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Total Polyphenol, Polyphenol Oxidase, Antioxidant Activity and Color Profiles of Some Wheat Varieties from Bangladesh

[1]A.S.M.G. Masum Akond, [2]Laila Khandaker, [3]Khwaja G. Hossain and [4]Y. Furuta

[1,2,3)]Division of Math and Science, Mayville State University, Mayville, ND 58257, USA,
[4]Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

Abstract: Nine wheat varieties (Shatabdi, Showrav, Protiva, Ahgrani, Kanchan, Akbar, Barkat, Sonalika, Kheri) from Bangladesh were analyzed for determination of total polyphenol (TP), polyphenoloxidase (PPO), antioxidant activities (AA) and color index (CI). The highest TP (1.316 mg /gm GAE) and AA (14.58%) determined in Shatabdi and PPO (0.295 U × ml^{-1} × min) in Barkat. The lowest TP (1.280 mg /gm GAE), PPO (0.145 U × ml^{-1} × min) was determined in Akbar and the lowest AA (7.64%) was in Sonalika . Least color index was obtained in Akbar but good color parameters for wheat like brightness and yellowness values were found higher in Shatabdi. There was stronger correlation found between AA and TP than among TP, AA, PPO and CI. Comperatively new released varieties, Protiva, Shourav (BARI Gom-19) and Shatabdi (BARI Gom- 21) have higher level of TP, AA with brighter color profile.

Key words: Wheat; antioxidant activity; total polyphenol; polyphenol oxidase activity, color index.

INTRODUCTION

Wheat is the second important cereal crop after rice, as a source of energy and protein in Bangladesh. Over the last 35 years, the wheat improvement programs of Bangladesh Agricultural Research Institute have been very successful in developing and releasing so far 24 commercial wheat varieties. These released varieties are providing tremendous impacts on wheat production of the country. Manufacturing standards in Bangladesh has two types of flour, 'Atta' and 'Maida'. The flat breads are made with high-extraction flour known as 'Atta', whereas breads and pastries are made with various grades of white flour, known as 'Maida'. However, those standards do not specifically allow for micronutrient status and flour color.

The flour color and micronutrient status of wheat have been attributed to the unique phytochemicals of grains that complement beneficial effects to human health. Whole grain consumption has been associated with reduced risk of chronic diseases, such as cardiovascular diseases and cancer[1]. Major phytochemicals phenolics or polyphenols known as a class of nutrients, include bioflavonoids, organic acids and phenolic acids and cereal grains are rich in phenolic acids[20]. Quality trait Polyphenol oxidase (E.C 1.14.18.1 and E.C 1.10.3.2, syn; tyrosinase, catecholase, o-diphenol oxidase), an oxidative enzyme present mainly in the bran of wheat[10], is a major factor in wheat products discoloration. High kernel PPO and Flour protein content affects noodle brightness[5,11]. Anti-oxidants are natural or synthetic substances in the form of vitamins and minerals that have been linked in removing harmful molecules called free radicals in the body to help fight against infection and other conditions including cancer, coronary artery disease, muscular degeneration, and other serious eye diseases[18]. Anti-oxidant activity varied both among species[19,12] and among genotypes within a species[8]. Antioxidants also play an important role in preventing undesirable changes in flavor and nutritional quality of foods.

So far there has been no report about the inherent varietals differences in polyphenolics, antioxidants, polyphenoloxidase, and color profile of wheat from Bangladesh. Information on these health beneficiary phytochemical profiles could increase consumer awareness, food processing industries and breeders. The objective of the current study was to evaluate the total polyphenol, polyphenoloxidase, total antioxidant activities and color property of 9 wheat varieties from Bangladesh.

MATERIALS AND METHODS

Grain Samples and Sample Preparation: Descriptions of the nine popular wheat (*Triticum aestivum*) varieties used in the study are given in Table 1. The wheat varieties were collected from Wheat Research Centre, Bangladesh Agricultural Research Institute (Table 1).

Corresponding Author: A.S.M.G. Masum Akond, Division of Math and Science, Mayville State University, Mayville, ND 58257, USA; Tel: 1-701-788-4674;
E-mail: masu.akond@mayvillestate.edu.

All samples were milled to a fine powder using 60 mesh size, mixed thoroughly to assay the phytochemical profiles. Whole grain was used for the assessment of polyphenoloxidase activity and color index.

Determination of Total Polyphenols (TP): Total phenolic content in wheat variety was determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton[21] using gallic acid as a standard phenolic compound. Briefly, 50 µL of wheat grain extract solution was placed in a test tube, then 1ml of Folin-Ciocalteu reagent (previously diluted by distilled water; Reagent: Water = 1: 4) was added and the content was mixed thoroughly. After 3min, 1mL of Na_2CO_3 (10%) was added, the mixture was allowed to stand for 1 h in the dark. Absorbance was measured at 760 nm using a (U-1800, HITACHI, Tokyo, Japan) spectrophotometer. The concentration of total phenolic compounds in leaf extracts was determined as micrograms of gallic acid equivalent using an equation obtained from a standard gallic acid graph. Results are expressed as mg/100 g gallic acid equivalent (GAE) of dry mass.

Determination of Polyphenol Oxidase Activity (PPO): PPO activity of wheat varieties was assessed following the methods proposed by Mahoney and Ramsay[3] and Bernier and Howes[4] with minor modifications. Both methods were based on a spectrophotometric assay in the presence of tyrosine as a substrate. Three whole seeds for each variety were incubated in 5 ml of 0.01 M disodium tyrosinate solution with 0.2% Tween 80 at 37°C for 19 hours. After removing the seeds, the absorbance of the solution was measured at 405 nm with a UV/VIS spectrophotometer (Lambda 40, Perkin Elmer,USA). Experiments were done with 3 replications. One unit of PPO activity was expressed as a change of absorbance of 0.1 per min per ml enzyme extract (Unit × ml^{-1} × min).

Determination of Antioxidant Activity (AA): Antioxidant activity was measured by the diphenylpicrylhydrazyl (DPPH) radical degradation method[2]. Briefly, 10 µL of leaf extract solution (three replicates) was introduced into test tubes, and 4mL distilled water and 1mL of 250 µM DPPH solution was added. The tubes were mixed and allowed to stand for 30 min in the dark. Absorbance was read against a blank at 517 nm using a (U-1800, HITACHI) spectrophotometer. Antioxidant activity was calculated as the percent of inhibition relative to the control using the following equation: Antioxidant activity (%) = $(A_{blank} - A_{sample}/A_{blank}) \times 100$, where A_{blank} is the absorbance of the control reaction (control consisted 10ml, methanol instead of sample extract), and A_{sample} is the absorbance of the test compound.

Determination of Color Index (CI): The color measurements were made with a CS- Sharpener colorimeter (Toppan Co. Ltd, Japan) calibrated to a standard white reflective plate. The grain samples for color measurement were placed in the light impervious vessels covered by a glass plate in order to obtain perfectly flat surface. Every color measurement was made in five readings per sample. Lightness (L^*) and redness (a^*) and yellowness (b^*) were recorded. Color index (CI) was calculated through the equation CI= 1000 x a*/L x b*[14].

Statistical Analysis: A complete randomized design with five replications per variety was used for assay. Data were analyzed using Tukey's multiple comparison test ($P<0.05$) via ANOVA[15]. The PC software 'Excel Statistics, ver 5.0 (2000): Esumi Co. Ltd., Japan.' was used for the calculations.

RESULTS AND DISCUSSION

The total polyphenol content of 9 wheat varieties are shown in Table 1. The highest total phenolic content was found in the extract of wheat variety 'Shatabdi', and the lowest in 'Akbar' and that varied from 1.269 to 1.316 mg /gm GAE of whole grain flour. Statistically non significant differences ($p < 0.05$) were obtained among the tested wheat varieties, the range in mean values also did not vary greatly among the varieties. Total phenolic content for the wheat variety Shatabdi was similar to American varieties Atlas 66 and Chinese Spring assessed in our laboratory (unpublished data).

PPO activities in the wheat varieties tested for the enzyme activity. In this research the PPO activity Table 1was varied from 0.145 (Akbar) to 0.295 U × ml^{-1} × min (Barkat). Statistically ($p > 0.05$) the varieties: Sonalika, Kanchan, Showrab and Protiva have the similar level of PPO enzyme. Watanabe *et al.*,[22] measured PPO activity of durum wheat by similar methods and the PPO activity was for ICARDA advanced line Cham 1 (0.185 U) and Jennah Khetifa (0.302 U). It was well known that tetraploid durum wheat (*Triticum turgidum* L.) has significantly lower kernel PPO activity than hexaploid bread wheat cultivars (*Triticum aestivum* L.; Kruger[10]; Demeke and Morris[9]. Hence, we found the wheat variety studied here having similar or less level than that of advance and RI lines of *T. durum*. This result indicated that these wheat varieties have good flour color for bakery use, especially for white flour, locally known as 'Maida'.

The total antioxidant activities of nine wheat varieties are shown in Table 1. Total antioxidant activity among the wheat varieties were statistically different ($p > 0.05$) and ranged from 7.64% (Sonalika) to 14.58% (Shatabdi). Statistically the total antioxidant activity of Shatabdi (14.58%) was similar ($p > 0.05$) to Showrab (13.08%) and Barkat (12.66%). In general, there was a trend of increased antioxidant activity with increased total phenolic content. Variation of antioxidant activity among cultivars was much wider than that of total polyphenol content. An increased number of hydroxyl groups or amino groups in the phytochemical's molecular structure led to higher antioxidant activity[7].

The color index among wheat samples was varied (Table 2). Wheat varieties Shatabdi, Showrab, Protiva, Aghrani and Sonalika displayed greater *b** (yellowness) unit value than the others. This relates to greater yellow pigment and flavonoid contents in the flours. Those varieties also had higher unit of L^* value (brightness) and lower CI (color index) value. Colored fruit and vegetables such as black carrots, beetroot, red oak and black bayberry cultivars which had much higher antioxidant activity and polyphenols than less colored ones[2,15]. But our results didn't support strongly of their results.

Table 1: List of different wheat varieties from Bangladesh, depicting variable total phenolic contents, polyphenol oxidase and antioxidant activity.

Variety Name	Year of release	Source of germplasm	Pedigree	TP (mg/ g GAE)	PPO (U × ml^{-1} × min)	AA (%)
SHATABDI (BARI GAM-21)	2000	CIMMYT	MRN/BVC//BLO/PVN/3PJB-81	1.316a	0.282 d	14.58e
SOURAV (BARI GAM-19)	1998	CIMMYT	NL560=NAC/VEE	1.298a	0.275 bc	13.08de
PROTIVA	1993	Thailand	KU Head selection-12	1.293a	0.254 bc	10.55bc
AGHRANI	1987	Pakistan	INIA/3/SON64/p4160E//SON64	1.291a	0.253 ab	11.11cd
KANCHAN	1983	India	UP301/C306	1.289a	0.185 bc	8.68ab
AKBAR	1983	CIMMYT	PON/TOB	1.269a	0.145 a	8.33a
BARKAT	1983	CIMMYT	BB/GLL//CARP/3/PVN	1.309a	0.295 d	12.66de
SONALIKA	1973	India	1154-388/AN/3/YT54/N10B//LR64	1.271a	0.249 bc	7.64a
KHERI	-	Local collection	-	1.280a	0.207 c	9.28abc

Table 2: Coloral properties and index of different wheat varieties.

Variety	Brightness (L*)	Redness (a*)	yellowness (b*)	Color Index (CI)
SHATABDI (BARI GAM-21)	54.15a	3.99a	19.29abcd	3.82b
SOURAV (BARI GAM-19)	49.64c	4.03a	21.25ab	3.82b
PROTIVA	51.22abc	4.26b	21.30a	3.90b
AGHRANI	52.58abc	3.19b	20.16abc	3.01c
KANCHAN	49.54c	2.99b	17.71cde	3.40bc
AKBAR	52.95ab	2.92b	18.47bcd	2.99c
BARKAT	53.45a	4.08a	15.61e	4.89a
SONALIKA	51.74abc	3.33b	19.26abc	3.34c
KHERI	50.23bc	3.36a	14.16de	4.73bc

To understand relationships among the studied properties correlation analysis were established based on simple linear regression (Fig 1a- f). We found moderate significant correlations for antioxidant activity, total phenolics and polyphenol oxidase activity in relation to color index (Fig.4, 5, and 6). Color Index was seems to be a indicator of determine PPO activity in wheat, lowest PPO activity found in the variety Akbar, which had the lowest color index, on the other hand the variety Barkat showed the highest PPO having higher color index. Total phenolic content and antioxidant activity of wheat were highly correlated

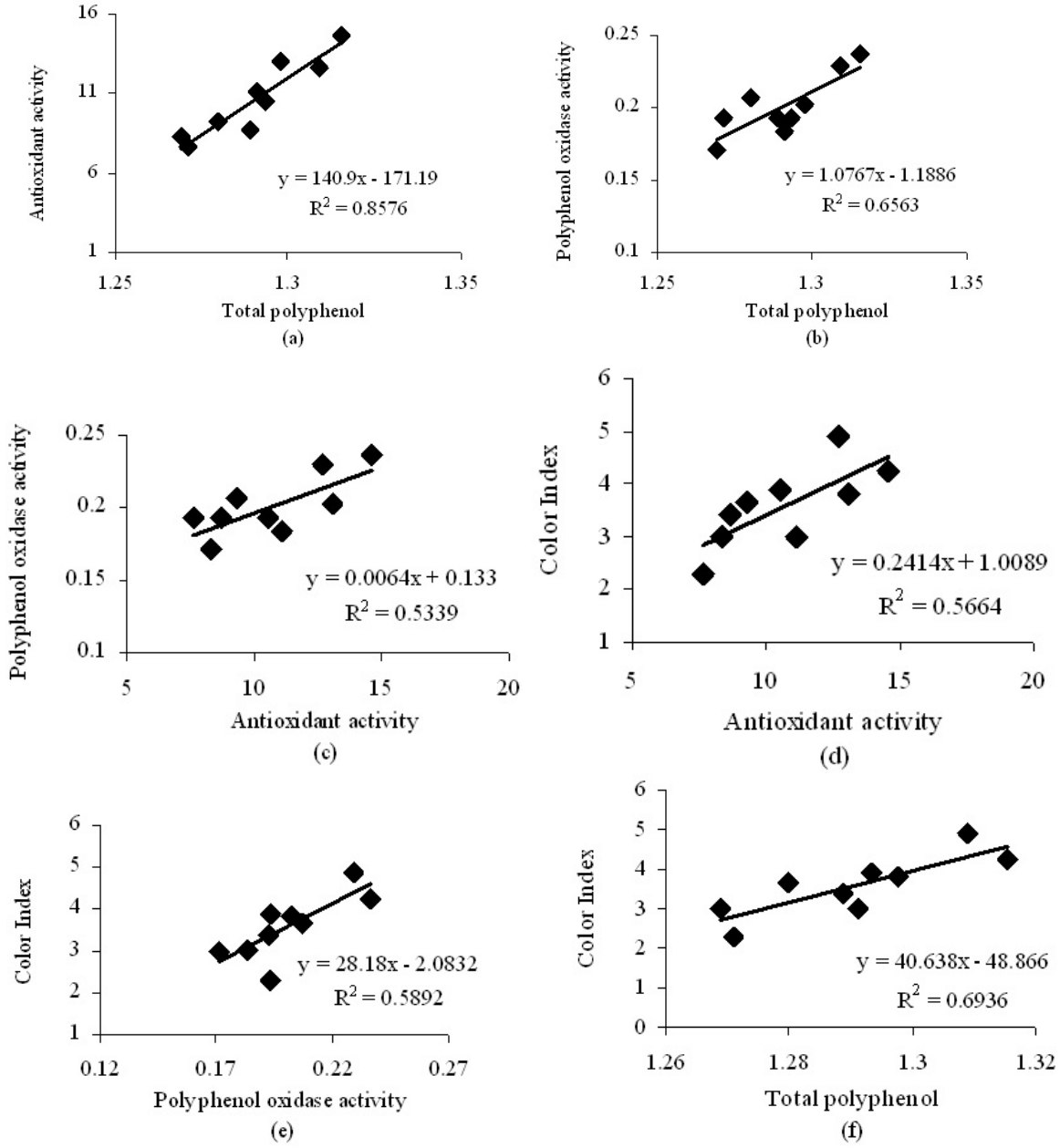

Fig. 1(a)-(f): Correlation based on simple regression method ($p>0.05$) among TP, PPO, AA and CI of different wheat varieties.

(R^2 = 0.8576), providing strong evidence that the predominant source of antioxidant activity derives from phenolic compounds in wheat. High correlation coefficients between the phenolic content and antioxidant activities have been reported for various food commodities such as sorghum, R^2 = 0.971 and cactus pear, R^2 = 0.970[6]. Thus, the phenolic content of wheat can be use as an indicator in assessing the antioxidant activity like other fruits and vegetables. The TP content was moderately correlated with PPO content (r^2 = 0.6563), indicated that TP does not all to have PPO activity in wheat. On the other hand the lower correlation was found between AA and PPO (r^2 = 0.5339).

Overall, comparatively latest varieties, Protiva, Shourav and Shatabdi have higher level of antioxidants with brighter color index. Those varieties are semi-dwarf, better disease resistance varieties produce more

than 8-10% higher yields than popular variety Kanchan (source Bangladesh Agricultural Research Institute, BARI. Web page http://www.bari.gov.bd), which covering 80% of the total wheat cultivated area in Bangladesh. The yield of Kanchan also has been reduced to a great extent due to its susceptibility to foliar diseases. This variety should be gradually replaced by new varieties like Shatabdi, Sourav and Protiva.

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