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EFFECT OF TEMPERATURE STRESS ON POLYPHENOL OXIDASE ACTIVITY IN GRAINS OF SOME WHEAT CULTIVARS

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Abstract

Color is a key quality trait of wheat-based products and polyphenol oxidase (PPO) is implicated to play a significant role in their undesirable darkening. Polyphenol oxidase catalyzes the oxidation of phenols to quinines, which auto oxidize and polymerize with amino acid of cellular proteins resulting brown and black pigmentation propounding reduced nutritional values. In present study, the PPO activity in 50 different Pakistani wheat cultivars was investigated and grouped into three categories *viz*; low, medium and high PPO activity cultivars. PPO is a heat labile enzyme. To investigate effect of heat stress, nine cultivars from each category were chosen for treatment at 30, 40 and 50°C for 30, 60, and 120 minutes each. A substantial change was experienced in PPO activity as compared to room temperature. Two wheat cultivar Wafaq-2001 and AS-2002 showed a compromising attitude of minimum PPO activity at 30°C for a period of 30 and 60 minutes of incubation. In general, an incubation of 30°C or 60°C (low or high) for a period of 30 minutes can be recommended for suppressing PPO activity.

Introduction

The cultivation of wheat (*Triticum* spp.) reaches far back into history. Wheat was one of the first domesticated food crops and for 8,000 years has been the basic staple food of the major civilizations of Europe, West Asia and North Africa. Today, wheat is grown on more land area than any other commercial crop (Kamran *et al*., 2009; Kayani *et al*., 2010). Being an important food, there are some concerns regarding the quality of the wheat grain. One of the quality parameters is the browning of wheat flour. These changes in physical characteristics are governed by oxidation of many phenolic compounds, catalyzed by polyphenol oxidase (PPO) (Simone *et al.*, 2002).

PPO is a copper containing enzyme. It is commonly distributed in plants. This enzyme catalyzes two reactions in the oxygen presence. In first reaction, monophenols are converted to *ortho*-diphenols (monophenolase activity). In second reaction, *ortho*-diphenols are oxidized to *ortho*-quinones (diphenolase activity) (Steffens *et al*., 1994). *Ortho-quinones* polymerize non-enzymatically into red, brown or black pigment (melanin). Its latter property makes it an important enzyme in food industry to cause browning in some of the wheat-based food products (Walker & Ferrar, 1998).

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Noodles are the major wheat based product all over the world. PPO is the major cause in their time-dependent darkening (Martin *et al*., 2005). In wheat grains, PPO activity is found to be located mainly in bran (Sullivan, 1946). Common substrates for PPO are tyrosine, catechol (McCaig *et al*., 1999) and 3, 4-dihydroxyphenyl alanine (L-DOPA) (Anderson & Morris, 2001). There are some endogenous phenolic acids to the wheat plant and grain which are potential substrates for PPO as well. They include ferulic acid, sinapic acid and vanillic acid (Hatcher & Kruger, 1997).

In plants, dormant PPOs are localized in plastids while their phenolic substrates are mainly located in the vacuole. So the enzymatic browning occurs only when sub-cellular compartment is ruptured in the presence of oxygen (Vaughn *et al*., 1998). PPOs also play a defense-related role in higher plants (VanGelder *et al*., 1997; Thipyapong *et al*., 2004).

In 1907, tyrosinase (the PPO which uses tyrosine as substrate) was discovered from the wheat grains. After it PPO has been extensively studied in wheat (Anderson & Morris, 2001). Immature seeds exhibited greatest PPO activity which may be the result from its 12 isozymes (Anderson & Morris, 2001). Wheat cultivars differ in PPO activity and plant breeders wish to select grains with low PPO activities to sow. Wheat cultivars differ considerably in PPO activity (Park *et al*., 1997; Anderson & Morris, 2001). Methods for the evaluation of PPO activity have been developed and the variation of PPO activity among wheat (*Triticum aestivum* L.) cultivars has been well documented.

PPO activity in wheat has commonly been measured either spectrophotometrically (production of colored products) or extent of the oxygen consumed (Marsh & Galliard, 1986). Half of the PPO is extracted and leached to solution from mechanically damaged seeds. This might cause variation in the PPO activity assay (McCaig *et al*., 1999). The expression of PPO activity is affected by genotype and growing conditions (Park *et al*., 1997). In hard red bread wheat, growing locality contributed more to variation in flour PPO activity than genotype (Park *et al*., 1997).

PPO inhibited by chelating agents, zinc and calcium ions but those additives may affect taste. According to Vadlamani & Seib (1996), PPOs are heat-labile and are inactivated by a short heat treatment at 70-90°C. They also found that heat treatment of wheat at 13-17% moisture content and 95-110°C for 4 to 12 minutes inactivate oxidative enzymes without altering the flour properties.

Milling of wheat grain at a higher flour extraction rate raises darkening effect in end products (Baik *et al*., 1994). Compulsory heating is an important step in the preparation of wheat based food products. It may affect the quality of wheat flour and its products. To check the qualitative characteristics of the wheat seeds after the heating treatment, PPO activity assay is the best criterion to formulate the recommendations to develop improved processing methodology. In the present study, PPO activities of 50 wheat cultivars (majorly grown in Pakistan) were analyzed and all of them were grouped into three main classes based on their low, medium and high PPO activity. On the basis of preliminary data, nine wheat cultivars were selected for analyzing the effect of temperate on PPO activity.

Materials and Methods

Arrangements of wheat seeds: Wheat (*Triticum aestivum* L.) grains were procured from National Agriculture Research Center (NARC) Islamabad, Pakistan.

PPO assay preparation: PPO assay was conducted as described by Anderson & Morris (2001) with some modifications. Initially, three healthy wheat grains were placed in

standard test tubes containing 2 ml of reacting buffer (50mM phosphate buffer of pH 6.5 containing 10mM L. tyrosine and pH adjusted at 8.0 by using 0.5 M tris [tris (hydroxyethyl) aminomethane] solution). The tubes were then incubated at room temperature with rotation at 160 rpm for 2 hours. Following incubation, solutions were removed from the tubes and the change in absorbance was compared with the absorbance of substrate solution. Change in absorbance was recorded at 475 nm. One unit of PPO activity was defined as a change in absorbance divided by the weight of the sample of grains taken. All reactions were conducted at room temperature. Initially fifty wheat varieties were assayed without any treatment and referred as control. Among these 50 varieties, 9 varieties were selected (by constructing a frequency table of three classes and three mean valued varieties from each class were selected). The nine selected varieties were then incubated at 30, 40 and 50 °C and for three time durations as 30, 60, and 120 minutes. During incubation, temperature was carefully adjusted.

Statistical analysis: Experiments were performed in a 4X3 factorial design consisting of temperature (room temperature, 30, 40 & 50°C) and time (30, 60 & 120 minutes) respectively. All experiments were done in triplicates. The data obtained was analyzed statistically (ANOVA & LSD) in MSTATC Program.

Results and Discussion

Categorization of wheat cultivars based on PPO activity: The wheat grains of 50 varieties were assayed for PPO activity. Varieties showed a wide variation in PPO activity ranging from 0.214 (Wadanak-85) to 4.710 (Indus-79) Enzyme Unit (EU) (Table 1). Three classes were made based on the PPO activity of the varieties as varieties with low, medium and high PPO activities (Table 2).

Effect of temperature on PPO activity: Three representative varieties from each group were treated with different temperatures (30, 40 & 50°C) and their PPO activity was compared with the enzyme activity checked at room temperature (control).

Effect of temperature on wheat cultivars with low PPO activity: AS-2002, Wafaq-2001 and Momal-2002 were selected as low PPO activity cultivars and were treated with 30, 40, 50°C and room temperature for a period of 30, 60 and 120 minutes. Maximum activity was observed for Momal-2002 (1.654 EU), significantly followed by that (0.896 EU) of Wafaq-2001 and AS-2002 (0.792 EU) (Table 3).

Significant decrease in PPO activity was observed when wheat grains of different low PPO activities cultivars were treated with different temperatures (Table 3). Maximum PPO activity (1.328 EU) was observed under control (room temperature), which was significantly decreased when treated with 40°C (1.160 EU) and 50°C (1.131 EU) Minimum activity (0.836 EU), significantly lowered to all other comparable means, was observed when wheat grains were treated with 30°C.

Maximum PPO activity (1.231 EU) was observed when wheat grains were incubated for a period of 60 minutes, non-significantly followed by that (1.083 EU) when incubated for 30 minutes and significantly followed by that (1.027 EU) when incubated for 120 minutes. The lowest activity has non-significantly different with the activity of those grains incubated for 30 minutes.

Table 1. Wheat (*Triticum aestivum* L.) cultivars with their polyphenol oxidase activities (varieties are arranged on the basis of increase in PPO activity).

S. No.	Wheat cultivars	PPO activities (EU)	S. No.	Wheat cultivars	PPO activities (EU)
1.	Wadanak 85	0.214	26.	NR 271	2.600
2.	A. S. 2002	1.111	27.	Shahkar 95	2.641
3.	Bakhar 2001	1.132	28.	Bahawalpur 94	2.677
4.	Kohistan 97	1.186	29.	Kohinoor 83	2.683
5.	Margalla 99	1.209	30.	Punjab 96	2.802
6.	Wafaq 2001	1.261	31.	Mathar	2.806
7.	Sandal	3.803	32.	NR 270	2.864
8.	Momal 2002	1.610	33.	Nowshera 96	2.902
9.	Saleem 2000	1.656	34.	Chakwal 97	3.007
10.	Marwat. J. 2001	1.682	35.	G. A. 2002	3.076
11.	M. H. 97	1.701	36.	Zarlashta 99	3.079
12.	Tatara	1.724	37.	Soghat 90	3.084
13.	Suleman 96	1.854	38.	Kohsar 95	3.100
14.	Mehran 89	1.904	39.	Sutlej 86	3.363
15.	Khaghan 93	2.023	40.	Inqalab 91	3.487
16.	Chenab 2000	2.082	41.	Barani 83	3.493
17.	Pirsabak 85	2.124	42.	Sariab 92	3.569
18.	Auqab 2002	2.247	43.	Pawan	3.607
19.	S. H. 2003	2.251	44.	Khayber 87	3.791
20.	Iqbal 2000	2.286	45.	Zamindar 80	3.901
21.	Zarghoon 79	2.321	46.	Sindh 81	4.218
22.	Shalimar 88	2.340	47.	Pari 73	4.282
23.	NR 267	2.465	48.	Rohtas 90	4.460
24.	Anmol 91	2.486	49.	Drawar 96	4.607
25.	NR 268	2.590	50.	Indus 79	4.710

Table 2. Simple statistics of PPO activities of different wheat cultivars.

Mean	**2.64142**	**Range**	**4.496**
Standard error	0.142593571	Minimum	0.214
Median	2.6205	Maximum	4.71
Standard deviation	1.008288813	Sum	132.071
Sample variance	1.01664633	Largest (1)	4.71
Kurtosis	-0.291476097	Smallest (1)	0.214
Skewness	0.070832811	Confidence level (95.0%)	0.286552505

Momal-2002 showed maximum PPO activity (1.822 EU) when treated with 30°C while Wafaq-2001 exhibited minimum activity (0.318EU) when treated with 30°C. Momal-2002 showed significantly lowered PPO activity under all other conditions as compared to 30°C. Wafaq-2001 exhibited significantly lowered PPO activity (0.319, 0.979 & 1.025 EU) when treated with 30, 40 and 50°C, respectively, than control (1.260 EU). Almost all the durations of incubation showed significantly lowered PPO activity than control. Maximum activity (1.467 EU) was seen at 40°C for a duration of 60 minutes, while minimum value (0.711) was obtained at 30°C for 30 minutes.

Table 3. Effect of temperature on polyphenol oxidase activities in wheat varieties with low PPO activities (Group 1).

Varieties (V)	Temperature (T)				Mean
	Control	30 °C	40 °C	50 °C	
AS -2002 (V_1)	1.118 cd	0.368 g	0.856 ef	0.827 f	0.792 B
Wafaq-2001 (V_2)	1.260 c	0.319 g	0.979 d-f	1.025 de	0.896 B
Momal-2002 (V_3)	1.607 b	1.822 a	1.644 b	1.542 b	1.654 A
Duration (D)	**D X T**				**Mean**
30 minutes (D_1)	1.328 ab	0.711 e	1.297 a-c	0.995 d	1.083 AB
60 minutes (D_2)	1.328 ab	0.998 d	1.467 a	1.132 cd	1.231 A
120 minutes (D_3)	1.328 ab	0.800 e	0.714 e	1.267 bc	1.027 B
Interaction	**V X T X D**				**Mean**
V_1D_1	1.118 c-f	0.421 jk	0.926 e-h	0.665 h-j	0.782 D
V_1D_2	1.118 c-f	0.358 k	0.957 e-g	0.893 f-h	0.832 CD
V_1D_3	1.118 c-f	0.324 k	0.686 hi	0.923 e-h	0.763 D
V_2D_1	1.260 cd	0.415 jk	1.225 cd	1.090 c-f	0.998 C
V_2D_2	1.260 cd	0.288 k	1.272 cd	0.823 gh	0.911 CD
V_2D_3	1.260 cd	0.255 k	0.440 i-k	1.161 c-e	0.779 D
V_3D_1	1.607 b	1.298 c	1.742 b	1.230 cd	1.469 B
V_3D_2	1.607 b	2.347 a	2.172 a	1.680 b	1.952 A
V_3D_3	1.607 b	1.821 b	1.018 d-g	1.716 b	1.540 B
Mean	**1.328 A**	**0.836 C**	**1.160 B**	**1.131 B**	**1.114*** **

Means followed by similar letters are not significant to each other at $p \leq 0.05$
LSD for varieties: 0.1504
LSD for temperature: 0.1285
LSD for variety X temperature: 0.1711
LSD for duration: 0.1504
LSD for variety X duration: 0.1681
LSD for temperature X duration: 0.1711
LSD for variety X temperature X duration: 0.2639

Its evident from the means of V X T X D interactions that maximum PPO activity was obtained by Momal-2002 (1.952 EU) at 60 minutes of incubation and minimum activity is presented by AS-2002 (0.763 EU) at 120 minutes of incubation at 120 and 30 minutes of incubation respectively (Table 3). It is obvious that AS-2002 (0.832 EU) & Wafaq-2001 (0.911 EU) have non-significant difference in PPO activity at 60 minutes of incubation, after Wafaq-2001 (0.998 EU), which showed non-significant difference to them at 30 minutes.

V X T X D interactions shows that the minimum (0.255 EU) was showed by Wafaq-2001 at 120 minutes of incubation at 30 °C. AS-2002 showed a significantly increased activity (0.324, 0.686 & 0.923 EU) at temperature treatment of 30, 40 and 50°C respectively. Wafaq-2001 exhibited an increased PPO activity (1.260 EU) at control than low PPO activities (1.161, 0.440 & 0.255 EU) accordingly at 50, 40 and 30°C at 60 minutes of incubation period. Means followed by similar letters are non-significantly different to each other in PPO activity.

Table 4. Effect of temperature on polyphenol oxidase activities in wheat varieties with medium PPO activities (Group 2).

Varieties (V)	Temperature (T)				Mean
	Control	30 °C	40 °C	50 °C	
Iqbal-2000 (V_1)	2.287 d	1.652 f	2.743 c	2.985 b	2.417 B
Mathar (V_2)	2.806 bc	1.960 e	3.363 a	2.810 bc	2.735 A
N. R. -267 (V_3)	2.464 d	2.506 d	1.986 e	3.241 a	2.549 AB
Duration (D)	**D X T**				**Mean**
30 minutes (D_1)	2.519 d	1.899 f	2.379 de	3.030 ab	2.457 B
60 minutes (D_2)	2.519 d	1.954 f	2.770 c	2.882 bc	2.531 AB
120 minutes (D_3)	2.519 d	2.265 e	2.944 a-c	3.124 a	2.713 A
Interaction	**V X T X D**				**Mean**
V_1D_1	2.287 I-l	1.433 p	2.450 g-j	2.657 f-h	2.207 D
V_1D_2	2.287 I-l	1.713 op	2.655 f-h	3.073 c-e	2.437 CD
V_1D_3	2.287 I-l	1.811 no	3.124 c-e	3.206 b-d	2.607 BC
V_2D_1	2.806 e-g	1.860 m-o	3.276 b-d	2.650 f-I	2.648 ABC
V_2D_2	2.806 e-g	1.971 l-o	3.525 ab	2.424 h-j	2.681 AB
V_2D_3	2.806 e-g	2.050 k-o	3.289 b-d	3.350 bc	2.875 A
V_3D_1	2.464 g-j	2.405 h-k	1.409 p	3.784 a	2.515 BC
V_3D_2	2.464 g-j	2.179 j-m	1.129 j-n	3.128 c-e	2.475 BC
V_3D_3	2.464 g-j	2.933 d-f	2.420 h-j	2.811 e-g	2.657 ABC
Mean	**2.519 C**	**2.039 D**	**2.698 B**	**3.012 A**	**2.567****

Means followed by similar letters are not significant to each other at $p \leq 0.05$
LSD for varieties: 0.2078
LSD for temperature: 0.1775
LSD for variety X temperature: 0.2364
LSD for duration: 0.2078
LSD for variety X duration: 0.2323
LSD for temperature X duration: 0.2364
LSD for variety X temperature X duration: 0.3646

Effect of temperature on wheat cultivars with medium PPO activity: Iqbal-2000, Mathar and N. R.-267 were selected as medium PPO activity cultivars and treated with 30, 40, 50°C and room temperature for a period of 30, 60 and 120 minutes. Maximum activity (2.735 EU) was observed for Mathar while Iqbal-2000 showed minimum activity (2.417 EU). A significantly increased PPO activity was observed when treated the wheat grains of different medium PPO activity cultivars except 30°C (Table 4). Maximum PPO activity (3.012 EU) was observed under 50°C significantly decreased when treated with 40°C (2.698 EU), control (2.519 EU) and 30°C (2.039 EU). Minimum activity (2.039 EU), significantly lowered to all other comparable means was observed when wheat grains were treated with 30°C.

Maximum PPO activity (2.713 EU) was observed when wheat grains were incubated for a period of 120 minutes and minimum PPO activity (2.457 EU) at 30 minutes of incubation. Mathar showed maximum PPO activity (3.363 EU) when treated with 40°C while Iqbal-2002 exhibited minimum activity (1.652 EU) when treated with 30°C. Iqbal-2002 showed significantly lowered PPO activity (1.652, 2.743 & 2.985 EU) under all other temperatures of incubation as compared to control (2.287 EU). N. R.-267 showed

significantly high PPO activity (3.241 EU) at 50°C than all other temperatures (2.464, 2.506 & 1.986 EU). N. R.-267 showed non-significant difference in PPO activity at control and 30°C. Maximum PPO activity (3.124 EU) was shown by 120 minutes of incubation at 50°C while minimum (1.899 EU) by 30 minutes of incubation at 30°C. 30 minutes of incubation showed maximum activity (3.030 EU) at 50°C significantly different from others (2.519, 1.899 & 2.379 EU). 60 minutes of incubation showed an increase PPO activity (2.882 EU) at 50°C. Maximum activity (1.954 EU) at 60 minutes of incubation was exhibited by 30°C. Significant increase in PPO activity (2.944 & 3.124 EU) was observed except 30°C (2.265 EU) at 120 minutes of incubation as compared to control (2.519 EU).

On the comparison of V X T X D interactions, it is evident that maximum PPO activity (2.875 EU) is displayed by Mathar at 120 minutes of incubation and Minimum PPO activity exhibited by Iqbal-2000 (2.207 EU) at 30 minutes of incubation. Maximum PPO activity (3.784 EU) was shown by N. R.-267 at 50 minutes of incubation while minimum (1.409 EU) exhibited by itself at incubation of 30 minutes. Iqbal-2000 showed maximum PPO activity (2.657 EU) at 50°C of incubation for 30 minutes that decreases with decreasing temperature of incubation (2.450 & 1.433 EU), while at control it exhibited (2.287 EU) PPO activity. Iqbal-2000 exhibited an increasing pattern in PPO activity when incubated for 60 and 120 minutes of incubation at the temperatures given. Mathar at 120 minutes of incubation for 30, 40 and 50°C showed an increased PPO activity (2.405, 3.289 & 3.350 EU).

Effect of temperature on wheat cultivars with high PPO activity: Barani-83, Rohtas-90 and Pari-73 were selected as high PPO activity cultivars treated with 30, 40, 50°C and room temperature for a period of 30, 60 and 120 minutes. Maximum PPO activity (3.728 EU) was observed for Pari-73 non-significantly followed by Rohtas-90 (3.563 EU). Barain-83 exhibited minimum activity (2.777 EU). Control showed maximum PPO activities. A significantly decreased PPO activity was observed when treated the wheat grains of different high PPO activities cultivars with different temperatures (2.593, 3.423 & 3.329 EU). Maximum PPO activity (3.428 EU) was observed for 120 minutes of incubation (Table 5) and minimum PPO activity (3.226 EU) was observed for 30 minutes of incubation period. Incubation periods showed interesting results by exhibiting reduced PPO activities from control. Maximum PPO activity (4.459 EU) was observed by Rohtas-90 in control, while minimum (3.493 EU) by Barani-83 at 30°C of incubation non-significantly different from Pari-73 (4.285 EU) at control. Pari-73 showed an increase in PPO activity (2.908, 3.713 & 4.004 EU) on 30, 40 and 50°C respectively. Maximum and same PPO activity (4.079 EU) showed by control at three temperatures significantly followed by 30 minutes of incubation (3.781 EU) at 40°C Minimum PPO activity (2.031 EU) exhibited by 30 minutes of incubation at 30°C.

On the comparison of V X T X D interactions, it is clear that maximum PPO activity (3.992 EU) is shown by Pari-73 at 120 minutes of incubation and minimum PPO activity (2.341 EU) exhibited by Barani-83 at 120 minutes which is significantly followed by itself (2.947 EU) at a duration of 30 minutes, which intern is followed non-significantly by itself again (3.043 EU) at a duration of 60 minutes. Rohtas-90 showed non-significant difference in PPO activity (3.348 & 3.389 EU) at 30 and 60 minutes of incubation. These interactions reveal that maximum PPO activity (4.823 EU) showed by Pari-73 at 50°C at a duration of 120 minutes, while minimum (1.310 EU) by Barain-83 at 120 minutes of incubation at 30°C. Pari-73 showed maximum (4.285 EU) PPO activity for 60 minutes of incubation at control, decreased at 30°C treatment (2.797 EU) and then increased (3.918 & 4.230 EU) at 40 and 50°C respectively. These interactions showed diversified results.

Table 5. Effect of temperature on polyphenol oxidase activities in wheat varieties with high PPO activities (Group 3).

Varieties (V)	Temperature (T)				Mean
	Control	30 °C	40 °C	50 °C	
Barani -83 (V_1)	3.493 cd	1.955 g	3.043 e	2.617 f	2.777 B
Rohtas-90 (V_2)	4.459 a	2.914 e	3.513 cd	3.366 d	3.563 A
Pari-73 (V_3)	4.285 a	2.908 e	3.713 c	4.004 b	3.728 A
Duration (D)	**D X T**				**Mean**
30 minutes (D_1)	4.079 a	2.031 g	3.781 b	3.013 ef	3.226 A
60 minutes (D_2)	4.079 a	2.774 f	3.319 cd	3.481 c	3.413 A
120 minutes (D_3)	4.079 a	2.973 e-f	3.169 de	3.493 c	3.428 A
Interaction	**V X T X D**				**Mean**
V_1D_1	3.493 g-k	2.315 pq	3.362 I-k	2.620 n-p	2.947 C
V_1D_2	3.493 g-k	2.241 pq	3.320 j-l	3.119 k-m	3.043 C
V_1D_3	3.493 g-k	1.310 r	2.446 o-q	2.112 q	2.341 D
V_2D_1	4.459 ab	1.605 r	3.864 d-g	3.462 h-k	3.348 B
V_2D_2	4.459 ab	3.285 j-l	2.719 m-o	3.092 k-m	3.389 B
V_2D_3	4.459 ab	3.851 d-h	3.955 c-e	3.544 f-j	3.952 A
V_3D_1	4.285 bc	2.172 q	4.116 b-e	2.958 l-n	3.383 B
V_3D_2	4.285 bc	2.797 m-o	3.918 c-f	4.230 b-d	3.807 A
V_3D_3	4.285 bc	3.757 e-I	3.105 k-m	4.823 a	3.992 A
Mean	**4.079 A**	**2.593 C**	**3.423 B**	**3.329 B**	**3.356*** **

Means followed by similar letters are not significant to each other at $p \leq 0.05$
LSD for varieties: 0.2290
LSD for temperature: 0.1956
LSD for variety X temperature: 0.2605
LSD for duration: 0.2290
LSD for variety X duration: 0.2560
LSD for temperature X duration: 0.2605
LSD for variety X temperature X duration: 0.4018

Wheat flour has scanty amount of glucose and fructose. They may react with free amino groups of proteins to produce Maillard browning. In this reaction, Amadori compounds are produced which are degraded to give highly reactive dicarbonyl sugar derivatives (DCSD). DCSD reacts with Aminoguanidine to produce triazine that stops browning (Hirsch *et al*., 1992). PPO is heat-labile enzyme (Vadlamani & Seib 1996). Kihara *et al*., (2005) purified PPO from Japanese wheat (*Triticum aestivum* cv. Tohoku 198) bran, using 4-methylcatechol substrate. They found that the purified enzyme was stable at 6.5-7.5 pH at 4°C for 24h. PPO remained stable after heat treatment at 60°C for 10min. Heat treatment at 70°C for 10 min decreased 30% PPO activity.

QuindeAxtell *et al*., (2006) found that browning of barley-based food products may be reduced successfully by lowering total polyphenol content or PPO activity through heat treatment. The optimum temperature for the chestnut PPO was determined to be 40°C (Xu *et al*., 2004). Optimum temperature measurements for PPO activity lie in the range of 30-40°C (Madani *et al*., 1999). Chestnut PPO activity decreased by 10% after incubation at 40°C for 30 min (Xu *et al*., 2004) however, 90% PPO activity reduced 8% when incubated at 70°C for 30 min. PPO is inactivated at 70-90°C for a short period of time in plant tissues (Vadlamani & Seib 1996).

The present study revealed that there were different wheat cultivars with different PPO activities. In response to heat treatment, different wheat cultivars behaved differentially. Low-PPO-activity containing cultivars responded well and declared as PPO is heat-labile enzyme. On the other hand the cultivars with high-PPO-activity also responded well but comparatively these were containing more PPO activity and heat-labile proteins were more in quantity. So, in response to heat treatment, they showed overall decrease in total catalytic proteins. The cultivars with heat resistance in this stage may not be recommended for overall cultivation due to less adaptability on yield basis in farming system. The other cultivars with high PPO activity, although showing low category in quality, produce high yield in our farming system.

Conclusions

In conclusion, among 9 of the local cultivars, Wafaq-2001 and AS-2002 exhibited a compromising manner of minimum PPO activity at 30°C (the lowest temperature selected) for a period of 30 and 60 minutes of incubation. Furthermore it was also concluded, according to the general behavior of the selected cultivars, that the treatment with 30°C for a period of 30 minutes incubation can be recommended for suppressing the PPO activity.

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