

PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY IN MILLING FRACTIONS OF RYE

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Abstract: Phenol compounds are a class of plants' metabolites with role in protecting against ultra violet radiations and pathogen agents. Rye 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 rye grain. The aim of this paper is to determine the distribution of phenol compounds in the fractions resulted from grinding the rye cultivars grown in our country. Three rye cultivars of the 2008-harvest were used. The samples were ground by the 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, complying with the limits specified by the literature in the field. 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 reducing capacity increases as the fraction ground contains a higher percentage of aleuronic tissue.

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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 [1]. The primary sources of naturally available antioxidants are whole cereals, fruits and vegetables [2]. 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 [3]. 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 [2]. 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 [4]. 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 [5].

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 [6]. Rye antioxidants can also form chelating complexes with transitive metals in order to reduce the availability of these ones as catalyzers to produce free radicals [7].

2. Experimental

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

The preparation of rye 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, ȘR1, ȘR2, ȘR3, and T, were weighed by the analytical balance Mettler Toledo.

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

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 [8].

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.

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.

The method Folin-Ciocalteu was used to determine the total phenol content [9].

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 $\mu\text{g/ml}$ (Pearson correlation coefficient: $r^2= 0.9917$) accordingly to the standardized method ISO 14502-1[93].

Determination of reducing activity was made using the method DPPH [10].

Dilutions of 1: 100 of ultra pure water for each sample analyzed were made. 200 μl of sample or standard were taken and introduced into Eppendorf tubes and 1.4 ml DPPH solution 80 $\mu\text{mol}/100\text{ ml}$ 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:

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

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.

Statistical Analysis

The program Excell of Microsoft Office 2003 was used to determine the correlation coefficient [11] between total content of antioxidants and the antioxidizing 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 0,2140 and 0,9527 $\mu\text{g GAE./ml}$ extract.

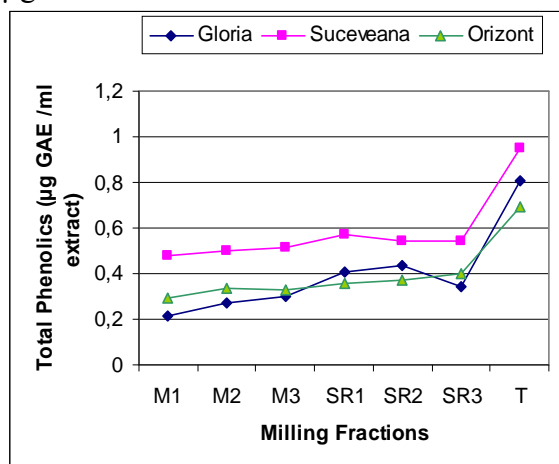


Figure 1: Total Phenolics content in milling fractions

The total phenolic content of the cultivars Gloria and Orizont registered near values in the milling fractions resulted from breaking and grinding, the cultivar Suceveana having a higher content of phenol compounds. It has been observed that in the case of fractions resulted from grinding, the cultivar Gloria has the lowest content of phenol substances and in the case of grists the lowest polyphenol content is registered by the cultivar Orizont. In all the three cultivars analyzed the total polyphenol quantity increases slightly as the fractions contain more ground material resulting from the aleuronic layer. A significant increase of the total polyphenol content in bran is registered as compared with the rest of fractions obtained from grinding.

Comparing the total polyphenol content in bran we can see that the cultivar Orizont had the minimum values, the cultivar Gloria the medium ones and the cultivar Suceveana the maximum ones. The antioxidant activity of the three cultivars analyzed can be seen in the graph of figure 2. As a whole the fractions ground from the rye cultivar Orizont have a higher reducing activity than those corresponding to the cultivars Suceveana și Gloria. All three cultivars analyzed show in the fractions resulting from milling [M1, M2, M3] a more reduced antiradical activity as compared with the values obtained from the grists and bran, fact explained by the

higher percentage of starch poor in antioxidants.

The maximum values of antiradical activity were registered in bran, as it was expected, the minimum ones corresponding to the cultivar Gloria, the medium ones to Suceveana and the maximum to Orizont.

The calculation of correlation coefficient between total content of antioxidants and antioxidant activity of the cultivars analyzed led to values of $r = 0,993864$ for the cultivar Gloria and $r = 0,895667$ for Suceveana and $r = 0,950117$ for Orizont. These values show a strong correlation in the same sense of the parameters analyzed in the case of all the cultivars analyzed.

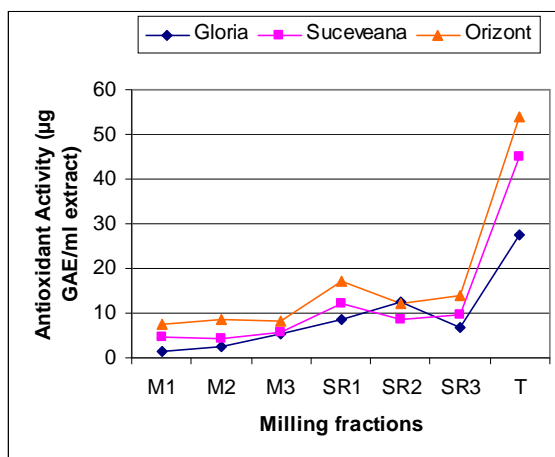


Figure 2 Antioxidant Activity in milling fractions

4. Conclusion

In the rye cultivars analyzed, the total polyphenol quantity had relatively near values in the cultivars Gloria and Orizont, Suceveana registering some higher values. 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. The different values of total phenol content obtained from breaking can be explained as well by the presence of their different proportions of bran, aleuronic layer and germs. In the fractions processed the

endosperm having a reduced content of polyphenols decreases the share of antioxidant substances present in bran and consequently the total phenol content of whole cereals is lower, as compared with the bran fraction taken individually. The quantity of polyphenols found in the cultivars analyzed subscribes to the limits of the literature in the field.

As regards the antioxidant activity, even if we expected that there would be a direct proportionality between the total phenol content and antioxidant activity, we can see that the antioxidant activity of the fractions of the cultivar Suceveana hold the second position though its has registered a

maximum polyphenol content. It is obvious that the antioxidant activity of the Romanian rye cultivars analyzed registers comparable values to those cited in the literature of the field at world level.

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.

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