EVALUATION OF ANTIOXIDANT PROFILE OF COLD PRESSED WALNUT OIL (*JUGLANS REGIA* L.)

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Abstract: *The objective of this study was to determine the antioxidant properties of the oil obtained from the kernel of walnut variety belonging to the species Calaras. The antioxidant profile of walnut oil samples were evaluated by reaction kinetics and reducing power of the oil samples towards 1,1-diphenyl-2-picrylhydrazyl stable free radical (DPPH•). The principle of DPPH assay is to measure the free radicals generated from oils directly. The obtained results showed that antioxidant properties of walnut oil were in the range of 24.11 – 54.73 %. The DPPH assay is rapid and easy to use, feasible and accessible to be use for antioxidant properties evaluation of walnut oil.*

Key words: *antioxidant profile, reaction kinetics, DPPH assay, processing technology, walnut oil.*

1. Introduction

Walnut is a crop of a high economic interest for the food industry. Walnut kernel is highly appreciated nut because of its unique organoleptic characteristics, biological and nutritional value. Walnuts generally contain about 60% oil, but this can vary from 52 to 70% depending on the cultivar, location grown, and irrigation rate. The major constituents of the oil are triacylglycerols; free fatty acids, diacylglycerols, monoacylglycerols, sterols, sterol esters, and phosphatides are all present in only minor quantities. The major fatty acids found in walnut oil are oleic (18:1), linoleic (18:2), and linolenic (18:3) acids. The ratios of these to each other are important to the economic and nutritional value of the nut. Walnuts also possess numerous polyphenolic compounds with potent free radical scavenging ability and therefore, capable to break the propagation chain of lipoperoxidation [3, 5, 10, 11].

Recently, much attention has been paid to walnut oil antioxidant profile and its possible role as a functional food or food ingredient. Reseacher Orhan et al. (2011) propose a rapid, simple and accessible method to measure antioxidant capacity of food involves the use of the free radical 1.1-diphenyl-2-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of oils. DPPH is a purple-colored free stable radical; when reduced, it becomes the yellow-colored diphenylpicrylhydrazine. The antioxidant activity of various oils can be determined accurately, conveniently, and rapidly using DPPH testing. The trend in antioxidant activity obtained by using the DPPH method is comparable to trends found using other methods reported in the literature [4, 8].

In view of the fact, that walnut oil is to be effectively used in the food industry and human nutrition, it is extremely important to elaborate technology of walnut oil extraction. This trial was set up to determine the effective method of technological treatment on quality of walnut oil. The effectiveness of the applied treatments was assessed by measuring the antioxidant potential of walnut oil.

2. Materials and methods

2.1. Sample preparation and extraction procedure

Walnut fruits (Juglans regia L.) were obtained from agency Moldsilva, which is the central public administration body on state policy in forestry and hunting in the Republic of Moldova (http://www.moldsilva.gov.md/). At full maturity, fruits were hand-picked directly from the trees. After harvest fruits were transported to the laboratory. Before oil extraction, the walnuts were manually cracked and shelled. Then, kernels were chopped in a KEM 36 mill. Walnut oil extraction was carried out at 20±2 ^{0}C using an electrical press (Model PCU-125). The oil obtained was subjected to different technological treatments as centrifugation, dehydration, heat processing and their combination. Walnut oil without applied technological treatments was used as reference sample.

2.2. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH˙) as free radical form (90% purity) was supplied by Sigma-Aldrich. Methanol (99.8%) was supplied by Eco-Chimie (Chisinau, Moldova). All the chemicals used were of HPLC or analytical grade. Distilled water was used throughout.

2.3. Determination of the basic quality properties

Acidity of the walnut oil samples were determined by potassium hydroxide titration as described in AOCS Official Method Cd 3d-63 (AOCS, 1999). Peroxide value was determined according to AOCS Official Method Cd 8-53 (AOCS, 2003). The formation of the secondary oxidation products accumulation in the investigated oil samples were characterized by the 2-thiobarbituric acid value (2-TBA) and p-anisidine value in accordance with AOCS Official Method Cd 19-90 (AOCS, 2009) and AOCS Official Method Cd 18-90 (AOCS, 1997) respectively.

2.4. DPPH assay

The radical scavenging activity of walnut oil samples as well as the kinetics of inhibition of free radicals were studied in terms of radical scavenging ability using the stable DPPH˙ method [1, 6]. Briefly, 0.1 ml of the sample was added to 3.9 ml of 60 µM solution of DPPH˙ in methanol. The reaction was carried in dark and the absorbance was recorded at 515 nm to determine the concentration of remaining DPPH˙. Methanol as instead of DPPH˙ solution was used as blank solution. The values of $[DPPH^{\bullet}]_t$ at each reaction time were calculated according to the standard curve. Concentration range of DPPH was of 0.38-38 µg/ml ($A_{515\ nm}$ = 0.0293 $[DPPH^{\bullet}]_t$ – 0.0072), where the concentration $[DPPH^{\bullet}]_t$ is expressed in µg/ml). The coefficient of linear correlation of the above relation is R = 0.9999. The radical scavenging activity was calculated using the equation:

$$RSA = 100\% \bullet ([DPPH^{\bullet}]_0 - [DPPH^{\bullet}]_{30})/ [DPPH^{\bullet}]_0 \quad (1)$$

where $[DPPH^{\bullet}]_0$ is the concentration of the DPPH˙ solution (without sample) at t=o min and $[DPPH^{\bullet}]_{30}$ is the remained DPPH˙ concentration at t=30 min. Lower $[DPPH^{\bullet}]_t$ in the reaction mixture indicates higher free radical scavenging activity.

2.5. Statistical analysis

Variance analysis of the results was carried out by least square method with application of coefficient Student and Microsoft Office Excel program version 2007. Differences were considered statistically significant if probability was greater than 95% (p-value <0.05). All assays were performed by triplicate at room temperature 20 ± 1 ^{0}C. Experimental results are expressed as average ± SD (standard deviation).

3. Results and discussion

The health benefits of walnut oil are attributed to its chemical composition, especially to a high level of polyunsaturated fatty acids. Walnut oil has a limited shelf-life, i.e. nutritional and organoleptic changes due to losses of essential fatty acids. It is well known, that primary oxidation products of vegetable oils are peroxides, which can be transformed into secondary oxidation products. The physicochemical properties of walnut oil samples are shown in table 1.

Table 1. Quality characteristics of cold pressed walnut oil samples (±SD*)

№	Quality characteristics	Samples of the walnut oil			
		Reference sample	Dehydrated and thermally treated sample	Dehydrated sample	Thermally treated sample
1.	Acid value, [mg KOH/g oil]	0.39±0.01	0.46±0.01	0.63±0.05	0.29±0.03
2.	Peroxide value, [mmol/kg oil]	2.49±0.18	3.33±0.01	1.66±0.01	5.73±0.13
3.	p-Anisidine value, [c. u.]	6.10±0.57	3.26±0.18	5.96±0.27	0.88±0.17
4.	2-TBA value, [mg/kg oil]	0.07±0.01	0.11±0.01	0.11±0.04	0.10±0.02
5.	TOTOX value, [c. u.]	11.08±0.93	9.91±0.89	9.27±0.26	12.34±0.53

*Average concentration of three measurements ± standard deviation.

The antioxidant profile of any oil sample comes from the combined synergic action of a mixture of compounds, including phenolics, carotenoids, tocopherols, etc [2, 9]. The knowledge of the kinetics of atom

transfer is important because free radicals in the organism are short-lived species, what implies that the impact of a substance as an antioxidant depends on its fast reactivity towards free radicals. A kinetic assay following the changes in the absorbance of DPPH• reagent was applied to evaluate the level of antioxidant properties of the walnut oil samples in the current study (figure 1).

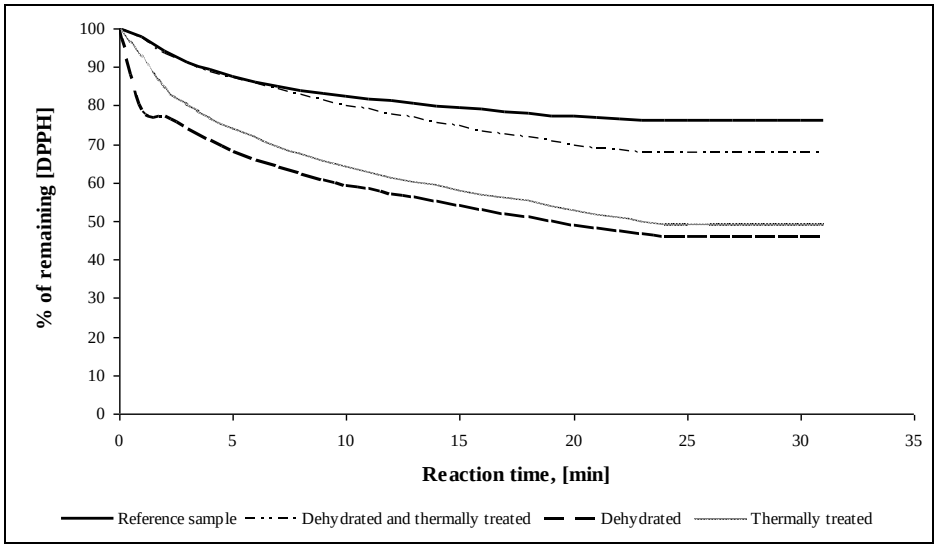

Fig. 1. *Reaction kinetics of DPPH• with walnut oil samples*

It is well known that the absorbance decreases as a result of a colour change from purple to yellow when the DPPH radical is scavenged by antioxidants through donation of hydrogen to form the stable DPPH-H molecule. A more rapid decrease of the absorbance means more potent antiradical activity, expressed in terms of hydrogen donating ability of the compounds. The DPPH• scavenging capacity of biologically active compounds is mostly related to their phenol hydroxyl groups [7].

The dehydrated walnut oil sample had the highest efficiency to scavenge DPPH• radicals. However, no significant difference was found in the inhibiting behavior of the thermally treated oil sample. The reference sample had the lowest efficiency to scavenge DPPH• radicals. The antioxidant potential of walnut oil samples was expressed as radical scavenging activity (RSC) and was influenced by the applied technological treatment (figure 2).

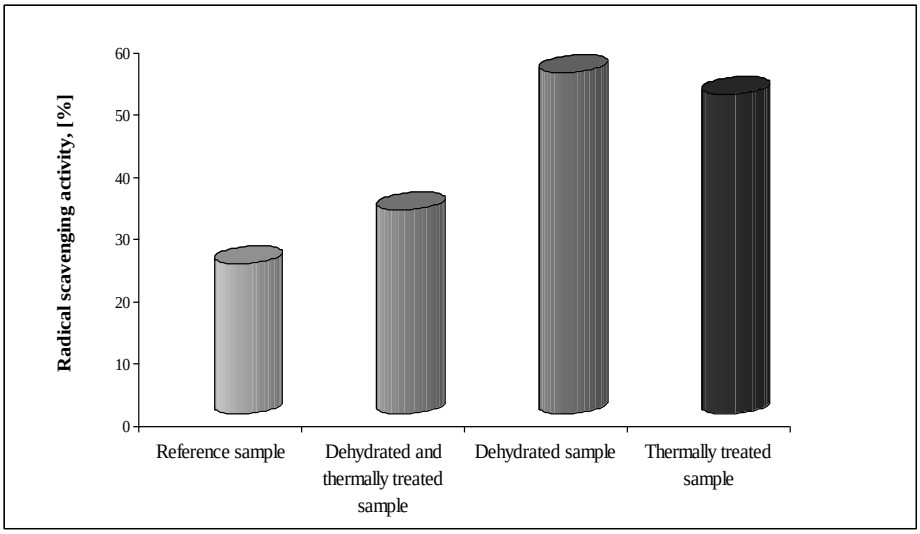

Fig. 2. *Radical scavenging activity DPPH• of walnut oil samples*

In general, this parameter was in the range of 24.11 – 54.73 %. The highest antioxidant function (minor amounts of DPPH•) was found in dehydrated walnut oil and sample treated in combination of dehydration and thermal processing. Antioxidant potential of walnut oil may be attributed to the phenolic compounds and tocopherols present in the oil samples.

4. Conclusions

Today, walnut oil has been extracted on a small scale to obtain edible vegetable oil in Europe. However, walnuts can be used to produce high quality oil. The results of this research showed the influence of dehydration, thermal treatment and their combination on the quality properties (primary and secondary oxidation products) and antioxidant potential cold pressed walnut oil.

Antioxidant properties of walnut oil samples were in the range of 24.11 – 54.73 %. The highest antioxidant function was found in dehydrated walnut oil and sample treated in combination of dehydration and thermal processing.It is important to note that the DPPH assay is rapid and easy to use, feasible and accessible to be use for antioxidant properties evaluation of walnut oil.

It was demonstrated that walnut oil possess high quality after dehydration treatment and quality deteriorates after combined dehydration and heat treatment. It is important to underline, that obtained results of this study are intermediate and could help to describe the scheme of walnut oil oxidation process and also to elaborate improved technology for walnut oil stabilization.

5. Acknowledgements

This work was benefited from support through the 11.817.04.40 A project, "*Elaboration of methods to protect walnut lipids (Juglans regia L.) from oxidative degradation*", funded by the Academy of Science of Moldova and Moldavian Government.

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