VEGETABLE OILS: DO THEY HAVE ANTIOXIDANT ACTIVITY?

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Abstract

The main aim of this paper is to start the discussion about the potential antioxidant activities of oil extracts and oils from various vegetable raw materials. Many publications reported that the seed oils and essential oils possess the high antioxidant activities. In our opinion it is not so. We want to share the results of own investigations and our view at this topic. We tested: a) oils extracts from seeds of apricot, grape, peach, pumpkin, fenugreek, white bryony, sea buckthorn and rosehip; b) walnut oils (eight samples of oil); c) essential oils from needles of juniper and pine. Antioxidant activities of oils and oil extracts were appreciated by two spectrophotometric methods: method based on model oxidation reaction of N,-diphenyl-n-phenylenediamine by azo-bis-(isobutyronitrile) as the initiator of free radicals; and procedure of DPPH free radicals scavenging. Trolox was used as standard antioxidant. Tested oil extracts from seeds did not show antioxidant capacities; on the contrary, they took part in reaction enhancing of free radical oxidation. Walnut oils demonstrated a neutral effect against free radicals. They did not enhance and did not reduce oxidative processes. Thus, the antioxidant capacities of walnut oils also were not identified. Freshly obtained essential oils from needles of juniper and pine possessed the antioxidant activity, which decreased by 2-8 times during one mount of storage. The oil extracts, vegetable and essential oils have others nutritional values and should not be considered as antioxidant. Moreover, to extend shelf-life they need to be fortified by antioxidants.

Keywords: *oil extract, vegetable, essential oil, antioxidant activity*

Introduction

Vegetable oils are valuable sources of high-calorie fats and essential fatty acids, phospholipids, carotenoids and other physiologically active components. Many publications reported that the seed oils and essential oils possess the high antioxidant activities (Poiana et al., 2009; Xie et al., 2015; Jorge et al., 2016; Akbari et al., 2018; Shinagawa et al., 2018). Taking into account the beneficial effects of antioxidants on human health, many researchers and manufacturers endeavour to find the high antioxidant activity of vegetable oils. In this regard, in 2007, the Armenian company Hagenas LLC asked us to study the antioxidant properties of oil extracts from various seeds, which were intended for sport nutrition. The results of these investigations were not so positive as to be shared in a wide public publications or commercial promotion. That is why we can write about it just now. Subsequent testing of other vegetable oils allowed us to confirm that the quality of the vegetable oils should not be focused on its antioxidant activity. Some authors, describing the qualitative parameters of cold-pressed oils from vegetable seeds, studied the antioxidant activity of methanolic extracts from the corresponding seeds, but no these oils (Kulaitiene et al., 2018), which is not the same thing. The purpose of this paper is to start the discussion about the potential antioxidant activities of oil extracts and oils from various vegetable raw materials based on our comparative researches.

Materials and methods

The oil extracts from seeds of apricot, grape, peach, pumpkin, fenugreek, white bryony, sea buckthorn and rosehip were produced by HAGENAS LLC (Yerevan, Armenia); and were stored during one year at 20±2ºC. In plus the samples of grape seed oils and beta-carotene oil solution were purchased from Moldovan markets.

The walnut oil samples were obtained by cold pressing using an electrical press (Model PSU-125) in laboratory of the Technical University of Moldova. Tatarov & Popovici (2014) described the general scheme of procedure for walnut oil extraction. The eight samples of walnut oil obtained in different times were tested.

The essential oils from needles of *Juniperus sabina* (juniper), *Pinus sylvestris* (scots pine) and *Pinus nigra* (black pine) were extracted by hidrodistillation using Ginsberg method. (Rassem et al., 2016) in laboratory of Institute of Genetics. Physiology and Plant Protection.

The chemicals: ethanol, N,-diphenyl-n-phenylenediamine, azo-bis-(isobutyronitrile), 2,2-diphenyl-1-picrylhydrazyl, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) of HPLC, spectrophotometric or analytical grade were supplied by Sigma-Aldrich.

Spectrophotometric methods for antioxidant activity determination of oils and oil extracts were used. One of them, the method that was proposed for standardization of individual and complex herbal remedies, including hydrophobic ones (Dadali &Dadali, 2015). The essence of the method consists in applying a model oxidation reaction of N,-diphenyl-n-phenylenediamine (DPPD) dissolved in dimethylformamide (DFA). Azo-bis (isobutyronitrile) was used as the initiator of free radical oxidation of DPPD. The result of the oxidation reaction is the formation of N, N-diphenyl-n-benzoquinone-diimine (DPBQD), which has a stable chemical structure and could be quantified at 450 nm. The reaction was carried out at a temperature of 60°C for 4-5 hours. Other method was widely reported procedure that based on scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals (Clarke et al., 2013) with absorption at 515 nm. Trolox was used as standard antioxidant substance and antioxidant activity was expressed in trolox equivalent (TE).

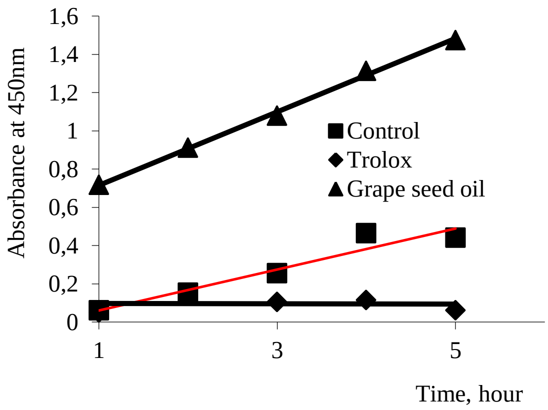
***The statistical analysis** of the results was carried out by EXCEL software. All assays were performed at least by triplicate.*

Results and discussion

During 4-5 hours of the reaction for oxidation of DPPD free radicals, the DPBQD formation and accumulation had the greatest approximation according the Beer-Lambert's law (R^2=0.951-0.998). The linear dependence of DPBQD absorbance on time, that very evident is shown in fig.1, allowed us to recalculate its content. The amount of accumulated DPBQD in reaction with tested samples was compared with amount of DPBQD accumulated in reaction without antioxidants (control). Thus, using the curves of absorbance the antioxidant activity of standard substance - trolox (fig.1a) and beta-carotene (fig. 1b) was calculated. The freshly prepared solution of trolox in concentration of 0.39 mg/ml inhibited 75–80% of free radicals, while the oil solution of beta-carotene in concentration of 0.01% scavenged only 5-10% of free radicals.

As shown in fig.1 the accumulation of DPBQD in reaction with Armenian oil extracts from grape (fig.1a) and fenugreek (fig.1b) seeds was proceeded at an increased rate. The tested oil extracts contributed to a greater accumulation of DPBQD or, in other words, they increased the number of free radicals involved in the reaction as an initiator of free radical oxidation. According our results, the oil extracts of fenugreek and grape seed enhanced of the reaction of free radical oxidation of DPPD to DPBQD by 3.6 and 2.4 times, respectively. Akbari with coauthors (2018) reported that the fenugreek seed oil possesses the high antioxidant activity. Index of antioxidant activity IC_{50} (concentration that inhibit 50% of free radicals) determined by DPPH radical scavenging procedure was equal to 172.6±3.1 μg/ml (Akbari et al., 2018).

This fact can be explained by excellent prevention of powdered seeds and extracted oil from air and light oxidation before determination of antioxidant activity. Moreover, the oil extraction was performed so that the polyphenolic content of oil (38.97±0.34 mgGAE/g) was analogic to water-ethanolic extracts of fenugreek seed (Melikyan et al., 2008). It is known (Kulaitiene et al., 2018) that antioxidant activity directly depends on the content of polyphenols in vegetable extracts, but it is necessary to indicate that these substances are soluble in water (no fat soluble).
Many authors (Shi et al., 2003; Ivanova & Casian, 2008; Ross et al., 2011) described the rich content of polyphenols in grape seeds. However, the property of grape seed oils to exhibit the antioxidant activity (from 53.41 to 205.52µM TE/100g) was saved through maintaining the samples at temperature -20°C into amber glass bottles and under nitrogen gas (Shinagawa et al., 2018). The highest values of antioxidant activity and polyphenols amount were obtained foe the fresh unrefined grape seed oil (Poiana et al., 2009). The pumpkin seed and orange seed oils after extraction were also stored under nitrogen gas at -18°C and oils with liquid nitrogen were analyzed (Jorge et al., 2016; Kulaitiene et al., 2018), which is not quite suitable for public markets.



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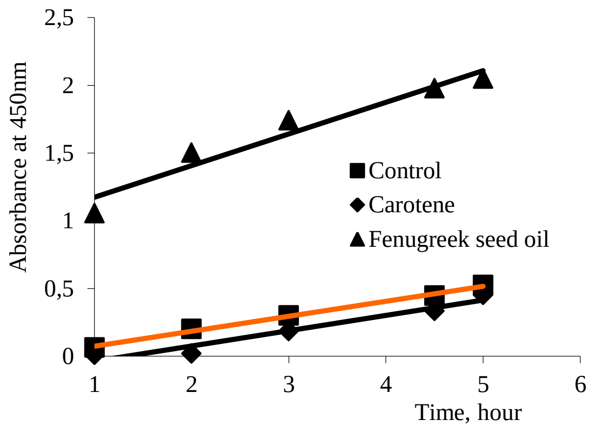

b

Figure 1. Dependences of DPBQD absorbance on time in reactions without antioxidant (control), with antioxidant trolox (a) and in presence of oil extracts: carotene (b), Armenian grape (a) and fenugreek seeds (b).

The other tested by us oil extracts from seeds also exhibited no antioxidant properties. Taking into account their capacities to increase the reaction of free radical oxidation (an increase by calculated % compared to control) the oil extracts from seeds can be represented by the following sequence: fenugreek (363%) > grape (Armenia) (238%) > white bryony (229%) >

sea buckthorn (138%) > rosehip (136%) > pumpkin (97%) > peach (65%) > grape (Moldova) (39%) > apricot (25%). Thus, the studied oil extracts from various seeds took part in the reaction and contributed to the enhancement of free radical oxidation of the reaction components.

The presence of walnut oils (it were tested eight samples of oil) in the reaction mixture did not significantly increase or decrease the oxidation processes (fig.2). Three samples showed the radical scavenging activity equal to 7.14-13.39%, two samples increased the content of oxidation products by 14.29-16.07%, and three samples practically did not differ from the control (0.89-1.79%). This fact indicated that the presence of walnut oil does not interfere with the normal course of the reaction. Since the values of radical scavenging activities, constituting less than 20% in this method are not statistically significant, it can be argued that walnut oils do not exhibit antioxidant activity. Therefore, it was showed, that these oils were unstable during the storage (Tatarov et al., 2017), and to increase their shelf life they needed to be fortified with some antioxidant ingredients.

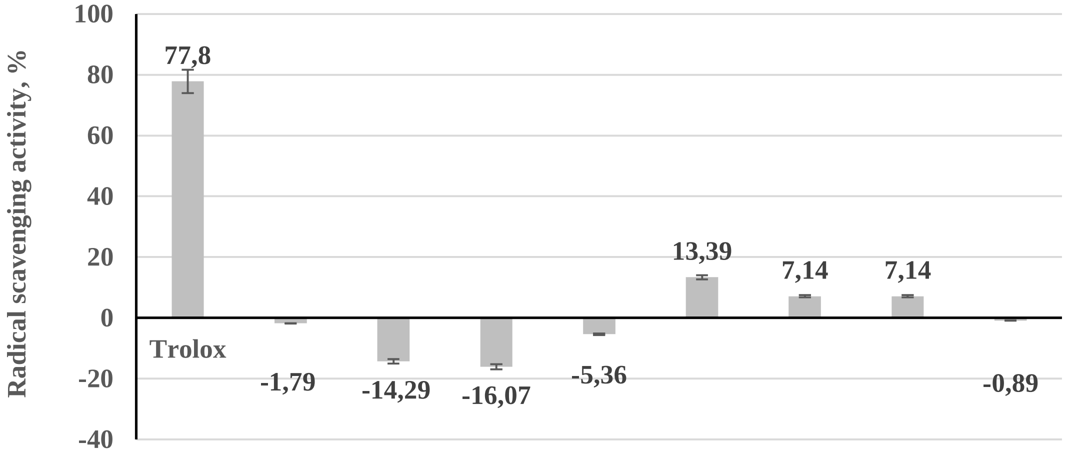

Figure 2. Radical scavenging activity (%) of trolox and various samples of walnut oil (total 8) determined by DPBQD method

It necessary to mention, that the main components of chemical composition of vegetable oils are saturated and unsaturated fatty acids, which easily are exposed to oxygen oxidation and according their chemical structures could not act as antioxidant. Protection against oxidation of oils is achieved by the action of antioxidants both natural (γ-tocopherol, β-carotene, lycopene) and synthetic (butylated hydroxytoluene), which are soluble in lipids. Influences of γ-tocopherol content on resistance during storage of vegetable oils were reported (Guseva et al., 2010; Radvzievska & Melnik, 2016). Moreover, the dependences of antioxidant activity values on total content of antioxidants (γ-tocopherol and carotenoids) are the extreme character and can be described by the function of the standard normal distribution. Because of that, the maximal antioxidant activity demonstrates only in certain concentration intervals of antioxidant substances contained in vegetable oils (Lobaeva et al., 2004).

Index of antioxidant activity IC_{50} for trolox determined by DPPH method was equal 140.02±0.24μg/ml. The essential oils from needles of juniper, scots and black pine collected in different areas of Slovak Republic (SR) and Republic of Moldova (RM) possessed the antioxidant activity. The antioxidant activities of tested essential oils expressed in trolox equivalent (mgTE/g) were equal to: 1.79±0.08 for *Juniperus sabina* (SR), 1.57±0.07 for *Juniperus sabina* (RM), 1.20±0.03 for *Pinus sylvestris* (RM); 1.63±0.02 for *Pinus nigra* (RM).

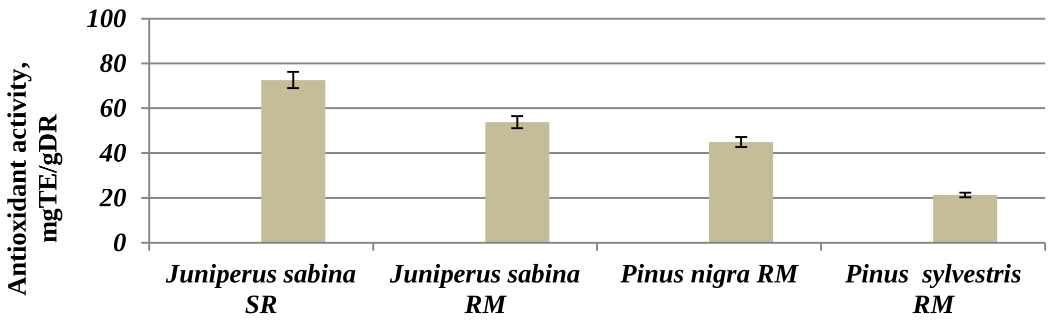

Figure 3. Antioxidant activity in trolox equivalent of water-ethanolic extracts from needles of juniper and pine determined by DPPH method

The essential oils from needles of six China endemic Pinus taxa exhibited antioxidant activity, which authors name as "acceptable" (Xie et al., 2015). However, they reported that antioxidant activities of pine oils were lower than some popular plant species such as Camellia sinensis and Oenocarpus bacaba. Low antioxidant activity of essential oils from different parts of Juniperus spp. was been explicated by specific constituents of the oil, which can be inactive in DPPH test, and the trace amounts of compounds that act as antioxidants (Emami et al., 2010). Samusenko A.L. (2011) concluded that the antioxidant activity of essential oils in a complex way depends on oils constituents, concentration and ratio between the most active components.

In our experiments, the trolox equivalents of essential oils were 17-40 times less than analogical indexes of water-ethanolic extracts from the same vegetal materials (fig. 3). It should be to mention that the storage of essential oils during one mount led to decreasing 2-8 times of their antioxidant activity. Due to the high content of no antioxidant biologically active constituents (for example α – pinene), the juniper essential oil, as natural product, has a perspective in ecological agriculture as insecticide against harmful insect species ((Elisovetcaia & Brindza, 2018) in medicine as a potential anti-tumor drug, in food and cosmetic industries as donor of aroma and odour (Salamon & Petruska, 2017).

Conclusion

The high nutritional and biological value of vegetable oil extracts and essential oils is due to the content of biologically active components, such as polyunsaturated essential fatty acids, terpenes etc., which do not act as antioxidants. Some vegetable oil extracts contain fat-soluble antioxidant compounds (tocopherols, carotenoids) which are useful and at the same time consumed to prevent oils from oxidation. Because of this, many long-stored vegetable oils lose or do not possess antioxidant capacity. To extend the shelf life of vegetable oils, they must be enriched with fat-soluble antioxidants in concentrations and ratio determined for the predicted shelf life

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References

Akbari S., Abdurahman N.H., Yunus R.M., Alara O.R., Abayomi O.O. (2018) Extraction characterization and antioxidant activity of fenugreek (*Trigonella foenum-graecum*) seed oil. Materials Science for Energy Technologies. DOI:10.1016/j.mset.2018.12.001.

Clarke G.1., Ting K.N., Wiart C., Fry J. (2013). High correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. Antioxidants (Basel). 2(1), 1-10. DOI: 10.3390/antiox2010001.

Elisovetcaia D.S., Brindza J. (2018). Effect of secondary metabolites *Juniperus sabina* L. (Cupressaceae) on the survival and nutrition of caterpillars Galleria mellonella L. (Pyralidae). Contemporary Problems of Ecology, Russia, 11(6), 594–603.

Emami S.A., Abedindo B.F., Hassanzadeh-Khayyat M. (2010). Antioxidant activity of the essential oils of different parts of *Juniperus excelsa* M.Bieb. subsp. *excelsa* and *J. excelsa* M.Bieb. subsp. *polycarpos* (K.Koch) Takhtajan (Cupressaceae). Iranian Journal of Pharmaceutical Research, 10(4), 799-810.

Guseva D.A., Prozorovskaya N.N., Shironin A.V., Sanjakov M.A., Evteeva N.M., Rusina I.F., Kasaukina O.T. (2010). Antioxidant activity of vegetable oils with different omega-6/omega-3 fatty acids ratio. Biomedi*tsinskaya himiya* (Biomedicinal Chemistry*)*, Russia, 56(3), 342-350.

Ivanova R., Casian I. (2008). Determination of antioxidant activity and characterization of antioxidant phenolics in the grape seed extracts. Journal of EcoAgriTourism, Romania, 4(1-2), 243—246.

Jorge N, da Silva A.C., Aranha C.P.M. (2016). Antioxidant activity of oils extracted from orange (*Citrus sinensis*) seeds. Anais da Academia Brasileira de Ciências ((Annals of the Brazilian Academy of Sciences), 88(2), 951-958. http://dx.doi.org/10.1590/0001-3765201620140562.

Kulaitiene J., Cerniauskiene J., Jariene E., Danilcenko H., Levickiene D. (2018). Antioxidant activity and other quality parameters of cold pressing pumpkin seed oil. Not Bot Horti Agrobo, 46(1):161-166. DOI: 10.1535/nbha46110845.

Lobaeva T.A., Gluchshenko N.N., Bogoslovskaj O.A. (2004). Interdependences between antioxidant content and antioxidative activity of oily phytodrugs. Vesnik RUDN (RUDN University Messenger), series Medicine, speciality Pharmacy, Russia, 4, 286-291.

Melikyan L.A., Ivanova R.A., Dascaliuc A.P., Alexanyan H.P., Hovhannisyan A.S., Gabrielyan E.S., Makai P., Makai S. (2008). Comparative study of antiradical activity of *Trigonella foenum-graecum* L. seed extracts from Armenian, Hungarian and Indian samples. Medical Science of Armenia, 48(1), 55-61.

Poiana M-A., Jianu C., Jianu I., Rinovetz A. (2009). The storage conditions: impact on the oxidative stability and antioxidant properties of grape seed oil. Journal of Food, Agriculture & Environment, 7(2), 50-53.

Radvzievska I., Melnik O. (2016). Investigation of oxidative degradation of vegetables oils of various degrees of saturation with tocopherol. Scientific paper of National University of Food Technologies, Ukraine, 22(3), 206-216.

Rassem H.H.A., Nour A. H., Yunus R.M. (2016). Techniques for extraction of essential oils from plants: A review. Australian Journal of Basic and Applied Sciences. 10(16), 117–127. http://www.ajbasweb.com/old/ajbas/2016/November/117-127.pdf.

Ross C.F., Hoye C.J., Fernandez-Plotka V.C. (2011). Influence of heating on the polyphenolic content and antioxidant activity of grape seed flour. Journal of Food Science. 76(6), 884-890. DOIi: 10.1111/j.1750-3841.2011.02280.x.

Salamon I., Petruska P. (2017). Quality of juniper essential oil (oleum juniperi) in the South Slovakia and its curative and industrial utilization. Indian Journal of Pharmaceutical Education and Research, 51(3), 479-482.

Samusenko A.L. (2011). Research of antioxidant activity of essential oils of lemon, rose grapefruit, coriander, clambers and their mixtures by the method of capillary gas chromatography. Himiya rastitelnogo syirya (Chemistry of vegetable raw material), Russia, 3, 107-112.

Shi J., Yu J., Pohorly J.E., Kakuda Y. (2003). Polyphenolics in grape seeds-biochemistry and functionality. Journal of Medicinal Food, 6(4), 291-299. DOI: 10.1089/109662003772519831.

Shinagawa F.B., de Santana F.C., Araujo E., Purgatto E., Mancini-Filho J. (2018). Chemical composition of cold pressed Brazilian grape seed oil. Food Science and Technology (Campinas), 38 (1), 164-171. http://dx.doi.org/10.1590/1678-457x.08317.

Tatarov P., Popovici C. (2014). Evolution of fatty acids and peroxydes content in walnut oil (*Juglans regia* l.) during storage. Meridian Ingineresc, Republic of Moldova, 4, 46-50. http://repository.utm.md/bitstream/handle/5014/2141/MI_2014_4_pg_46_50.pdf?sequence=1&isAllowed=y.

Tatarov P., Sandulachi E., Ivanova R., Baerle A. (2017). Quality changes in oil of walnut *(Juglans regia L.)* during storage. Lucrari stiintifice seria Agronomie (Scientific works, series Agrimony), Romania, 60(2), 187-190.

Xie Q., Li Z., Li Z. (2015). Chemical composition and antioxidant activity of essential oil of six *Pinus* taxa native to China. Molecules, 20, 9380-9392. DOI: 10.3390/molecules20059380.