EVOLUTION OF FATTY ACIDS AND PEROXYDES CONTENT IN WALNUT OIL (*Juglans regia* L.) DURING STORAGE

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INTRODUCTION

Walnut oil (*Junglas regia* L.) is high-quality oil due to its important physical and biochemical properties. In addition, walnut oil has significant economical value and medicinal importance for human health because of its chemical composition. Walnut oil contains approximately 7% saturated, 20% monounsaturated and 73% polyunsaturated fatty acids [8, 13].

The cold-pressing procedure involves neither heat nor chemical treatments, and it is becoming an interesting substitute for conventional practices because of consumers' desire for natural and safety food products. Cold pressing is simple, ecological and does not require much energy. Such factors as geographical location, species and processing technique may influence the final chemical composition of oils [10].

A number of experiments have been carried out on the oxidation stability of walnut oil. Temperature, light, moisture and exposure to oxygen have been found to be the main contributing factors to oxidation [5, 9]. Stark et al. found that walnut oil stored at room temperature in the dark, in sealed bottles, showed only small rises in peroxide values after four months of storage and remained an acceptable product in terms of its organoleptic properties [11].

Oxidation of walnut lipids is linked to the appearance of unpleasant odors and flavors. Tocopherol isomers provide some protection against oxidation. Walnut oil, which is cold pressed from the dried walnuts, has a strong and distinctive walnut flavor [6]. If the cold pressed walnut oil is to be effectively used in the food industry and human nutrition, it is important to determine how long it can be stored for without any deterioration. *The objective of this study* was to investigate the changes of fatty acids and peroxides content in dependence of applied manufacturing technology and storage time of walnut oil.

1. MATERIALS AND METHODS

1.1. 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 essentially following the procedure presented in figure 1.

Figure 1. Extraction procedure of walnut oil.

In particular, walnut oil expression was carried out at 20±2 °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.

1.2. Chemicals

Ethanol (99.9%), chloroform, 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.

1.3. Acid Value

Acid value was determined by potassium hydroxide titration as described in AOCS Official Method Cd 3d-63 (AOCS, 1999). The method was based on the number of milligrams of potassium hydroxide necessary to neutralize the free acids in 1 gram of oil sample. Results were expressed as milligram of potassium hydroxide per gram of walnut oil sample [1].

1.4. Peroxide Value

Oxidation rate was studied by determination of the peroxide value (PV). This was determined according to AOCS Official Method Cd 8-53 (AOCS, 2003). Peroxide value was expressed as millimoles peroxide per kilogram of walnut oil [2].

1.5. Statistical analysis

Variance analysis of the results was carried out by least square method with application of coefficient Student. Differences were considered statistically significant if probability was greater than 95% (p-value<0.05). All assays were performed by triplicate at room temperature. Experimental results are expressed as average ± SD (standard deviation).

2. RESULTS AND DISCUSSION

Today, vegetable oils are developed and subsequent production controlled with knowledge of their composition, structural and functional properties, and the expected reactions obtained through the application of scientific research. In this work walnuts as a perspective and valuable raw material were proposed for oil extraction. Prior to chemical analysis, the walnuts were manually cracked and shelled and then milled into a fine powder in an electric mill (Braun, Germany). Oil was extracted using cold pressing with an electrical lab press PSU - 125. General scheme of walnut oil extraction procedure is given in figure 2.

Walnuts → Cracking → Grinding → Oil pressing → Oil collection → Dehydration → Antioxidant incorporation → Nitrogen treatment of oil → Oil filtration → Oil Packaging → Oil storage in nitrogen.

Figure 2. Experimental scheme of cold pressed walnut oil extraction

Hydrolysis of oils is a major cause of their deterioration, and hydroperoxides formed by the reaction between oxygen and the unsaturated fatty acids are the primary products of this reaction. Hydroperoxides have no flavor or odor but break down rapidly to form aldehydes, which have a strong, disagreeable flavor and odor (figure 3).

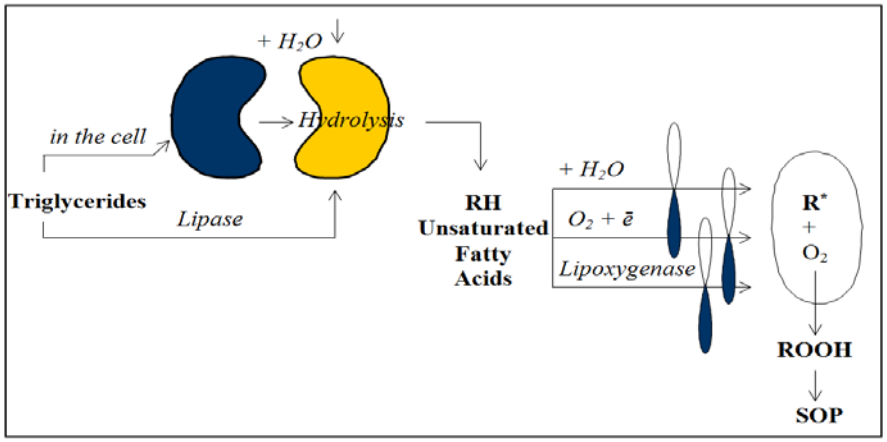


Figure 3. Autooxidation processes and enzymatic hydrolysis of the walnut oil.

The peroxide concentration is a measure of oxidation or rancidity in its early stages. But peroxide determination does not provide a full and unqualified evaluation of oils flavor because of the transitory nature of peroxides and their breakdown to nonperoxide materials.

Progress in the utilization of oils for the production of useful products is dependent upon a thorough knowledge of the characteristics of the raw materials, the changes effected by each process, and the requirements of the individually prepared food product. Physical, chemical, and performance analyses are the tools available to fats and oils processors for the purchase of raw materials, development of new products, and evaluation of the products produced. Statistical analysis of experimental data obtained for acid values of walnut oil during storage are given in table 1.

Table 1. Statistical analysis of the experimental data for walnut oil acidity

No	Parameters	Calculation Model	Calculation Value
1.	**Acid number of the experimental sample of walnut oil**	The average value of acid number: $\bar{X}$	$\bar{X}$ = 0.38 mg KOH/g oil
		Square deviation: $\sigma = \sqrt{\frac{\sum (X_m - x)^2}{n}}$	σ = 0.07
2.	**Error (ξ) reflects the limit in which the obtained AN value corresponds to real content of walnut oil acidity**	$\xi = \frac{\sigma}{\sqrt{n}}$	$\xi = \frac{0.07}{\sqrt{9}} = 0.023$
3.	**Confidence limit, 95% probability. Coefficient Student is t = 3 (from special tables)**	Square deviation σ = 3: $^{*}L_{max} = \bar{X} + 3\times\xi$ $^{**}L_{min} = \bar{X} - 3\times\xi$	$L_{max} = 0.38 + 3\times0.023 = 0.43$ $L_{max} = 0.38 - 3\times0.023 = 0.31$
4.	**Acid number of the walnut oil after 180 days of storage**	$X = \bar{X} \pm \sigma$	$X = 0.38 \pm 0.07$

$^{*}L_{max}$ – upper limit of acidity;
$^{**}L_{min}$ – lower limit of acidity.

Figure 4 shows the effect of storage time on evolution of acidity of walnut oil sample. The initial acid number of fresh walnut oil sample was 0.39 mg KOH/g oil. Changes in acid number were in the range from 0.39 to 0.53 mg KOH/g oil during 180 days of storage.

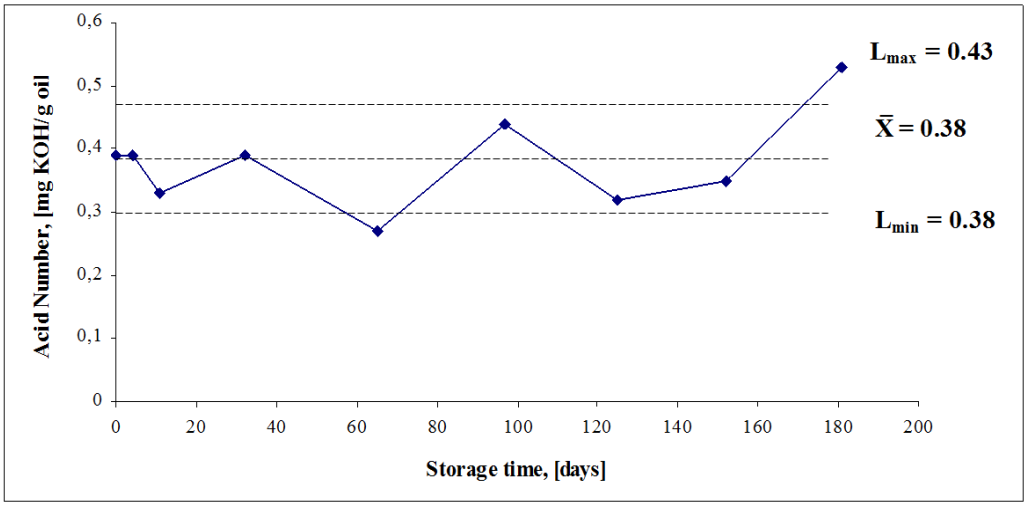
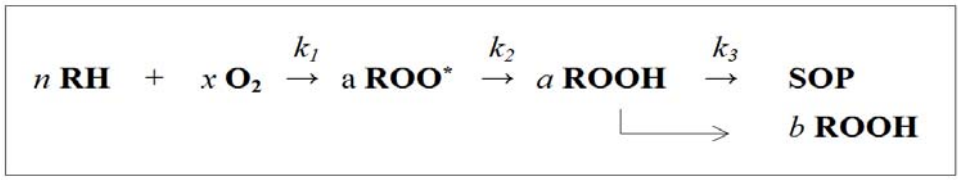


Figure 4. Numerical value of acid number for walnut oil sample
(X = 0.38 ± 0.07, $\bar{X}$ - average value of acid number)

Oxidation is a radical chain reaction. After an induction period, it may run very fast under certain circumstances. A chemical attack on the alkyl group is followed by a chain reaction, resulting in a hydroperoxide group (-OOH) in the chain. The chain reaction is started by peroxy-, alkoxy- and alkyl-radicals. The chain reaction proceeds by reaction with oxygen or RH. It is accelerated by branching of the chain.The chain reaction ends by combination of two radicals (figure 5).



Fifure 5. The kinetic model of the lipid oxidation process (**R**H – native lipids; **ROO*** - peroxides, hydroperoxides, primary oxidation products; **SOP** – secondary oxidation products; $\mathbf{k_1}$, $\mathbf{k_2}$, $\mathbf{k_3}$ – reaction rate constants).

It is well known, that primary oxidation products of oils are peroxides, which can be transformed induced by environmental factors such as humidity, temperature and oxygen content into secondary oxidation products such as aldehydes, ketones, oxidized fatty acids and other compounds. The oil quality data from walnut kernels pressed at cold indicated variations for all parameters evaluated, including peroxide values. It can be explained, that peroxides represent unstable intermediate compounds of lipid oxidation process.

The hydroperoxides formed react further to aldehydes, ketones and fatty acids, all of which represent secondary oxidation products and negatively influence on oil quality. Hydroperoxides content is determined by the reaction rate constants k_1/k_3. Secondary oxidation products can be determined as a function of primary oxidation products: **SOP** = f (**ROOH**).

Evolution of peroxides content in walnut oil has a variable character. Being primary products of oxidation process, the peroxides decomposition is observed up to a critical level during storage. Further, the rate of peroxides formation predominates in comparison with the rate of degradation. Therefore, the evolution of peroxides content is going in two stages: stage of formation and increase of peroxides content and stage of peroxides degradation. Overall, during oil storage process, the reaction rate of peroxides formation dominates, which generally leads to increasing of oxidation degree of walnut oil. For example, changes in peroxide concentration are shown in figure 6.

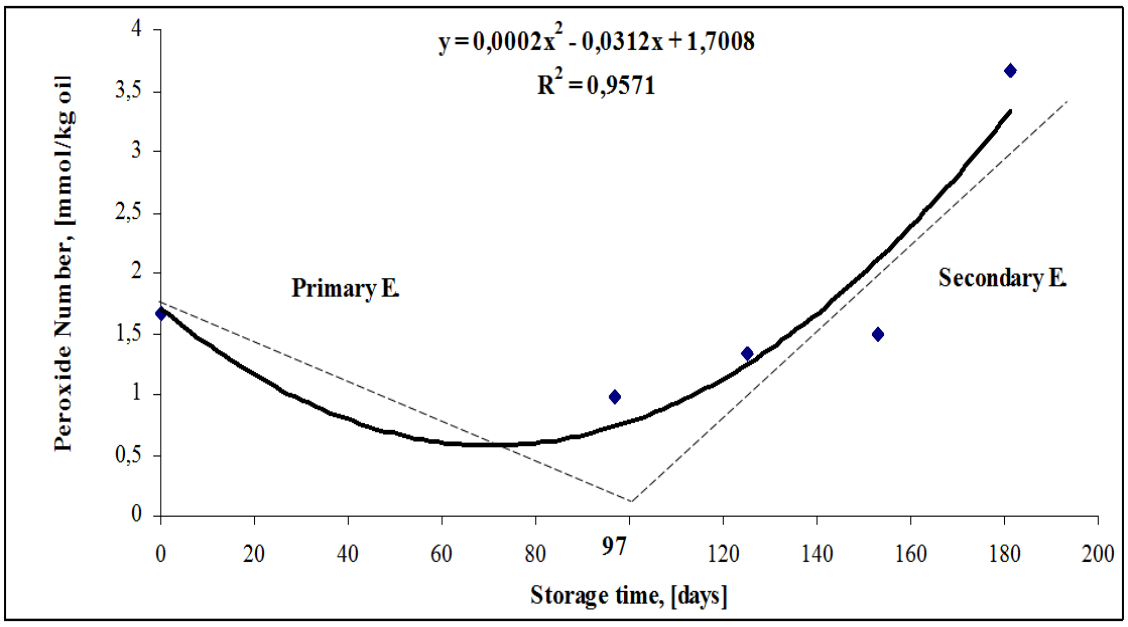


Figure 6. Dynamic changies of the peroxides content in walnut oil

Figure 6 shows the effect of storage time on primary oxidation products accumulation in walnut oil sample. The initial peroxide number of fresh walnut oil sample was 1.66 mmol/kg oil. Changes in peroxide concentration can be described in two steps. Changes in peroxide content were in the range from 1.66 to 0.99 mmol/kg walnut oil in first period of storage till 97 days. Second step includes storage time from 97 to 180 days. Peroxides concentration increases from 0.99 to 3.66 mmol/kg oil during this step. Rate of peroxides formation is 2.25 times more than the rate of their degradation.

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 manufacturing technology and storage time on the intensity of hydrolysis processes and primary oxidation products accumulation in cold pressed walnut oil. It was demonstrated that walnut oil retains acceptable quality after 180 days of storage. It is important to underline, that obtained results of this study are intermediate and could help authors to describe the scheme of walnut oil oxidation process and also to elaborate improved technology for walnut oil stabilization.

ACKNOWLEDGEMENTS

This work was benefited from support through the 11.817.04.40A 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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Recommended for publication:26.08.2014.