PHYSICOCHEMICAL CHANGES OF WALNUT OIL (JUGLANS REGIA L.)

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Abstract. Among the representatives of oil crops, fruits of walnut (Juglans regia L.) are differ by the richest in lipid content 65...75%, proteins 15…17%, vitamins, minerals, polyphenolic compounds etc. Processing of nuts along with the organization of industrial production of walnut oil is among the most promising directions for the national economy of the Republic of Moldova. Walnut oil is a product of an exceptionally high nutritional value, with high content of biologically active substances, including essential unsaturated fatty acids, the ω-3 and ω-6. During storage, walnut oil is extremely unstable, subject to physical and chemical changes with the accumulation of primary and secondary oxidation compounds.
Keywords: walnut (Juglans regia L.), oil, saturated and polyunsaturated fatty acids, oxidation

Walnut fruit (Juglans regia L) is one of the most valuable oleiferous raw materials from nutritional and health benefits point of view. Walnut cultivation is possible in geographic regions with specific climatic conditions and soil composition. In Europe walnut cultivation is developed in France, Italy, Spain, Romania, Bulgaria, Hungary and Moldova. The cultivation of walnut is also developed in the U.S.A and China. In the last decade the Republic of Moldova has enlarged the walnut plantations, increased walnuts harvesting and export. Results of study on walnut quality have shown that the nutritional components of this raw material are characterized by significant biological and dietetic value. Walnut kernels contain between 60…75% lipids. Walnuts provide appreciable amounts of proteins 15…18%, carbohydrates 12…16%, fiber 1.5…2.0% and minerals up to 1.8 %. Walnut oil contains approximately 10 % saturated, 18 % monounsaturated and 72 % polyunsaturated fatty acids [7]. Compared with most other nuts, with contain mostly monounsaturated fatty acids (MUFA), walnuts are highly enriched in omega-6 (ω-6) and omega-3 (ω-3) polyunsaturated fatty acids (PUFA), which are essential dietary fatty acids. In fact, MUFA and PUFA composition can influence various physiological and biochemical processes, including blood pressure regulation, glucose metabolism, lipids metabolism, platelet aggregation, and erythrocyte deformability [1, 2].

Taking into account the importance of lipids and their changes on the quality of walnuts and walnut products, the aim of our research was to estimate the oxidative changes of oil extracted from walnuts during storage.

Research started with a comparative analysis of chemical compositions of walnut oils produced in France, Hungary, Italy, Spain and oil from Moldova walnuts.

The data presented in table 1 show that the composition of fatty acids in the oil from walnuts produced in European countries [7] and walnut oil from Moldova according to normative documentation [9] is practically identical. For example, analyzed oils are rich in unsaturated fatty acids, content of linoleic acid (ω-6) equal with 53...70%, oleic acid from group of MUFA acids presents in quantity of 14...30%.

Table 1. *Fatty acid composition of walnut oil (percent of total fatty acids)*

Symbol	Fatty acid	Walnut oil from countries				
		France	Hungary	Italy	Spain	Moldova
C14:0	Myristic	-	-	-	-	0.1
C16:0	Palmitic	6.5-7.3	5.8 - 7.7	7.3 -8.1	7.1 -7.5	2.9 - 7.0
C16:1	Palmitoleic	0.1	0.1	0.1	0.1	0.1
C17: 0	Margaric	0.1	-	0.1	0.1	-
C18:0	Stearic	1.7 - 2.9	2.1 - 2.2	2.2 - 2.9	1.9 - 2.8	0.9 - 2.5
C18:1	Oleic	15.1- 18.9	17.4 - 22.2	14.5 -15.3	14.3- 19.2	14.0 - 30.0
C18:2	Linoleic	57.4 - 64.3	58.3 - 60.8	60.2 - 63.1	57.6 - 62.5	53.0 - 70.0
C18:3	Linolenic	11.3 -15.4	10.8 - 11.6	11.8 -14.3	12.4 - 13.2	9.8 - 13.0
C20:0	Arachidic	0.1	0.1	0.1	0.1	-
C20:1	Eicosenoic	0.2 - 0.3	0.2	0.2	0.2	1.7
C22:1	Docosenoic	-	-	-	-	3.8

Compared with other edible vegetable oils (olive, sunflowers, soybeans oils), the walnut oil is the only oil containing linolenic acid (ω-3) over 10%. In compared oils the ratio of polyunsaturated to saturated fatty acids varies between 7.2 …7.8 (table 2).

Table 2. *Ranges of saturated, monounsaturated, and polyunsaturated fatty acids in walnut oil*

Fatty acid	Walnut oil from countries				
	France	Hungary	Italy	Spain	Moldova
Saturated	8.5 – 9.7	8.3 – 9.9	10.2 – 10.5	9.5 – 10.2	4.1 - 9.8
Monounsaturated	15.4 – 19.1	17.7 – 22.5	14.8 – 15.5	14.6 – 19.5	14.3-19.2
Polyunsaturated	71.2 – 75.5	69.1 – 72.4	74.3 – 74.9	70.7 – 75.2	62.8 - 75.7
Polyunsaturated / Saturated, ratio	7.8	7.8	7.2	7.5	7.7

Presented data denotes that walnuts oils have a high biological value independently of country of their production due to the PUFA content. However, walnut oil is unstable, and during storage it starts oxidizing in a short time. The PUFA acids are first of all oil components that are exposed to oxidation.

The rate of oxidation of fatty acids increases in relation to their degree of unsaturation. The relative rate of autoxidation of oleate, linoleate, and linolenate is in the order of 1:100–1200:2500 on the basis of oxygen uptake and 1:12:25 on the basis of peroxide formation [3, 5].

According to presented data, the initiation of oxidation reaction of linolenic acid starts after 1.34 hours of oil extraction. The linoleic acid oxidation occurs after 19 hours (table 3). The rate of oil oxidation is accelerating in foods with increased water activity, in

presence of metals of variable valence, under the influence of prooxidants and other factors [4, 8].

Table 3. *Induction period and relative rate of oxidation for fatty acids at 25 ^{0}C [3]*

Fatty acid	Number of allyl groups	Induction period (h)	Oxidation rate (relative)
Stearic, (C^0_{18})	0	-	1
Oleic, (C^1_{18})	1	82	100
Linoleic, (C^2_{18})	2	19	1200
Linolenic, (C^3_{18})	3	1,34	2500

In addition, the highly polyunsaturated nature of walnut lipids makes them prone to oxidative instability. Despite the oxidative problem, oil extracted from walnuts is sold and enjoyed as high-quality specialty oil in many parts of the world. The three major components involved in lipid oxidation are unsaturated fatty acids, oxygen and water [4].

Autooxidation reaction of lipids in walnut kernels is initiated by biochemical reactions, via specific actions of the enzymatic system, including with involvement of oxidases. Oxidation of oil extracted from walnuts is carried out by biochemical and pure chemical reactions. During storage the walnut oil is subject to autooxidation by chemical reaction, catalyzed by radiation, metal of variable valence and water present in the oil composition. It is considered that in the beginning stage of autooxidation process the primary compounds of reaction - peroxides and hydroperoxides are formed. Primary compounds are unstable. "Shelf life" of hydroperoxides doesn't exceed 1.0 s.

Rapid changes of hydroperoxides are followed by the extension of autooxidation process which leads to accumulation of secondary compounds of oxidation. These compounds include aldehydes, ketones, alcohols, low-molecular weight fatty acids, volatile compounds and others. The decomposition of hydroperoxides to secondary compounds is shown in figure 1 [6]. The mechanism of process consists in bond cleavage of oxygen molecules in hydroperoxide radical and formation the intermediate free radicals of hydroxyl and alkoxy (R-O-¦-O-H → RO• + HO●). After that these free radicals by chemicals reactions are transformed in secondary compounds and generate some new free radicals of alkyl type R●.

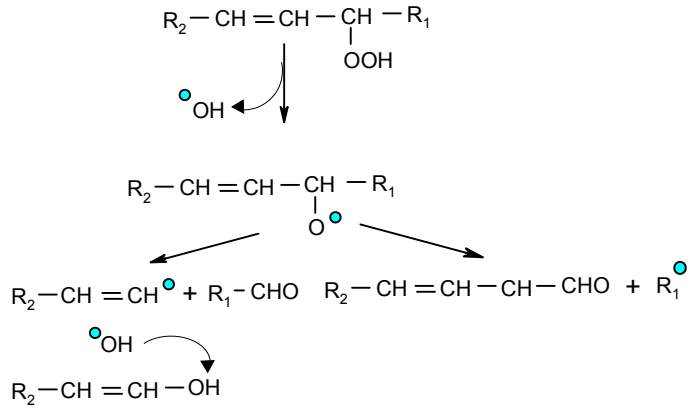

Fig. 1. The scheme of reactions of hydroperoxides decomposition and secondary compounds formation in autiooxidation process of lipids

Based on previously mentioned formation of primary and secondary compounds, the autooxidation of lipids in oil represents one integrated process. These chemical reactions are interrelated and reflect the dynamics of the overall process. The generalized scheme of lipid autooxidation process is presented in this paper (fig.2). It is an attempt to explain the dynamics of process of polyunsaturated fatty acids autooxidation in walnut oil.

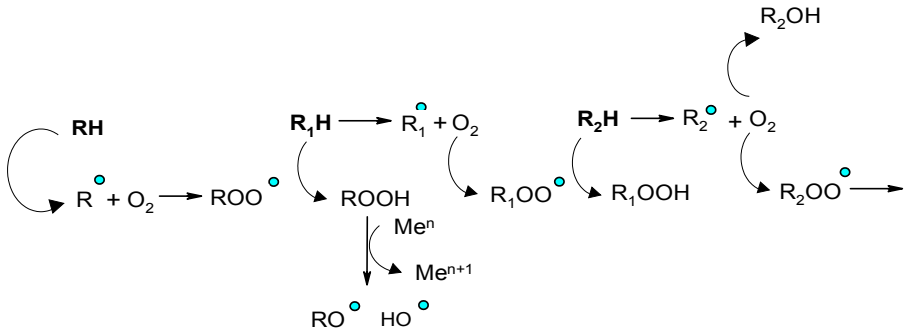

Fig. 2. The scheme of process of polyunsaturated fatty acids autooxidation in walnut oil: RH; R_1H; R_2H - polyunsaturated fatty acids; $ROO^{\bullet}$- peroxide radical, ROOH – hydroperoxide, $R^{\bullet}$, $R_1^{\bullet}$, $R_2^{\bullet}$ – fatty acids radicals, $RO^{\bullet}$; $HO^{\bullet}$ – alkoxy radical and hydroxyl; Me^{n}; Me^{n+1}- metals of variable valence.

According to the presented scheme, the PUFA autooxidation is an irreversible process. The reactions of this process occur consecutively. The hydroperoxides are intermediate compounds in the process of fatty acids autooxidation. They execute the function of substrate in subsequent reactions that lead to their transformation into secondary compounds of oxidation process. The acceleration of hydroperoxides decomposition occurs by involving in reactions the metals of variable valence which brings to formation of new radicals such as alkoxy (RO●) and hydroxyl (HO●). The next stage of the process is activated, on the one hand, for involving in reactions of new fatty acids (R3H) and, on the other hand, accumulation of final secondary compounds. Such oxidative changes of PUFA are continuous [6].

The conformity of the real process of oil oxidation with presented scheme was verified by experimental research. For this purpose the walnuts harvested in 2011 were used. The oil was obtained by pressing the walnut kernel, filtered and placed in sealed bottles for 7 months of storage at + 4…6ºC temperature. During storage the dynamics of oil autooxidation process was studied. There were determined modifications of PUFA content in oil, indices of peroxide value (PV), secondary compounds concentration by2-thiobarbituric acid value or TBA test. The changes of these indices in oil during storage are shown on Figure 3.

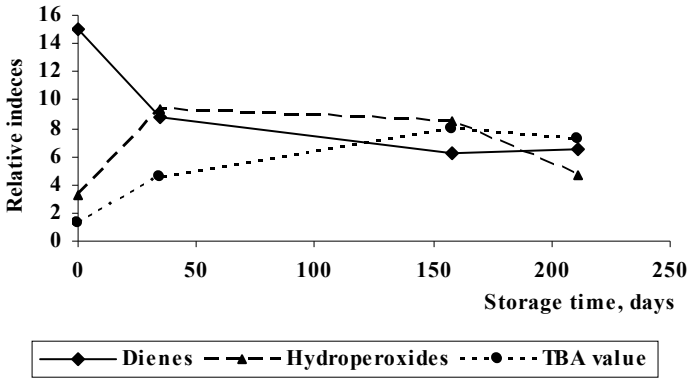

Fig. 3. Dynamics of primary and secondary compounds formation in the autooxidation of linoleic acid in walnut oil during storage

It was found that during storage of oil the PUFA concentration has been permanently decreased at a rapid rate in the first 30 days of storage. The process of hydroperoxides accumulation has been variable. Initial concentration has increased; reached the maximal value and after 150-200 days the hydroperoxides concentration has constantly decreased. Simultaneously, during oil storage the concentration of secondary compounds has been steadily growing.

Character of hydroperoxides concentration changes shows that these compounds are intermediates in PUFA oxidation reaction and represent the substrate of reactions for secondary compounds formation. It was also found that PUFA autooxidation in walnut oil takes place by consecutive reactions in which the hydroperoxides are intermediate compounds. Generally, one of the possible pathways of PUFA oxidation process in oil can be presented as follows:

$$RH \xrightarrow{k_1} ROOH \xrightarrow{k_2} RCHO$$

where: **RH -** polyunsaturated fatty acids,
ROOH - hydroperoxides,
RCHO - aldehydes,
$\mathbf{K_1}$ **-** rate constant of reaction of hydroperoxides formation**,** 1/h
$\mathbf{K_2}$ **-** rate constant of reaction of secondary compounds formation**,** 1/h

The rate constants of reactions depend on several internal and external factors. Complexity of the oxidation process consist in transformation of isomeric configuration cis/ cis of PUFA in configuration cis/trans, trans/cis of hydroperoxides, which generate not only aldehydes and ketones, but alcohols, low molecular weight fatty acids, volatile compounds. Walnut oil contains the natural antioxidant γ-tocopherol in quantities of 250… 500 mg/kg. [7]. However, γ-tocopherol in these quantities is not possessed the necessary activity to reduce the oxidative degradation of walnut oil.

In conclusion**,** walnut (*Juglans regia* L.) is characterized by increased biological and nutritional value. Autooxidation of polyunsaturated fatty acids (PUFA) in walnut oil is an irreversible process. Formation of primary and secondary compounds occurs simultaneously with consecutive reactions in which hydroperoxides are intermediate compounds. Formation of secondary compounds is dependent on the concentration of hydroperoxides.

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