THE INFLUENCE OF NATURAL AND SYNTHETIC ANTIOXIADANTS ON THE OXIDATION STABILITY OF HEAT TREATED WALNUT OIL (*JUGLANS REGIA* L.)

Popovici C.[1*]
Alexe P.[2], Dmitrieva E.[1], Deseatnicova O.[1]

[1]Faculty of Technology and Management in Food Industry, Technical University of Moldova,
[2]Faculty of Food Science and Engineering, Dunarea de Jos University of Galati, Romania
[*]Cristina Popovici, e-mail: popovici.kristina@gmail.com

Abstract: This paper was executed within the framework of improving the quality and increasing the oxidative stability of walnut oil by introducing into it a natural extract of the walnut green husk and synthetic antioxidant butylhydroxytoluene (BHT). The analysis of modern methods for the determination of primary and secondary oxidation products of walnut oil were carried out. There are given suggestions to stabilize the oil by using a natural extract of walnut green husk in this study. Substitution of the natural antioxidant for synthetic one may be an alternative solution to stabilize the walnut oil.
Keywords: walnut oil, green husk extract, butylhydroxytoluene, primary and secondary oxidation products, oxidative stabilization.

Introduction

Walnut oil (*Juglans regia* L.) is a product of vegetable origin and has a special value. The oil contains several times more linoleic and linolenic acid relative to other plant oils. Linoleic acid together with linolenic relates to essential fatty acids. These fatty acids get in human organism with food, mainly in the form of complex lipids - triglycerides and phosphatides. The chemical composition of the oil is also represented by carotenoids, biologically active compounsd, fat-soluble vitamins, macro-and micronutrients (Zn, Cu, I, Ca, Mg, Fe, P, Co) [7, 11].

But in spite of all the positive things, walnut oil is not a stable substance. The products of oil oxidation are formed during the oil's heat treatment, which leads to deterioration of the product. Tocopherol which is the main natural antioxidant part of the walnut oil is not able to bind all the free radicals which initiate the oxidation process.

At the moment the current trends of development of the food industry are to produce the natural antioxidants extracted from vegetable raw materials. This is a new and promising trend in the food industry, specifically aimed at improving the structure of nutrition, health and prevention of diseases prevalent in the society [6, 8].

Products of vegetable origin are the best source of antioxidants such as vitamins and polyphenols. Namely formation of phenolic compounds - one of the most important features of a plant cell. Polyphenols have strong antioxidant activity due to their structural characteristics. Polyphenolic molecule is composed of two or more atoms of benzoic acid and additional hydroxyl groups, which determine the effect and force of polyphenols.

It is known that the walnut green husk is characterized by high biological and nutritional value due to a significant content of antioxidants including ascorbic acid as

well as, tocopherol, β-carotene, flavonoids, phenolic compounds. In this paper, as a source of natural antioxidants was investigated walnut green husk, on the basis of economic reasons and accessibility for the Republic of Moldova.

The main purpose of this paper is the enrichment of walnut oil by natural antioxidant, namely extract of walnut green husk to increase the oxidative stability of the oil compared with synthetic antioxidant. It was necessary to develop technologies for getting the extract and determine the optimal conditions for its receipt to achieve this goal.

Materials and methods

Chemicals

2-thiobarbituric acid (4,6-dihydroxy-2-mercaptopyrimidine), butylhydroxytoluene (BHT) were obtained from Alfa Aesar. 1,1-Diphenyl-2-picrylhydrazyl (DPPH˙) as free radical form (90% purity) was supplied by Sigma-Aldrich. Ethanol (99.9%), chloroform, 1-butanol, glacial acetic acid, potassium hydroxide, phenolphthalein, potassium iodide, sodium thiosulfate ($Na_2S_2O_3 \times 5H_2O$) and starch were supplied by Eco-Chimie (Chisinau, Moldova). All the chemicals used were of HPLC or analytical grade. Distilled water was used throughout.

Experimental

Refined walnut oil was obtained from a local producer in the Republic of Moldova. Walnut oil samples with antioxidants and without were heated during 20 min at different temperatures: 120°C, 160°C and 180°C (Figure 1). After heat treatment walnut oil samples were used in the experiment.

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) [1]. Peroxide value was determined according to AOCS Official Method Cd 8-53 (AOCS, 2003) [2]. Conjugated dienes were detected by a spectral method of analysis (λ=232 nm) according to the AOCS Official method Ti la 64 (AOCS, 1993) [3]. The formation of the secondary oxidation products accumulation in the investigated walnut oil samples were characterized by p-anisidine value in accordance with AOCS Official Method Cd 18-90 (AOCS, 1997) [4] and the 2-thiobarbituric acid value according to AOCS Official Method Cd 19-90 (AOCS, 2009) [5].

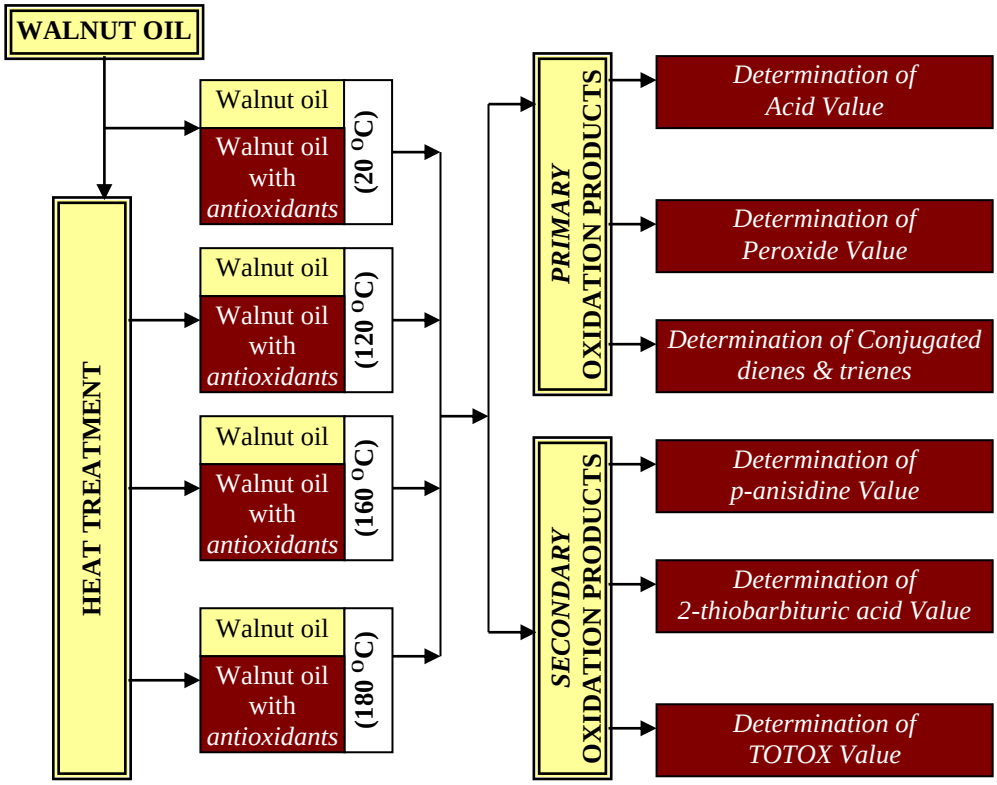

Fig. 1. Experimental design of the study

Statistical analysis

Variance analysis of the results was carried out by least square method with application of coefficient Student and Microsoft Office Excel program. 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).

Rezults and discussion

Influence of heat treatment on the oxidation stability of walnut oil

Physico-chemical characteristics of vegetable oils play an important role in the technology of obtaining food products and depend on their chemical composition and structure. The need for research in this area is to obtain some new oils with increased biological, nutritional value and high oxidative stability. For the purpose of these studies were determined quality properties of walnut oil , treated at different temperatures, namely at 120 °C, 160 °C and 180 °C.

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

№	Quality characteristics	Samples of the walnut oil			
		20 °C	**120 °C**	**160 °C**	**180 °C**
1	Acid value, [mg KOH/g oil]	0.94±0.01	0.97±0.01	0.97±0.01	1.01±0.01
2	Peroxide value, [mmol/kg oil]	5.81±0.02	19.97±0.02	23.96±0.02	26,55±0.01
3	Conjugated dienes, [μmol/g oil]	6.23±0.05	8.98±0.11	11.99±0.07	12.74±0.05
4	Conjugated trienes, [μmol/g oil]	1.58±0.05	2.54±0.05	4.04±0.07	6.25±0.03
5	p-Anisidine value, [c. u.]	2.98±0.11	3.24±0.07	12.02±0.07	16.91±0.07
6	2-Thiobarbituric acid value, [mg/kg oil]	0.03±0.01	0.03±0.01	0.04±0.01	0.045±0.01
7	TOTOX Value, [c. u.]	14.59±0.13	43.18±0.09	59.94±0.09	70.01±0.08

*Average concentration of three measurements ± standard deviation.

During the heat treatment process of vegetable oil there are various processes that influence on quality of the oil. Oils are easily oxidized and represent a system of organic complex with a large number of components that are involved in all stages of oxidation - the initiation, development and termination.

Analyzing the experimental data of table 1, it can be concluded that with increasing of temperature of oil processing the peroxide number increases. The highest peroxide value 26 mmol/kg oil is typical for the sample oil heated at 180°C.

Based on these results, it was found that during the thermal treatment of samples of walnut oil takes place the formation and growth of conjugated dienes and trienes in the range of 6.23 - 12.75 mmol/g oil for dienes and 1.58-6.25 mmol/g of oil for trienes.

The energy accumulation of aldehydes in samples of walnut oil was expressed by p-anisidine value. It was found that in the first two samples of oil increasing the number of p-anisidine value happened insignificantly, it speaks about the slow formation and a slight accumulation of aldehydes in these samples.The highest value of the accumulation of secondary oxidation products were found in samples of walnut oil heated at 160°C and 180 °C, which reached a value of 12.02 and 16.91 c.u. respectively.

The index of the total number of oil oxidation (TOTOH Value) was calculated for comparative analysis of the accumulation of primary and secondary oxidation products of oil for all the samples. The value of this parameter for the thermally treated walnut oil varied within 14.59 - 70.01 c. u.

Influence of antioxidants on the oxidation stability of heat treated walnut oil

The technology for obtaining an extract of walnut green husk has been developed for getting natural antioxidants. According to the suggested scheme was obtained a natural extract, which was evaluated from the standpoint of the total content of polyphenols and antioxidant activity by DPPH• method [9, 10]. The effectiveness of the use of natural extracts of walnut green husk to stabilize the oxidation processes of walnut oil was evaluated in comparison with the synthetic antioxidant butylhydroxytoluene. Figure 2 shows a graphical representation of introduced antioxidants on the acid number of walnut oil.

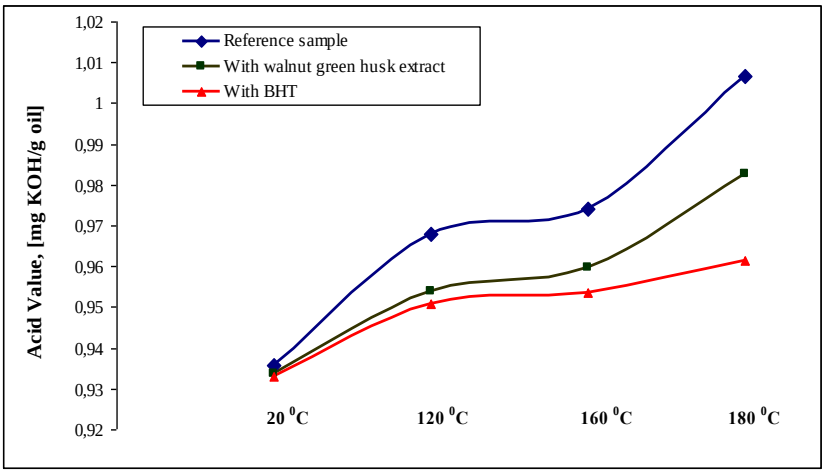

Fig. 2. Influence of introduced antioxidants on acid value of walnut oil samples

Analyzing the Figure 2 it can be concluded that oil samples with an extract of green husk and butylhydroxytoluene neutralize free fatty acids, thereby preventing oxidation of the oil. Synthetic antioxidant shows the best value.

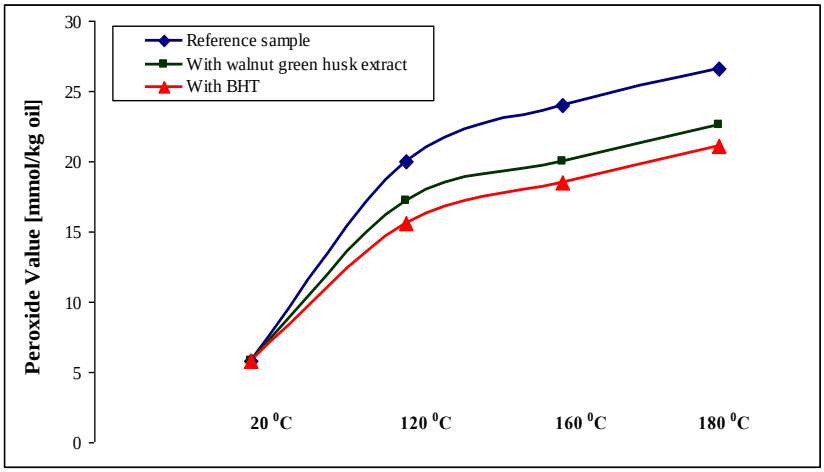

Fig. 3. Influence of introduced antioxidants on peroxide value of walnut oil samples

Analyzing the figure 3 it can be concluded that oil samples with an extract of green husk and butylhydroxytoluene show almost equal values of 20 and 22 mmol/g of oil, respectively. This indicates the effectiveness of the use of these two antioxidants to reduce oxidation of the oil.

Quality properties of vegetable oils, expressing secondary oxidation products include p-anisidine value and 2-thiobarbituric value. The experimental data of these parameters in the samples of walnut oil are shown in Figures 4 and 5.

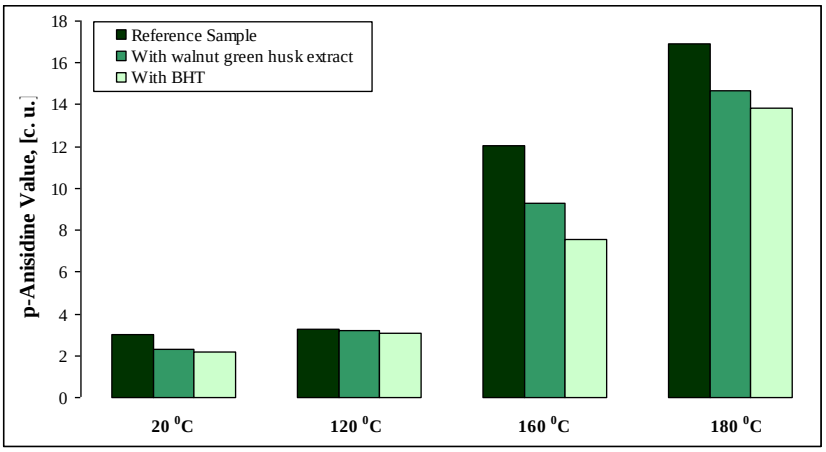

Fig. 4. Influence of introduced antioxidants on p-anisidine value of walnut oil samples

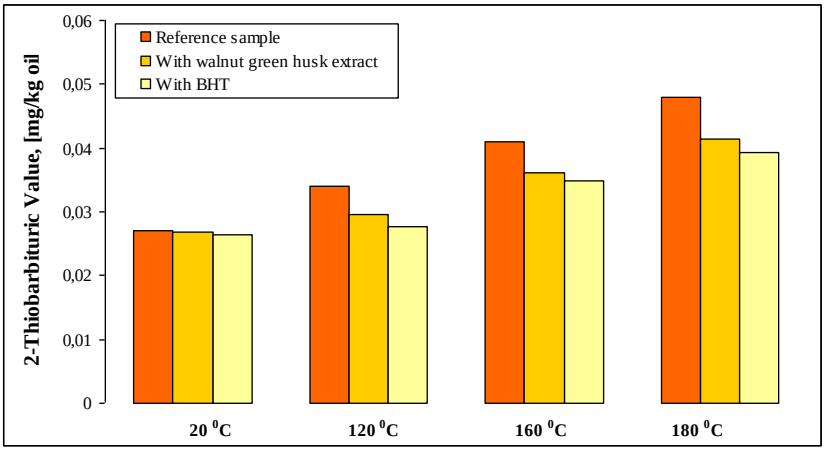

Fig. 5. Influence of introduced antioxidants on 2-thiobarbituric acid value of walnut oil samples

Figures 4 and 5 shows a graphical representation of influence of introduced antioxidants on intensity of accumulation the secondary oxidation products of walnut oil subjected to heat. In figure 6 it can be seen that the oil samples at 20 °C are characterized by a relatively equal values of 2-thiobarbituric value. The introduction of antioxidants in the oil samples treated with heat has a positive effect on the process of reducing the accumulation of oxidation products.

Conclusion

This study investigated the change of the accumulation of primary and secondary oxidation products of walnut oil, depending on the applied temperature. It was found that in samples of walnut oil which were thermally treated at a temperature of 120 °C and 160 °C the amount of these compounds is not significantly different relative to the control

sample. In a sample of walnut oil heated at 180 °C was established significant increase of the concentration of peroxides, conjugated dienes and trienes, aldehydes.

The effectiveness of the introduction of natural extracts of walnut green husk and butylhydroxytoluene on the processes of oxidative stabilization of walnut oil, treated thermally was also studied in this paper.

The experimental data showed that the effect of introduced antioxidants leads to reduction of oil oxidation. The obtained data showed that the effect of introduced antioxidants leads to reduction oil oxidation.

The use of a natural extract of walnut green husk with a high antioxidant potential in the composition of walnut oil contributes to the stabilization of oxidation processes that occur during heat treatment of oil, and the replacement of natural antioxidant for synthetic one may be an alternative solution to stabilize the walnut oil.

References

[1] AOCS, 1999. Official Methods and Recommended Practicles of the American Oil Chemists' Society. *Method Cd 3d-63*. Champaign: AOCS Press.

[2] AOCS, 2003. Official Methods and Recommended Practicles of the American Oil Chemists' Society. *Method Cd 8-53*. Champaign: AOCS Press.

[3] AOCS, 1993. Official Methods and Recommended Practicles of the American Oil Chemists' Society. *Method Ti la 64*. Champaign: AOCS Press.

[4] AOCS, 1997. Official Methods and Recommended Practicles of the American Oil Chemists' Society. *Method Cd 18-90*. Champaign: AOCS Press.

[5] AOCS, 2009. Official Methods and Recommended Practicles of the American Oil Chemists' Society. *Method Cd 19-90*. Champaign: AOCS Press.

[6] Calvoa P., Lozanoa M., Espinosa-Mansillab A. In-vitro evaluation of the availability of ϖ-3 and ϖ-6 fatty acids and tocopherols from microencapsulated walnut oil. Food Research International, Volume 48, Issue 1 (2012) 316–321.

[7] Gharibzahedi S.M.T., Mousavi S.M., Hamedi M., Rezaei K. Evaluation of physicochemical properties and antioxidant activities of Persian walnut oil obtained by several extraction methods. Industrial Crops and Products, 45 (2013) 133-140.

[8] Martínez M.L., Penci M.C., Ixtaina V., Ribotta P.D., Maestri D. Effect of natural and synthetic antioxidants on the oxidative stability of walnut oil under different storage conditions. LWT - Food Science and Technology, 51 (2013) 44 – 50.

[9] Popovici C. Soxhlet extraction and characterisation of natural compounds from walnut (*Juglans regia* L.) by-products. Ukrainian Food Journal, Vol. 2, Issue 3, 2013, p. 328 - 336. Available on Internet: http://www.ufj.ho.ua/EN/.

[10] Popovici C., Gîtin L., Alexe P. Characterization of walnut (*Juglans regia* L.) green husk extract obtained by supercritical carbon dioxide fluid extraction. Journal of Food and Packaging Science, Technique and Technologies, no. 1, 2012, p. 5-9. Available on Internet: https://mahvp.uft-plovdiv.bg/en.

[11] Tsamouris, G., Hatziantoniou, S., Demetzos C., (2002). Lipid analysis of greek walnut oil (Juglans regia L.). Z. Naturforsch, 57c, 51-56.