Antioxidant Activity of Polyphenols from Sea Buckthorn Fruits *(Hippophae rhamnoides)*

CAMELIA PAPUC*, CRISTIANA DIACONESCU, VALENTIN NICORESCU, CARMEN CRIVINEANU
University of Agronomical Sciences and Veterinary Medicine, 59 Marasti Blvd., 011466, Bucharest, Romania

Lipid oxidation in foods can be retarded by the addition of antioxidants. Certain plants rich in compounds with antioxidant activity manifest an increasing interest in food industry because they retard oxidative degradation of lipids. Sea buckthorn (Hippophae rhamnoides), alcoholic extract was investigated for antioxidant activity. Sea buckthorn fruits were extracted with ethanol using a Soxhlet extractor and the crude extract was analyzed in order to establish total soluble phenolics and the antioxidant activity. The alcoholic extract of Sea buckthorn was found to contain phenols with antioxidant activity. The extract was studied for 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), superoxide anion and hydroxyl radical scavenging activity. The extract was also studied for lipid peroxidation assay by thiobarbituric acid-reactive substances (TBARS) method. The results indicated that Sea buckthorn annihilates free DPPH radicals, the superoxide anion and the hydroxyl radical and it has an inhibitory effect upon lipid peroxidation process.

Keywords: sea buckthorn, DPPH radical, superoxide anion, hydroxyl radical, lipid peroxidation

The reaction of molecular oxygen with organic molecules is a process of considerable interest. The organic molecules with high susceptibility for oxygen attack are polyunsaturated fatty acids and acyl-lipids containing polyunsaturated fatty acids. In foods, lipid oxidation is the major chemical factor responsible for the loss of wholesomeness by deterioration of flavor and aroma, as well as the decay of nutritional qualities and food safety. Thus, the new volatile odorous compounds, hydroxy acids and the condensation between oxidation products and proteins are responsible for aroma changes, flavor and texture modifications.

The mechanism of autoxidation of lipids is a free radical reaction which involves three stages: initiation, propagation and termination [1].

Initiation $RH + ? \rightarrow R^{\cdot}$

$R - CH = CH - R + O_2 \rightarrow 2ROOH$

Propagation $R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$

$ROO^{\cdot} + RH \rightarrow ROOH + R^{\cdot}$

$ROOH \rightarrow RO^{\cdot} + OH^{\cdot}$

Termination $R^{\cdot} + R^{\cdot} \rightarrow$ Nonradical products

$2ROO^{\cdot} \rightarrow R - CO - R + R - CHOH - R + O_2$

The generation of the primary radicals is facilitated by the presence of transition metals, oxidants, various homolysis-prone substances or enzymes.

The oxidation of unsaturated fatty acids in foods can be retarded by the addition of antioxidants. Synthetic antioxidants such propyl, octyl and dodecyl gallate, 2,6-di-tert-butyl-p-hydroxytoluene (BHT) and tert-butyl-hydroxyanisole (BHA) are suspected like possible carcinogenic to humans, and from this reason the attention of the researchers is guided to natural antioxidants. Certain plants contain phenolic compounds (anthocyaninis, flavanols, flavonols, isoflavones, flavan-3-ols, proanthocyanidis, hydroxybenzoic acids, hidroxycinamics acids, stilbenes, gallotanninis) with antioxidant activity. Antioxidant activity of polyphenols from plants is based on the radical-scavenging capacity and on the capacity to chelate transition metals ions.

Sea buckthorn *(Hippophae rhamnoides)* is a fruit bush from the spontaneous flora of Europe and Asia. Even from ancient times, sea buckthorn fruits were used for therapeutic purposes. The sea buckthorn fruits are rich in vitamins A, C and E, bioflavonoids, lycopene, organic acids, kaemferol, triglycerides, glycerophospholipids, phytosterols, and minerals [2- 7]. In this study, our goal is to obtain an alcoholic extract of sea buckthorn, to emphasize the total soluble phenolics, as well as to determine the radical-scavenging activity and the antioxidant character of the extract against lipids from rat brain.

Experimental part

Obtaining vegetal extracts. In order to obtain vegetal extracts, the dry sea buckthorn fruits (10g) were ground and then subdued to a solid-liquid extraction in a Soxhlet installation with ethanol 60%. The alcoholic extract obtained was evaporated at rotavapor and the obtained residue was dissolved in 20 mL of ethanol. In order to determine the results, the following dilutions of alcoholic : ethanol were used: 1:9, 2:8, 3:7, 4:6 and 5:5 (v:v).

* *email: cami_papuc@yahoo.com*.

Determination of total phenolics

The total phenolics content was determined by mixing 10 µL extract with 2.5 mL 10% Folin Ciocalteu reagent (v/v) and 2 mlL of 7.5% sodium carbonate solution. The reaction mixture was incubated at 45^{0}C for 40 min, and the absorbance was measured at 765 nm using an UV-VIS spectrophotometer. Tanninic acid was used as standard phenol [8].

The annihilation of DPPH radicals

The ability of the alcoholic extract to annihilate the DPPH radicals (1,1-diphenil-2-picrylhydrazyl) was investigated by the method described by Lie-Fen Shyur [9]. Equal volumes of diluted extract were mixed with an equal volume of DPPH 6x10^{-3} M in absolute ethanol, and the obtained mixtures were kept at room temperature for 30 min. Then, the absorption of the mixtures at 520 nm was determined, in comparison with the control solution (maxim absorption). The annihilation activity of free radicals was calculated in % inhibition according to the following relation:

% Inhibition = (A control – A test) . 100/A control

In order to achieve the control test, the same operations were made, with the single exception that instead of the extracts, the solvent (ethylic alcohol) was used.

The annihilation of superoxide anions

The superoxide anions generated by the phenazin methosulfate (PMN)/nicotinamid-adenin-dinucleotid-phosphat, reduced form (NADPH) system, were detected by the reaction with chloride of 2,2'-di-p-nitrophenyl)-5,5'-diphenyl-(3,3'-dimethoxy-4,4'-diphenylene) ditetrazolium chloride (nitro blue tetrazolium – NBT) [10]. The mixture of reaction contained: 2.7 mL PBS 50 mM *p*H 7.8, 50 µL Na_2EDTA 0.1 M, 100µL NBT 0.6 M, 50 µL phenasin metosulphat 1mM, 50 µL NADPH 0.5 mM and 50 µL alcoholic plant extract.

At the same time, control tests were prepared. The results were expressed in % inhibition.

The annihilation of hydroxyl radicals

For the investigation of the annihilation process of hydroxyl radicals, the modified method described by Aruoma and col. [11] was used. Diluted extract was added to a reaction mixture containing 120µL 20 mM deoxyribose, 400µL 0.1M phosphate buffer *p*H 7.4, 40µL 20 mM hydrogen peroxide and 40µL 50µM $FeCl_3$ premixed with 100µM EDTA before addition to the reaction mixture and 100µL distilled water. Ascorbate (100µM) was added to start the reaction. The tubes were incubated for one hour at 37^{o}C. After incubation, the reaction was stoped by the addition of 0.5mL 2.8% trichloroacetic acid and 0,4 mL of 1% thiobarbituric acid. Then, the tests were maintained on water bath at 100^{o}C for 20 min and then the extinction was read at 532 nm. The obtained results were expressed in % inhibition.

The inhibition of the lipid peroxidation process

The effect of different concentrations of alcoholic extract of sea buckthorn on lipid peroxidation process inducted by the Fe^{3+}/ascorbic acid system on the phospholipids from rat brain homogenate was determined through the thiobarbituric-acid reactive substances (TBARS) method [12]. This method is based on the reaction of the TBA with the malondialdehyde (MDA), the secondary product of the lipid peroxidation process. Rat brain homogenate 25% (w/v) was used. At 200 µL of suspension there were added 500 µl phosphates buffer *p*H 7.4, 50 µL alcoholic extracts, 100 µL solution $FeCl_3$ 1mM and 100 µL ascorbate 1mM. After incubating the mixtures at 37^{o}C, for 60 min, the following were added: 50 µL butylated hydroxytoluen (BHT) 2%, 1 mL trichloracetic acid (ATA) 2.8% and 1 ml thiobarbituric acid (TBA) 1%. The mixtures that resulted from this process were maintained for 20 min at 80^{o}C. The resulting colored complex was extracted in buthanol and then, the extinction was read at 535 nm.

The inhibition of peroxides formation was expressed in % inhibition.

Results and discussions

Determination of total phenolics

The results revealed that *Hippophae rhamnoides* alcoholic extract contains 24.84 ± 0.06 mg/100 mL tanninic acid equivalent.

The annihilation of free DPPH radicals. DPPH radicals react with suitable reducing agents losing color stoichometrically with the number of electrons consumed which is measured spectrophotometrically at 517 nm. The obtained results for the five dilutions are shown in figure 1. The annihilation of DPPH radicals, expressed in % inhibition, was 11.9 % for the smallest dilution and 94.7 % for the largest dilution. For the other dilutions, intermediary values were obtained.

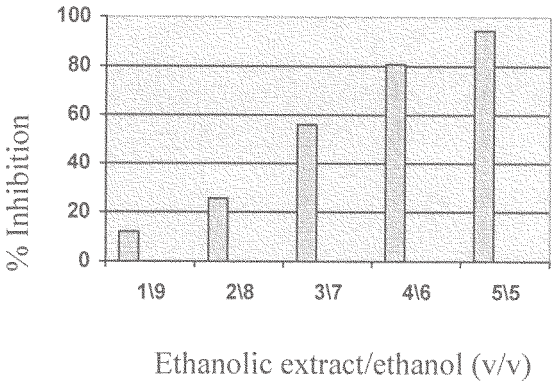

Fig. 1. Annihilation of the free DPPH radicals by the alcoholic extract of sea buckthorn

The annihilation of superoxide anions

The obtained results have emphasized the capacity of alcoholic sea buckthorn extract to annihilate the superoxide anions generated in PMS-NADPH-NBT system (fig. 2). The capacity of annihilating superoxide anions depended on the dilution relation of the extract, for the great values of the concentration in the extract being registered the reduction of capacity of alcoholic extract to annihilation superoxide anions. These results were probably obtained due to the presence of riboflavin in the alcoholic extract.

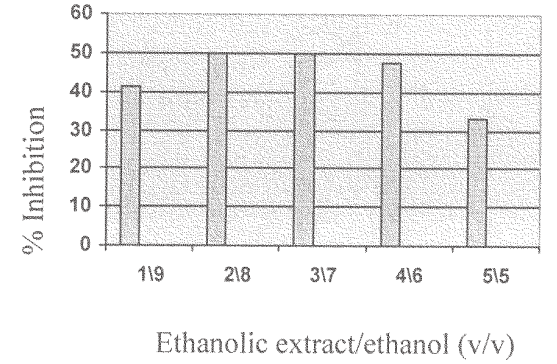

Fig. 2. Annihilation of the superoxide anions by the alcoholic extract of sea buckthorn

The annihilation of the hydroxyl radicals

The deoxyribose method evaluates the ability of hydroxyl radicals to damages the carbohydrates. In our researches, highly reactive hydroxyl radicals ($HO^{\cdot}$) were generated by a mixture of ascorbate and $FeCl_3$-EDTA at *p*H 7.4. Figure 3 shows the results of the deoxyribose damage by hydroxyl radicals, in the presence of the 5 dilutions taken in work. The annihilation of hydroxyl radicals presented a variation depending on dilution relation. The highest value was obtained by the dilution relation of 1: 9.

The inhibition of lipid peroxidation

The alcoholic extract of Sea buckthorn has shown a high degree of inhibition of the lipid peroxidation process of phospholipids found in rat brain. From the data presented in figure 4, it can be noticed that the diluted alcoholic extract 1:1 offers protection against peroxidation in proportion of 48.3 ± 0.02%.

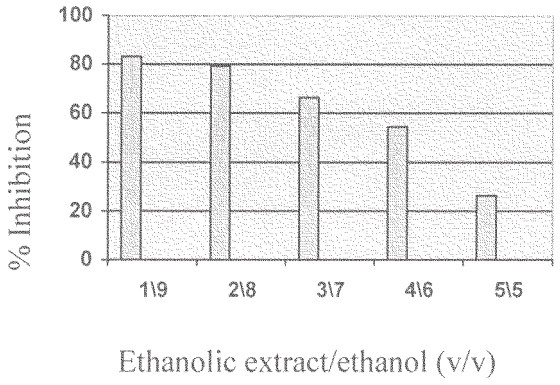

Fig. 3. Annihilation of the hydroxyl radicals by the alcoholic extract of sea buckthorn

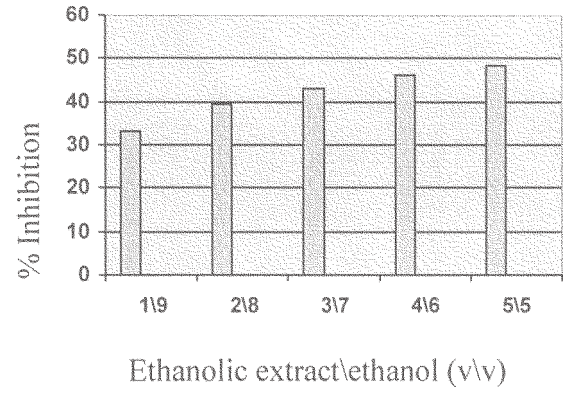

Fig. 4. The inhibitor effect of the alcoholic extract of sea buckthorn on the lipid peroxidation process from brain homogenate

The obtained results demonstrate that the alcoholic extract of sea buckthorn contains polyphenolic compounds which confer antioxidant properties to the extract. The alcoholic extract of sea buckthorn annihilates the free DPPH radicals, superoxide anions and hydroxyl radicals. This capacity of annihilating free radicals determines the ability of the sea buckthorn alcoholic extract to inhibit the lipid peroxidation process (oxidative deterioration of polyunsaturated fatty acids).

Conclusions

The alcoholic extract of sea buckthorn contains polyphenols with antioxidant activity.

The alcoholic extract annihilated DPPH radicals, superoxide anion and hydroxide radical.

The alcoholic extract significantly reduced the malondialdehyde content, which is a measure of lipid peroxidation and shows the antioxidant activity.

The polyphenols extracted from sea buckthorn have potential to be used as natural antioxidants in food industry.

Acknowledgements: The researches have been performed according to the researching Contract PN II _ ID / 256.

Bibliography

1.ERIKSON C. E., 1982, Food Chemistry, **9**, p.3
2.ROSH D., BERGMANN M., KNORR D., KROH L.W., 2003, J. of Agric. And Food Chemistry, **51**, p. 4233
3.GAO X., OHLANDER M., JEPPSSON N., BJORK L., TRAJKOVSKI V., 2000, J. of Agric. and Food Chemistry, **48**, p. 1485.
4.JEPPSSON N., GAO X.Q., 2000, Agric. Food. Finland, **9**, p. 17
5.ZADERNOWSKI R., NACZK H., NOWAK-POLAKOWSKA, 2002, Journal of Food Lipids, **9**, nr. 4, p. 249
6.NEGI P.S., CHAUHAN A.S., SADIA G.A., ROHINISHREE Y.S., RAMTEKE R.S., Food Chemistry, **92**, nr. 1, p.119
7.JINLING F., XIAOLIN D., WENZING G., 2007, Food Chemistry, **102**, nr.1, p. 168
8.SINGLENTON V.L., ORTHOFER R., LAMUELA-RAVENTOS R.M., 1999, Method Enzym. **299**, p.152
9.SHYUR L.F., TSUNG, J.H., CHEN J. H., CHIU Y., CHIU C. Y., CHIU P. L., 2005, Int. J. Appl. Sci. Eng., **3**, p.195
10.WANG., S. Y., KUO Y. H., CHANG H. N., TSAY H. S., LIN K. F., YANG S. H., SHYUR L. F., 2002, J. Agric. Food Chem., **50,** p. 1859
11. ARUOMA O.I., A. MURCIA, J. BUTLER, B., HALLIWELL, 1993, J. Agric. Food chem. **41**, P. 1880
12.ARUOMA O. I., SPENCER J. P. E., ROSSI R., HALLIWELL B., 1993, Food Chem. Toxic. **34**, p. 449

Manuscript received: 12.11.2007