QUALITY CHANGES IN OIL OF WALNUT *(JUGLANS REGIA L.)* DURING STORAGE

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Abstract

The purpose of investigation was to appreciate the changes in quality of walnut oil obtained by cold pressing of kernels. The kinetics of chemical reactions of oil oxidation was studied. The accumulation of the primary and secondary compounds of oxidation process depending on the storage life was analyzed. The oil quality is subjected to permanent changes predominantly in the direction of nutritional quality losses. It has been established that the chemical and biochemical reactions of the oxidation of polyunsaturated fatty acids in the triglyceride structure are irreversible and consecutive. At the same time, it has been determined that during oil storage the reaction of primary compounds (hydroperoxides) formation by oxidation is of zero order, but the reaction rate is variable. At the initial stage of storage (20-21 days) the increasing of reaction rate constant (K, days^{-1}) of hydroperoxides formation from K_3=0.042days^{-1} at t=3°C to K_{60}=1.82days^{-1} at t=60°C has been detected. The activation energy of the reactions was equal to 52.1kj/mol. However, the acidity index of oil increased in limited diapason from 0.12mgKOH/g to 0.19mgKOH/g.

Key words: walnut, oil, quality, change, storage

Walnut (*Juglans regia* L.) is one of the most valuable nuts rich in nutrients and biologically active substances. The kernels of walnuts have immense contents in lipids (55…70%); essential polyunsaturated fatty acids: linoleic, linolenic, docosahexaenoic, including eicosapentaenoic acids (omega-6, ωmega-3); proteins (15…18%); phenolic and mineral substances; microelements (Tatarov P, 2015). It is well known the extraordinary biological importance of walnut kernel compounds, which demonstrate a positive impact on protecting of human physiological state.

New results have been reported concerning to the technology of walnut processing, appreciation of antioxidant capacity of biologically active substances which belong to the group of phenolic compounds, and identification of secondary compounds of lipid oxidation (Baerle A. *et al*, 2016; Vanhanen L.P., Savage G.P., 2006; Decker E. A. *et al*, 2010; Ivanova R. *et al*, 2012). The technologies for obtaining walnut oil by cold pressing of kernels, or extraction of lipids from the walnut kernels with supercritical liquid of carbon dioxide (CO_2) at critical temperatures are wide applied (Louli V. *et al*, 2004).

Besides the methods of oil extracting from walnut kernels, there is the problem of protecting the quality of lipids from oxidative changes, in particular, preventing the oxidation of polyunsaturated fatty acids. Accumulation of the primary and secondary compounds of lipid oxidation leads to losses of nutritional and sensory qualities of kernels and oil (Vanhanen L.P., Savage G.P., 2006; Buranasompoba A. *et al*, 2007; Tatarov P, 2012). At the beginning, the reactions of lipid autooxidation can be initiated in raw materials, in the walnut kernels. Autooxidation of lipids continues during the processing of walnuts and storage of finished products. Therefore, under such conditions, the lipids in the walnut kernels are already oxidized. The causes that direct to these negative consequences occur because of reduced control over the parameters of technological processes during harvesting, primary processing and storage of walnuts (Leahu A. *et al*, 2016; Samotyja U., Malecka M., 2015; Popovici S. *et al*, 2016). The compounds formatted in oxidation process of walnut lipids have an unpleasant taste, known as rancid taste.

The shelf life of walnut oil in dependence on peroxide index values has been examined. It was demonstrated that external factors that have dominant influence on lipid oxidation are: increased temperatures, humidity and oxygen (Nikovska K., 2010; Baerle A. *et al*, 2016; Eliseeva L. *et al*, 2016). The shelf life of walnut oil, reported by different authors, is variable. Vanhanen L.P. and Savage G.P. (2006) reparted

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that the oil quality was acceptable for consumption after four months of storage in the glass bottles at room temperature 24°C in the dark. At the same time other published data (Eliseeva L. *et al*, 2016) denoted that an increase in storage temperature from 20°C to 25°C has been reduced shelf life of walnut oil by 20-30%. The formation and accumulation of peroxides at temperatures of 10…70°C varies within the diapason of 4.9…25.7meq. O_2/kg (Decker E A. *et al*, 2010).

It has been also determined that the rate of oxidation reactions increases as the temperature rises. The acceleration of lipid oxidation rate under temperature rising was analyzed using Arrhenius equation. It was found that the reaction rate constant of peroxide formation in dependence on temperatures corresponds to the zero or first order (n=0: n=1).

Taking into account the variability of walnut oil quality during storage, the purpose of this research was to determine the dynamics of the walnut oil oxidation during storage in dependence on storage temperatures.

MATERIAL AND METHODS

The walnuts oil has been obtained by cold pressing (t=18…20°C) of walnuts varieties Calarasi and Cogilniceanu, harvested in 2014 and 2015. Procedures of walnut oil obtaining in laboratory conditions were following: crushing the walnuts → kernels elimination → kernels grinding → pressing → oil separation → oil packing and storage.

Experimental oil samples were stored in the dark at temperatures of 3°C, 20°C, 40°C and 60°C. Temperature of 3°C was used as etalon temperature. During storage, the modification of acidity index (***Ia***) of oil was determined by Official methods and recommended practices of the AOCS (2009). The acidity index reflects the losses of oil quality due to the hydrolysis of triacylglycerols and accumulation of free fatty acids. The peroxide value (PV) as the index of oxidative stability was appreciated using method officially accepted by the American Oil Analytical Communities (AOAC) (Nielsen S.S., 2010). The changes in oil quality as result of autoxidation and accumulation of hydroperoxides, primary oxidation compounds, were analyzed. Analysis of hydroperoxides formation and decomposition was carried out using the Arrhenius life-stress model (Leahu A. *et al*, 2016; Vanhanen L.P., Savage G.P., 2006; Louli V. et al, 2004). The kinetics of hydroperoxides formation and decomposition reactions corresponds to the Arrhenius equation (van Boekel M.A.J.S., 2008).

$$K = K_A \exp.\left(-\frac{E_A}{RT}\right) \quad (1)$$

where:

K – rate constant of peroxides formation, h^{-1};

K_A - Arrhenius constant;

E - activation energy, kj/mol;

T - Kelvin absolute temperature, ^0K

R – gas universal constant (R=8,31 kj/mol. grad).

The increasing of hydroperoxides concentration leads to loss of quality and reducing of shelf life of the oil. The Arrhenius life - stress model was used to assess the shelf life of walnut oil quality. Based on the Arrhenius equation, through the indirect proportional variation of equation (1) a new relationship could be obtained:

$$1/K = 1/K_A \cdot 1/\exp.\left(-\frac{E_A}{RT}\right) \quad (2)$$

$$L(T) = C\,e^{B/S} \quad (3)$$

where:

L (T) = 1/K - time of oil shelf life at certain temperature (hours, days);

$C = 1/K_A$ - constant parameter indirect proportional to Arrhenius constant, (C>0);

S = T – temperature as stress *factor* of oil storage, ^0K

B = E/R – ratio between activation energy and gas universal constant.

Relationship (3) represents the shelf life of oil. In fact, the numerical value of parameter C in relation (3) is unknown. Following the exclusion of parameter C from relations (4), we obtain the relation (5) which shows the Factor of Reaction Acceleration (A_F). Factor (A_F) is calculated as the exponential ratio between the B/S_U values of stored oil at the etalon temperature and B/S_A values of oil stored at the experimental temperatures.

$$L_{etalon} = C\,e^{B/S_u} \qquad L_{texperimental} = C\,e^{B/S_A} \quad \mathbf{(4)}$$

$$A_F = e^{(B/S_u - B/S_A)} \quad \mathbf{(5)}$$

where:

A_F - acceleration factor of reaction in dependence on oil temperatures;

B/S_u – energetic parameter at etalon temperature;

B/S_A – energetic parameter at experimental temperature.

RESULTS AND DISCUSSIONS

The concentration of primary lipid oxidation compounds are an important feature in assessing the shelf life of walnut oil. The initiation of oxidation reactions and formation of primary compounds (hydroperoxides) are usually observed beginning with the first days of storage after obtaining the walnut oil. The dynamics of the oxidation process has a variable character that occurs through the consecutive irreversible reactions of formation and decomposition of the hydroperoxides (Tatarov P., 2012). The kinetic model of consecutive reactions for formation and decomposition of hydroperoxides which directly influence on lipids quality is presented in *figure1*.

$$n\,RH + x\,O_2 \xrightarrow{k} a\,ROO^{\bullet} \xrightarrow{k_1} a\,ROOH \xrightarrow{k_3} (a-\triangle)\;ROOH \xrightarrow{k_4} b\,ROOH \ldots \xrightarrow{k_n} P.secondary$$

$$\downarrow k_2 \qquad\qquad \downarrow k_5$$

$$\triangle\,ROOH \qquad\qquad \triangle\,ROOH$$

Figure 1 **General scheme of kinetic model of formation and decomposition of hydroperoxides in processes of lipid oxidation: RH- native lipids; ROO•; ROOH – peroxides and hydroperoxides; k ; k_1; k_2 ; k_3. . . k_n – rate constants of reactions for formation and decomposition of hydroperoxides; *n, a*, (a -△) , b – stoichiometric coefficients; P. secondary – secondary compounds of lipids oxidation.**

According to the kinetic model of irreversible consecutive reactions (*figure 1*), at the initial stage of oil storage, the reaction of hydroperoxides formation takes place with the rate constant of this reaction k_1, which leads to the accumulation of the hydroperoxides in excessively critical concentrations (a ROOH). Excess of hydroperoxides (Δ ROOH) is unstable and decomposes with the rate constant k_2. In continuation the equilibrium of compounds formed through the oxidation moves in the direction of hydroperoxides formation extension with the reaction rate constant k_3. It has been found that during the oil storage one or more critical hydroperoxides concentrations are formed. Their excess decomposes, but the dynamics of hydroperoxides concentration increasing is maintained.

The phenomenon of formation and decomposition of hydroperoxides excess in walnut oil has a legislative character and carries out at various storage temperatures. The dynamics of hydroperoxides formation processes and the decomposition of their excess in dependence on storage temperature are shown in *figure 2*.

According to the mode of hydroperoxides modification during walnut oil storage at t=3°C (*figure 2 a*), after three days the peroxide excess was in quantities of 1.15µmol/kg, the curve had one pick. During 5 days the hydroperoxides concentration decreased to 0.82µmol/kg. In next days of storage, the increasing of hydroperoxides concentration is maintained. At temperature 20°C of oil storage (*figure 2 b*), the curve of hydroperoxides concentration had two peaks (after 5^{th} and 11^{th} days of storage). The most profound changes in hydroperoxides contents were found at temperatures of 40 and 60°C (*figure 2 c, d)*. After 9^{th} and 11^{th} days of storage, the hydroperoxides concentration suddenly increased and reached quite high critical concentrations. The decomposition of hydroperoxides excess was fast and took place only 2…3 days. This fact indicates that at high temperatures the hydroperoxides are not stable and very easily decomposed by transforming into various secondary compounds of walnut oil oxidation.

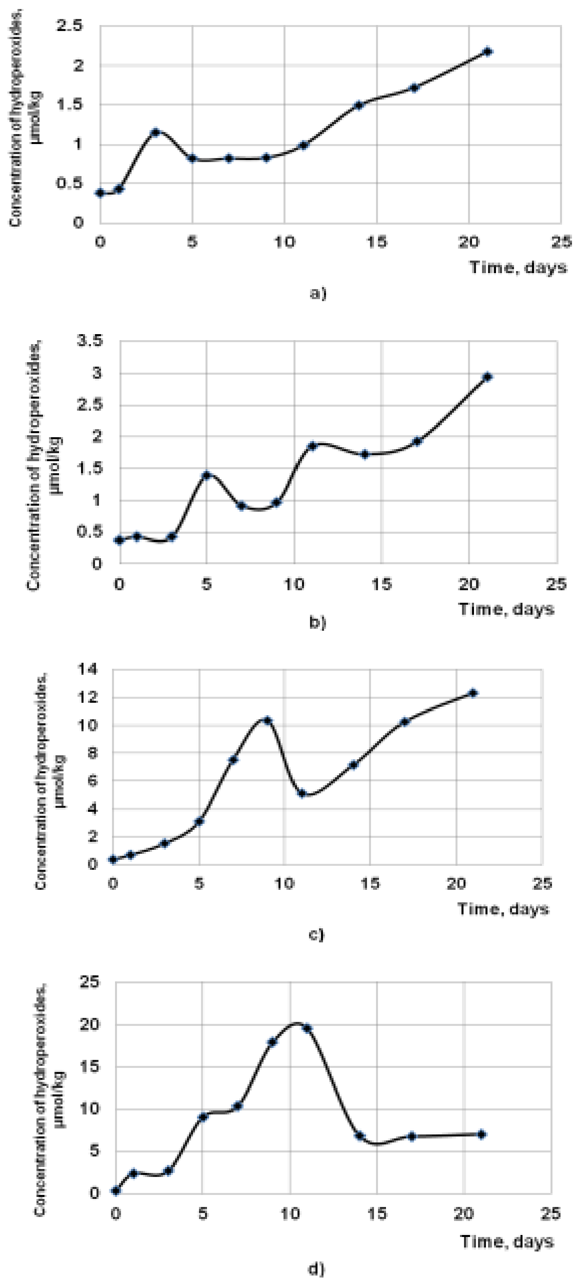

Figure 2 **Dynamics of formation and decomposition of hydroperoxides excess in initial stage of oil storage at different temperatures: a) t =3^{0}C; b) t=20^{0}C; c) t=40^{0}C; d) t=60 ^{0}C**

The experimental data processed according to the kinetic model, allowed to find that the formation of hydroperoxides in oil during storage corresponds to the Arrhenius equation (1) with reaction order equal to zero (n=0). The rate constant (K, days^{-1}) of hydroperoxides formation depends directly on temperature (*table 1).* The activation energy of reaction for hydroperoxides formation was determined (E=52.1kj/mol).

Table 1

Constants of reaction rate for hydroperoxides formation

Temperature, ^{0}C /(T^{0}K)	Constant of reaction rate, *K,* days^{-1}	ln K	1/T
3 / (276)	**0.042**	**-3.17**	**0,0036**
20 / (293)	**0.120**	**- 2,08**	**0,0034**
40 / (313)	**0,560**	**-0,53**	**0,0032**
60 / (333)	**1,820**	**0,6**	**0,0030**

The factor of reaction acceleration of hydroperoxides accumulation in the walnut oil was determined by application of relationships (*3, 4 and 5*). The reactions for hydroperoxides formation at temperature of 20°C were accelerated by 3.7 times in comparison with the same index at 3°C, the etalon temperature of storage. The acceleration factors of reactions for hydroperoxides formation at 40°C (A_{F40}) and 60°C (A_{F60}) were equal to 14.6 and 50.0 times, respectively.

The modifications of walnut oil acidity were analyzed simultaneously with the studying of hydroperoxides formation. It has been found that the hydrolysis of triacylglycerols and accumulation of free fatty acids was quite slow and not depended on storage temperatures. During the first 21 days of walnut oil storage at various temperatures of 3....60°C, the acidity index demonstrated a constant increasing in limited diapason from 0.12mgKOH/g to 0.19mgKOH/g. These non significant changes in acidity lead to accumulation of small concentrations of free fatty acids, especially polyunsaturated fatty acids. Therefore, as results could be created some favorable conditions to prevent the formation of compounds with low molecular weight such as aldehydes, ketones, and the secondary products formation of oxidation.

CONCLUSIONS

This study showed that the dynamics of lipid oxidation processes in walnut oil had a variable character. The oxidation processes realize through the formation and decomposition of hydroperoxides excess and could be described by equation of Arrhenius life - stress model.

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