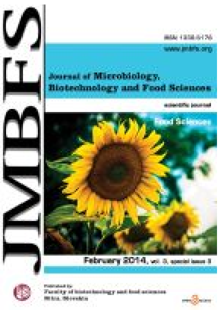

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FREE PHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF WINTER WHEAT IN SUSTAINABLE FARMING SYSTEMS

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ABSTRACT

The objective of this work was to evaluate the free phenol content and antioxidant activity of winter wheat white flour, whole grain flour and bran in ecological and integrated farming system. The experiment was established on a scientific research base Dolná Malanta in western Slovakia during the years 2009 - 2011. Free phenol content was determined by Folin-Ciocalteu's method and antioxidant activity by DPPH radical scavenging method. Whole grain flour and bran had higher content of free phenols in ecological farming system. Antioxidant activity was not affected by farming systems. The content of free phenols and antioxidant activity was affected by growing year and forecrop. Fertilisation had no effect on the content of free phenols and antioxidant activity. White flour contain two times less free phenols and antioxidant activity than whole grain flour. The highest free phenol content and antioxidant activity was determined in wheat bran.

Keywords: antioxidant activity, free phenols, winter wheat, ecological system

INTRODUCTION

Epidemiological studies have strongly suggested that diets play a crucial role in the prevention of chronic diseases such as heart disease, cancer, diabetes, and Alzheimers's disease **(Willet, 1994; Temple, 2000)**. Minimizing oxidative damage may, be one of the most important approaches to the primary prevention of these aging associated diseases and health problems. Recent research demonstrates that wheat grain contains significant level of natural antioxidants. Wheat is an important agricultural commodity and a primary food ingredient worldwide and contains considerable beneficial nutritional components. Wheat and wheat-based food ingredients rich in natural antioxidants can ideally serve as the basis for development of functional foods designed to improve the health of millions of consumers **(Yu, 2008)**.
Wheat contains a diverse array of bioactive compounds that may contribute to its antioxidant capacity. These bioactive components include carotenoids, tocopherols, tocotrienols, phenolic acids, phytic acid, phytosterols and flavonoids **(Yu *et al.*, 2005)**. It is estimated that flavonoids account for approximately two thirds of the phenolics in our diet and the remaining one third are from phenolic acids **(Liu, 2004)**.
The content of biologically active components are influenced by various environmental factors and management practices. **Holmboe-Ottesen (2010)** divided the sources of variability to two groups. Environmental factors, such as latitude, location, climatic variations, altitude, and soil type affect the composition of nutrients and secondary metabolites. But interested are the agricultural production practices which also affect plant composition, such as choice of variety/cultivar, fertilizer regime, pesticide application, soil preparation methods and different cultivation practices, such as crop rotation, cover crops, crop mixing and timing of harvest. Therefore, different food production methods may result in differences in the content of secondary metabolites **(Matt *et al.*, 2011)**.
The aim of this work was to evaluate the effect of integrated and ecological farming system on the content of free phenols and antioxidant activity in different milling fraction of winter wheat.

MATERIAL AND METHODS

Field experiments were conducted at the Research Experimental Station Dolná Malanta, Western Slovakia during 2009 - 2011 on a Haplic Luvisol developed at proluvial sediments mixed with loess. The altitude of the experimental field was 178 meters above sea level. The location has a continental climate with an average temperature 19.7 °C in July and -1.7 °C in January, an average annual precipitations are 561 mm. The aim of this work was to evaluate the free phenol compounds and antioxidant activity of winter wheat (*Triticum aestivum* L.). A split - plot design was used with two main treatments, ecological (ES) and integrated (IS) cropping systems. The ecological system was composed of a six course crop rotation: beans + alfalfa – alfalfa – winter wheat – peas – maize – spring barley. The integrated system consisted of the crop rotation: winter wheat – peas – winter wheat – maize – spring barley – alfalfa (3 years at the same plot). Subplots were fertilized and unfertilized variants. The fertilized variant in ES was based on 40 t of manure while the IS also received 40 t of manure plus synthetic fertilizers (Tab. 1). Experiment was replicated four times. Sowing and harvesting dates, rainfall and average temperature calculated for vegetative period of the crop, synthetic fertilizer inputs (kg.ha^{-1}) applied in the IS are shown in the table 1. Nitrogen fertilizers were applied in three split applications.

Table 1 Crop management data for winter wheat 2009 – 2011

Year	Sowing date	Harvest date	Rainfall (mm)	Average temperature (°C)	Nitrogen (kg.ha^{-1})	Phosphorus (kg.ha^{-1})	Potassium (kg.ha^{-1})
2009	13/10/08	15/07/09	426	9,6	82,5	37,5	20,0
2010	7/10/09	28/07/10	610	8,8	62,5	7,5	40,0
2011	28/10/10	13/07/11	304	8,53	76	30	120

The biologically active components – free phenols and antioxidant activity were determined in winter wheat samples grown in 2009, 2010 and 2011 vegetative periods.

Sample preparation method

Grain of winter wheat was milled on a laboratory mill Quadrumat Senior from the company Brabender, which allows to obtain 4 fractions: I, II - flour, III, IV - brans. Whole wheat flour was obtained by grinding at the mill PSY MP.

Extraction

Free phenols of winter wheat milling fractions were extracted according to the methods of **Van Hung *et al.* (2009)**, with a modification. Wheat milling fractions (2 g) were mixed with 15 mL of 80 % chilled methanol, using sonication with a Bandelin sonorex digitec sonicator (35 kHz, 350 W). The suspension was centrifuged and the supernatant (free phenol extract) was collected.

Determination of free phenol content

Free phenol content in the extract of winter wheat fractions were immediately determined using the Folin-Ciocalteu's method by **Van Hung *et al.* (2009),** with a modification. To the 150 µL of the extract was added 750 µL of distilled water. This solution was oxidized with 300 µL of Folin-Ciocalteu's reagent and then neutralized with 300 µL of 20 % sodium carbonate solution (20 % w/v). Thoroughly mixed samples were allowed to stand for 120 min at dark place at ambient temperature. The solution was centrifuged for 10 min at 12000 rpm and the absorbance of the clear supernatants was measured at 765 nm using a spectrophotometer (UV-1800, Shimadzu, Osaka, Japan). A standard calibration was prepared using gallic acid and the content of free phenolics in each extract was calculated and expressed as micrograms of gallic acid equivalent (GAE) per gram of the sample. Tests were carried out in four replications.

Determination of antioxidant activity method

DPPH radical scavenging capacities of winter wheat extracts were determined according to the method by **Huang *et al.*, 2005, Van Hung *et al.* 2009**. The extract (0.6 mL) was mixed with 0.9 mL DPPH solution (12.5 mg of DPPH in 100 mL 80 % methanol), kept in the dark at ambient temperature and the absorbance of the mixtures was recorded at 515 nm for exactly 30 min. Blank was made from 0.9 mL of DPPH and 0.6 mL of 80 % methanol and measured the absorbance at t = 0. The scavenging of DPPH was calculated according to the following equation: $\%\ DPPH\ scavenging = (Abs_{(t=0)} - Abs_{(t=30)}) / Abs_{(t=0)} \times 100$. Where, $Abs_{(t=0)}$ = absorbance of DPPH radical + 80 % methanol at t = 0 min; $Abs_{(t=30)}$ = absorbance of DPPH radical + extract at t = 30 min.

The data were statistically evaluated in program Statgraphic 5.0, using analysis of variance (ANOVA), and significant differences were evaluated by LSD test ($P \leq 0.05$)

RESULTS AND DISCUSSION

Phenols are present in cereals in the free and conjugated forms. The highest concentration of phenolic acids and flavonoids is in the aleurone layer of cereal grains, but these compounds are also found in embryos and seed coat of grains **(Shirley, 1998)**. Free phenols (FP) content and antioxidant activity (AA) of the milling fractions of winter wheat were statistically different (Table 2). The FP content and AA of whole grain flour was significantly higher than in the white flour. White flour contain 48 % less FP than whole grain flour. Similar result was in AA when white flour had two times lower AA than whole grain flour. The highest FP content and AA was determined in wheat bran. **Ivanišová *et al.* (2012)** observed that flour showed the lower proportion of the total antioxidant potential and bran showed higher AA. Higher FP content in outer layer of waxy wheat reported also **Van Hung *et al.* (2009)**.

Table 2 Free phenol content and antioxidant activity of winter wheat milling fractions

	Free phenolic content ($\mu g\ GAE/g^{-1}$)	DPPH (%)
White flour	97.04 a	23.26 a
Whole grain flour	185.57 b	49.57 b
Bran	493.93 c	76.47 c

Kim *et al.* (2006) observed that the total phenolic content of the bran that contains mainly the phenolic acids and polyphenols is 3362–3967 mg/g^{-1} gallic acid equivalent, in which only 15 % are free phenolic and rest 85 % of the total phenolic content in wheat bran is in bound form. In a case of waxy wheat **Van Hung *et al.* (2009)** observed that from total phenolic content, 30 % of phenols are free in whole grain flour.
Although there have been many reports on polyphenols in cereal grain, data on the effect of cultivation/fertilization on their content are scarce. Most of them indicate that phenolic compounds content is higher in grain from organic cultivation **(Konopka *et al.*, 2012)**. According to **Zuchowski *et al.* (2011)** the observed higher concentration of phenolic acids in organic wheat is caused mainly by the smaller kernel size**.** In presented work farming system had significant effect on the content of FP only in the whole grain flour and bran (Table 3). The farming system had no effect on concentration of FP in white flour. Whole grain flour in the ES contain almost 18 % more of FP than whole grain flour in the IS. Similar result was in the case of bran which contain 13 % more of FP in ES. The regime of fertilization or year had no effect on the content of FP in any of milling fraction.

Table 3 Free phenol contents in different milling fractions

		Free phenol content (µg GAE/g)		
		White flour	**Whole grain flour**	**Bran**
Farming system	**Integrated system**	95.61 a	173.03 a	470.15 a
	Ecological system	100.45 a	210.64 b	541.51 b
Year	**2009**	85.36 a	172.71 a	486.16 ab
	2010	102.95 a	178.26 a	467.55 a
	2011	103.34 a	205.73 a	528.09 b
Forecrop	**Fabaceae – pea**	109.93 b	184.92 ab	502.87 b
	Fabaceae - lucerne	100.45 ab	210.64 b	541.51 c
	Spring Barley	81.28 a	161.15 a	437.42 a
Fertilization	**Fertilized**	108.8 a	185.12 a	497.30 a
	Unfertilised	85.63 a	186.02 a	490.56 a

Growing year had no effect on the concentration of FP in the white flour and whole grain flour, but had an effect on FP in bran. Significant differences were recorded between years 2010 and 2011. Bran in the year 2010 contain 12 % less of FP, than bran in the year 2011.
In the work by **Stracke *et al.* (2009)** was compared ecological and conventional produced wheat, and was pointed out that climate variations in different observed years had a greater influence on the biological active components concentration in whole wheat than the production method. Organically produced wheat exhibited no statistically significant differences in the biologically active components concentrations as compared to the conventionally grown ones.
Forecrop had significant effect on FP in white flour, whole grain flour and bran. The lowest concentration of FP were after spring barley in all fractions. White flour after spring barley contain 26 % less of FP than white flour after fabaceae - pea. Whole grain wheat after spring barley contain 13 % less of FP than whole grain wheat after fabaceae in ES. Whole grain wheat and bran contain the highest concentration of FP after fabaceae - lucerne. Concentration of FP in the bran decreased with forecrop in order: fabaceae - lucerne > fabaceae - pea > spring barley.

Significant effect had an interaction farming system and regime of fertilization in white flour in IS, when using of manure plus artificial fertilizers lead to almost 36 % increase of FP (Table 4).

Table 4 Free phenol content in interaction farming system and fertilization – white flour

Farming system	Free phenol content (µg GAE/g)	
	Unfertilised	**Fertilized**
Integrated system	74.68 b	116.53 a
Ecological system	107.55 a	93.34 a

Significant effect had an interaction farming system and growing year. Bran in ES in years 2009 and 2010 contain 31 % more of FP than bran in IS in the same years (Table 5).

Table 5 Free phenol content in interaction farming system and growing year in winter wheat

Year	Free phenol content (µg GAE/g)	
	Integrated farming system	**Ecological farming system**
2009	444.85 a	568.78 b
2010	431.27 a	540.10 b
2011	534.32 a	515.63 a

AA of milling fractions of the winter wheat with different factors are given in Table 6. Farming system did not affect AA of white flour, whole grain flour neither bran. But AA significantly increased in order: white flour < whole grain flour < bran.

Table 6 Antioxidant activity in different milling fractions of winter wheat

		Antioxidant activity (%)		
		White flour	**Whole grain flour**	**Bran**
Farming system	**Integrated system**	19.83 a	49.49 a	77.45 a
	Ecological system	20.55 a	51.16 a	76.77 a
Year	**2009**	24.79 b	46.08 a	74.74 a
	2010	21.73 b	53.06 b	78.20 b
	2011	13.69 a	51.00 b	78.73 b
Forecrop	**Fabaceae – pea**	21.08 a	53.15 a	78.31 a
	Fabaceae - lucerne	20.55 a	51.16 a	76.77 b
	Spring Barley	18.58 a	45.83 b	76.59 b
Fertilization	**Fertilized**	19.95 a	49.22 a	77.49 a
	Unfertilised	20.19 a	50.87 a	76.96 a

Growing year had significant effect on AA but results were not consistent. White flour in year 2011 had around 41 % lower AA in comparison with years 2009 and 2010. In case of whole grain flour and bran the lowest AA was in year 2009, in comparison with years 2010 and 2011

Farming system had significant effect on AA in interaction with regime of fertilisation in white flour (Table 7). Using of manure in ES lead to 26 % increase of AA in white flour, compared to variant without fertilisation. On the other side, using manure and artificial fertilization in IS lead to 16 % decrease of AA in the white flour. This effect of fertilization was not observed neither in whole grain flour, nor in bran.

Table 7 White flour antioxidant activity interaction of farming system and fertilisation

Farming system	White flour antioxidant activity (%)	
	Unfertilised	**Fertilized**
Ecological (ES)	17.49 a	23.61 b
Integrated (IS)	21.53 b	18.13 a

AA of white flour was not affected by forecrop. AA of whole grain flour and bran were not consistent. AA of whole grain flour was affected by forecrop in order: fabaceae pea = fabaceae lucerne > spring barley. While AA of bran was affected by forecrop in order: fabaceae - pea > spring barley = fabaceae - lucerne.

Langenkämper *et al.* (2006) assessed the antioxidative capacity and soluble and total phenolic compounds of the various wheat samples (bio-dynamic, bio-organic and two conventional systems) with a high and low fertilization doses. For none of these parameters no significant difference was found between wheat samples from the farming systems using fertiliser. None-fertilised wheat, however, had increased levels of soluble phenolic compounds. **Langenkämper *et al.* (2006)** conclude, that results did not provide evidence that wheat of one agricultural system or the other would be better or worse.

CONCLUSION

In presented work, the content of free phenols and antioxidant activity of winter wheat, in white flour, whole grain flour and bran fractions after milling were evaluated. Winter wheat was cultivated in ecological and integrated arable farming systems. Whole grain flour and bran had higher content of free phenols in ecological farming system. Antioxidant activity of winter wheat was not affected by farming system. White flour contain two times less free phenols and two time lower antioxidant activity than whole grain flour. By milling process most of the free phenols and highest antioxidant activity remain in bran fraction. At the content of free phenols and antioxidant activity had effect growing year and forecrop. Using of fertilisation had no effect on the content of free phenols and antioxidant activity.

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