OXIDATIVE STORAGE STABILITY OF COLD PRESSED WALNUT OIL

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Abstract. *The aim of the work was to evaluate the influence of storage time on quality indices related to walnut oil oxidative stability. Oxidative storage stability was assessed by measuring the primary and secondary oxidation products in cold pressed walnut oil samples immediately after pressing up to 30 weeks of storage. Primary oxidation products of walnut oil samples were evaluated by measuring peroxide value (PV) and conjugated dienes content (CD). Secondary oxidation products of walnut oil samples were evaluated by measuring p-anisidine value (p-AV) and 2-thiobarbituric acid value (2-TBA). Besides, the walnut oil has been chemically characterized using UV/Vis spectra. This method allowed the determination of the main compounds present in walnut oil. Obtained data should help to describe oxidation mechanism of cold pressed walnut oil. However it is necessary to note that these data are not enough to make a final conclusion about the behavior of walnut oil during storage time.*

Key Words: walnut oil, primary and secondary oxidation products, storage stability, shelf life.

I. Introduction

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 safe food products. The consumption of new and improved products such as cold-pressed oils may improve human health and may prevent certain diseases [15].

Over the last few years, increased interest in cold-pressed plant oils has been observed as these oils have better nutritive properties than those after refining. Cold pressing is simple, ecological and does not require much energy. The disadvantage of this process is low productivity and difficulties in obtaining a product of constant quality [15]. Such factors as geographical location, species and processing technique may influence the final chemical composition of plant oils [5, 15].

Walnut kernel (*Juglans regia* L.) 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 [11]. 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 [15]. The ratios of these to each other are important to the economic and nutritional value of the nut. Lower linoleic and linolenic acid content oils may have a longer shelf life, and monounsaturated fatty acids may be more desirable because of their potential health benefits [7, 12]. The high linoleic acid content of walnut oil makes it undesirable for use in cooking as it is more prone to charring. But walnuts also possess numerous polyphenolic compounds with potent free radical scavenging ability and therefore, capable to break the propagation chain of lipoperoxidation [13]. Walnuts are a perfect ingredient in a variety of breads, muffins, cakes, and biscuit.

High levels of polyunsaturated fatty acids make walnut oil prone to oxidation and may mean that oil has a limited shelf-life. Some 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 [6, 11, 13]. It was 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 sensory properties.

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 meat of dried walnuts, has a strong and distinctive walnut flavor. It is generally used as a flavoring for baked goods and for some sauces. It can provide a bold flavor to salad dressing or it can be added to mildly flavored oils to create a subtle taste [8].

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. This storage trial was set up to determine the storage life of walnut oil. The quality of cold pressed walnut oil was assessed by measuring the primary and secondary oxidation products accumulation during 30 weeks of storage.

II. Materials and methods

2.1. Plant material

Walnut (*Juglans regia* L.) healthy fruits were manually collected in October 2011 in Chisinau, Central Moldova. The walnut fruits were stored at room temperature, packed in bags in order to protect them from light until extraction.

2.2. Walnut 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 1.

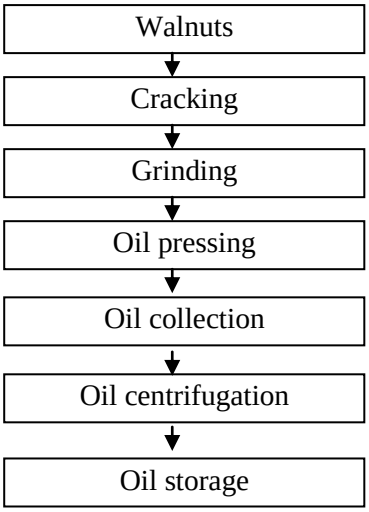

Figure 1. *Experimental scheme of cold pressed walnut oil obtaining*.

Extracted oil sample had a light yellow colour and very characteristic nutty flavour. Oil sample was collected, centrifuged and stored in dark polypropylene tube at 18±2 ^{0}C until analysis.

2.3. Chemical and reagents

Ethanol (99.9%), methanol (99%), potassium hydroxide, phenolphthalein, potassium iodide, sodium thiosulfate ($Na_2S_2O_3 \times 5H_2O$) and starch were supplied by Eco-Chimie (Chisinau, Moldova). Chloroform, 1-butanol and glacial acetic acid were purchased from Sigma-Aldrich. 2-thiobarbituric acid (4,6-dihydroxy-2-mercaptopyrimidine) and p-anisidine were obtained from Alfa Aesar. All the chemicals used were of HPLC or analytical grade. Distilled water was used throughout.

2.4. 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 [3].

2.5. UV/Vis spectra analysis

The UV/Vis spectra were recorded using UV/Vis spectrophotometer HACH-LANGE DR-5000 (Germany) in the range of 200 - 750 nm using quartz cuvette 10×10 mm. Walnut oil sample was dissolved in isooctane. There were identified the maxima wavelengths specific for different compounds.

2.6. Conjugated dienes & trienes

The experiment was carried out according to the AOCS Official method Ti la 64 (AOCS, 1993) with minor modifications [1, 9]. Approximately 0,02 g of walnut oil was placed into a 25 ml volumetric flask. The sample was dissolved in chloroform, brought to volume and mixed thoroughly. Absorbance of the dissolved walnut oil was measured in UV/Vis spectrophotometer HACH-LANGE DR-5000 (Germany) at 232 nm and 270 nm using quartz cuvette 10×10 mm. Results were expressed in micromole conjugated dienes/trienes per gram of walnut oil.

2.7. p-Anisidine Value

The p-anisidine value of walnut oil samples was measured following the methodology described in AOCS Official Method Cd 18-90 (AOCS, 1997) [2, 10]. This value was determined by the amount of aldehydes (principally 2-alkenals and 2,4-dienals) in walnut oil samples after reaction in an acetic acid solution of the aldehydic compounds in the walnut oil and the p-anisidine mixture. Absorbance of the samples was measured in UV/Vis spectrophotometer HACH-LANGE DR-5000 (Germany) at 350 nm using quartz cuvette 10×10 mm.

2.8. 2-Thiobarbituric acid Value

The 2-thiobarbituric acid was determined according to the AOCS Official Method Cd 19-90 (AOCS, 2009) [4]. The method is based on the spectrophotometric quantitation of the pink complex formed after reaction of one molecule of malondialdehyde (MDA), product of oxidation, with two molecules of 2-thiobarbituric acid added to the walnut sample.

2.9. 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).

III. Results and discussion

Today, fats and oils products are developed and subsequent production controlled with a knowledge of their composition, structural and functional properties, and the expected reactions obtained through the application of scientific research. Progress in the utilization of fats and 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.

In this work walnuts as a perspective and valuable raw material were proposed for oil extraction. Walnuts chemical composition is presented in figure 2.

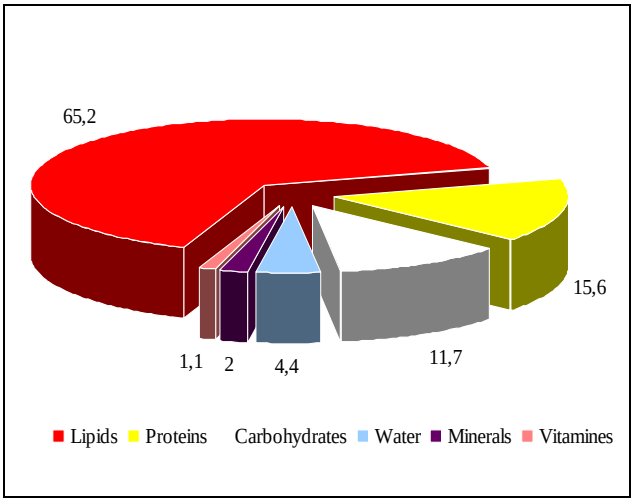

Figure 2. *Chemical composition of walnuts*

It is important to note, that walnut lipids contain high amount of polyunsaturated fatty acids: linoleic (18:2) and linolenic (18:3), which contribute to its biological and nutritional value. But on the other side, this makes it prone to oxidation.

The health benefits of walnut oil are attributed to its chemical composition. Walnut oil contains approximately 7% saturated, 20% monounsaturated and 73% polyunsaturated fatty acids [16]. These high levels of polyunsaturated fatty acids make walnut oil prone to oxidation and may mean that oil has a limited shelf-life, i.e. nutritional and organoleptic changes due to losses of essential fatty acids and formation of volatile compounds from subsequent degradation of hydroperoxides.

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:

$$R\text{-}OO^{\bullet},\ R\text{-}O^{\bullet},\ R^{\bullet} \qquad (1)$$

The chain reaction proceeds by reaction with oxygen or RH:

$$R^{\bullet} + O{=}O + R\text{-}OO^{\bullet} \qquad (2)$$

$$R\text{-}OO^{\bullet} + R\text{-}H + R\text{-}OOH + R^{\bullet} \qquad (3)$$

$$R\text{-}O^{\bullet} + R\text{-}H + R\text{-}OH + R^{\bullet} \qquad (4)$$

It is accelerated by branching of the chain:

$$R\text{-}OOH + R\text{-}O^{\bullet} + {}^{\bullet}OH \qquad (5)$$

$$2\ R\text{-}OOH + R\text{-}O^{\bullet} + R\text{-}OO^{\bullet} \qquad (6)$$

The chain reaction ends by combination of two radicals. It is well known, that this negative reactions can be stopped by the antioxidants.

In this study, UV/Vis spectra of the investigated walnut oil were analyzed in the wavelength range of 200 - 750 nm (Figure 3).

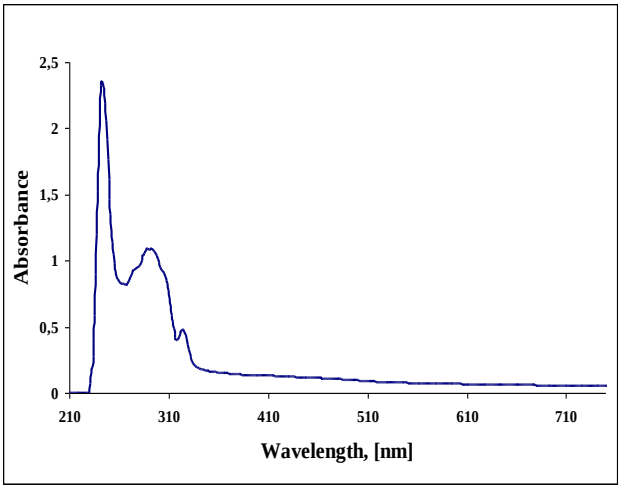

Figure 3. *UV/Vis spectra of cold pressed walnut oil*

From identification of bioactive compounds by UV/Vis spectra, it clearly revealed that walnut oil contains juglone (245 nm), tocopherol (295 nm) and retinol (325 nm). Probably, these compounds can play protective role in oxidation process of walnut oil.

Oxidation of lipids 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.

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. Therefore, conjugated dienes and trienes content were evaluated parallel with peroxides concentration. Experimental results are presented in figure 4.

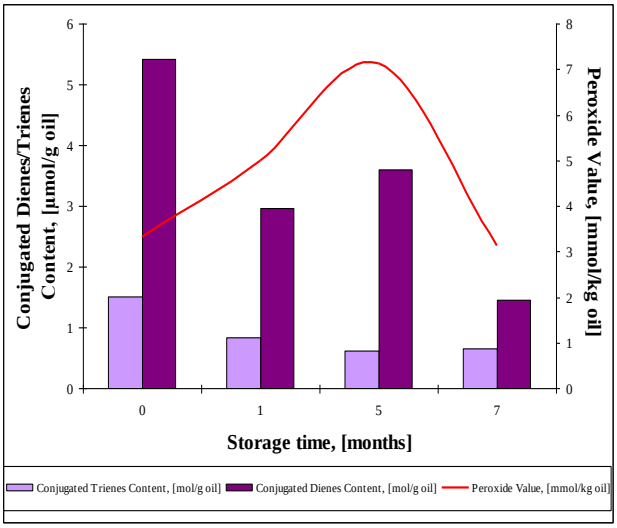

Figure 4. *Primary oxidation products accumulation in walnut oil during storage*

It is well known, that primary oxidation products of vegetable 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. These trends were registered for conjugated dienes content of the investigated walnut samples

The hydroperoxides formed react further to aldehydes, ketones and fatty acids, all of which represent secondary oxidation products and nefatively influence on oil quality. Anisidine value is a measure of secondary oxidation (the amount of α and β unsaturated aldehydes), and therefore is useful in determining the quality of oils and the efficiency of processing procedures. Changes in secondary oxidation products accumulation were expressed by p-anisidine and 2-thiobarbituric acid values. Obtained experimental results of p-anisidine values in walnut oil samples are shown in figure 5.

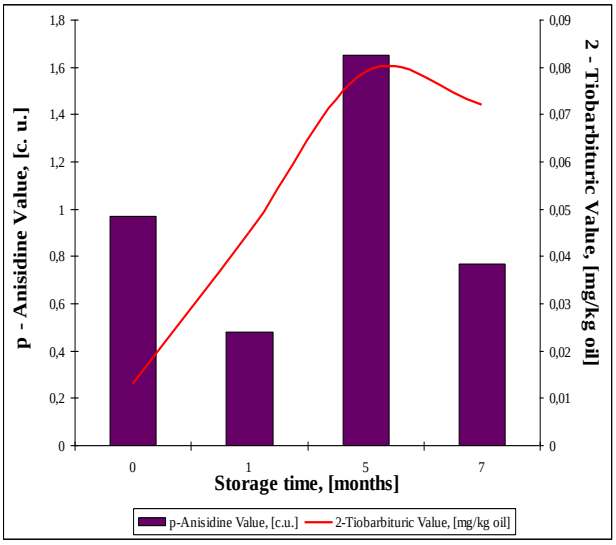

Figure 5. *Secondary oxidation products accumulation in walnut oil during storage*

As can be seen from figure 5, there were evaluated 2-tiobarbituric value of walnut oil sample as an important indicator of secondary oxidation products accumulation. Significant increase of p-anisidine and 2-tiobarbituric values were registered after 5 months of walnut oil storage.

For better understanding of obtained experimental results there was calculated total oxidation value (TOTOX) for walnut oil sample. TOTOX value represents the sum of primary (peroxides) and secondary (aldehydes) oxidation products accumulation in vegetable oils. To compare the influence of storage time on the oxidation process of cold pressed walnut oil, changes in TOTOX value are indicated in figure 6.

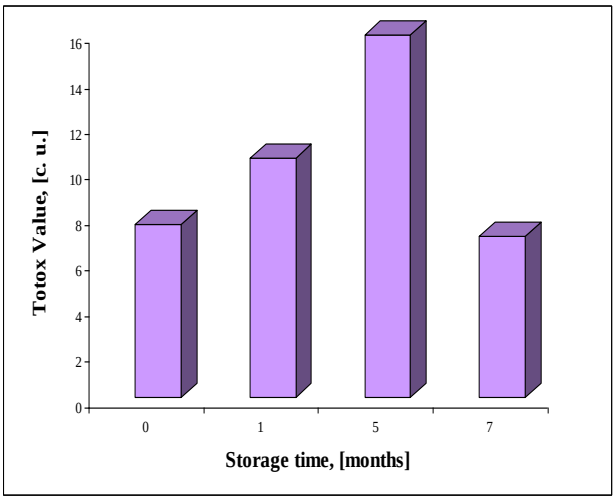

Figure 6. *Changes in TOTOX value of walnut oil during storage*

TOTOX value represents the sum of primary (peroxides) and secondary (aldehydes) oxidation products accumulation in vegetable oils. It is obvious, that increasing of storage time of cold pressed walnut oil leads to an increase of the TOTOX value.

IV. 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 vegetable oil. The results of this research showed the influence of storage time on the intensity of primary and secondary oxidation products accumulation in cold pressed walnut oil. It was demonstrated that walnut oil retains acceptable quality after 7 months 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.

Acknowledgments

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