한작지(Korean J. Crop Sci.), 53(2): 187~195(2008)

Effect of Polyphenol Oxidase Activity on Discoloration of Noodle Dough Sheet Prepared from Korean Wheats

Chon-Sik Kang*, Young-Keun Cheong*, Sun-Lim Kim**, Dae-Ki Kim***, Jung-Gon Kim*, and Chul Soo Park*†

*Homan Agricultural Research Institute, NICS, Iksan 570-080, Korea
**National Institute of Crop Science, RDA, Suwon 441-857, Korea
***Chonbuk National University Medical School, Jeonju 561-180, Korea

ABSTRACT Polyphenol oxidase (PPO) is implicated in discoloration of white salted noodles and other wheat based foods. PPO activity was evaluated to determine the effect on discoloration of noodle dough sheets prepared from 25 Korean wheat flours during storage and to screen experimental lines with low PPO activity in 52 Korean wheats. PPO activity was assayed with whole-seed and performed with L-dihydroxyphenylalanine (L-DOPA) as substrates. Absorbance by L-DOPA assay of 25 Korean wheats was from 0.285 to 1.368 at 475 nm. PPO activity was significantly related with grain characteristics, including 1000-kernel weight and grain colors. In flour characteristics, PPO activity positively correlated with ash and protein content (r = 0.658, $P < 0.001$ and r = 0.424, $P < 0.05$, respectively) and negatively correlated with L* value of flour (r = 0.412, $P < 0.05$). In the changes of color of noodle dough sheet, L* and b* values consistently decreased and a* value increased during storage. PPO activity negatively correlated with L* value of noodle dough sheet during storage (r = 0.566, $P < 0.01$ at 2 hr, r = 0.547, $P < 0.01$ at 24 hr, and r = 0.509, $P < 0.01$ at 48 hr). But, no significant relationship was found in between PPO activity, a* and b* values during storage. The 52 Korean wheat lines examined in this study were divided into 3 different groups, low (< 0.500), medium (0.501-0.999) and high level (> 1.000), on the basis of the level of PPO activity. Twenty two Korean wheat lines showed low level of PPO activity and Suwon 252, 277 and 280 showed lower PPO activity (< 0.200) than others.

Keywords : wheat, polyphenol oxidase (PPO), discoloration, noodle quality

†Corresponding author: (Phone) +82-63-840-2153
(E-mail) pcs89@rda.go.kr <Received February 13, 2008>

Color is one of the major quality criteria in noodles due to the first consideration of consumers in evaluating noodle quality. Discoloration of raw noodles is undesirable because bright white color is generally favored by consumers. Discoloration of noodles has been associated primarily with the activity of polyphenol oxidase (PPO) (Baik *et al.*, 1995; Park *et al.*, 1997; Bhattacharya *et al.*, 1999; Morris *et al.*, 2000). PPO is mostly located in the aleurone layer of wheat grain; hence, flour PPO activity increase with flour extraction rate (Hatcher and Kruger, 1993). PPO activity was influenced by cultivar rather than growing conditions (Baik *et al.*, 1994; Park *et al.*, 1997).

PPO is a copper-containing metalloprotein that catalyzes the hydroxylation of *o*-monophenols to *o*-diphenols (monophenolase activity) and the oxidation of *o*-dihydroxyphenols to *o*-quinones (diphenolase activity) (Steffens *et al.*, 1994). The quinine products react with amines and thiol groups or self-polymerization to form complex colored products (melanins) (Feillet *et al.*, 2000). This is the basis for PPO-mediated discoloration of many food products and then the extent of discoloration of noodles depends on PPO. Many test with different substrates (phenol, L-tyrosine, catechol, methylcatechol, L-DOPA and caffeic acid) confirmed L-dihydroxyphenylalanine (L-DOPA) and catechol were the best substrates, that resulted in the highest enzyme activities in whole seed assay (Anderson and Morris, 2001).

Small-scale whole seed assay for PPO have been studied to eliminate high PPO lines and to select wheat breeding lines with superior noodle color potential in wheat breeding programs (Bernier and Howes, 1994; Kruger *et al.*, 1994; Morris *et al.*, 1998; McCaig *et al.*, 1999; Anderson and Morris, 2001; Bettge, 2004). Recently, enhancement of end-use quality of Korean wheat has been very important consideration to increase consumption of Korean wheat products. However, there was little effort to reduce the levels of PPO in Korean wheat breeding programs. Therefore, the consideration of noodle color and related properties should be required for the better noodle quality of Korean wheats. The objectives of this study were to determine the effect of PPO on discoloration of noodle dough sheets prepared from Korean wheat flours during storage and to screen wheat lines with low PPO activity in breeding program.

MATERIALS AND METHODS

Materials

Twenty five Korean wheat cultivars and 52 experimental lines were harvested Iksan (Upland Crop Experimental Farm of Honam Agricultural Research Institute, NICS, RDA) in 2007. The seed was sawn on October 20 and mean temperature (9.7℃) was higher than mean value (8.7℃) for 10 years and precipitation was lower (387.6 mm) than mean value (492.0 mm) for 10 years. Korean wheat cultivars were milled using Bühler experimental mill, and flour of about 60% extraction was prepared by blending millstreams.

Analytical methods

Test weight and 1000-kernel weight were measured by Grain Scale (Seedburo Equipment Co., USA) and Seed Counter (Pfeuffer GmbH, Germany), respectively. Moisture, protein and ash content of wheat flour were determined according to Approved Methods 44-15A, 46-30 and 08-01 (AACC 2000). The color of grain, flour and noodle dough sheet was measured by Minolta CM-2002 (Minolta Camera Co., Ltd, Japan) with an 11 mm measurement aperture. The color differences of noodle sheets were recorded as CIE-LAB L* (lightness), a* (redness-greenness) and b* (yellowness-blueness) values.

L-dihydroxyphenylalanine (L-DOPA) assay

The procedure for L-DOPA assay followed that of Anderson and Morris (2001). A 1.5 mL aliquot of 5 mM L-DOPA in 50 mM MOPS [3-(N-morrpholino) propanesulfonic acid] solution, pH 6.5, was added to five seeds in a 2.0 mL microcentrifuge tube. The tubes were rotated for 1 hr to allow the reaction to take place. Absorbance was measured on 1 mL of the incubated solution at 475 nm with spectrophotometer (Shimadzu Biospec-1601, Shimadzu Co., USA).

Preparation of noodle dough sheet

Flour (100 g, 14% mb) was mixed with sodium chloride solution and the concentration was adjusted to 2.0% with 35% water absorption of noodle dough in a pin mixer (National Mfg. Co., Lincoln, NE) for 4 min. High water absorption (39%) was applied for making noodle dough with waxy wheats, including Shinmichal and Shinmichal1, because high water absorption of waxy wheat flours, which absorb and retain more water during dough mixing than non-waxy wheat starches, could be mainly due to their starch properties (Park and Baik, 2004). Dough was passed through the rollers of a noodle machine (Ohtake Noodle Machine Mfg. Co., Japan) at 8 rpm and a 3 mm gap; dough was folded and put again into the sheeting rollers. The folding and sheeting were repeated twice. The dough sheet was rested for 1 hr and then put through the sheeting rollers three times at progressively decreasing gaps between rollers to 2.40, 1.85 and 1.30 mm. In order to measure the color change of noodle dough sheets during storage, a piece of noodle sheet was kept in a plastic bag to prevent surface drying and stored at 21℃. The color of each sheet was measured after 2, 24 and 48 hr. Measurements were made with three replications at three random locations on the surface of each sample.

Statistical analysis

Statistical analysis of data was performed by the SAS software (SAS Institute, Cary, NC) using Fisher's least significant difference procedure (LSD), analysis of variance (ANOVA) and Pearson correlation coefficient.

RESULTS AND DISCUSSION

PPO activity of Korean wheat cultivar

Test weight, 1000-kernel weight, grain color and PPO activity of 25 Korean wheat cultivars are summarized in Table 1. Test weight and 1000-kernel weight of Korean wheat cultivars was 787.00-844.50 g and 33.45-51.15 g, respectively. In grain color, L* value was 44.82-53.03, a* value was 5.82-8.90, and b* value was 20.20-29.99. PPO activity

Table 1. Test weight, 1000-kernel weight, grain color and PPO activity of 25 Korean wheats cultivars.

Cultivar	Test weight (g)	1000-Kernel weight (g)	PPO Activity†	Color of grain‡		
				L*	a*	b*
Alchanmil	828.00	38.04	0.300	50.28	8.63	28.04
Anbaekmil	831.00	47.64	1.195	47.19	7.63	23.43
Chungkyemil	828.50	37.33	0.327	49.05	7.86	26.99
Dabunmil	811.50	37.89	0.659	51.27	5.86	25.20
Dahongmil	816.00	33.45	0.314	49.36	8.88	28.39
Eunpamil	839.00	37.48	0.763	44.82	7.06	22.95
Geurumil	797.50	51.15	1.209	45.62	7.07	23.08
Gobunmil	842.00	42.40	1.312	48.14	7.11	23.62
Jinpummil	819.50	39.69	0.303	49.89	8.55	28.04
Joeunmil	844.50	38.76	1.190	44.93	7.16	20.20
Jokyoungmil	814.00	48.93	1.047	51.21	5.96	25.66
Jonongmil	825.00	45.51	0.854	49.06	6.74	23.49
Jopummil	817.50	38.02	0.776	47.11	6.75	21.60
Keumkangmil	831.50	47.46	0.830	52.62	5.82	26.48
Milseoungmil	815.50	36.14	0.373	51.87	8.92	29.99
Namhaemil	787.00	37.59	0.338	50.46	8.21	27.51
Olmil	795.00	39.60	0.786	50.55	7.68	28.57
Olgeurumil	821.00	41.52	0.759	49.24	7.41	23.29
Saeolmil	825.00	38.60	0.422	48.15	7.90	26.48
Seodunmil	823.50	39.74	0.298	48.89	7.95	26.68
Shinmichalmil	818.00	35.86	0.551	51.29	8.31	27.47
Shinmichal1	842.50	34.40	0.370	46.37	8.00	24.66
Tapdongmil	830.50	38.87	0.614	45.41	6.66	22.59
Urimil	792.00	39.72	0.398	48.54	8.15	24.84
Younbaekmil	820.00	38.67	0.413	53.03	5.93	28.21
LSD§	4.02	1.39	0.114	0.68	0.20	0.52

†PPO activity was assayed L-DOPA as substrates using whole-seed and absorbance was measured at 475 nm.
‡L* = lightness; a* = redness-greenness; b* = yellowness-blueness.
§Least significant difference ($P < 0.05$). Differences between two means exceeding this value are significant.

performed with L-DOPA assay was 0.285-1.368 at 475 nm. This range was a similar to that of U.S. wheats (Anderson and Morris, 2001; Bettege, 2004). Alchanmil, Chungkyemil, Dahongmil, Jinpummil, Milseoungmil, Namhaemil, Saeolmil, Seodunmil, Shinmichal1, Urimil and Younbaekmil showed lower level of PPO activity (< 0.500), which is similar to low level PPO activity of U.S. wheats (Bettege, 2004) and referred as low PPO activity in this study, than other cultivars. Anbaekmil, Geurumil, Gobunmil, Joeunmil and Jokyoung mil showed higher level of PPO activity (> 1.047), referred as high PPO activity in this study, than others. Korean wheat cultivars with low PPO activity showed lower 1000-kernel weight (< 39.74 g) than Korean wheat cultivars with high PPO activity (> 42.40 g), except in Joeunmil (38.76 g). Korean wheat cultivars with white color, including Dabunmil, Jokyoungmil, Keumkangmil and Younbaekmil, showed higher L* value (> 51.21) than others. However, Dabunmil, Jokyoungmil and Keumkangmil showed higher PPO activity (> 0.659) than those of red wheat cultivars with low PPO activity.

PPO activity positively correlated with 1000-kernel weight (r = 0.669, P < 0.001; Fig. 1-A). But, there was no significant relationship between PPO activity and test weight. Baik *et al.* (1994) proposed that large, fully-matured wheat grains have higher levels of PPO activity than small, immature wheat grains. Park *et al.* (1997) reported on a negative correlation between 1000-kernel weight and flour PPO activity in white and red wheats. The PPO activities of U.S. hard red wheats were significantly higher than those of the other classes of wheats; soft white, club and hard white wheats had lower PPO activities (Baik *et al.*, 1994). PPO activity negatively correlated with L* value of grain (r = -0.418, P < 0.05; Fig. 1-B). PPO activity also negatively correlated with a* and b* values of grain (r = -0.530, P < 0.01 and r = -0.665, P < 0.001, respectively). Lee *et al.* (1999) reported PPO activity of grain negatively correlated with grain colors. Park *et al.* (1997) also reported that grain PPO activity was related to variation in grain color observed among hard white samples.

The relationship between PPO activity and flour characteristics

Ash, protein and color of 25 Korean wheat flours are summarized in Table 2. Ash and protein content of Korean wheat flours was 0.39-0.69% and 8.43-13.96%, respectively. Korean wheats with high PPO activity was higher ash content (> 0.56%) than low PPO activity (< 0.52%), except in Shinmichall (0.57%) and Younbaek (0.59%). Korean wheats with high PPO activity were higher protein content (> 11.51%), except in Jokyoungmil (10.85%) than low PPO activity (< 11.28%). Ash content and protein content of flour positively correlated with PPO activity (r = 0.658, P < 0.001 and r = 0.424, P < 0.05, respectively; Fig. 2-A and B). These results are in agreement with many previous reports (Hatcher and Kruger, 1993; Baik *et al.*, 1994; Park *et al.*, 1997; Lee *et al.*, 1999; Chang and Lee, 2004) which showed positive correlation between physical properties of flour, especially ash and protein, and PPO activity.

In flour color of Korean wheat cultivars with low PPO activity, L* value was 89.26-91.61, a* value was -1.58 – -1.20, and b* value was 7.92-11.08. Korean wheats with high PPO activity showed lower L* value (< 89.07, except in Jokyoungmil and Gobunmil) but there was no significant difference in a* and b* values of flour between high and low PPO activities. L* value of flour negatively correlated with PPO activity (r = -0.412, P < 0.05; Fig. 2-C). This result is in agreement with the reports by Lee *et al.* (1999) reported that significant relationship between PPO activity and flour colors.

The changes of color of noodle dough sheet during storage

The changes of color of noodle dough sheet during storage in 25 Korean wheat cultivars are summarized in Table 3. The L* value of noodle dough sheet decreased during storage for 48 hr from 75.71-82.77 to 65.16-77.18. Alchanmil, Dahongmil, Milseongmil and Urimil showed higher L* value of noodle dough sheet during storage than others. The L* value of noodle dough sheet from these wheat cultivars was > 82.23 at 2 hr, > 77.79 at 24 hr and > 75.73 at 48 hr. These

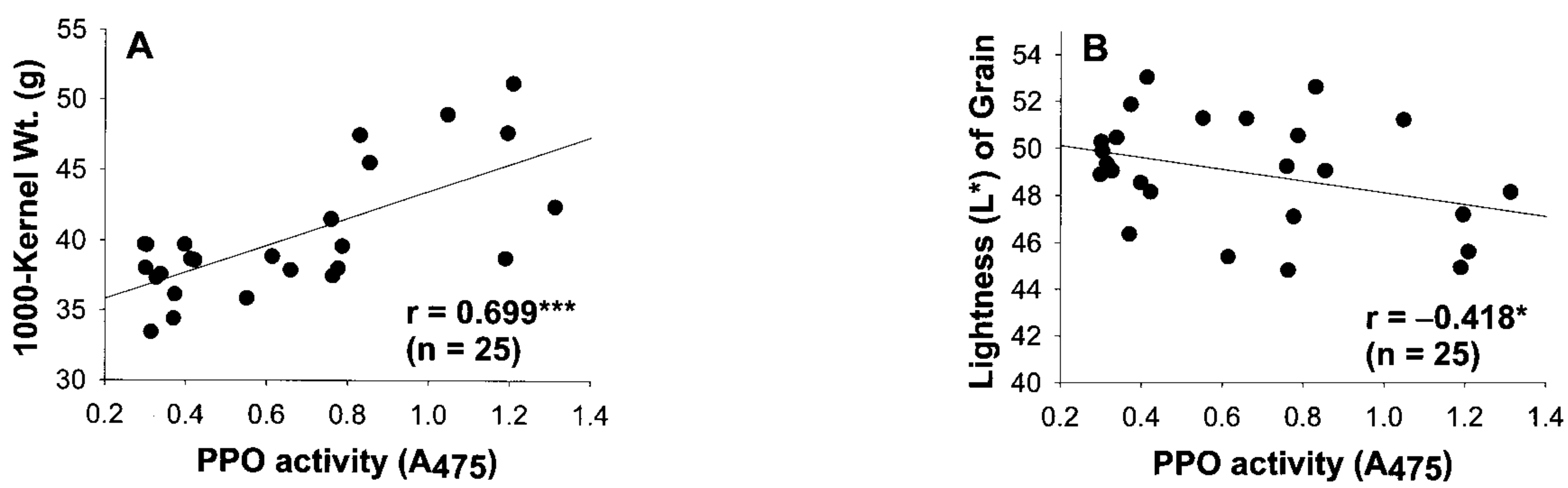

Fig. 1. The relationships between 1000-kernel weight (A), Lightness of grain (B) and PPO activity assayed L-DOPA as substrates using whole-seed. r = correlation coefficients, n = 25: Korean wheat cultivars. * and *** mean P = 0.05 and P = 0.001, respectively.

Table 2. Ash, protein and color of 25 Korean wheat flours.

Cultivar	Ash (%)	Protein (%)	Color of flour†		
			L*	a*	b*
Alchanmil	0.49	9.31	89.92	-1.20	8.67
Anbaekmil	0.56	12.02	89.07	-1.58	10.47
Chungkyemil	0.51	10.70	89.98	-1.51	8.64
Dabunmil	0.48	11.23	88.70	-1.49	10.41
Dahongmil	0.48	9.86	90.69	-1.56	8.31
Eunpamil	0.52	12.32	88.38	-1.42	10.39
Geurumil	0.69	13.92	87.70	-1.18	9.89
Gobunmil	0.59	11.51	89.31	-1.36	10.03
Jinpummil	0.50	9.95	89.48	-1.71	10.78
Joeunmil	0.59	13.95	88.92	-1.10	9.57
Jokyoungmil	0.56	10.85	90.56	-1.58	10.54
Jonongmil	0.58	13.10	91.02	-1.09	7.78
Jopummil	0.52	13.96	89.69	-1.35	9.32
Keumkangmil	0.45	11.86	89.89	-1.40	9.60
Milseoungmil	0.48	9.84	90.64	-1.55	8.96
Namhaemil	0.50	10.93	90.79	-1.35	7.92
Olmil	0.39	10.15	90.32	-1.57	9.32
Olgeurumil	0.39	9.91	90.71	-1.36	7.85
Saeolmil	0.44	10.30	90.80	-1.35	7.94
Seodunmil	0.52	9.34	89.30	-1.58	11.08
Shinmichalmil	0.60	11.78	90.96	-1.40	7.97
Shinmichal1	0.57	11.28	89.26	-1.48	10.58
Tapdongmil	0.52	11.83	89.12	-1.28	8.61
Urimil	0.42	8.43	91.61	-1.47	8.00
Younbaekmil	0.59	11.24	89.83	-1.44	10.77
LSD‡	0.04	0.25	0.25	0.05	0.12

†L* = lightness; a* = redness-greenness; b* = yellowness-blueness.
‡Least significant difference ($P < 0.05$). Differences between two means exceeding this value are significant.

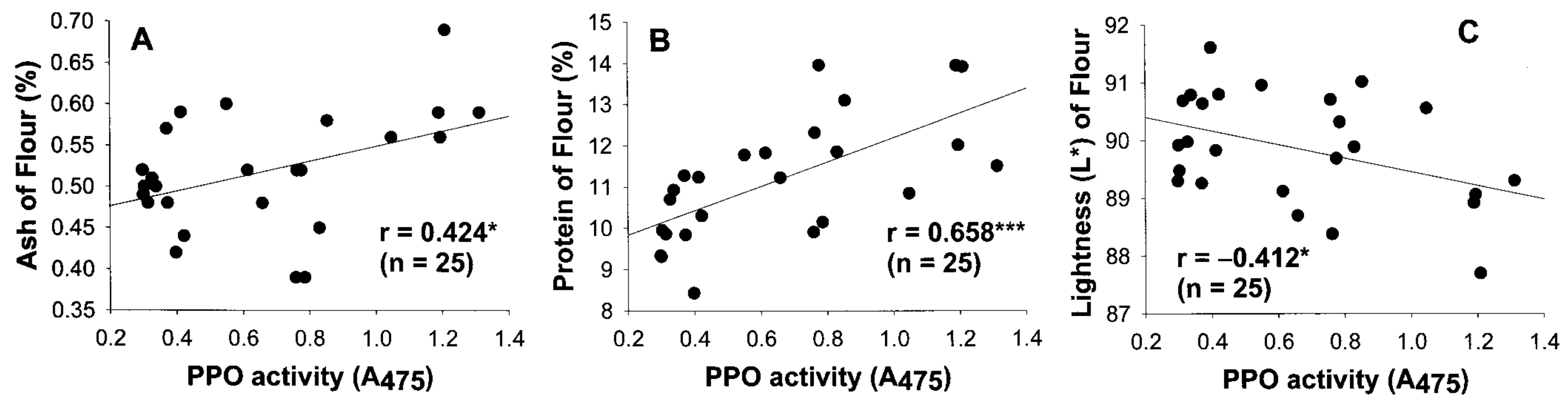

Fig. 2. The relationships between ash (A), protein (B), L* of wheat flour (C) and PPO activity assayed L-DOPA as substrates using whole-seed. r = correlation coefficients, n = 25: Korean wheat cultivars. * and *** mean $P = 0.05$ and $P = 0.001$, respectively.

Table 3. The changes of color of noodle dough sheet during storage in 25 Korean wheat cultivars.

Cultivar	Color of noodle dough sheet[†]								
	Lightness (L*)			Redness-Greenness (a*)			Yellowness-Blueness (b*)		
	2 hr	24 hr	48 hr	2 hr	24 hr	48 hr	2 hr	24 hr	48 hr
Alchanmil	82.36	78.71	77.12	-2.42	-1.32	-1.74	15.84	15.10	15.60
Anbaekmil	78.59	74.50	72.49	-2.90	-2.26	-1.90	18.36	17.28	17.11
Chungkyemil	81.02	75.59	73.01	-2.37	-1.53	-1.16	17.61	16.40	16.29
Dabunmil	80.23	76.29	74.68	-3.16	-2.63	-2.41	20.00	19.71	19.30
Dahongmil	82.77	78.95	77.18	-2.64	-1.61	-1.36	17.22	15.64	15.74
Eunpamil	79.24	73.37	70.41	-2.15	-1.32	-0.97	17.69	16.37	16.39
Geurumil	79.23	72.37	69.12	-2.67	-1.94	-1.55	18.29	16.83	15.88
Gobunmil	80.46	74.66	71.53	-2.23	-1.70	-1.50	17.35	16.30	16.35
Jinpummil	81.16	76.28	73.23	-2.70	-2.00	-1.71	19.15	18.02	17.64
Joeunmil	77.31	69.50	65.16	-1.55	-1.03	-0.75	17.89	16.99	16.78
Jokyoungmil	80.87	76.48	74.61	-2.98	-2.45	-2.18	18.47	17.30	17.41
Jonongmil	79.09	70.96	66.63	-1.86	-1.04	-0.54	16.39	15.18	14.79
Jopummil	77.37	72.33	70.42	-2.55	-1.83	-1.62	19.28	18.07	17.76
Keumkangmil	80.44	75.80	73.71	-2.86	-2.19	-1.93	18.20	17.42	17.19
Milseoungmil	82.24	77.79	75.73	-3.23	-2.40	-2.04	17.79	17.11	17.01
Namhaemil	81.62	74.18	71.17	-1.75	-1.01	-0.76	16.29	16.21	16.28
Olmil	80.65	75.51	73.19	-2.77	-1.92	-1.44	19.31	18.31	18.08
Olgeurumil	82.15	77.47	75.19	-2.64	-2.07	-1.79	17.39	17.16	16.98
Saeolmil	81.08	75.39	73.23	-2.66	-1.73	-1.42	17.15	16.20	16.33
Seodunmil	80.79	75.13	71.88	-2.71	-2.18	-1.95	18.95	17.99	17.96
Shinmichalmil	78.24	73.34	72.17	-3.37	-2.57	-2.13	19.34	17.90	17.17
Shinmichal1	76.71	69.54	67.61	-3.22	-2.62	-2.36	21.56	19.15	18.56
Tapdongmil	78.75	72.43	69.82	-1.90	-1.17	-0.77	18.54	16.87	16.97
Urimil	82.23	77.97	76.52	-2.61	-1.73	-1.41	17.41	16.59	16.37
Younbaekmil	80.85	77.89	76.81	-3.00	-2.49	-2.29	17.40	16.50	16.16
LSD[‡]	0.83	0.86	0.92	0.32	0.46	0.26	0.89	0.82	0.79

[†]Optimum water absorption of noodle dough.
[‡]Least significant difference (P < 0.05). Differences between two means exceeding this value are significant.

cultivars showed lower PPO activity (< 0.398), 1000-kernel weight (< 39.74 g) and protein content of flour (< 9.86%) than others (Table 1 and 2). These cultivars could be considered for making white salted noodles because higher values of L* are preferred with consumers (Nagao, 1996; Hou, 2001). Anbaekmil, Geurumil, Gobunmil and Joeunmil showed lower L* values during storage than others. The L* value of noodle dough sheet from these wheat cultivars was < 78.59 at 2 hr, < 74.66 at 24 hr and < 72.49 at 48 hr. These cultivars showed higher PPO activity (> 1.190) and protein content of flour (> 11.51%). The L* value of noodle dough sheet at 2 hr storage positively correlated with L* value at 24 and 48 hrs (r = 0.917, P < 0.001 and r = 0.816, P < 0.001, respectively). Waxy wheat cultivars, Shinmichalmil and Shimichal1, showed lower L* values during storage in spite of low PPO activity (< 0.551) and protein content (< 11.78%). These results indicate that waxy starch could influence on higher water absorption during making noodle dough and this higher water absorption in noodle dough could effect on lower L* value of noodle dough sheet during storage.

There was a consistent increase during storage for 48 hr from -3.37 − -1.55 to -2.41 − -0.54 in a* value of Korean wheat cultivars. Jonongmil, Joeunmil, Namhaemil and Tapdongmil showed higher a* value during storage, which was > -1.90 at 2 hr, > -1.17 at 24 hr and > -0.77 at 48 hr, than others. Dabunmil, Jokyoungmil, Milseongmil, Shinmichalmil, Shinmichal1 and Younbaek showed lower a* value during storage than, which was < -2.98 at 2 hr, < -2.40 at 24 hr and < -2.04 at 48 hr, others. Extreme on either side of the a* value of noodle dough sheet for making alkaline noodles are considered deleterious (Hatcher *et al.*, 1999). The a* value of noodle dough sheet at 2 hr positively correlated with a* value at 24 and 48 hrs (r = 0.949, $P < 0.001$ and r = 0.917, $P < 0.001$, respectively). The b* value of noodle dough sheet decreased during storage for 48 hr 15.84-21.56 to 14.79-19.30. Dabunmil and Shinmichal1 showed higher b* value during storage, which was > 20.00 at 2 hr, > 19.15 at 24 hr and > 18.56 at 48 hr, than others. Alchanmil and Jonongmil showed lower b* value during storage, which was < 16.39 at 2 hr, < 15.18 at 24 hr and < 15.60 at 48 hr, than others. Moderate b* value of noodle dough sheet for white salted noodles is preferred with consumers (Nagao, 1996; Hou, 2001). The b* value of noodle dough sheet at 2 hr storage positively correlated with a* value at 24 and 48 hrs (r = 0.923, $P < 0.001$ and r = 0.861, $P < 0.001$, respectively).

The relationship between PPO activity and color of noodle dough sheet

The relationship between grain characteristics and color of noodle dough sheet during color are summarized in Table 4. Test weight of wheat grains showed negative correlation with L* value of noodle dough sheet at 24 and 48 hrs but there was no significant relationship between 1000-kernel weight and L* value during storage. No significant relationship between 1000-kernel weight and a* and b* values of noodle dough sheet during storage was found. However, Chang and Lee (2004) reported that single kernel weight significant relationship with a* and b* values of noodle dough sheet during storage. PPO activity negatively correlated with L* value of noodle dough sheet during storage (r = -0.566, $P < 0.01$ at 2 hr, r = -0.547, $P < 0.01$ at 24 hr, and r = -0.509, $P < 0.01$ at 48 hr). These results are in agreement with many previous reports (Baik *et al.*, 1995; Lee *et al.*, 1999; Chang and Lee, 2004), which reported negative correlation between PPO activity and L* value of

Table 4. Correlation coefficients for PPO activity, characteristics of grain and flour and changes of color of noodle dough sheet during storage in 25 Korean wheat cultivars.

Parameters†	Color of noodle dough sheet								
	Lightness (L*)			Redness-Greenness (a*)			Yellowness-Blueness (b*)		
	2 hr	24 hr	48 hr	2 hr	24 hr	48 hr	2 hr	24 hr	48 hr
Test Weight	-0.394	-0.409*	-0.396*	0.144	0.059	0.006	0.105	-0.048	0.001
1000-Kernel Weight	-0.172	-0.213	-0.204	0.028	-0.073	-0.018	-0.115	-0.091	-0.164
PPO activity	-0.566**	-0.547**	-0.509**	0.203	0.047	0.123	0.036	0.029	-0.034
L* of Grain	0.217	0.281	0.292	-0.094	-0.009	-0.031	-0.118	-0.007	-0.057
a* of Grain	0.325	0.326	0.228	-0.049	0.142	0.097	-0.147	-0.201	-0.171
b* of Grain	0.662***	0.693***	0.705***	-0.477*	-0.316	-0.360	-0.108	-0.043	0.008
Ash	-0.652***	-0.531**	-0.557**	0.076	-0.035	-0.006	0.114	-0.070	-0.214
Protein	-0.816***	-0.789***	-0.732***	0.313	0.190	0.276	0.152	0.053	-0.080
L* of Flour	0.546**	0.393	0.430*	-0.093	0.038	0.058	-0.365	-0.300	-0.259
a* of Flour	-0.379	-0.487*	-0.498*	0.601**	0.582**	0.522**	-0.421*	-0.459*	-0.528**
b* of Flour	-0.288	-0.147	-0.162	-0.303	-0.472*	-0.489*	0.525**	0.502*	0.507**

†Optimum water absorption of noodle dough.

*indicates significance at the 0.05 level, ** at the 0.01 level, and *** at the 0.001 level.

Table 5. Classifications of 52 Korean experimental lines with respect to the level of PPO activity.

Level of PPO activity	No of line	Korean wheat experimental line†
Low level (< 0.500)	22	Suwon280 (0.125), Suwon252 (0.171), Suwon277 (0.191), Suwon263 (0.204), Iksan311 (0.231), Suwon264 (0.235), Suwon260 (0.239), Suwon289 (0.243), Suwon296 (0.250), Suwon278 (0.270), Suwon286 (0.273), Iksan307 (0.303), Iksan308 (0.358), Suwon271 (0.370), Suwon293 (0.388), Suwon270 (0.409), Iksan313 (0.419), Suwon295 (0.424), Suwon294 (0.426), Suwon305 (0.439), Suwon266 (0.490)
Medium level (0.501-0.999)	22	Suwon265 (0.507), Suwon302 (0.509), Suwon269 (0.527), Iksan309 (0.547), Suwon258 (0.548), Suwon259 (0.572), Suwon262 (0.586), Suwon288 (0.615), Suwon301 (0.649), Iksan310 (0.761), Suwon283 (0.776), Suwon273 (0.795), Suwon285 (0.797), Suwon282 (0.807), Suwon287 (0.812), Suwon291 (0.822), Suwon297 (0.834), Suwon275 (0.841), Suwon276 (0.874), Iksan317 (0.952), Iksan319 (0.965), Suwon268 (0.969)
High level (> 1.000)	8	Iksan318 (1.017), Iksan315 (1.047), Iksan316 (1.048), Iksan314 (1.062), Suwon281 (1.123), Suwon272 (1.179), Suwon299 (1.263), Iksan320 (1.347)

†Parenthesis indicates that PPO activity was assayed L-DOPA as substrates using whole-seed and absorbance was measured at 475 nm.

noodle dough sheet during storage. There was no significant relationship between PPO activity and a* and b* values during storage, which are in agreement with report of Lee *et al.* (1999). The b* value of grain color positively correlated with L* value of noodle dough sheet during storage but no significant relationship was found between other grain colors and colors of noodle dough sheet during storage.

The relationship between flour characteristics and color of noodle dough sheet during color are also summarized in Table 4. Ash and protein content of flour negatively correlated with L* value of noodle dough sheet during storage, which are in agreement with many previous reports (Miskelly, 1984; Crosbie, 1990; Kruger *et al.*, 1994; Baik *et al.*, 1995; Bhattacharya *et al.*, 1999; Hatcher *et al.*, 1999; Morris *et al.*, 2000; Chang and Lee, 2004; Wang *et al.*, 2004). Ash and protein content did not significantly correlated with a* and b* values of noodle dough sheet during storage. The L* value of flour positively correlated with L* value of noodle dough sheet at 2 and 48 hr storages ($r = 0.546$, $P < 0.01$ and $r = 0.430$, $P < 0.05$, respectively). The a* value of flour also positively correlated with a* value of noodle dough sheet during storage and negatively correlated with L* and b* values of noodle dough sheet during storage. The b* value of flour also positively correlated with b* value of noodle dough sheet during storage and negatively correlated with a* value of noodle dough sheet during storage. These results are agreement with report of Wang *et al.* (2004).

The distribution of PPO activity of Korean experimental lines

The distribution of PPO activity for the 52 Korean wheat experimental lines was shown in Table 5. The 52 Korean wheat lines examined in this study were divided into 3 different groups on the basis of the level of PPO activity. Twenty two Korean wheat lines showed low level of PPO activity, which was performed with L-DOPA assay at 475 nm and lower than 0.500. The PPO activity of other 22 lines was 0.507-0.969 and referred as medium level of PPO activity in this study. Eight lines showed higher PPO activity (> 1.017) and referred as high level of PPO activity. Among Korean wheat lines with low level of PPO activity, Suwon 252, 277 and 280 showed lower PPO activity (< 0.200) than others. Therefore, these lines could be used as genetic resources for improving noodle color in Korean wheat breeding programs.

CONCLUSION

PPO assay with whole-seed could be applied for selection of wheat lines with better end-use quality for noodle in Korean wheat breeding programs. PPO activity showed significant relationship with grain characteristics, including 1000-kernel weight and grain colors, flour characteristics, including ash, protein content and L* value of flour and L* value of noodle dough sheet during storage.

Alchanmil, Dahongmil, Milseongmil and Urimil showed lower PPO activity and higher L* value of noodle dough sheet during storage than others. These cultivars showed lower 1000-kernel weight, ash and protein content of flour than others. Among experimental lines, Suwon252, 277 and 280 showed lower PPO activity than those cultivars. These lines could be used for improving noodle color of Korean wheats.

REFERENCES

American Association of Cereal Chemists. 2000. Approved Methods of the AACC. 10th ed. Method 08-01, 38-10, 44-15A, 46-30 and 54-40A. The Association: St. Paul, MN.

Anderson, J. V. and C. F. Morris. 2001. An improved whole-seed assay for screening wheat germplasm for polyphenol oxidase activity. Crop Sci. 41 : 1697-1705.

Baik, B. K., Z. Czuchajowska, and Y. Pomeranz. 1994. Comparison of polyphenol oxidase in wheats and flours from Australian and U.S. cultivars. J. Cereal Sci. 19 : 291-296.

Baik, B. K., Z. Czuchajowska, and Y. Pomeranz. 1995. Discoloration of dough for oriental noodles. Cereal Chem. 72 : 198-205.

Bernier, A. M. and N. K. Howes. 1994. Quantification of variation in tyrosinase activity among durum and common wheat cultivars. J. Cereal Sci. 19 : 157-159.

Bettge, A. D. 2004. Collaborative study on L-DOPA - Wheat polyphenol oxidase assay (AACC Method 22-85). 49 : 338-342.

Bhattacharya, M., Q. Luo, and H. Corke. 1999. Time-dependent changes in dough color in hexaploid wheat landraces differing in polyphenol oxidase activity. J. Agric. Food Chem. 47 : 3579-3585.

Chang, H. G. and Y. T. Lee. 2004. Relationships among polyphenol oxidase activity, quality characteristics and alkaline noodle color of hard white wheats. Food Sci. Biotechnol. 13 : 498-503.

Crosbie, G. B., D. M. Miskely, and T. Dewan. 1990. Wheat quality for the Japanese flour milling and noodle industries. Western Aust. J. Agric. 31 : 83-88.

Feillet, P., J. C. Autran, and Icard-Verniere. 2000. Pasta brownness: an assessment. J. Cereal Sci. 32 : 215-233.

Hatcher, D. W., S. J. Symons, and J. E. Kruger. 1999. Measurement of the time-dependent appearance of discolored spots in alkaline noodles by image analysis. Cereal Chem. 76 : 189-194.

Hatcher, D. W. and J. E. Kruger. 1993. Distribution of polyphenol oxidase in flour millstreams of Canadian common wheat classes milled to three extraction rates. Cereal Chem. 70 : 51-55.

Hou, G. 2001. Oriental noodles. Adv. Food Nutr. Research 43 : 141-193.

Kruger, J. E., D. W. Hatcher, and R. DePauw. 1994. A whole seed assay for polyphenol oxidase in Canadian prairie spring wheats and its usefulness as a measure of noodle darkening. Cereal Chem. 71 : 324-326.

Lee, W. J., S. H. Kim, D. R. Shelton, and M. J. Kim. 1999. Relationship of wheat polyphenol oxidase activity to color of Asian raw noodles. Food Sci. Biotechnol. 8 : 361-366.

McCaig, T. N., D. Y. K. Fenn, R. E. Knox, J. M. DePauw, J. M. Clarke, and J. G. McLeod. 1999. Measuring polyphenol oxidase activity in a wheat breeding program. Can. J. Plant Sci. 79 : 507-514.

Miskelly, D. M. 1984. Flour components affecting paste and noodle colour. J. Sci. Food Agric. 35 : 463-471.

Morris, C. F., J. V. Anderson, A. D. Bettge, R. J. Rogahn, and M. E. Correll. 1998. Distribution of PPO activity among a large number of hexaploid wheat genotypes using an improved L-DOPA assay. Cereal Foods World. 43(7) : 518.

Morris, C. F., H. C. Jeffers, and D. E. Engle. 2000. Effect of processing, formula and measurement variable on alkaline noodle color-toward an optimized laboratory system. Cereal Chem. 77 : 77-85.

Nagao, S. 1996. Processing technology of noodle products in Japan. In: J.E. Kruger *et al.* (ed) Pasta and noodle technology. Am. Assoc. Cereal Chem. St. Paul, USA, pp. 169-194.

Park, C. S. and B. K. Baik. 2004. Cooking Time of White Salted Noodles and Its Relationship with Protein and Amylose Contents of Wheat. Cereal Chem. 81 : 165-171.

Park, W. J., D. R. Shelton, C. J. Peterson, T. Martin, S. D. Kachman, and R. L. Wehling. 1997. Variation in polyphenol oxidase activity and quality characteristics among hard white wheat and hard red winter wheat samples. Cereal Chem. 74 : 7-11.

Rani, K. U., U. J. S. P. Rao, K. Leelavathi, and P. H. Rao. 2001. Distribution of enzymes in wheat flour mill streams. J. Cereal Sci. 34 : 233-242.

SAS. 2000. SAS User's Guide. The Institute: Cary, NC.

Steffens, J. C., E. Harel, and M. D. Hunt. 1994. Polyphenol oxidase. In: Ellis *et al.* (eds) Genetic engineering of plant secondary metabolism. Plenum Press, New York, USA, pp. 275-313.

Wang, C., M. I. P. Kovacs, D. B. Fowler, and R. Holly. 2004. Effects of protein content and composition on white salted noodle making quality: Color. Cereal Chem. 81 : 777-784.