# OXIDATION OF WALNUT OIL (*Juglans regia* L.) AT HEAT TREATMENT: PRIMARY AND SECONDARY OXIDATION PRODUCTS ACCUMULATION

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**Abstract.** *In this work heat treatment experiment was set up to determine the intensity of oxidation products accumulation in walnut oil with particular reference to the walnut oil sample at room temperature. The influence of the heat treatment conditions was assessed by measuring the primary and secondary oxidation products in oil samples immediately after treatment up to the highest temperature of 180 $^0$C. Primary oxidation products of walnut oil samples were evaluated by measuring peroxide value and conjugated dienes & trienes content. The peroxide value was in the range of 5.81 – 26.55 mmol/kg of walnut oil. The conjugated dienes content of the walnut oil samples ranged from 6.23 to 12.75 µmol/g, while the conjugated trienes content ranged from 1.58 to 6.25 µmol/g. Secondary oxidation products of walnut oil samples were evaluated by measuring p-anisidine value and 2-thiobarbituric acid value. The p-anisidine value was in the range of 2.98 – 16.91 c.u. The 2-thiobarbituric acid value of the walnut oil samples ranged from 0.027 to 0.048 mg/kg. Walnut oil retained acceptable quality for heat treatment at 120 $^0$C and quality deteriorated significantly for heat treatment of walnut oil at 160 $^0$C and 180 $^0$C.*

**Key Words:** walnut oil, heat treatment, primary and secondary oxidation products, quality characteristics.

## 1. Introduction

The walnut tree (*Juglans regia* L.) is cultivated commercially throughout southern Europe, northern Africa, Eastern Asia, the USA and western South America [11]. Green walnuts, shells, kernels and seeds, even bark and leaves, are used in food, pharmaceutical and cosmetic industries [15, 23].

The walnut seed (kernel) represents from 40 to 60% of the nut weight, depending mainly on the variety. The kernels contain high levels of oil between 52% and 70% [8, 10, 12, 17]. Walnut oil is prized as a specially oil because of its potential health benefits and organoleptic properties [8, 17].

The composition of walnut oils from different geographic origins has been reported [7, 14, 24, 25]. Their major constituents are triglycerides, in which monounsaturated (oleic acid mainly) and polyunsaturated fatty acids (PUFAs, linoleic and α-linolenic) are present in high amounts [13, 16].

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 [9, 21, 22]. 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 organoleptic properties.

The aim of this study was to investigate the intensity of primary and secondary oxidation products accumulation in walnut oil under heat treatment conditions. The analyses were performed on four different temperatures in order to evaluate the influence of traditional heat treatment conditions on changes in quality characteristics of walnut oil.

## 2. Materials and methods

### *2.1. Materials*

Refined walnut oil was obtained from a local producer in the Republic of Moldova in April 2011. Walnut oil was heated during 20 min at different temperatures: 120$^0$C, 160 $^0$C and 180$^0$C. After heat treatment walnut oil samples were used immediately in the experiment.

### *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, 2,2,4-trimethylpentane (isooctane), 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. Acid Value*

Acid value was determined by potassium hydroxide titration as described in AOCS Official Method Cd 3d-63 (AOCS, 1999) [1]. 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.

*2.4. Refractive Index*

The refractive index of walnut oil samples was measured following the process described in AOCS Official Method Cc 7-25 (AOCS, 1998) [2]. This index is related to degree of saturation but is affected by other factors such as acid value, oxidation, and heat treatment. Refractive index was determined using digital handheld refractometer Krüss Optronic DR 301-95 (Germany).

*2.5. Peroxide Value*

Oxidation rate was studied immediately after walnut heating by determination of the peroxide value (PV). This was determined according to AOCS Official Method Cd 8-53 (AOCS, 2003). PV was expressed as millimoles peroxide per kilogram of walnut oil [3].

*2.6. Conjugated dienes and trienes*

The experiment was carried out according to the AOCS Official method Ti la 64 (AOCS, 1993) with minor modifications [4, 18]. Approximately 0.02 g of walnut oil was placed into a 25 ml volumetric flask. The sample was dissolved in 2,2,4-trimethylpentane, 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 and 273 nm using quartz cuvette 10×10 mm. The CD and CT values were calculated using the following equations:

$$C_{CD/CT} = A_{236/273} / (\varepsilon \times l) \text{ and } CD/CT_{value} = \\ = [C_{CD/CT} \times (2.5 \times 10^4)] / W$$

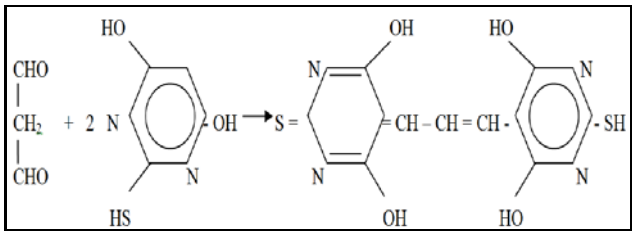
where $C_{CD/CT}$ is the CD/CT concentration in mmol/ml (i.e., the molar concentration), $A_{236/273}$ is the absorbance of the oil solutions at 236 nm and 273 nm, $\varepsilon$ is the molar absorptivity (i.e., the extinction coefficient) of linoleic acid hydroperoxide ($2.525 \times 10^4$ $M^{-1} \cdot cm^{-1}$), l is the path length of the cuvette in cm (1 cm), $2.5 \times 10^4$ is a factor that encompasses the volume of 2,2,4-trimethylpentane (25 ml) used to dissolve the oil sample as well as a unit conversion (1000 µmol/mmol) so that the content of CDs and CTs can be expressed in µmol, and W is the weight of the walnut oil in gram. Results were expressed in micromole conjugated dienes and 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) [5, 19]. 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) [6]. The basic reaction of this method is presented below:




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) (Snedecor *et al.*, 1989).

## 3. Results and discussion

It is well known, that primary oxidation products of vegetable oils are peroxides, which can be transformed into secondary oxidation products such as aldehydes, ketones, oxidized fatty acids and other ompounds during heat treatment. The kinetics of vegetable oil oxidation products accumulation are presented in figure 1.

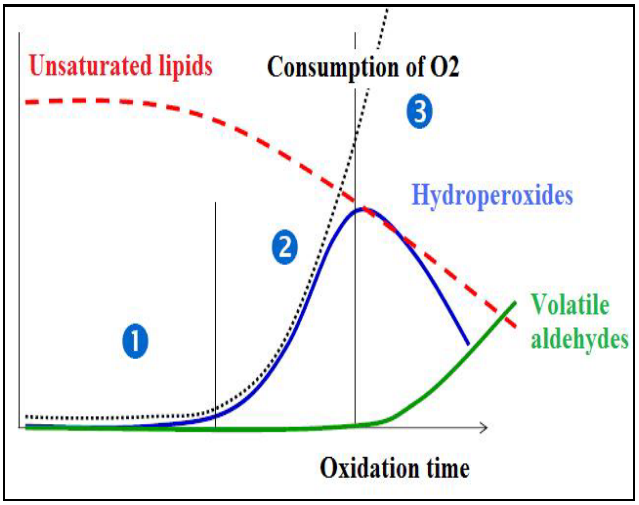



**Figure 1.** *The kinetics of primary and secondary oxidation products accumulation in vegetable oil*

*3.1. Primary oxidation products*

Primary oxidation products of walnut oil samples were evaluated by measuring peroxide value, refraction index and conjugated dienes & trienes content. Changes in these parameters are shown in table 1.

Table 1 shows the effect of heat treatment temperature on primary oxidation products accumulation in walnut oil samples. The initial acid value of fresh walnut oil sample was low (0.94 mg KOH/g walnut oil). Acid value of heated walnut oil samples is 0.96 mg KOH/g walnut oil for sample heated at 120 $^0$C, 0.97 mg KOH/g walnut oil for sample heated at 160$^0$C and 1.01 mg KOH/g walnut oil for sample heated at 180 $^0$C. These values are higher that those of the fresh walnut oil sample.

Changes in peroxide value were in the range from 5.81 to 26.55mmol/kg walnut oil. The observation is that given heat treatment temperatures lead to a rapid lipid oxidation of walnut oil. The difference from the acid value and peroxide value, heat treatment did not significantly affect refraction index. Respectively refraction index was 1.4514 for walnut oil sample heated at 120 $^0$C, 1.4516 for walnut sample heated at 160 $^0$C and finally 1.4518 for walnut oil sample heated at 180 $^0$C.

**Table 1.** *Changes in primary oxidation products of walnut oil as a function of heat treatment at 120 $^0$C, 160 $^0$C and 180$^0$C.*

| Quality characteristics | Walnut oil samples | | | |
|---|---|---|---|---|
| | 20 $^0$C | 120 $^0$C | 160 $^0$C | 180 $^0$C |
| **Acid Value, mg KOH/g oil** | 0.94±0.03 | 0.96±0.07 | 0.97±0.02 | 1.01±0.03 |
| **Refractive Index** | 1.4510±0.0001 | 1.4514±0.0002 | 1.4516±0.0001 | 1.4518±0.0002 |
| **Peroxide Value, mmol/kg oil** | 5.81±0.11 | 19.97±0.17 | 23.96±0.13 | 26.55±0.14 |
| **Conjugated Dienes, µmol/g oil** | 6.23±0.05 | 8.98±0.03 | 11.99±0.07 | 12.75±0.03 |
| **Conjugated Trienes, µmol/g oil** | 1.58±0.03 | 2.54±0.07 | 4.04±0.07 | 6.25±0.05 |

As with acid value and peroxide value, heat treatment significantly affected conjugated dienes & trienes content. Walnut oil sample heated at 120 $^0$C had following conjugated dienes & trienes content - 8.98 µmol/g walnut oil and 2.54 µmol/g walnut oil respectively. Walnut oil sample heated at 160 $^0$C had following conjugated dienes & trienes content - 11.99 µmol/g walnut oil and 4.04 µmol/g walnut oil respectively. And finally walnut oil sample heated at 180 $^0$C had following conjugated dienes & trienes content - 12.75 µmol/g walnut oil and 6.25 µmol/g walnut oil respectively. As with all previous obtained results, the highest content of conjugated dienes & trienes were recorded for walnut samples heated at 180 $^0$C and the lowest conjugated dienes & trienes content, for fresh walnut samples.

Figure 2 illustrates the UV/Vis spectra of the walnut oil samples heated at different studied temperatures in the wavelength range 190 - 1100 nm. The spectrum of the oil samples display strong peaks, typical for conjugated dienes and trienes at 236 and 273 nm respectively. It is important to note, that

during heat treatment of walnut oil samples there were not significant change in the intensity of absorption values, what can be seen from figure below.

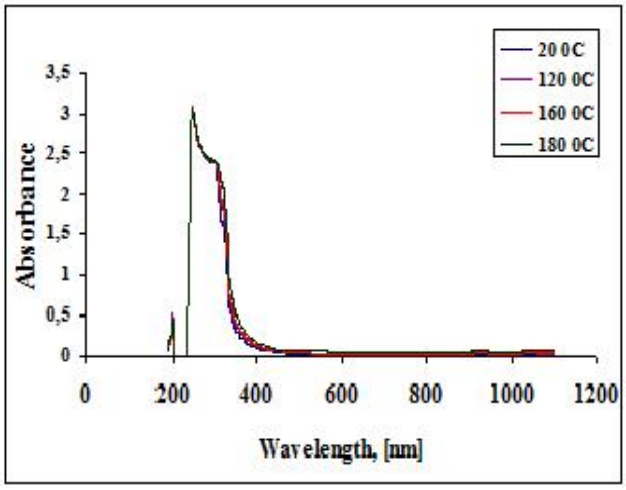



**Figure 2.** UV/*Vis spectra of walnut oil samples as a function of heat treatment at 120 $^0$C, 160 $^0$C and 180$^0$C.*

Comparison of data in table 1 and figure 2 leads to the conclusion that as the heat treatment was more severe the effect of temperature increased. Also for all walnut oil samples the effect of temperature was more pronounced at temperature regimes 160 $^0$C and 180 $^0$C.

*3.2. Secondary oxidation products*

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 3.

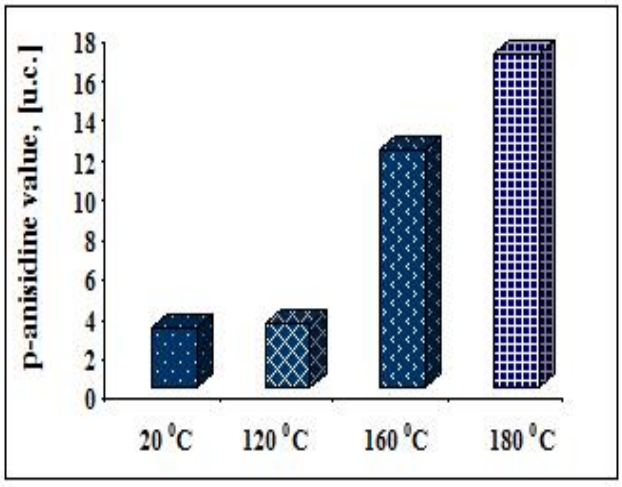



**Figure 3.** *Changes in p-anisidine values of walnut oil samples as a function of heat treatment at 120 $^0$C, 160 $^0$C and 180$^0$C.*

Figure 4 shows the effect of heat treatment conditions on 2-thiobarbituric acid values changes in walnut oil samples.

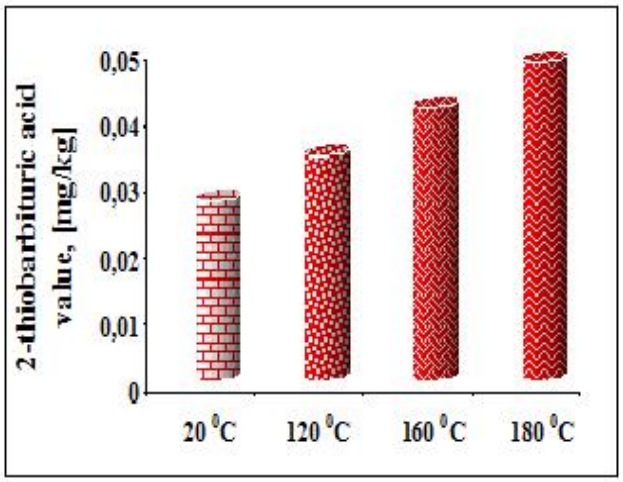



**Figure 4.** *Changes in 2-thiobarbituric acid values of walnut oil samples as a function of heat treatment at 120 $^0$C, 160 $^0$C and 180$^0$C.*

It can be seen, that the initial p-anisidine and 2-thiobarbituric acid values were 2.98 c.u. and 0.027 mk/kg of walnut oil respectively.

Figures 3 and 4 show the effect of heat treatment conditions on secondary oxidation products accumulation in walnut oil. After heat treatment at 180 $^0$C values of p-anisidine and 2-thiobarbituric acid were high enough 16.91 c.u. and 0.048 mg/kg. Differences are attributed to the accelerated process of the walnut oil lipid oxidation.

To compare the influence of heat treatment conditions on the oxidation process of walnut oil were calculated TOTOX value. Obtained data are indicated in figure 5.

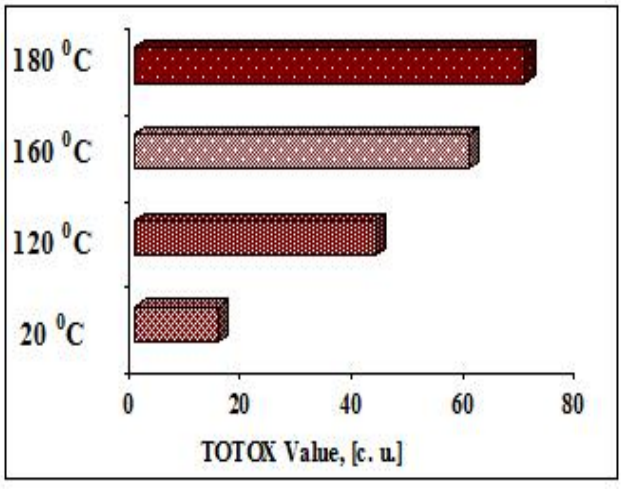



**Figure 5.** *Changes in TOTOX value of walnut oil samples as a function of heat treatment at 120 $^0$C, 160 $^0$C and 180$^0$C.*

TOTOX value represents the sum of primary (peroxides) and secondary (aldehydes) oxidation products accumulation in vegetable oils. This value of the walnut oil samples ranged from 14,59 to 70,01 c.u. It is obvious, that increasing of heat treatment temperature of walnut oil leads to a significant increase of the TOTOX value.

## 4. Conclusions

The results of this research showed the influence of heat treatment conditions on the intensity of primary and oxidation products accumulation in walnut oil as a function of temperature value. It was investigated that walnut oil retains acceptable quality for heat treatment at 120 $^0$C and quality deteriorates for heat treatment of walnut oil at 160 $^0$C and 180$^0$C.

All these quality characteristics (peroxide value, conjugated dienes & trienes content, p-anisidine and 2-thiobarbituric acid values) of walnut oil were similar to those of sunflower oil, which is one of the most commonly produced and consumed oils in the world. 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.

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## References

[1] AOCS, 1999. Official Methods and Recommended Practicles of the American Oil Chemists’ Society. *Method Cd 3d-63*. Champaign: AOCS Press.

[2] AOCS, 1998. Official Methods and Recommended Practicles of the American Oil Chemists’ Society, 5th ed. *Method Cc 7 - 25*. Champaign: AOCS Press.

[3] AOCS, 2003. Official Methods and Recommended Practicles of the American Oil Chemists’ Society. *Method Cd 8-53*. Champaign: AOCS Press.

[4] AOCS, 1993. Official Methods and Recommended Practicles of the American Oil Chemists’ Society. *Method Ti la 64*. Champaign: AOCS Press.

[5] AOCS, 1997. Official Methods and Recommended Practicles of the American Oil Chemists’ Society. *Method Cd 18-90*. Champaign: AOCS Press.

[6] AOCS, 2009. Official Methods and Recommended Practicles of the American Oil Chemists’ Society. *Method Cd 19-90*. Champaign: AOCS Press.

[7] Arranz, S., Cert, R., Perez-Jimenez, J., Cert, A., Saura-Calixto, F., (2008). Comparison between free radical scavenging capacity and oxidative stability of nut oils. Food Chemistry, 110, 985-990.Snedecor, G. W., Cochran, W. G. 1989. *Statistical Methods*. The Iowa State University Press, 8th edition. Ames.

[8] Bada, J.C., Leon-Camacho, M., Prieto, M., Copovi, P., Alonso, L., (2010). Characterization of walnut oils (*Juglans regia L.*) from Asturias, *Spain. J Am Oil Chem Soc*, 87, 1469-1474.

[9] Crowe, T.D., White, P.J., (2003). Oxidative stability of walnut oils extracted with supercritical carbon dioxide. *JAOCS*, 80(6), 575-578.

[10] Greve, L. C., McGranhan, G., Hasey, J., Snyder, R., Kelly, K., Goldhamer, D., (1992). Variation in polyunsaturated fatty acids composition of Persina walnut. *Journal of the American Horticultural Science*, 117, 518–522.

[11] Labuckas, D.O., Maestri, D.M., Perello, M., Martinez, M.L., Lamarque, A.L., (2008). Phenolics from walnut (*Juglans regia* L.) kernels: antioxidant activity and interactions with proteins. *Food Chemistry*, 107, 607-612.

[12] Martınez, M. L., Mattea, M., Maestri, D. M., (2006). Varietal and crop year effects on lipid composition of walnut (Juglans regia) genotypes. *Journal of the American Oil Chemists’ Society*, 83, 791–796.

[13] Martinez, M.L., Mattea, M.A., Maestri, D.M., (2008). Pressing and supercritical carbon dioxide extraction of walnut oil. *Journal of Food Engineering*, 88, 399-404.

[14] Mexis, S.F., Badeka, A.V., Riganakos, K.A., Karakostas, K.X., Kontominas, M.G., (2009). Effect of packaging and storage conditions on quality of shelled walnuts. *Food Control*, 20, 743-751.

[15] Oliveira, I., Sousa, A., Ferreira, I.C.F.R., Bento, A., Estevinho, L., Pereira, J.A., (2008). Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. *Food and Chemical Toxicology*, 46, 2326-2331.

[16] Ozcan, M.M., (2009). Some nutritional characteristics of fruit and oil of walnut (*Juglans regia* L.) growing in Turkey. Iran. *J. Chem. Chem. Eng*., 28(1), 57-62.

[17] Pereira, J.A., Oliveira, I., Sousa, A., Ferreira, I.C.F.R., Bento, A., Estevinho, L., (2008). Bioactive properties and chemical composition of six walnut (*Juglans regia* L.) cultivars. *Food and Chemical Toxicology*, 46, 2103-2111.

[18] Popovici, C., Alexe, P., Deseatnicova, O., (2012). Primary oxidation products accumulation in walnut oil during heat treatment. *Journal of EcoAgriTourism*, Vol. 8, 2 (25): 212-216.

[19] Popovici, C., Capcanari, T., Deseatnicova, O., Sturza, R., (2012). Rheological properties and a new functional mayonnaise microstructure of enriched by grape seeds oil. *Proceeding of Engineering Academy of Armenia*, 9 (1): 192-196.

[20] Popovici, C., (2012). Extraction of bioactive compounds from walnut (*Juglans Regia* L.) by-products and extract application for vegetable oil stabilization. *The Annals of the 78th scientific conference* “Scientific achievements of young scientists for solving problems of nutrition humanity in the XXI century, Kiev, Ukraine, p. 291.

[21] Rabrenovic, B.E., Dimic, M. Maksimovic, S. Sobajic, L. Gajic-Krstajic., (2011). Determination of fatty acid and tocopherol compositions and the oxidative

stability of walnut (*Juglans regia* L.) cultivars grown in Serbia. *Czech J. Food Sci*, 29(1), 74–78.

[22] Salcedo, C.L., Lopez de Mishima, B.A., Nazareno, M.A., (2010). Walnuts and almounds as model system of foods constituted by oxidisable, pro-oxidant and antioxidant factors. *Food Research International*, 43, 1187-1197.

[23] Stampar, F., Solar, A., Hudina, M., Veberic, R., Colaric, M., (2006). Traditional walnut liqueur – cocktail of phenolics. *Food Chemistry*, 95, 627-631.

[24] Tsamouris, G., Hatziantoniou, S., Demetzos C., (2002). Lipid analysis of greek walnut oil (*Juglans regia* L.). *Z. Naturforsch*, 57c, 51-56.

[25] Vanhanen, L.P., Savage, G.P., (2006). The use of peroxide value as a measure of quality for walnut flour stored at five different temperatures using three different types of packaging. *Food Chemistry*, 99, 64-69.