

PHENOL CONTENT AND ANTIOXIDANT ACTIVITY IN MILLING FRACTIONS OF BREAD WHEAT CULTIVARS

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Abstract

Phenol compounds are a class of plants' metabolites with role in protecting against ultra violet radiations and pathogen agents. Wheat contains phenol compounds that have raised the specialists' interest especially due to their antioxidant action with benefic effects in reducing the incidence of cancer and cardiovascular diseases. Antioxidants are not homogeneously distributed in the wheat grain. The aim of this paper is to determine the distribution of phenol compounds in the fractions resulted from wheat grinding. Two cultivars of bakery wheat of the 2008- harvest recommended by the National Institute of Agricultural Research- development Fundulea (the cultivar Boema and the cultivar Faur) were used to test their resistance to the new climate conditions from our country. The samples were ground by a pilot mill Buhler and the fractions resulted were submitted to the extraction method. The extracts were filtered and analyzed further on to determine the total phenol content and total antioxidant capacity. Total phenol content was determined using the method Folin –Ciocâlțeu in all the fractions resulted from grinding. Total antioxidant capacity was spectrophotometrically determined on phenol extracts by the method DPPH. The results obtained did not show notable differences between the cultivars analyzed as regards the total phenol content. As for the distribution on milling fractions, the quantity of total phenols is higher as the fractions contain more milling material coming from the aleuronic layer and bran.

When determining the antiradical capacity we noticed that the fractions ground from the wheat Faur have a higher reducing capacity than those from the wheat Boema and the reducing capacity increases as the fraction ground contains a higher percentage of aleuronic tissue. These data underline the importance of big extractions which have a higher content in bran and aleuronic layers in order to obtain some physiologically important and benefic products for the body's daily consumption due to the intake of antioxidants.

Keywords: Phenol Content, Antioxidant Activity, Wheat, Milling Fractions

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1. INTRODUCTION

Phenol compounds are a class of plants' metabolites with role in protecting against ultra violet radiations and pathogen agents (Alvarez-Jubete et al., 2010). The primary sources of naturally available antioxidants are whole cereals, fruits and vegetables (Moure et al., 2001). Wheat contains phenol compounds that have raised the specialists' interest especially due to their antioxidant action with benefic effects in reducing the incidence of cancer and cardiovascular diseases (Beta et al., 2005). Antioxidants are a group of small molecular weight –phytochemicals that can be especially found in produces of vegetal origin. These ones include carotenoids, tocopherols, lignans and

phenolic acids. These antioxidant components can prevent the oxidation of some enzymes and DNA by different mechanisms. The antioxidants from food play an important role as protecting factor. It has been scientifically proved that they reduce the incidence risk of cancer and cardiovascular diseases (Moure et al., 2001). Vitamin C, vitamin E, phenolic acids, phytates and phytoestrogenes have been acknowledged as potential factors in preventing the incidence risk of cancer and cardiovascular diseases (Halliwell, 1996). The antioxidants in cereals and cereal products are already known but their potential contribution to health by diet has been essentially ignored. Their capacity to fight against free radicals makes them being of special interest at this research moment in the field of foods which can provide certain

solutions regarding the intake of antioxidants in consumers' diet (Baublis et al., 2000).

Studies have shown that wheat antioxidants can directly react with the oxygen reactive species such as hydroxyl radicals or singlet oxygen molecules, diminishing their attack upon biological molecules (Chandrika and Shahidi, 2007). Wheat antioxidants can also form chelating complexes with transitive metals in order to reduce the availability of these ones as catalyzers to produce free radicals (Rice-Evans et al., 1996).

By investigating significant levels of natural antioxidants in cereals, the research results in the field have shown that antioxidants are not homogenously distributed in grains (Lloyd et al., 2006).

The antioxidant activity of different fractions in cereals resulted from the milling process has little been studied and it is useful in the manufacturing of functional foods with important role in ensuring consumers' health condition.

2. MATERIALS AND METHODS

The cereal samples to be analyzed are from Romania, the harvest of 2008, recommended by the National Institute of Agricultural Research- development Fundulea (cultivar Boema and cultivar Faur) for their resistance to new climate conditions from our country and to the attack of specific pests.

The preparation of wheat samples for grinding: the samples were brought to a moisture of 13,5% 24 hours before grinding and about 30 minutes before grinding to a moisture of 15%. The moisture content was determined by the help of the electronic humidometer T1.

The samples were ground in the pilot mill Buhler. The seven fractions of cereals obtained (three from milling, three from grist and bran) symbolized as M1, M2, M3, ŞR1, ŞR2, ŞR3, T and I, were weighed by the analytical balance Mettler Toledo. The ash content of the fractions resulted was determined by the method STAS 90-88 by incineration at 550 °C. 2.5 g from each sample were taken and put into recipients on which 64% ethanol solution was

poured. The samples were placed into an ultrasound bath of Sonica 2200 type at the temperature of 60 ° C, for 25 minutes. After that, the extracts from the cereal fractions were vacuum filtered to get the clear watery extract, without any impurity, ready for further determinations. The extracts were further filtered and analyzed to determine the total phenol content and oxidizing capacity. All the reagents used were of analytical purity and all tests were made in duplicates.

2.1. Determination of total phenol content

The method Folin-Ciocalteu was used to determine the total phenol content (Singleton et al., 2002). The extracts were diluted to the proportion of 1:3 by ultra-pure water obtained by the help of the Water Ultra purifying system TKA SMART 2 PURE, then 1 ml of diluted extract sample was transferred to a test tube containing 5 ml of Folin-Ciocalteu 1/10 solution into water. Then 4 ml of sodium carbonate 7.5 %(w/v) solution was added to neutralize. This operation was repeated for all the fractions of cereals analyzed.

The test tubes were maintained at room temperature for 60 minutes; afterwards the absorbance was measured at the wavelength of 765 nm by the spectrophotometer JASCO model V 530, using ultra-pure water as control sample.

The total phenol content was expressed in equivalents of gallic acid (GAE) in g/100 g material, using a standard curve of gallic acid, with concentrations varying between 0 -50 µg/ml (Pearson correlation coefficient: $r^2=0.9917$) accordingly to the standardized method ISO 14502-1(93).

2.2. Determination of reducing activity was made using the method DPPH (Molyneux, P, 2004)

Dilutions of 1: 100 of ultra pure water for each sample analyzed were made. 200µl of sample or standard were taken and introduced into Eppendorf tubes and 1.4 ml DPPH solution 80 µmol/100 ml was added.

The control sample consists of 200 µl ethanol plus 1.4 ml DPPH solution. The samples were centrifuged at 15000 RPM by the Universal 320R centrifuge, for 10 minutes, at the temperature of 18 ° C in order to get some homogeneity and remove possible impurities left in. The absorbance of samples is read at the minute 0 and minute 30.

For the quantitative determination, a calibrating plot was made, represented by the intensity variation of absorption peak of DPPH at 517 nm under different concentrations of Trolox (6-hydroxy - 2,5,7,8 tetramethylecroman-2-carboxylic acid, a synthetic analogue of vitamin E).

The comparative analysis of samples was made by calculating the antiradical activity (% RA_{DPPH}), standing for the relative decrease of absorbance in the samples analyzed. The absorbance inhibition percentage of DPPH solution was calculated using the following equation:

$$\% \text{ RA}_{\text{DPPH}} = \left[\frac{(\text{Abst}_{0 \text{ min}} - \text{Abst}_{30 \text{ min}}) / \text{Abst}_{0 \text{ min}}}{\times 100} \right] \quad (1)$$

Where $\text{Abst}_{0 \text{ min}}$ is the absorbance PPH at the moment zero and $\text{Abst}_{30 \text{ min}}$ is the absorbance DPPH after 30 de minutes of incubation.

2.3. Statistical Analysis

The program Excell of Microsoft Office 2003 was used to determine the correlation coefficient [10] between total content of antioxidants and the antioxidantizing activity of the cultivars analyzed.

3. RESULTS AND DISCUSSION

The results of experimental data regarding the total phenol content are presented in the graph of figure 1. The total phenol content varies between 0,3451 and 0,5172 µg/ml GAE.

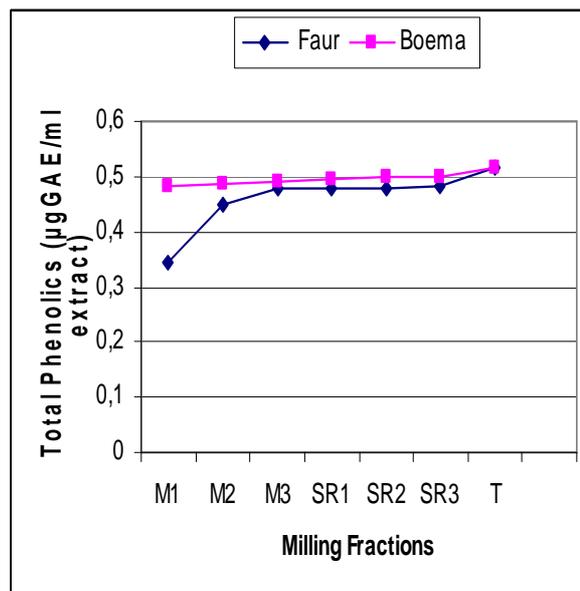


Figure 1: Total Phenol Content in milling fractions

It can be clearly noticed that the total quantity of phenols is higher as the fractions contain more ground material resulting from the aleuronic layer and bran in both cultivars analyzed. If we compare the total values of phenol compounds in the wheat samples analyzed, we can draw the conclusion that the lowest total phenol content is registered by the flour fractions resulting from first milling and increases as the cereal fractions have a higher percentage of bran.

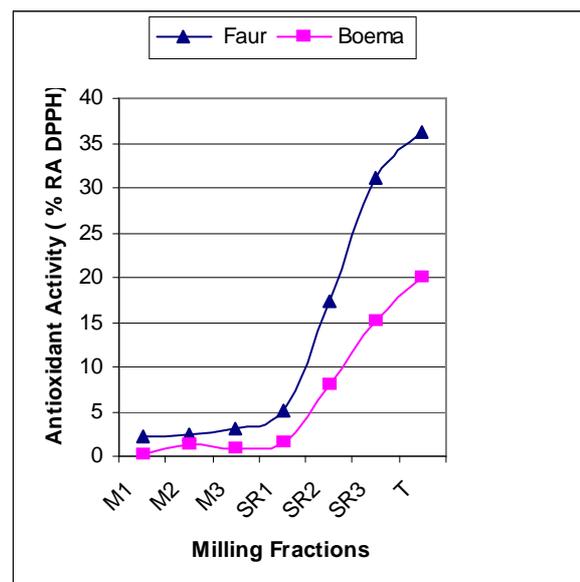


Figure 2. Antioxidant Activity in milling fractions

As a whole the fractions ground from the wheat Faur have a higher reducing activity than those corresponding to the wheat Boema. The cultivar Faur, in the fractions M1, M2, M3, has a relatively reduced antiradical activity as compared with the values obtained from the grists 2, 3 and bran, fact explained by the higher percentage of starch poor in antioxidants.

For the wheat Boema, as it was expected, the maximum value of antiradical activity registered in bran is about 1,8 times lower than that corresponding to the Faur cultivar. Comparing the arithmetic means of the values of antiradical activity at milling we get a proportion of 2, 4: 1 between the cultivars Faur and Boema. In the case of grists, the ratio of arithmetic means is 2,1:1.

The calculation of correlation coefficient between total content of antioxidants and antioxidant activity of the cultivars analyzed led to values of $r = 0,846$ for the cultivar Boema and $r = 0,757$, showing a strong correlation in the same sense of the parameters analyzed in the both cases of the cultivars Boema and Faur.

4. CONCLUSIONS

In wheat, the total polyphenol quantity had near values in the cultivars analyzed (wheat Boema, with values ranging between 0.4815 $\mu\text{g/ml}$ GAE in the fraction M1 and up to 0.5148 $\mu\text{g/ml}$ GAE, and the cultivar Faur, between 0.3451 $\mu\text{g/ml}$ GAE in the fraction M1, up to 0.5172 $\mu\text{g/ml}$ GAE, in the bran fraction. It has also been noticed that the bran had the highest percentage of total polyphenols, and the fractions resulted from milling had the lowest one due to the higher content of endosperm.

Out of the different types of cereals used at milling, the bran had a higher share of phenol compounds as compared with the endosperm producing flour fractions.

The highest quantities of phenol compounds in the cereal fractions are in bran and grists as well resulted from breaking and can be explained by the presence of different proportions of bran (the farthest layers of cereal

core, including the aleuronic one) and germs (embryos from cereals). In non-processed fractions, the endosperm diminishes the antioxidant substances present in bran and consequently the total polyphenol content in whole cereals is lower as compared with the bran fraction taken individually.

Total polyphenol content has varied in cereal fractions as the processing degree has been more advanced. The proportion of bran and germs in the whole grain is of 14% and 1% respectively, according to the data of literature, the cereal fractions may contain 10% bran residue whereas 20% may contain a mixture of endosperm, aleuronic layer and bran. From the experiment made in the lab, it is obvious that the aleuronic layer contributes significantly to total polyphenol compounds and therefore especially to wheat antioxidant capacity.

Phenol compounds have been concentrated in bran fraction, whereas the endosperm has also contributed to the concentration of phenol compounds. The tests bring the argument that phenol compounds in wheat are generally concentrated in the cell walls of aleuronic layer. It has also been demonstrated that the cereal fractions, bran especially, are rich sources in phenol compounds.

As regards the antioxidant activity, in the breaking passages $\text{\textcircled{R}}1$, $\text{\textcircled{R}}2$, $\text{\textcircled{R}}3$, and bran as well, the reducing power increases as the fraction ground contains a higher percentage of bran. In the cultivar Faur the antiradical activity reaches 36,3% in bran whereas in the cultivar Boema, it reaches 19,9. These data highlight the importance of bigger extracts having a high content in bran and aleuronic layers in order to obtain nutritional important products benefic for the body's daily consumption due to their intake of antioxidants.

The study shows that the processing degree should be kept at a minimum level in order to maintain high the value of bioactive compounds in cereals.

The results bring some additional confirmation on the location of phenol compounds in external layers of cereal grains.

5. REFERENCES

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