PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY IN OAT

Cristian SEREA, Octavian BARNA
Biochemistry Department, Faculty of Food Science and Engineering, Galati,
„Dunărea de Jos ” University, 111 Domneasca St., 800201 Galati,Romania
cristian_serea@yahoo.com;octavian.barna@yahoo.com

Abstract
The aim of our research was to study the content of antioxidants, their composition and antioxidant activity of five specific Romanian oat cultivars: Jeremy, Lovrin 1, Lovrin 27-T., Mures and Comun. The total phenolic content was determined by the method Folin - Ciocâlteu.
The total antioxidant activity was estimated by spectrophotometric method using the reagent DPPH (2,2-DIPHENYL-1-PICRYLHYDRAZIL), as generating system of a free radical. The antioxidant activity is expressed in percentage as antiradical activity. Following the investigations made, we established that all oat cultivars have antioxidant activity due to a wide range of active substances formed both in free and bound compounds. The statistic analysis by calculating the correlation coefficient Pearson (r^2) was used to estimate the influence of free and bound compounds upon the antiradical activity determined by the method DPPH. Significant differences from the quantitative point of view between the five oat cultivars analyzed were found. Pure cultivars lack certain phenolic compounds which are normally present in the commercial ones.. The commercial cultivar Comun showed the highest quantity of phenolic compounds, followed by the cultivars Mures, T27, Lovrin and Jeremy. The experimental data confirm the high quantity and diversity of antioxidant substances in Romanian oat and the possibility of using it as food source with important role in ensuring the consumers' health.

Keywords: Phenol Content, Antioxidant Activity, Oat

Submitted: 06.08.2011 Reviewed: 20.09.2011 Accepted: 25.10.2011

1. INTRODUCTION

The presence of compound with antioxidant properties in human nutrition is an intensely studied and discussed issue by the sciences of nutrition at present due to the correlation existing between the oxidative stress and some of the pathologies the humankind is facing with.
The most important sources of natural antioxidants are vegetables, fruits and cereals as well (Petersen, 2001). The presence of antioxidants in cereals is a consequence of the fact that all biological systems, including cereals, have a natural tendency of minimizing the destructive potential of oxidation reactions and consequently they developed their own multifunctional defence systems (Shahidi, 2000). For a long time cereals have not been considered important sources of antioxidants despite the fact they are an important component of diet for a large part of the planet population (Duve, 1991). Antioxidants in cereals have the advantage of keeping their antioxidant capacity inside the human body, too, and not only in the plant have they derived from.
It is known that the distribution of antioxidant substances in the cereal grain is not uniform (Petersen, 2001) and consequently their contribution to the fund of nutritional antioxidants depends on the way they are processed.

2.MATERIALS AND METHODS

The samples of oat analyzed were cultivated under the same agro-technical conditions, coming from the harvest of the year 2009. The cultivars Jeremy, Lovrin 1, Lovrin 27-T were registered as oat cultivars in the years 2005, 2002 and 2005 respectively at the Research and Agricultural Development Station Lovrin, in Timisoara where they were purchased from whereas the cultivar Mures was purchased from the Research and Agricultural Development Station Turda, in Turda, Cluj – Napoca where it was registered as distinct oat

cultivar in 1991. The oat Comun is not a pure cultivar but a commercially common one.
The oat was ground by a lab disk-mill of type IKA A10 IKAWERKE GmbH and Co. KG. Staufen Germany, for 5 minutes at 15 ^{o}C, particles < 0.6mm being obtained.

Extraction of antioxidant activity substances

Free compounds

4 g of each sample were taken and put into receptacles over which 40 ml solution of 4/1 methanol/water was poured. The samples were placed into an ultrasound bath of type Sonica 2200 at the temperature of 60 ^{o} C for 10 minutes. After being centrifuged at 1000g for 10 minutes, the separated liquid was removed and the extraction was repeated once more. The separated liquids were gathered and concentrated in a vacuum evaporator at 40 ^{o} C and reconstituted with 4ml of solution 1/1 methanol /water (v/v).
The extracts were further analyzed to determine the total phenolic content and antioxidant capacity. All the reagents used were of analytical purity and all the tests were made in replicates 4 times (n =4) and extracts were stored at –18 C until use.

Bund compounds

Alkaline hydrolysis was used to determine antioxidant activity bound compounds. The residue resulted from the methanol extraction of antioxidant activity free compounds was treated with 200ml solution of NaOH 2M and shaken for 20 hours at room temperature under nitrogen atmosphere. The mixture was acidified up to pH 2-3 by adding 500 ml of hexane. Hydrophilic fraction was extracted by 100ml solution 1/1 diethyl ether/ethyl acetate (v/v), by repeating the operation five times. Organic fractions were gathered and evaporated to dry. Then, the solution was reconstituted by adding 4 ml solution methanol/water 1/1 (v/v).

Determination of total phenolic content

The method Folin-Ciocâlteu was used to determine the total phenolic content (*Current Protocols in Food Analytical Chemistry* (2002) I1.1.1-I1.1.8, by John Wiley & Sons, Inc)
Extracts were diluted in proportion of 1:3 with ultra pure water obtained by the help of the water ultra purification System TKA SMART 2 PURE, and then each 1 ml of diluted extract was transferred into a tube containing 5 ml of solution Folin-Ciocâlteu 1/10 in water. 4 ml of sodium carbonate solution 7.5%(w/v) were added for neutralization. The operation was repeated for all the cereal fractions analyzed.
The tubes were kept at room temperature for 60 minutes; afterwards, absorbance was measured at the wavelength of 765 nm by the help of a spectrophotometer JASCO model V 530, ultra pure water being the blank sample.
Total polyphenol content was expressed as galic acid equivalents (GAE) in mg/kg product, using a standard curve of galic acid, with concentrations varying between 0 -50 µg/ml complying with the standardized method ISO 14502-1(93).

Determination of antioxidant activity (DPPH method) (10)

Dilutions of 1: 100 of ultra pure water were made from each sample prepared to be analyzed. 200µl of sample or standard were taken, put into Eppendorf tubes and 1.4 ml solution DPPH 80 µmol/100 ml was added. The blank sample consisted 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 18 degrees Celsius, in order to get some homogeneity and remove possible impurities left in. The absorbance of samples was read at the minute 0 and minute 30.
A calibrating plot was made for quantitative determination, represented by the intensity variation of absorbance 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).
The comparative analysis of samples was made by calculating the antiradical activity (% RA_{DPPH}), which stands for the relative decrease of absorbance in the samples analyzed. The

percentage of absorbance inhibition of DPPH solution was calculated using the following equation:

$$\% RA_{DPPH} = [(Abst_{0\ min} - Abst_{30\ min}) / Abst_{0\ min}] \times 100$$

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

Statistical analysis

The results presented in this study are the average of 4 determinations. We used the program Statistical analises Excel of Micrisoft Office 2003 to determine the correlation coefficient Pearson $p<0.05$ (Hedges, 1985) between the total content of antioxidant activity substances and their antioxidant activity in the cultivars analyzed.

3. RESULTS AND DISCUSSION

Taking into consideration the fact that phenol substances my exist in oat both in free or combined form with other compounds, within the research on the quantity of substances with total antioxidant role of this cereal, a two-step evaluation was necessary, namely one for free phenol compounds and the other for the bound ones. The determination of the content of total free phenols using the method Folin-Ciocâlteu led to results synthetically expressed in figure 1.

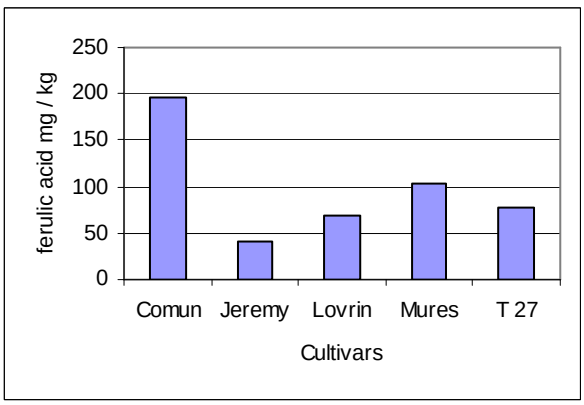

Fig. 1 – Content of total free phenols, quantified by the method Folin-Ciocâlteu, of Romanian oaten wholemeal (ferulic acid mg / kg)

As can be seen in figure 1, the highest quantity of phenol compounds was found in the commercial cultivar Comun, followed by the cultivars Mureş, T27, Lovrin and Jeremy.

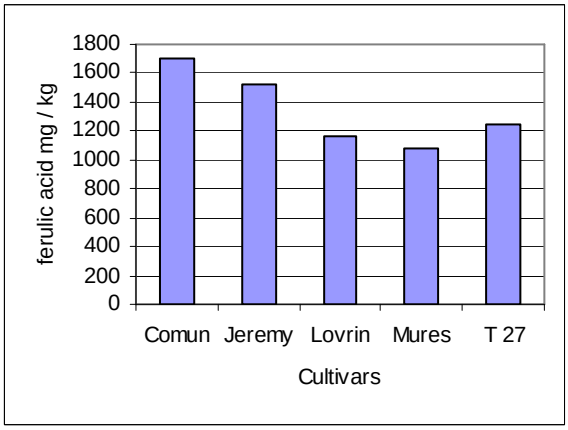

Fig. 2 – Content of total bound phenols, quantified by the method Folin-Ciocâlteu, of Romanian oaten wholemeal

As regards the total quantity of phenolic compounds, in this case too, the commercial variety (the cultivar Comun) has registered the highest quantity, followed by the cultivar Jeremy. The other three cultivars analyzed did not show significant differences in terms of the indicator analyzed.

The quantity of total phenols was correlated with the antioxidant activity determined by the method DPPH.

The results obtained when determining the antioxidant activity of free phenol extracts are shown in the graph of figure 3.

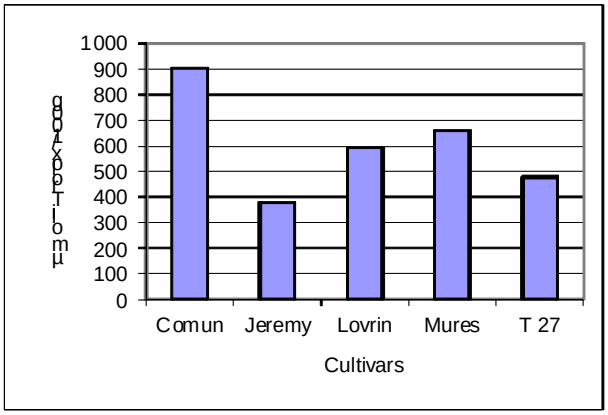

Fig. 3 –Antioxidant activity of free phenol extracts in Romanian oaten wholemeal (µmoles of Trolox/100g)

.

From the graph of figure 3 the same tendency indicated by the value of total free phenols can be noticed as well as in the evaluation of antioxidant activity, the cultivar Comun getting a maximum value of the indicator analyzed, followed by the cultivars Mureş, Lovrin, T27 and Jeremy.
The determination of the antioxidant activity of bound phenol extracts led to the data shown in figure 4.

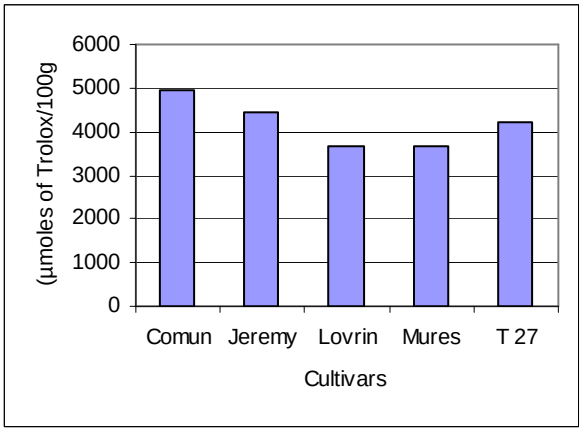

Fig. 4 – Antioxidant activity of bound phenol extracts in Romanian oaten wholemeal (µmoles of Trolox/100g)

This time, too, higher values in the antioxidant activity were registered by the oaten samples Comun, but the differences noticed between this cultivar and the others analyzed are not so obvious. The cultivar Lovrin distinguished itself by the lowest antioxidant activity of the bound compounds.
The antioxidant activity of free and bound compounds by DPPH method was statistically analyzed by calculating the correlation coefficient Pearson $r^{2.}$
The calculation of correlation coefficient between total free compounds and antioxidant activity of the cultivars analyzed led to values of r^2= 0.9305 ($p<0.001$) and r^2= 0.7297 ($p<0.01$) for total bound compounds .
These values show a strong correlation in the same sense of the parameters analyzed .

4. CONCLUSIONS

Following the investigations made, we have established that all oat cultivars have antioxidant activity due to a wide range of active substances existing both in free and bound form.
There is strong correlation between the total concentration of phenolic compounds and total antioxidant capacity in cereal extracts. Therefore, one may draw the conclusion that the level of phenols in cereals is a relevant indicator for their reducing capacity.
The tests made show differences in values of total polyphenol level and total antioxidant capacity of different Romanian oat cultivars. The results of the present study demonstrate that the values of the total content of phenolic substances and antioxidant activity obtained are within the limits specified in literature for the oat cultivars from other European countries, fact which shows that the Romanian oat can also be successfully used as a source of nutritional antioxidants.

5. REFERENCES

Journals:

[1] Duve,K.J.; White, P.J., Extraction and Identification of Antioxidants in Oats, JAOCS, 1991, 68 (4), 365-370
[2] Gray, D.A.; Clarke, M.J.; Baux, C.; Bunding, J.P.; Salter, A. M., Antioxidant Activity of Oat Extracts added to Human LDL particles and in Free radical Trapping Assay, Jornal of Cereal Science, 2002, 36, 209-218.
[3] Halliwell, B., Antioxidants in Human Health and Disease, Annual Review of Nutrition, 1996; Vol. 16: 33-50 .
[4] Karadag A., *et al*, *Review of Methods to Determine Antioxidant Capacities*, Food Analytical Methods, 2009,2:41–60.
[5] Martinez-Tome, M.; Murcia , M.A.; Frega, N.; Evaluation of Antioxidant Capacity of Cereal Brans, J.Agric. Food Chem., 2004, 52,4690-4699.
[6] Molyneux, P. The use of the stable free radical di-phenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J. Sci. Technol., 2004; v. 26, n. 2, p. 211-219.
[7] Moure, A., Cruz, J.M., Franco,D., Dominguez, J.M., Natural antioxidants from residual sources, Food Chemistry, 2001; vol 72, Issue 2, Feb, 145-171.
[8] Petersen, D.M.; Oat Antioxidants, Journal of Cereal Science,2001, 33,115-129.
[9] Peterson, D. M.; Emmons, C. L ; Phenolic Antioxidants and Antioxidants Activity in Pearling Fractions of Oat Groats; Journal of Cereal Science, 2001, 33, 97-103
[10] Rice-Evans, C. A., Miller, N. J., & Paganga, G. *Structure antioxidantActivity relationships of flavonoids*

and phenolic acids.Free Radical Biology and Medicine, 1996; 20, 933–956.
[11]. Shahidi F, Antioxidants in food and food antioxidants. *Nahrung* 2000 44:158–163 (2000).
[12]. Simonsen H. T. *et al.*, *Molecular Interactions between Barley and Oat b-Glucans and Phenolic Derivatives*, Journal of Agric. Food Chemistry. 2009 57, 2056–2064.
[13]. Vaya, J.; Aviram, M.; Nutricional Antioxidants Mechanism of Action, Analyses of Activities and Medical Applications, Curr.Med. Chem-Imm, Endoc.Metab.Agents, 2001, vol1, no1, 99-117.
[14] Verardo, V., Bonoli,M., Marconi, E., Caboni, M.F., Distribution of bound hydroxicinamic acids and theyr glycosil esters in barley, Journal of Agricultural and Food Chemistry 2008,56, 11, 900-905,

Books:
[15] Hedges, L.V., Olkin,I., Statistical Methods for Meta-Analysis, Hartcourt Brace Jovanovich Publishers, New York, 1985.

Chapters:
[16] Singleton et all., *Current Protocols in Food Analytical Chemistry* I1.1.1-I1.1.8, by Andrew L. Waterhouse University of California, 2002.