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REGULAR ARTICLE

FREE FLAVONOID 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 flavonoid content and antioxidant activity of winter wheat white flour, whole grain flour and bran in ecological and integrated farming system in the years 2009-2010. The experiment was established on a scientific research base Dolná Malanta in western Slovakia during the years 2009 and 2010. Content of free flavonoids and antioxidant activity of white flour and whole grain flour was not affected by farming systems. Antioxidant activity of whole grain flour was more effected by a year and by a forecrop. White flour contain two times less free flavonoids and antioxidant activity than whole grain flour. By milling process most of the free flavonoids and other compounds with higher antioxidant activity remain in outer layers of grain.

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

INTRODUCTION

Antioxidant activity of grains reported in the literature has been underestimated since only unbound antioxidants are usually studied. 90 % of the wheat antioxidants are bound.

Bound biologically active compounds could survive stomach and intestinal digestion, but would then be released in the large intestine and potentially play a protective role **(Adom and Liu 2002)**. The total antioxidant activity of wholegrain products is similar to that of fruits or vegetables on a per serving basis **(Miller *et al.*, 2001)**. Biological active components and antioxidants in whole grains have not received as much attention as the phytochemicals in fruits and vegetables although the increased consumption of whole grains and whole grain products has been associated with reduced risk of developing chronic diseases such as cardiovascular disease, type 2 diabetes, some cancers and all-cause mortality **(Liu, 2007)**. A large number of studies have shown that wheat whole grain and wheat bran extracts possess antioxidant properties against oxidation of biologically important molecules such as DNA, proteins and membrane lipids **(Yu *et al.*, 2005)**. Thus consumption of whole wheat foods such as whole wheat bread or pasta besides other whole grain foods has been recommended for healthy diets **(Wood, 2004)**.

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 of interest here 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, 2011)**.

The aim of this study was determining of free flavonoid contents and antioxidant activity of winter wheat in sustainable farming systems.

MATERIAL AND METHODS

Field experiments were conducted at the Research Experimental Station Dolná Malanta, Western Slovakia during 2009 and 2010 on a Haplic Luvisol developed at proluvial sediments mixed with loess. The altitude of the experimental field was 178 m. 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 paper was to evaluate the free flavonoid 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 (F) and unfertilized (N). 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). Fertilizing 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 – 2010

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

The biologically active components – free flavonoids and antioxidant activity were determined in winter wheat samples grown during 2009 and 2010 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 flavonoids 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, after that the samples were placed for 15 min to ultrasound bath. The suspension was centrifuged and the supernatant (free flavonoid extracts) was collected.

Determination of free flavonoid content method

Flavonoid contents in the extract of winter wheat fractions were determined using the aluminum chloride colorimetric method by **Van Hung *et al.* (2009)**. The extracts (150 µL) were mixed with 450 µL of 80% methanol, followed by 40 µL of 10% aluminum chloride, 40 µL of 1 M potassium acetate and 820 µL of distilled water. The mixture was incubated for 30 min at room temperature. Before measuring an absorbance samples were centrifuged at 21 000 g in 10 min, measured was at 415 nm with a Shimadzu UV-1800 spectrophotometer (Osaka, Japan). The flavonoid content was calculated using a standard calibration of rutin methanol solution and expressed as micrograms of rutin equivalent (RE) per gram of sample.

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 flavonoid extracts (0.6 mL) were 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 + flavonoid extracts at t = 30 min.

RESULTS AND DISCUSSION

Phenolic acids and flavonoids 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 flavonoid (FF) content and antioxidant activity (AA) of the milling fractions of winter wheat were statistically different (Table 2). The free flavonoid contents and AA of whole grain flour was significantly higher than in the white flour. White flour (240.53 $\mu g.g^{-1}$) contain 51 % less FF than whole grain flour (490.81 $\mu g.g^{-1}$). Similar result was in AA when white flour (23.26 %) had two times lower AA than whole grain flour (49.57 %). The highest FF content (661.00 $\mu g.g^{-1}$) and AA (76.47 %) 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 antioxidant activity. Higher free flavonoid content in outer layer of waxy wheat reported also **Van Hung *et al.* (2009)**.

Table 2 Free flavonoid contents and antioxidant activity of winter wheat milling fractions.

	Free flavonoid content ($\mu g.g^{-1}$)	**DPPH (%)**
White flour	240.53 a	23.26 a
Whole grain flour	490.81 b	49.57 b
Bran	661.00 c	76.47 c

Some studies have concluded that ES production methods lead to increase in nutrients, particularly organic acids and polyphenolic compounds. However, other studies did not demonstrate differences in nutrients between ES and conventional production methods **(Winter and Davis 2006)**. FF contents of winter wheat milling fractions as influenced by farming system, forecrop, plant nutrition and year are given in Table 3. There were no significant differences in the contents of FF in the white flour, whole grain flour, experimental factor – farming system, fertilization, forecrop and year did not influenced the content of FF. Only interaction of farming system and year, had significant effect on FF content in white flour, when white flour in IS in a year 2010 (220 $\mu g.g^{-1}$) contain 22 % less of FF than ES in 2010 (280.72 $\mu g.g^{-1}$). But in the case of bran significant effect of framing system, year and forecrop on FF was determined. Bran in ES (706.61 $\mu g.g^{-1}$) contain almost 10 % more of FF than bran in IS (638.19 $\mu g.g^{-1}$). For production of FF in bran was better year 2010

(711.17 µg.g^{-1}) when there were accumulated at about 14 % higher amount of FF than in 2009. Forecrop had significant effect on FF content in the case of spring barley in IS and fabaceae in ES. Bran of winter wheat after fabaceae in ES (706.61 µg.g^{-1}) contain almost 14 % more FF than bran after spring barley in IS (609.97 µg.g^{-1}).

Table 3 Free flavonoid contents in different milling fractions

		Free flavonoid content (µg RE/g^{-1})		
		White flour	**Whole grain flour**	**Bran**
Farming system	**Integrated system (IS)**	232.40 a	479.98 a	638.19 a
	Ecological system (ES)	256.78 a	512.48 a	706.61 b
Year	**2009**	240.81 a	523.02 a	610.83 a
	2010	240.24 a	458.60 a	711.17 b
Forecrop	**Fabaceae IS**	233.68 a	514.19 a	666.41 ab
	Fabaceae ES	256.78 a	512.48 a	706.61 b
	Spring Barley IS	231.12 a	445.77 a	609.97 a
Fertilization	**With fertilization OR**	252.78 a	480.83 a	667.84 a
	Without fertilization O	228.27 a	500.79 a	654.15 a

AA of milling fractions of the winter wheat are given in Table 4. Farming system did not effect AA of white flour and whole grain flour but had small effect of this parameter on bran. Bran in IS (77.03 %) had only slightly higher antioxidant activity than bran in ES (75.35 %). Year had no significant effect on AA in white flour but had significant effect on AA in whole grain four and bran. Both whole grain flour and bran in year 2010 possess higher AA. In the case of whole grain flour it was 53.06 % in 2010 and 46.08 % in 2009 respectively. The best forecrop for AA in our study was fabaceae in IS for whole grain flour and bran. Forecrop had no effect on white flour. AA of whole grain flour decreased after forecrop in order: fabaceae IS (55.01 %) > fabaceae ES (50.24 %) > spring barley IS (43.46 %). Regime of fertilization had no effect on AA in white flour, whole grain flour and bran respectively.

Langenkämper *et al.* (2006) assess the antioxidative capacity and soluble and total phenolic compounds of the various wheat samples (bio-dynamic, bio-organic and two conventional systems) with high and low fertilization doses. For none of these parameters any 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, the results do not provide evidence that wheat of one agriculture system or the other would be better or worse.

Table 4 Antioxidant capacity in different milling fractions of winter wheat

		Antioxidant activity (%)		
		White flour	**Whole grain flour**	**Bran**
Farming system	**Integrated system (IS)**	22.85 a	49.23 a	77.03 b
	Ecological system (ES)	24.08 a	50.24 a	75.35 a
Year	**2009**	24.79 a	46.08 a	74.74 a
	2010	21.73 a	53.06 b	78.20 b
Forecrop	**Fabaceae IS**	23.98 a	55.01 c	78.29 b
	Fabaceae ES	24.08 a	50.24 b	75.35 a
	Spring Barley IS	21.72 a	43.46 a	75.78 a
Fertilization	**With fertilization OR**	23.05 a	48.01 a	76.62 a
	Without fertilization O	23.47 a	51.12 a	76.33 a

CONCLUSION

In presented work, the content of free flavonoids, 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. Content of free flavonoids and antioxidant activity of white flour and whole grain flour was not affected by farming systems. White flour contain two times less free flavonoids and antioxidant activity than whole grain flour. By milling process most of the free flavonoids and compounds with antioxidant activity remain in bran fraction. At content of free flavonoids and antioxidant activity of bran the highest effects of farming system, year and forecrop were determined

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