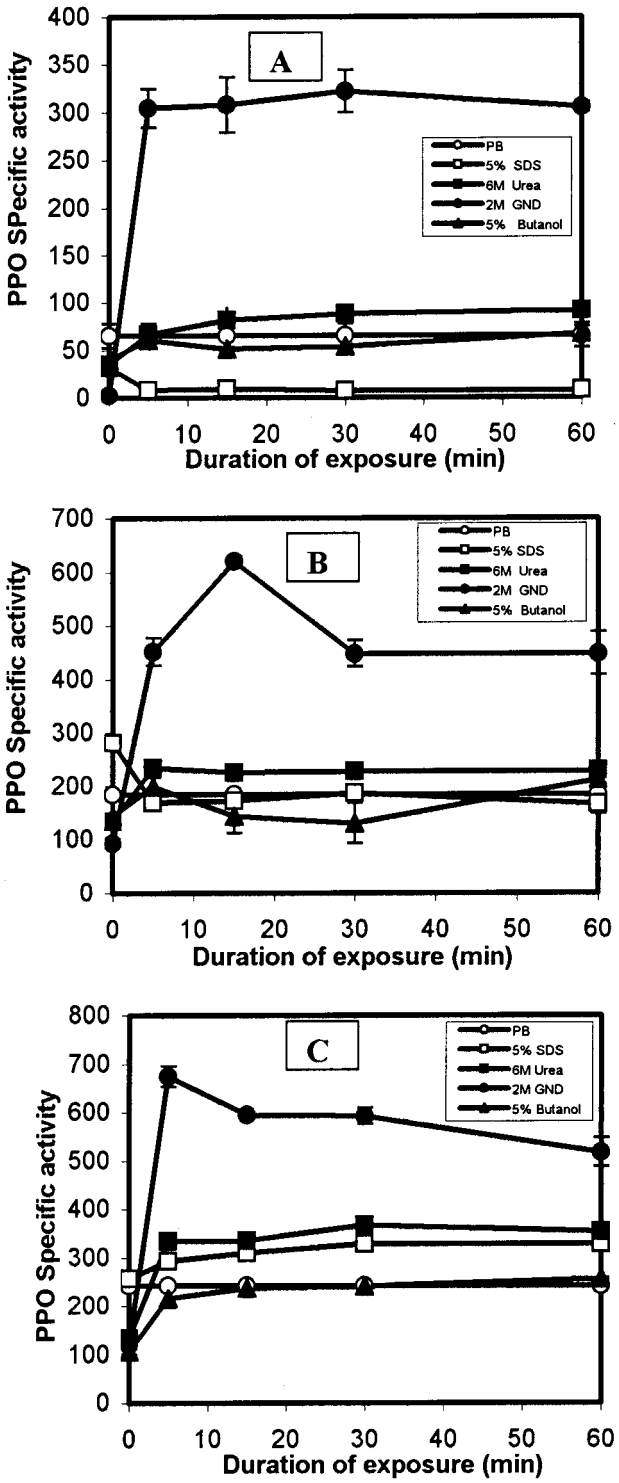


Figure 5. Activation of PPO in phosphate buffer extracts of bran from 5 cultivars of hard winter wheat: Lakin (A), Prowers (B), and Betty (C), as designated in the figure. Each sample was extracted with 0.1 M phosphate buffer, pH 6.5, and an aliquot of the supernatant containing 500 μg of protein treated with 5% SDS, 6 M urea, 2 M guanidine·HCl (GND), or 5% *n*-butanol before assaying for PPO activity using 10 mM DOPA substrate as described under Materials and Methods. Each sample was replicated at least 3 times. Vertical bars represent ± STD.

to hydrophobic interactions that induce a conformational change favoring binding of the substrate to the active site of the enzyme, removal of an inhibitor, or by other mechanism. The activation mechanism of latent mushroom tyrosinase by benzyl alcohol (*29*) was attributed to a conformational change of the enzyme molecule. The hydrophobic benzyl ring of this alcohol might have facilitated this process in the mushroom tyrosinase as well through hydrophobic interactions. The authors suggested a mechanism similar to that elicited by SDS reported earlier by Moore and Flurkey (*24*), who had suggested that the conformational change in the enzyme resulted from binding SDS monomers.

We further investigated whether wheat latent PPO could be activated by other denaturants. Detergents and chaotropes were tested for their ability to activate the PPO. Extraction of PPO from bran using phosphate buffer containing ionic and nonionic detergents showed that only extractions with buffers containing ionic detergents yielded PPO with higher specific activity than phosphate buffer alone. This suggests that the ionized nature of the detergents had influence on the activation of the enzyme. An anionic detergent, SDS, was a better activator than the cationic detergent, CTAB, while the nonionic detergent, Triton X-114 had no effect on the extracted PPO activity at a 0.5% concentration or less. It was not possible to compare the influence of nonionic versus ionic detergents on the enzyme activity at higher concentrations because of cloudiness that appeared in the solution when higher concentrations of Triton X-114 were used in the extraction buffer. The increases in enzyme activity following the extraction with buffers containing ionic detergents may be attributable to either more active enzyme being extracted, or more latent PPO molecules becoming activated during this treatment, or both of these factors. Activation of PPO by SDS has been reported in the enzyme preparations from other plant systems (*24, 31*−*33*) as well as in tyrosinase in mushroom (*26*). It is evident, therefore, that activation of latent PPO from wheat by SDS is likely to occur. Triton X-114 has been shown to be a nonactivator of mushroom tyrosinase (*34*), whereas SDS and CTAB were activators, an observation that led these authors to employ Triton X-114 in their PPO purification steps. It is not surprising, therefore, that in the present study Triton X-114 extracts of bran showed no increases in PPO activity.

Assuming that the activation mechanism of latent PPO was through a conformational change, representative chaotropes that are commonly used in disrupting protein structure were assessed for their ability to activate PPO when added to the phosphate extraction buffer. The results showed that inclusion of guanidine·HCl or urea induced the highest levels of PPO activity in the bran extracts of any of the chemical compounds tested. Guanidine·HCl was more efficient than urea at inducing higher activity at lower concentrations ($\leq$ 0.5 M) of these denaturants, but both chaotropes were equally efficient at concentrations higher than 0.5 M. However, 6 M guanidine·HCl was less effective than urea suggesting that a more extensive destabilization of PPO molecular structure resulted, leading to reduced substrate binding. These results suggest that wheat bran latent PPO activation mechanism is mainly through a conformational change of the enzyme molecules, as chaotropes are known to be potent destabilizers of protein structures, with guanidine·HCl being a stronger agent than urea.

More evidence was required to establish whether the increased PPO specific activity observed was due to improved efficiency of its extraction from bran aleurone layer with these denaturants or if it was due to latent enzyme activation. Results in **Figure 5A**,**B** and **C** showed that guanidine·HCl and urea are indeed efficient activators of wheat PPO when known quantities are added to extracts of bran that had been prepared previously with phosphate buffer alone. A time course activation profile was observed. However, prolonged exposure of the extracts to these denaturants did not yield any higher PPO activity after a

threshold was achieved, even after an overnight exposure (24 h). Although direct activation with chaotropes was evident, the levels of specific activity were lower than or equal to the levels when these compounds were included in the extraction buffer. This suggests that inclusion of chaotropes in the extraction buffer enhanced extraction efficiency in addition to activating the latent enzyme. Results on the direct activation by *n*-butanol and SDS were not conclusive, especially in the Lakin and Prowers samples. This may have been due to a phase separation, in the case of *n*-butanol, during the assay process. There was no phase separation when the samples were homogenized in the buffer containing *n*-butanol due to emulsification resulting from the homogenization process. It is unclear why direct activation of PPO from these 2 varieties was not feasible with SDS, although some activation was observed in Betty extracts.

In conclusion, we have established that higher wheat PPO activity can be induced by either extraction with buffers containing various chemicals or by direct treatment of the extracts with chemicals that destabilize protein structures. This suggests the existence of PPO in a latent form that can be activated by a conformational change or a removal of an inhibiting component from the latent enzyme. It appears that the stronger the destabilizing ability the compound has, the better it is as an activator, but only to a certain concentration threshold. At higher concentrations of the denaturants, the enzyme becomes denatured to an extent that renders it nonfunctional. The factors that govern the activation processes appear to be the chemical nature of the denaturants, their concentrations, and the contact time during the first 15 min. Under natural conditions, presumably, wheat PPO in a latent enzyme form ought to be activated for it to display catalytic functions or to induce food discoloration. We do not know the activation mechanism of latent PPO in this plant under natural conditions, a subject that is currently under investigation in our laboratory.

ABBREVIATIONS USED

PPO, Polyphenol oxidase; SDS, sodium dodecyl sulfate; CTAB, cetyltrimethylammonium bromide; DOPA, dihydroxyphenyl alanine; GND, guanidine·hydrochloride.

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