

Phenolic Content and Antioxidant Activity in Milling Fractions of Oat

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Received: 21 May 2011; Accepted: 04 August 2011

Abstract

The aim of the research is to study the antioxidant activity and concentration of phenolic substances with antioxidant role in milling fractions of two characteristic cultivars of Romanian oat. The method Folin – Ciocâlțeu has been used to determine the total content of phenolic substances. The total antioxidant activity was determined by spectrophotometric method using the reagent DPPH (2,2-Diphenyl-1-Picryl Hydrasil), as system of releasing the free radical DPPH. The antioxidant activity is expressed as antiradical activity of samples and as percentage. Both cultivars analyzed have registered the highest content of phenolic substances in bran, and the antioxidant capacity of the fractions analyzed has been strongly correlated with the content of phenolic substances and that of ash. The data resulted from the experiment confirm the positioning of substances with antioxidant role especially in the superior strata of oat grain and high quantity of substances with antioxidant role in oat.

Keywords: phenolic content, Antioxidant activity, oat, milling fractions

1. Introduction

The addition of antioxidants in foods can increase the stability of the latter ones while being stored for long periods of time, protecting essential fatty acids from destruction by spontaneous oxidation [1,2]. The data regarding the toxicity and carcinogen effect of synthesis antioxidants such as butyl hydroxitoluene (BHT) and butyl hydroxianisole (BHA) turned the specialists' interest to natural antioxidants [3]. The most important sources of natural antioxidants are vegetables, fruits and cereals [4]. The presence of antioxidants in cereals is explained by the fact that all biological systems including cereals display a natural tendency of minimizing the destructive potential of the oxidation reactions and consequently they have developed multifunctional defense systems [5].

For long time, cereals have been considered important sources of antioxidants despite the fact that they are an important component of diet for a large part of the planet population [6]. The antioxidants in cereals have the advantage of maintaining their antioxidant capacity inside the human body, too, and not only in the plants, they come from.

Besides the effect of increasing foods' stability, antioxidants are also known for their ascertained properties in preventing cardiovascular diseases and cancer [7,8].

It has been acknowledged that the distribution of antioxidant substances in cereals' grains is not uniform [9].

The aim of this research is to study the antioxidant activity and concentration of phenolic substances in milling fractions of two characteristic cultivars of Romanian oat.

2. Material and Methods

Materials: The oat sample studied are purchased from Romania, the harvest of year 2008, from the National Institute of Agricultural Research-Development Fundulea (the cultivar Mures and the cultivar Somesan) recommended for their resistance to the new climate conditions in our country and the attack of specific pests. The oat was ground in a mill with vertical disks and then separated into three fractions: coarse flour, fine flour and bran, by sieving for 5 minutes on a mechanic sieve with holes of 0.5mm and 0.25mm. The ash content in the fractions obtained was determined by the method STAS 90-88 by incineration at 550 °C. 2.5 g from each sample was taken and this quantity was put into recipients on which a solution of 64% ethanol was poured. The samples were placed into an ultrasound bath of type Sonica 2200 at the temperature of 60 ° C, for 25 minutes. Then, the fraction extracts from cereals were vacuum filtered to obtain a clear aqueous extract, without impurities, ready for further determinations. The extracts were further filtered and analyzed in order to determine the total content of phenols and antioxidant capacity. All the reagents used were of analytical purity and all the tests made were duplicated.

Determination of total phenolic content: The method Folin-Ciocalteu was used to determine the total phenolic content [10, 11]. The extracts were diluted in proportion of 1:3 with ultra pure water obtained by the help of the system of water ultra purification TKA SMART 2 PURE, and then each 1ml of diluted sample of extract was transferred into a test tube containing 5ml of a solution Folin-Ciocalteu 1/10 in water. Then 4 ml of soda salt solution 7.5 % (w/v) were added for neutralization. The operation was repeated for all the cereal fractions analyzed. The test tubes were kept for 60 minutes at room temperature; afterwards the absorbance at a wavelength of 765 nm was measured by the help of a spectrophotometer JASCO model V 530, having ultra pure water as blank sample.

The total phenolic content was expressed in equivalents of Gallic acid (GAE) in mg/kg product, using a standard curve of Gallic acid, with concentrations varying between 0 -50 µg/ml complying with the standardized method ISO 14502-1(93).

Determination of reducing activity using the method DPPH [10]: Dilutions of 1: 100 with ultra pure water for each sample to be analyzed were made. 200µl of sample or standard were taken off, put into Eppendorf tubes and 1.4 ml solution DPPH 80 µmol/100 ml were added in. The blank sample consists of 200 µl ethanol plus 1.4 ml solution DPPH. The samples were centrifuged at 15000 RPM by the help of the centrifuge Universal 320R, for 10 minutes, at the temperature of 18 degrees Celsius, in order to get some homogeneity and remove the possible impurities left in. The absorbance of samples is read at the minute 0 and the minute 30. A calibrating plot, represented by the intensity variation of the absorption maximum of DPPH at 517 nm in the presence of different concentrations of Trolox (6-hydroxi-2,5,7,8 tetramethylcroman-2-carboxylic acid, a synthetic analogue of Vitamin E), was made for quantitative determination. The comparative analysis of samples was made by calculating the antiradical activity (% RA_{DPPH}), which is the relative decrease of absorbance in the samples analyzed. DPPH solution's inhibition percentage of absorbance was calculated by the following equation:

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

Where $\text{Abst}_{0 \text{ min}}$ was the absorbance DPPH at the time zero and $\text{Abst}_{30 \text{ min}}$ was the absorbance DPPH after 30 minutes of incubation.

Statistical Analysis: The program Excel of Microsoft Office 2003 was used to determine the correlation coefficient Pearson [12] between the total content of phenol substances and antioxidant activity of the cultivars analyzed.

3. Results and Discussion

The results of experimental data regarding the total phenolic content were centralized in the graph from figure 1. The symbols for the milling fractions are the following: CF - coarse flour, FF- fine flour and B – bran.

The total content of phenolic substances in the fractions analyzed varies between 144 mg/kg and 361 mg/kg.

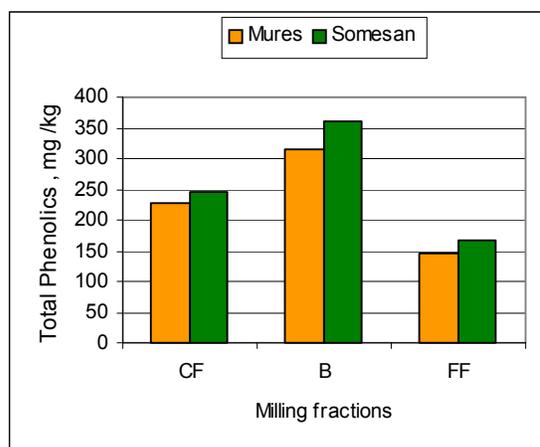


Figure 1. Total Phenolics content in milling fractions of oat

The highest content of phenolic substances was found in bran in both cultivars. The second position, in terms of total content of phenolic substances, is occupied by coarse flour, and the third one is taken by the fine flour, with fine grain. We can see a more significant difference between the content of phenolic substances in the fractions of the cultivar Somesan, this one having the highest content of phenolic substances proportional in all the milling fractions analyzed.

The determination results regarding the antioxidant activity of the fractions derived from the two oat cultivars were centralized in the graph from figure 2.

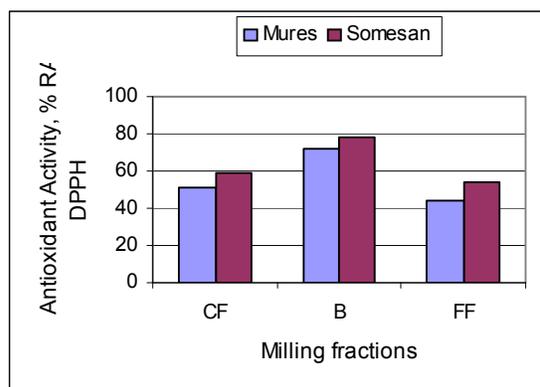


Figure 2. Antioxidant Activity in milling fractions

As it was expected, bran had the highest antioxidant activity, followed by the coarse flour and fine flour.

The graph presenting the antioxidant activity of milling fractions in the two cultivars keeps broadly the allure of representing the content of phenolic substances with some differences due to the fact that in oat there are compounds with antioxidant activity but without phenolic structure. The antioxidant activity varies between 44, 4 in the case of fine flour of the cultivar Mures and 77, 8 in the case of bran of the cultivar Somesan.

The calculation of Pearson correlation coefficients between the total content of phenolic substances and antioxidant activity of oat led to values of $r=0,96185$ in the case of the cultivar Mures and $r=0,97788$ in the case of the cultivar Somesan, showing that there is a very strong correlation in the same sense between the two parameters analyzed. The determination results of ash content can be seen in the table 1.

Table 1. Ash content in oat cultivars milling fractions

Oat cultivars/ Milling fractions	CF	B	FF
Mures	2,23	3,62	0,54
Somesan	2,47	3,73	0,68

The statistical analysis of experimental data indicates the existence of some strong correlations in the same sense between the total content of phenolic substances and ash content of the fractions analyzed, correlation coefficients having the values $r = 0,99801$ for the cultivar Mures and $r = 0,97622$ for the cultivar Somesan, fact which confirms once more the hypothesis according to which the substances with antioxidant activity are placed in the exterior strata of the oat grain, in bran and aleuronic stratum.

4. Conclusions

The phenolic compounds with antioxidant activity are mainly distributed in the exterior strata of oat grain, fact demonstrated by the strong correlation in the same sense between the quantity of phenolic compounds and ash content of the milling fractions analyzed.

From the nutritional point of view it is recommended that during oat processing these strata rich in substances with antioxidant activity should not be removed, so as to be found in as high as possible proportion in foods.

The values of total content of phenolic substances and antioxidant activity of milling fractions comply with the limits registered by the literature for oat cultivars from other countries, fact which shows that the Romanian oat can be successfully used as a source of antioxidants in foods with high nutritional activity.

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