EFFECT OF NITROGEN TREATMENT ON QUALITY OF COLD PRESSED WALNUT OIL

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Abstract: In this work nitrogen treatment experiment was set up to determine the intensity of oxidation products accumulation in cold pressed walnut oil. The influence of the nitrogen treatment was assessed by measuring the primary and secondary oxidation products in oil samples immediately after treatment up to the 30 weeks of storage. Primary oxidation products of walnut oil samples were evaluated by measuring peroxide value and conjugated dienes content. Secondary oxidation products of walnut oil samples were evaluated by measuring p-anisidine value and 2-thiobarbituric acid value. These data should help us to describe oxidation mechanism of walnut oil and to recommend optimal conditions for walnut oil stabilization. However it is necessary to note that these data are not enough to make a final conclusion about the behavior of walnut oil after nitrogen treatment during storage. Currently we obtained preliminary data about accumulation of primary and secondary oxidation products in walnut oil.
Keywords: walnut oil, nitrogen, oil stabilization, storage, quality characteristics.

1. Introduction

Walnut is a crop of a high economic interest for the food industry [8]. The walnut is highly appreciated nut because of its unique organoleptic characteristics, high level of 18:1, 18:2 and 18:3 fatty acids and hypocholesterolemic, and antihypertensive effects. Walnuts are utilized as shelled whole kernels or ground kernels being used as ingredients of many foodstuffs such as bakery products and confectionary as well as flavoring agents in beverages and ace-cream [9].

Walnut kernels have a lipid content of 65% of which 73% consists of polyunsaturated fatty acids, although values do vary between cultivars. Walnut kernels are consumed fresh or toasted, alone or in other edible products. Among the by-products of the walnut industry the oil has not yet obtained popularity, although it has been demonstrated that its consumption has a lot of nutritional benefits. The consumption of walnut oils from different geographic origins has been reported extensively. Their major constituents are tryglicerides, in which monounsaturated (oleic acid mainly) and polyunsaturated fatty acids (linoleic and α – linolenic acids) are present in high amounts. The presence of other bioactive minor components, such as tocopherols and phytosterols, has been also documented. Walnut oil is appreciated as specialty oil also because of its characteristic flavor, aroma and health benefits [7 - 9].

A major goal in walnut production is to find an appropriate method to stabilize lipids from walnut kernels. The oxidative stabilization of walnut oil is imperative to determine the feasibility of bringing it into commercial production. A number of storage experiments have been carried out on the storage of in-shell walnuts, walnut kernels and walnut oil [5]. Mexis et al. (2009) investigated the effect of packaging and storage conditions on quality of raw shelled walnuts. It was established, that the effect of parameters investigated are followed the sequence: temperature > degree of O_2 barrier > lighting conditions. Savage et

al. (2001) reported that fresh walnut kernels could be stored in-shell at room temperature (mean 24 ^{0}C) for up to 12 months with only modest rises in peroxide levels. Stark et al. (2000) found that walnut oil stored at room temperature (mean 24 ^{0}C) in the dark, in sealed bottle, showed only small rises in peroxide levels after four months storage and remained an acceptable product in terms of its organoleptic properties.

However, the addition of this type of oil in processed food is not usual due to its low stability. To avoid this stability problem, researcher Calvoa et al. (2012) investigated the possibility to introduce walnut oil to the processed food as its microencapsulated form. As result of this research work, walnut oil was protected while food processing and released after consumption by the digestive process. Tsamouris et al. (2002) extracted and analized total lipids of walnut oil by high performance thin layer chromatography (HPTLC)/FID and GC-MS. Unsaturated fatty acids were found as high as 85%, while the percentage of the saturated fatty acids was found 15%. On the base of this data, researchers decided to prepare liposomes, which were characterized as new formulations. As authors declare, these formulations may have future applications for encapsulation.

In view of the fact, that walnut oil is to be effectively used in the food industry and human nutrition, it is extremely important to determine how long it can be stored for with out any deterioration. This storage trial was set up to determine the storage life of walnut oil with particular reference to the walnut oil treated with nitrogen. The effectiveness of the nitrogen treatment was assessed by measuring the primary and secondary oxidation products accumulation in the walnut oil during storage up to a total duration of 30 weeks.

2. Materials and methods

2.1. Samples and extraction of oil

Walnuts (*Juglans regia* L.) were collected from the faculty yard Chisinau, the Republic of Moldova in October 2011. Walnuts were cracked and shelled. Oil extraction was carried out with mechanical press. Immediately after extraction oil was filtered, treated under different conditions and used for experiments.

2.2. Chemicals

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.

2.3. 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 [1].

2.4. Conjugated dienes

The experiment was carried out according to the AOCS Official method Ti la 64 (AOCS, 1993) with minor modifications [2, 10]. 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 236 nm using quartz cuvette 10×10 mm. Results were expressed in micromole conjugated dienes per gram of walnut oil.

2.5. 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) [3, 11]. 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.

2.6. 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.

2.7. 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).

3. Results and discussion

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 [12].

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 [9].

In order to prevent high level of walnut oil oxidation was proposed technological treatment of walnut oil immediately after oil extraction. In this study the benefits of combined treatment with temperature and nitrogen of walnut oil was investigated. Quality of walnut oil samples was characterized by determination of peroxide value (PV), conjugated dienes content (CD), p-anisidine value (p-AV) and 2-thiobarbiturc value (2-TBA). Obtained values of PV are shown in figure 1.

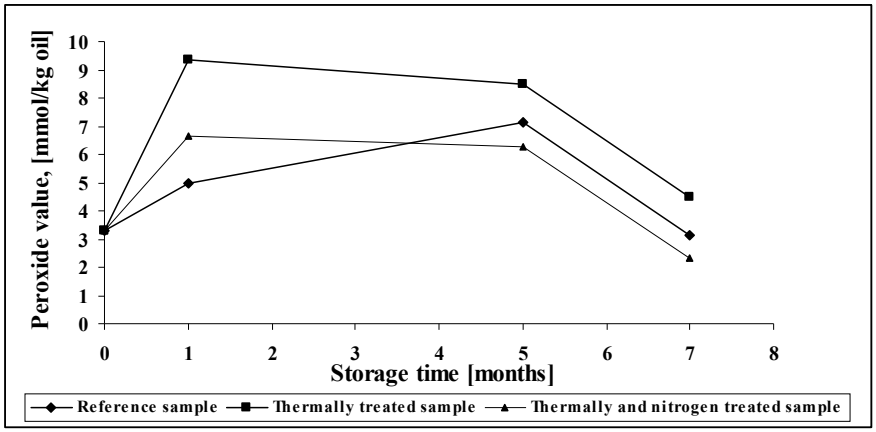

Fig. 1. Changes in peroxide value of walnut oil samples as a function of treatment conditions and storage time

The oil quality data from walnut kernels pressed at cold indicated significant variations for all parameters evaluated. The most outstanding feature was the decrease of PVs with increasing storage time of all investigated oil samples.

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 (Figure 2).

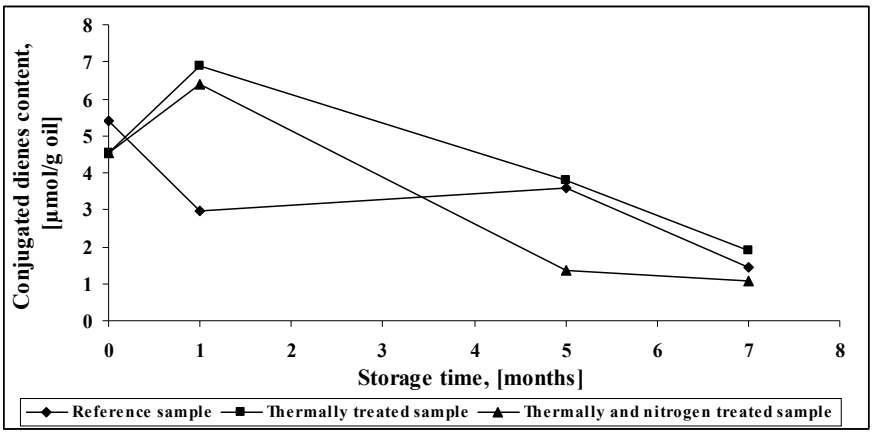

Fig. 2. Changes in conjugated dienes content of walnut oil samples as a function of treatment conditions and storage time

Further secondary oxidation products accumulation (p-anisidine and 2-thiobarbituric values) in walnut oil samples was investigated. Figure 3 shows the results of p-anisidine values changes. It can be seen, that significant increase of p-anisidine values were registered after 5 months of storage in all investigated oil samples. Considerable change of p-anisidine value was characterised for thermally treated oil sample, which is in accordance with theoretical data.

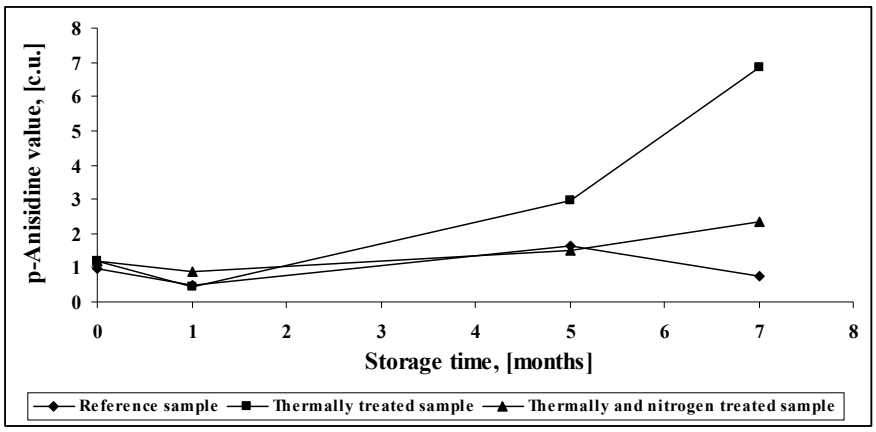

Fig. 3. Changes in p-anisidine value of walnut oil samples as a function of treatment conditions and storage time

For better understanding of obtained experimental results there was calculated total oxidation value (TOTOX) for all walnut oil samples, TOTOX value represents the sum of primary (peroxides) and secondary (aldehydes) oxidation products accumulation in vegetable oils. This value ranged from 7,58 to 7,81 c.u. for fresh oils and from 7,04 to 15,77 c.u. for walnut oils after 7 months of storage. It was obvious, that application of heat treatment of walnut oil led to a significant increase of the TOTOX value. But combination application of thermally and nitrogen treatment led to decrease of all investigated parameters (PV, CD, p-AV and 2-TBA).

4. Conclusion

The results of this research showed the influence of heat, nitrogen and their combination treatment on the intensity of primary and secondary oxidation products accumulation in cold pressed walnut oil as a function of storage time. It was demonstrated that walnut oil retains acceptable quality after combined heat and nitrogen treatment and quality deteriorates for reference sample (untreated walnut oil) and thermally treated sample. 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.

5. Acknowledgements

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