ANTIOXIDANT ACTIVITY OF CAROTENOIDS EXTRACTS FROM *HIPPOPHAE RHAMNOIDES*

Stanila Andreea, S. Stanila
University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, No.3-5 Manastur St., ,400372, Romania; email: andreea7s@yahoo.com

Abstract. *This work aimed to verify carotenoids antioxidant activity in raw sunflower oil, oxidized with Cu^{2+} ion. In order to evaluate this activity it was used standards of β-carotene, luthein and total carotenoids extract from Hippophae rhamnoides (seabuckthorn). Sunflower oil was treated with $CuSO_4$ solution 100mM necessary to induce peroxidation and antioxidant activity of carotenoids was evaluated by decreasing of their amount, measured at 445nm after 1h and 50^0C heating, 24h and 50^0C, 48h and 50^0C . The best antioxidant activity had luthein at higher concentration. Comparing with luthein, seabuckthorn extract has similar antioxidant activity with this standard. Peroxidation level was established by MDA-TBA test, using spectrofluorimetric method.*

Keywords: carotenoids, antioxidant activity, oil peroxidation.

INTRODUCTION

The paradox of life is that oxygen, which is vital for aerobic organisms, can be dangerous for their existence. The danger of these molecules is derived from the presence of unpaired electrons on their outer valence shell (Passoto *et al.,* 1995).

Chemically, they are classified in carotens, which have only hydrogen and carbons in molecule and oxicarotenoids with oxygen in molecule(Cambell *et al.*, 2000). Carotenoids have a widely varieties of functions: pro-vitamin A activity, in photosynthesis, protection of cells against the effects of light, antioxidants by reacting with active oxygen species (Quaglia *et al.,* 1998, Britton *et al.,* 1995).

The study of the antioxidant effect of carotenoids showed that these pigments delayed the formation of hydroperoxides. Carotenoids suppressing properties reside not only in the length of the conjugated dienes, but are also dependent on the functional groups (Eriksson, 1995). The carotenoids have quenching and scavenging effects on oxygen species, such as singlet oxygen radicals and hydroxyl radicals (Takashi *et al.,*1997). In order to reduce oil peroxidation, and subsequent stability increase, several researchers studied the influence of carotenoids on photooxidation of comestible oils (Berset *et al.,* 1996).

Malondialdehyde occurs in biological materials in the free state and in various covalently bound forms. The most widely employed method for the determination of MDA in biological materials is based on the reaction with TBA (thiobarbituric acid) to form a pink complex with an absorption maximum at 532-535nm (Gutteridge *et al.,*1990; Halliwell *et al.,* 1986).

This work aimed to verify carotenoid resistance in oxidative means, its antioxidant activity in activated sunflower oil and the level of malondialdehyde results as peroxidation of fatty acids from oil.

MATERIAL AND METHOD

The oil used for this experiment was fresh raw sunflower oil, solution of $CuSO_4$ 100mM, standard of β-carotene, luthein and natural extract from *Hippophae rhamnoides* at different concentrations. Analytical grade solvents and reagents were used.

The experimental procedure was conducted without artificial light or direct sunlight exposure to avoid oxidation. In order to get extract of carotenoids, fruits of *Hippophae rhamnoides* were cut and homogeneized with acetone, then filtered and transferred to ethyl acetate for extraction. Sunflower oil was treated with solution of $CuSO_4$ 100mM,1,5% in oil, which is the minimum concentration determinated to induce lipid peroxidation(PASSOTO et al.1992).

Concetration of carotenoids standards used were 100µM, 200µM and 1000 µM in ethyl acetate. Total carotenoids extract was quantified by UV-Vis spectrometry with a Jasco V-530 device. Quantification of pigment was carried out from maximum absorbance
obtained by means of visible absorption spectra, according with the next formulae (BRITTON et.al.,1995): µg carotenoid / ml = DO_{445nm} x dilution / 250

For the experiments were used two concentrations of total carotenoids calculated from the maximum absorbance at 445nm: 0,007mg total carotenoid /ml and 0,014mg total carotenoid/ml.

Oil samples (10ml) was treated with solution of copper ion at the concentration mentionated above and stirred for a few minute. Then, the samples were kept for one hour and 50^0C, 24h and 50^0C and 48h and 50^0C in the oven. Oxidation was monitored by fluorimetry, by means of the MDA-TBA test, to establish the amount of malonaldehyde resulted from lipid peroxidation. Fluorimetric method was made at λ_{ex}=515nm and λ_{em}=550nm, integration time=5sec and slits=2,5nm.

In the same conditions, oil samples were treated with carotenoids standards and *Hippophae rhamnoides* extract at concentrations presented above and heated for 1h, 24h, 48h and 50^0C. Then, peroxidation level were determined also by MDA-TBA test and compared with carotenoid-free samples. Sunflower oil was oxidized with metal ion solution of $CuSO_4$ 100mM and then the samples was treated with antioxidants: β-caroten, natural extracts of Hippophae rhamnoides).

After the addition of metal solutions and antioxidants, the samples was sonicated and mentained for one hour in the presence of oxygen at room temperature.

RESULTS AND DISCUSSION

It was verified that carotenoids presented differentiated degradation in this system, according with their chemical structure and different concentrations. Luthein, which is an oxycarotenoid, is much susceptible to oxidation , respectively to degradation, so has a better antioxidant activity(TAKASHI et al.1997) The reason

for which it was used *Hippophae rhamnoides* is that it's very rich in luthein and carotene, so the antioxidant activity of the extract could be compared with standards.

The degradation percent of each carotenoid standard and total carotenoid extract were calculated in relation to their control, which was considered the carotenoid-free sample.

Table 1

Percentages of decomposed carotenoids in oil oxidased with ion copper

No.	Samples	% after 1h 50^{0}C	% after 24h 50^{0}C	% after 48h 50^{0}C
1.	Oil+ β-carotene100µM	8.2	71.71	74.95
2.	Oil+ β-carotene200µM	3.52	**73.69**	**81.33**
3.	Oil+ β-carotene1000µM	39.93	61.42	71.11
4.	Oil+ luthein 100µM	0.5	51.27	49.93
5.	Oil+ luthein 200µM	30.12	49.6	57.37
6.	Oil+ luthein 1000µM	**43.20**	45.57	79.93
7.	Oil+ hip. 0.007mg carot.	6.7	40.5	57.7
8.	Oil+ hip. 0.014mg carot	19.6	63.1	74.5

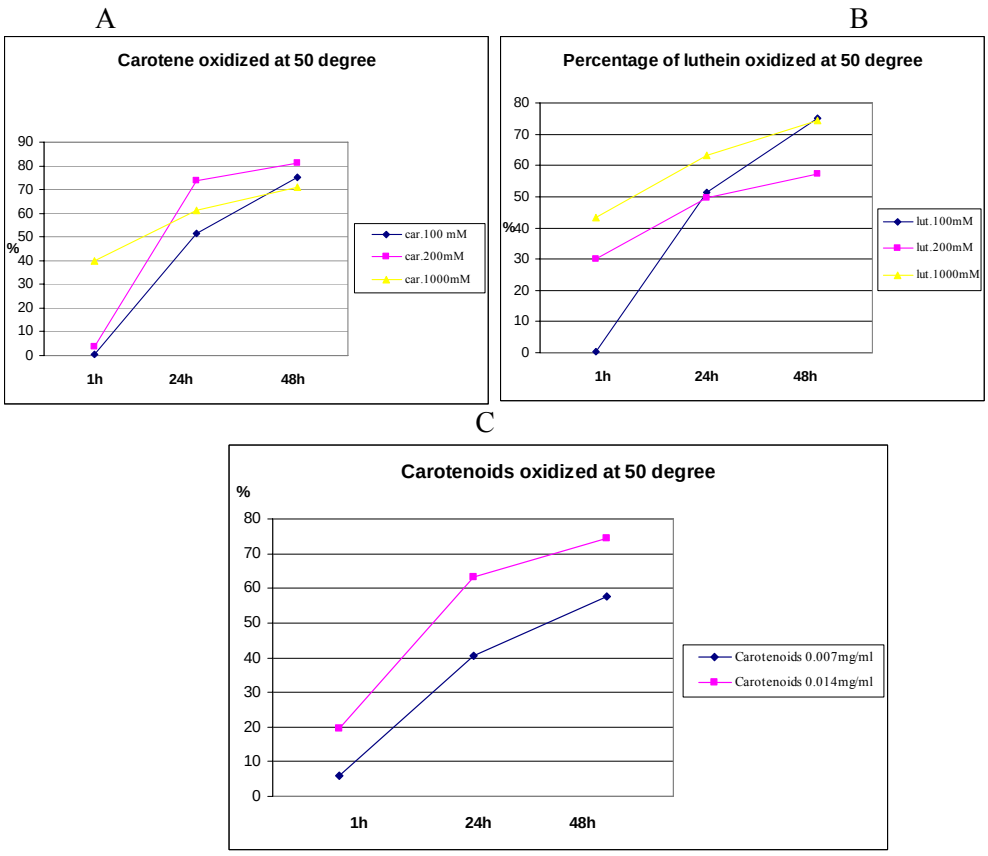


Fig.1. Percentage of β-carotene(A), luthein(B), carotenoids from seabuckthorn(C) decomposed

Several authors (Sunderman, 1986; Gutteridge *et al.,* 1990) have demonstrated that carotenoids inhibit the formation of singlet oxygen, essential to the development of oxidative reactions in photo-oxidation processes.

Results obtained by this analysis suggest that there is an interaction between these compounds with active radicals rapidly after their addition into the system. According with literature data, this allow us to suggest the existence of a mechanism by means of which carotenoids react directly to the peroxide radicals, this reactivity being faster than two peroxide radicals between themselves; this way the mechanism of the antioxidant action of the carotenoids may be explained by the better affinity of the free radicals for carotenoids, finishing the reaction of propagation (Halliwell *et al.*, 1986). It is probable that active radicals react in strategical points of the molecule of the carotenoids, especially at the end of the cromophore system, where there is a greater electronic density existence.

Associating the molecular structure of carotenoids to the antioxidant activity, it can be concluded that β-caroten is the most susceptible compound to oxidation and with the higher antioxidant activity especially after 24h and 48h of treatment.

By these means, concentrations of β-carotene 100 µM, 200µM and 1000 µM after 1 hour of treatment with copper ion decrease with 8,2%, 3,5% and 39,9%; after 24 hours the decreasing were 71,7%, 73,6% and 61,4%; after 48 hours the percentage were 74,95%, 81,3% and 71,1%.

Concentrations of luthein 100 µM, 200µM and 1000 µM after 1 hour of treatment with copper ion decrease with 0,5%, 30,1% and 43,2%; after 24 hours the decreasing were 51,3%, 59,6% and 45,5%; after 48 hours the percentage were 49,9%, 57,3% and 79,9%.

Concentrations of total amount of carotenoids extracted from seabuckthorn were 6,7% and 19,6% after 1 hour for the first (0,007mg car./ml), respectively second (0,014 mg car./ml) carotenoids concentrations used; after 24 hours the percents of carotenoids degradation were 40,5% and 63,1% ; after 48 hour the decreasing were 57,7%, respectively 74,5%. As it can be seen by the above results, after one hour the best antioxidant activity had luthein at higher concentration . Comparative with luthein , β-carotene had good effect at higher concentration with almost 40% degradation rate. After 24 hours of treatment very good results had carotene and carotenoids from seabuckthorn at higher concentration with a degradation percent between 60% and 73%. After 48h the best results had again β-caroten at all concentrations and also luthein and carotenoids from seabuckthorn at higher amounts.

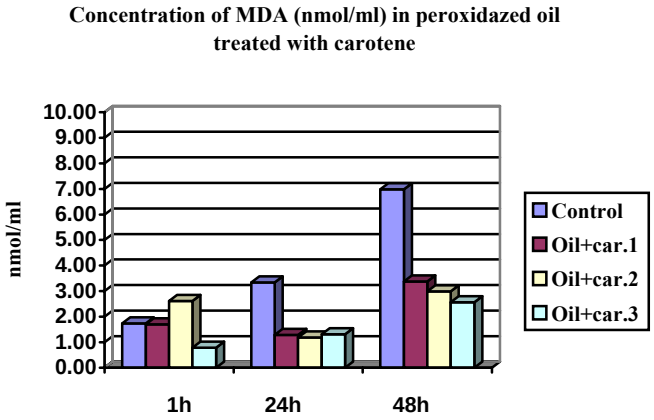
In table 2 it is presented the amounts of malondialdehyde resulted by peroxidation of fatty acids from oil before and after addition of antioxidants: β-carotene 100 µM, 200µM and 1000 µM, luthein 100 µM, 200µM and 1000 µM, and carotenoids from seabuckthorn 0.007mg/ml and 0.014mg/ml.

Table 2

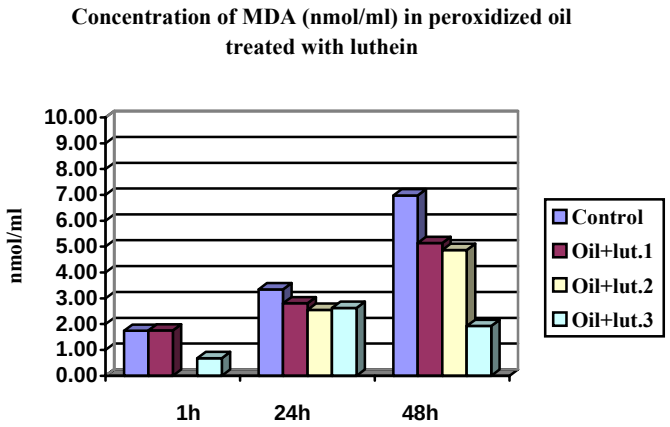
Amount of MDA resulted from oil peroxidation (nmol/ml)

No.	Samples	After 1h 50^{0}C	After 24h 50^{0}C	After 48h 50^{0}C
1.	Control (oil+ $CuSO_4$ sol.)	1.740	3.347	6.984
2.	Oil+ β-carotene100µM	1.705	1.295	3.383
3.	Oil+ β-carotene200µM	2.613	1.187	2.995
4.	Oil+ β-carotene1000µM	0.793	1.314	2.563
5.	Oil+ luthein 100µM	1.752	2.803	5.137
6.	Oil+ luthein 200µM	0.862	2.547	4.863
7.	Oil+ luthein 1000µM	0.685	2.620	**1.926**
8.	Oil+ hip. 0.007mg carot.	**0.100**	2.198	4.074
9.	Oil+ hip. 0.014mg carot	**0.414**	**1.677**	3.012

A



B



C

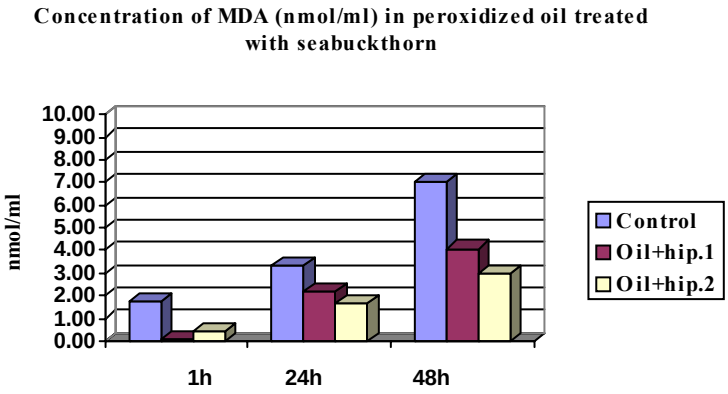


Fig. 2. Concentration of MDA in peroxidized oil before and after treatment with carotenoids: A – oil treatment with β-carotene 100 µM, 200µM and 1000 µM; B – oil treatment with luthein 100 µM, 200µM and 1000 µM; C – oil treatment with carotenoids from seabuckthorn 0.007mg/ml and 0.014mg/ml.

As it could be saw from the above data the best antioxidant activity after one hour of treatment have carotenoids from seabuckthorn at both concentrations; after 24 and 48 hours the lowest amounts of MDA were obtained after treatment with luthein and β-carotene at higher concentration. These experiments lead to the conclusion that all types of carotenoids used decrease the amount of MDA; carotenoids from seabuckthorn have good antioxidant activity after 1h and 24h of treatment which is important because this is a natural product with benefits for human health.

CONCLUSIONS

The results of these experiments lead to the conclusion that chemical groups of carotenoids have great influence in the prevention of lipidic peroxidation. The hydroxyl group of luthein contributed to the increasing of antioxidant activity, especially at the higher concentrations used in this work. Carotenoids extract from *Hippophae rhamnoides* showed similar antioxidant activity as luthein, which could be a basis for the next experiments, convenient from the economical and human health points of view (carotenoid standards are expensive and natural extract could have benefic effect on human organism).

Acknowledgements. This work was funded by the National Research Grant IDEI no.1114/2009 financed by CNCSIS – Romania.

REFERENCES

1. Berset, C. and M.-E. Cuvellier M-E. (1996). Methodes d'evaluation du degree d'oxydation de lipids et de mesure du pouvoir antioxidant, Sci.Aliments 16(3), 220-41.

2. Britton, G., S. Liaaen-Jensen and H. Pfander (1995). Carotenoids, vol.1B, 19-30.

3. Campbell, P., and A. Smith (2000). Biochemistry Illustrated, Churchill Livingstone, Elsevier Science.

4. Eriksson, C.H. (1995). Antioxidant agents in raw materials and processed foods; Free radicals and oxidative stress; Halliwell L.G.G.(Editors*)*, 221-68.

5. Gutteridge J. and B. Halliwell (1990). The measurement and mechanism of lipid peroxidation in biological system, TIBS 15, 129-34.

6. Halliwell, B. and J. Gutteridge (1986). Role of free radicals and catalytic metal ions in human diseases, Methods in Enzymology, vol.186, 2-21.

7. Palozza, P. and S. Moualla (1992). Effects of β-carotene on radical initiated peroxidation, Free Rad.Biology and Med., 13, 127-36.

8. Passoto, J.A., M.D. Penteado and J. Mancini-Filho (1995). Oxidative resistance and antioxidative activity of some carotenoids in food, Bull.Chim.Farmaceutico-Anno 134-n.6, 306-11.

9. Sunderman, F.W. (1986). Metals and lipid peroxidation; Acta Pharmacol. Toxicol., 59.

10. Quaglia, G., F. Paoletti, E. Finotti, C. Beye and A. Raffo (1998). Antioxidant role of minor compounds in vegetables with particular attention to oil, Recent Res. Devel. In Nutrition, 2.

11. Takashi, M. and S. Takayuki (1997). Antioxidative activities of natural compounds found in plants, J. Agric. Food Chem., 45, 1819-22.