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The Study of Oxidative Processes in Walnut Fats during Storage

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Abstract

Objectives: The aim of this paper was to explore chemical and sensory stability of walnuts during their storage. **Methods/ Statistical Analysis**: We have determined fatty acid composition, chemical parameters of lipid oxidation (peroxide and thiobarbituric values and conjugated dienes) during storage of walnuts. The accelerated storage of walnuts was used to reveal changes in sensory characteristics of walnuts, as well as significant increase of peroxide and thiobarbituric values and conjugated dienes contents. **Findings**: The results of our studies allowed establishing a correlation between physico-chemical parameters of oxidative damage of walnuts and their organoleptic characteristics. Determination of volatile compounds revealed the substances that caused the appearance of rancid taste and smell. Determination of fatty acid composition showed that during storage an increase of amount of saturated fatty acids and decrease of amount of polyunsaturated fatty acids occur. **Application/Improvements:** The research results can be used to predict shelf life of nuts, and to develop the methods of its increasing.

Keywords: Lipid Oxidation, Nuts, Peroxide Value, Storage, Thiobarbituric Value, Walnuts

1. Introduction

Walnut is one of the most popular nut species in the world. It is characterized by high nutritional value and exceptional influence on the human. Walnuts are used not only as snack, but also as the part of various meals.

Walnuts are the unique natural source of basic nutrients and minor biologically active compounds. They are rich in complete proteins, fats and have high energy value. Walnuts contain vitamines A, E and B group, as well as unique complex of micro- and macro-elements[1,2].

Walnuts contain up to 60% of fat rich in mono- and poly-unsaturated fatty acids that causes their rapid rancidity. The products of lipid oxidation have a carcinogenic and mutagenic effect on humans. Oxidative processes in fats attract great attention in assessment of nuts' quality[3,4].

The aim of this study was to explore oxidative processes that occur in fats during storage of walnuts.

2. Materials and Methods

For this research the sample walnuts (harvested in 2015) were purchased at Moscow retail markets. To study the oxidative damage, those walnuts were stored in thermostate at 30°C in the original package during 5 weeks. The measurements of oxidation's key parameters were performed every week.

The walnut oil was produced by cold pressing. We measured peroxide value (to show the content of primary oxidation products (peroxides and hydroperoxides), thiobarbituric value (to show the content of secondary oxidation products, i.e., malondialdehyde), conjugated dienes, volatile aromatic substances and fatty acid composition.

Peroxide Value (PV) was measured using State Russian quality methodology GOST R 51487-99 «Plant oils and animal fats. Peroxide value estimation». This method

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provides the reaction of a mixture of oil and chloroform-acetic acid 2:3 (v/v) with saturated potassium iodide solution in darkness. The iodine formed was titrated with 0.1 N sodium thiosulphate until the yellow colour disappeared. Then after adding starch indicator titration was continued until the blue colour just disappeared. Peroxide value (meq kg^{-1}) was calculated according to the formula: PV = volume of sodium thiosulphate × 0.1 N × 1000/mass of oil.

For measurement of Thiobarbituric Value (TV), 5 ml of oil was added to 5 ml of thiobarbituric acid solution and heated in a water bath at 80°C for 40 min for pink color development. Then the tube with mixture was cooled at room temperature for one hour, and absorbance at 532 nm was measured by spectrophotometer. Thiobarbituric value were calculated from a standard curve of malondialdehyde and expressed as mg of malondialdehyde per kg sample[5,6].

For measurement of Conjugated Dienes contents (CD) weighed oil samples were dissolved in 6 ml of n-hexane, and absorbance was measured at 232 nm by a spectrophotometer. The results were reported as the sample extinction coefficient *E* (1%, 1 cm)[7–9].

Fatty acid compositions were evaluated following the methods of State Russian quality methodology GOST 30418-96 "Plant oils. Method of estimation of fatty acid composition". The fatty acid methyl esters of total lipids were analyzed on gas-liquid chromatograph (Kristalljuks 4000 M) equipped with a flame ionization detector. We used HP FFAP capillary column (50 m × 0.2 mm × 0.3 nm). Column temperature was from 200 to 230°C. The carrier was nitrogen. The separated fatty acid methyl esters were identified by comparing their retention times with those of authentic samples.

Volatile compounds were determined by extraction of chopped walnuts with diethyl ether. The extract was chromatographed in Chromatograph Shimadzu GC 2010 with mass detector GCMS-QP 2010 on column MDN-1 (hard-connected methyl silicone 30 m × 0.25 mm) in temperature regime at following parameters: injector temperature 2000°C, interface temperature 2100°C, detector temperature 2000°C. The carrier gas was helium[10,11].

Group of certified tasters performed sensory analysis. All parameters were determined thrice, and then results were used for statistics analysis. Data were analyzed with ANOVA STATISTICA Base version 12.6 (2015) to calculate the main differences between values and Duncan test.

3. Results and Discussion

Assessment of walnuts smell was performed by profile method. We used following descriptions of smell: "oily", "fruity", "nutty", "sweet", "woody" and "rancid". The intensity of smell was assessed using the 10-points scale. Results are presented at Figure 1.

During the accelerated storage of walnuts the intensity of "oily" and "rancid" smells increased significantly while the intensity of "fruity" and "nutty" smells decreased.

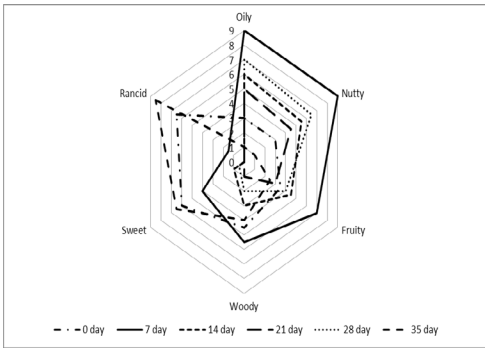
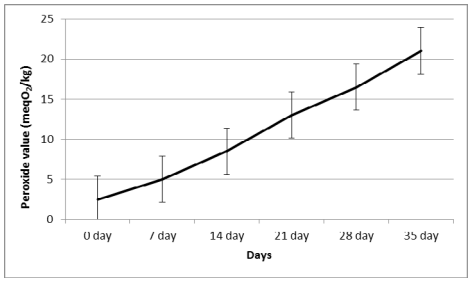


Figure 1. Intensity of walnuts' smell during the accelerated storage

The dynamics of peroxide value, thiobarbituric value and conjugated dienes contents is presented at Figure 2. It is clear that all the values increased during the accelerated storage.



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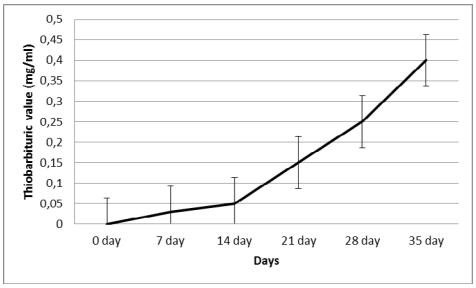

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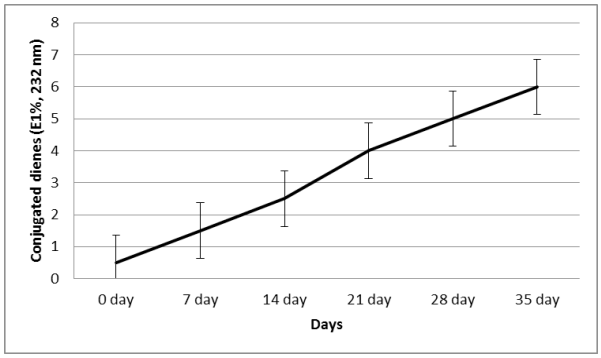


(c)

Figure 2. (a) Peroxide value. (b) Thiobarbituric value. (c) Conjugated dienes contents in walnut fats during storage.

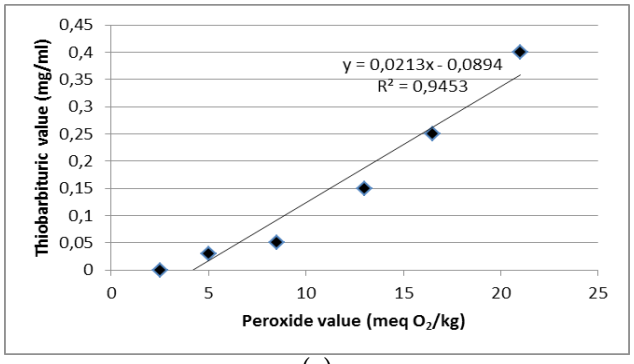
The PVs of walnut samples increased from 2.5 (day 0) to 21.0 (day 35). Similar data were obtained by other authors who stored the walnuts at 60°C during 12 days. They have reported the growth of peroxide values from 1.5 to 46.7 meqO_2/kg[12].

The TVs of walnut samples increased from 0.01 (day 0) to 0.4 (day 35). These values are comparable with ones published previously[13,14]. In this study authors compared 6 different varieties of walnut cultivated in Portugal.

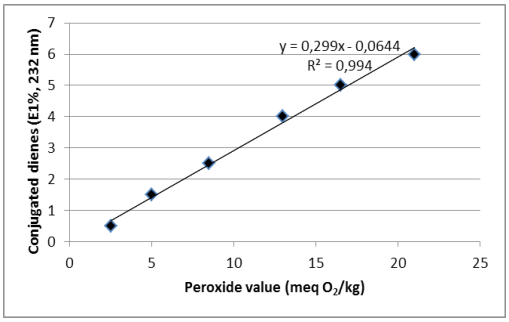
The CD contents of walnut samples varied from 0.5 (day 0) to 6.0 (day 35). Several authors[15,16] described the similar results, though some differences were observed due to geographical origin, environmental factors, storage condition and variety of walnut.

All the values sharply increased after 14 days of storage which indicates an increase of oxidative processes.

The correlation between peroxide value, thiobarbituric value and conjugated dienes contents is presented at Figure 3. Strong positive correlation of more than 0.9 between all three parameters is obvious. The maximum correlation was shown between peroxide value and conjugated dienes contents.



(a)



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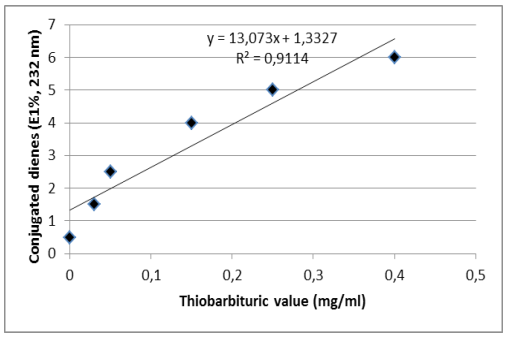

(c)

Figure 3. Relationships between. (a) peroxide value and thiobarbituric value. (b) Peroxide value and conjugated dienes contents. (c) Thiobarbituric value and conjugated dienes contents.

Figure 4 shows the chromatogram of volatile substances of walnut oil after 35 days of storage.

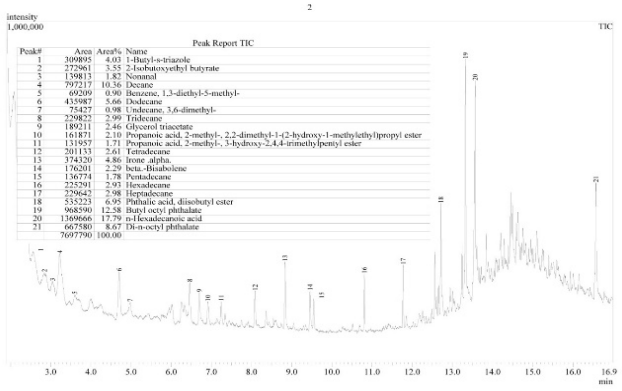

Figure 4. The chromatogram of volatile substances of walnut oil after 35 days of storage.

Results of identification of these volatile substances are presented in Table 1.

The data obtained shows that the smell of fresh walnuts is caused by the following main substances: butyl-

s-triazole, isobutyl-oxyethyl-butyrate, decane, dodecane, 2-nonenal, 2-dodecenal, tridecane, propanoic acid, tetradecan, cyclohexanone, phthalic acid, n-hexadecanoic acid, octadecadienoic acid and di-n-octyl phthalate.

Oxidative damage of walnut fats was accompanied by increase of butyl-octyl-phtalate, 1-hexanol, ethinamate, 2-heptenal, furan, heptadecane, hexanoic acid, 2-noneon-1-ol, 2-undecane-9-methyl, that caused the unpleasant smell of rancidity.

Some papers deal with the volatile compounds in walnut[17,18] and their authors identified 22 to 49 key odorants. However, the difference in key odorant profiles should be referred to regions of growing. Different walnut varieties contain the same substances causing the smell of fresh walnuts.

Table 1. Volatile substances in walnut samples

Substance	Content, %	
	0 day	35 day
Butyl-s-triazole	4.68	4.03
Isobutyl-oxyethyl-butyrate	4.12	3.55
Nonanal	2.11	1.82
Decane	12.04	10.36
Benzene	1.04	0.90
Dodecane	6.58	5.66
Undecane	1.14	0.98
Tridecane	3.47	2.99
Glycerol triacetate	2.86	2.46
Propanoic acid	3.04	2.61
Tetradecane	5.65	4.86
Irone alpha	2.66	2.29
Beta-bisabolene	2.06	1.78
Pentadecane	3.40	2.93
Hexadecane	3.47	2.98
Heptadecane	5.28	6.95
Phthalic acid	12.99	12.58
Butyl-octyl-phtalate	13.26	17.79
n-Hexadecanoic acid	5.71	8.67
Di-n-octyl phthalate	3.04	2.61
1-Hexanol	0.60	14.85
Ethinamate	1.23	12.26
2-Heptenal	0.05	6.98
Furan	1.87	32.78
Hexanoic acid	0.53	4.36
2-Noneon-1-ol	2.47	5.41
2-Nonenal	3.45	0.85
Octanoic acid	0.14	1.07
2,4-Nonadienal	0.36	1.05
Cyclohexanone	4.85	0.26
2-Dodecenal	6.54	0.80
Cyclohexene	0.08	0.95
Dodecadienal isomer	0.03	0.69
Decadienal	1.13	1.54
2-Octenal-butyl	0.07	0.23
Bicyclo	0.02	0.16
Dodecatrien	0.59	1.38
Octadecadienoic acid	3.84	0.08
Heptanoic acid	0.75	1.78
2-Undecane-9-methyl	6.24	11.28
2-Buten-1-amine	0.25	0.94

Fatty acid composition of walnut samples is presented in Table 2. These data show that walnut oil contains mainly polyunsaturated and monounsaturated fatty acids.

Predominant fatty acid is linoleic, the second one is linolenic. During storage, the content of polyunsaturated acids decreases while the content of saturated fatty acids significantly increases.

Data obtained correspond with results of other studies[19]. The monounsaturated fatty acid content in fresh walnuts was 1.5-2 g/100 g higher than in walnuts grown in Poland. The observed differences may be due to either genotype or growing seasons.

Table 2. Fatty acid composition of walnut oil

Fatty acid	Content, g/100 g	
	0 day	35 day
Total saturated fatty acids	10.97	15.71
6:0	0.16	0.24
10:0	0.06	0.12
11:0	0.26	0.42
14:0	0.02	0.05
16:0	8.31	10.62
17:0	0.03	0.07
18:0	2.05	4.03
20:0	0.08	0.16
Total monounsaturated fatty acids	16.00	17.83

16:1	0.08	0.24
18:1	15.42	17.59
Total polyunsaturated fatty acids	73.03	66.46
18:2	60.28	54.98
18:3	12.75	11.48

The fatty acid composition of walnuts is important for nutritional quality, health benefits offered by mono-unsaturated and polyunsaturated fatty acid, especially in relation to blood serum lipid profile, desirable flavors (attributed to several fatty acid), contribution to texture, and importance for storage.

4. Conclusion

Our research shows that all chemical indicators of oxidative damage change during the accelerated storage of walnuts. Significant changes were observed in walnut smell characteristics – during storage oily and rancid smells intensified while the nutty and fruity smells decreased.

During storage all the indicators of oxidative damage (peroxide value, thiobarbituric value and conjugated dienes content) significantly increased. Positive correlation between all these values was observed. Fatty acid composition substantially changed during accelerated storage of walnuts - the content of polyunsaturated acids decreased while content saturated fatty acids significantly increased. The volatile aromatic substances also changed considerably: Storage is accompanied by accumulation of substances with unpleasant smell of rancidity.

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