PRIMARY OXIDATION PRODUCTS ACCUMULATION IN WALNUT OIL DURING HEAT TREATMENT

C. POPOVICI[*] P. ALEXE[**] O. DESEATNICOVA[*]

Abstract: *During this study walnut oil was heated at different technological conditions - 120 ^{0}C, 160 ^{0}C and 180 ^{0}C. Free fatty acid, conjugated dienes and trienes, peroxide value and refractive index were investigated immediately after heat treatment. The data obtained from the experiment indicate that heat treatment cause initiation of walnut oil oxidation. The conjugated dienes (CD) content of the walnut oil samples ranged from 6.23 to 12.75 µmol/g, while the conjugated trienes (CT) content ranged from 1.58 to 6.25 µmol/g. The peroxide value (PV) was in the range of 5.81 – 26.55 mmol/kg of walnut oil. These data should assist in selecting conditions that are suitable and better for walnut oil heat treatment. However it is necessary to note that these data are not enough to make a final conclusion about the behavior of walnut oil in thermal oxidation conditions. Currently we obtained preliminary data about accumulation of secondary oxidation products in walnut oil during heat treatment. These data should help us to describe oxidation mechanism of walnut oil and to recommend optimal conditions for walnut heat treatment.*

Keywords: *walnut oil, heat treatment, primary oxidation products, quality indices.*

1. Introduction

Walnut (*Juglans regia* L) a member of Juglandaceae family is one of the finest nuts of temperate regions. It is the oldest cultivated fruit in the world (Ozcan, 2009). Walnut oil is prized as a specially oil because of its potential health benefits and organoleptic properties (Bada *et al.*, 2010; Pereira *et al.*, 2008).

The health benefits of walnut oil are usually attributed to its chemical composition. Walnut oil contains approximately 7% saturated, 20% monounsaturated and 73% polyunsaturated fatty acids (Rabrenovic et al., 2011; Tsamouris *et al.*, 2002). These high levels of polyunsaturated fatty acids make walnut oil prone to oxidation and may mean that oil has a limited shelf-life.

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 (Salcedo *et al.*, 2010; Mexis *et al.*, 2009; Aranz *et al.*, 2008; Martinez *et al.*, 2008; Vanhanen *et al.*, 2006; Crowe *et al.*, 2003;). Stark *et al*. (2000) 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 objective of this study was to investigate the intensity of primary oxidation products accumulation in walnut oil under different heat treatment conditions.

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 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, Republic of Moldova). Chloroform, 2,2,4-trimethylpentane (isooctane) and glacial acetic acid were purchased from Sigma-Aldrich. All reagents were of analytical grade. Distilled water was used throughout.

[*] Faculty of Technology and Management in Food Industry, Technical University of Moldova, e-mail: popovici.kristina@gmail.com
[**] Faculty of Food Science and Engineering, “Dunarea de Jos” University Galati, Romania, e-mail: petru.alexe@ugal.ro

2.3. 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.

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). 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.

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. 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:

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

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, □ is the molar absorptivity (i.e., the extinction coefficient) of linoleic acid hydroperoxide (2.525×10^4 $M^{-1} \cdot cm^{-1}$), l is the path length of the cuvette in cm (1 cm), 2.5×10^4 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. 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

3.1. Acid value

Lipid oxidation of walnut oil samples was evaluated by measuring peroxide value, refraction index and conjugated dienes & trienes content. Changes in acid value are shown in figure 1.

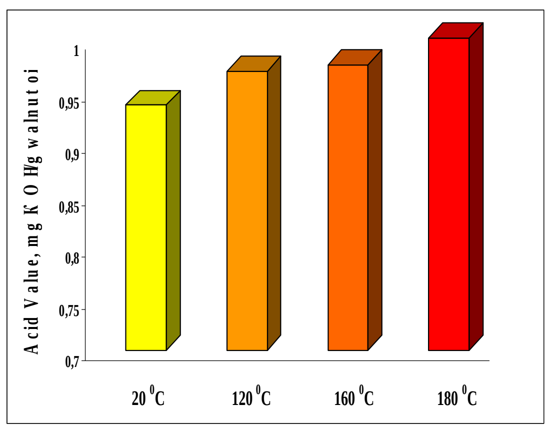

Fig. 1. *Changes in acid value of walnut oil samples as a function of heat treatment at 120 ^{0}C, 160 ^{0}C and 180^{0}C.*

Figure 1 shows the effect of heat treatment temperature on acid value of walnut 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. Differences are attributed to the accelerated process of the walnut oil lipid oxidation.

3.2. Peroxide value

Changes in peroxide value are shown in figure 2. The initial peroxide value of fresh walnut oil sample was very low (5.81 mmol/kg walnut oil). Figure 2 shows the effect of heat treatment on peroxide value at 120 ^{0}C, 160 ^{0}C and 180^{0}C.

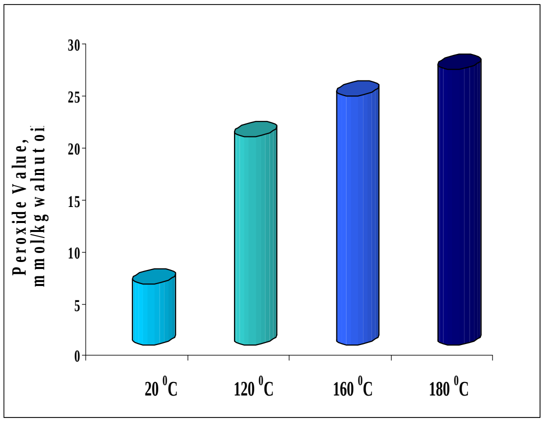

Fig. 2. *Changes in peroxide value of walnut oil samples as a function of heat treatment at 120 ^{0}C, 160 ^{0}C and 180^{0}C*

All heat treatment conditions significantly affected peroxide value of walnut oil samples. After 120 ^{0}C of heat treatment walnut oil sample had a very low peroxide value 19.97 mmol/kg walnut oil. Respective peroxide value was 23.96 mmol/kg and 26.55 mmol/kg walnut oil for walnut samples heated at 160 ^{0}C and 180^{0}C. The observation is that given heat treatment temperatures lead to a rapid lipid oxidation of walnut oil.

3.3. Refraction index

Changes in refraction indexes are shown in figure 3. The initial refraction index of fresh walnut oil sample was 1.4510. Figure 3 shows the effect of heat treatment on refraction index at 120 ^{0}C, 160 ^{0}C and 180^{0}C.

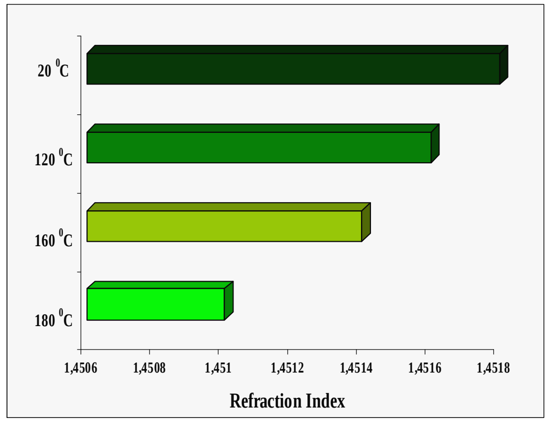

Fig. 3. *Changes in refraction index of walnut oil samples as a function of heat treatment at 120 ^{0}C, 160 ^{0}C and 180^{0}C*

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.

3.4. Conjugated dienes & trienes

Changes in conjugated dienes & trienes are shown in figure 4. The initial conjugated dienes & trienes of fresh walnut oil sample were 6.23 µmol/g walnut oil and 1.58 µmol/g walnut oil respectively. Figures 4 and 5 show the effect of heat treatment at 120 ^{0}C, 160 ^{0}C and 180^{0}C on conjugated dienes & trienes content.

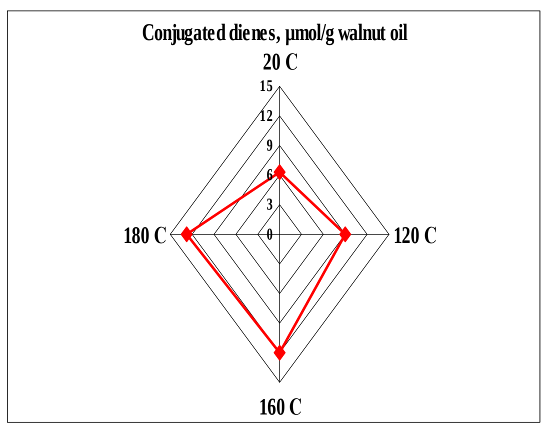

Fig. 4. *Changes in conjugated dienes of walnut oil samples as a function of heat treatment at 120 ^{0}C, 160 ^{0}C and 180^{0}C*

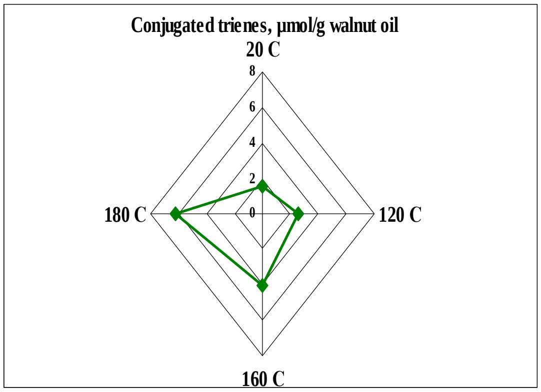

Fig. 5. *Changes in conjugated trienes of walnut oil samples as a function of heat treatment at 120 ^{0}C, 160 ^{0}C and 180^{0}C*

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. Comparison of data in figures 4 and 5 leads to the conclusion that as the heat treatment was more severe the effect of temperature increased. Also for all walnut samples the effect of temperature was more pronounced at 160 ^{0}C and 180 ^{0}C.

4. Conclusions

The present study investigated the influence of heat treatment conditions on the intensity of primary oxidation products accumulation in walnut oil as a function of temperature value. It was shown that walnut oil retains acceptable quality for heat treatment at 120 ^{0}C and quality deteriorates significantly for heat treatment of walnut oil at 160 ^{0}C and 180 ^{0}C.

Acknowledgments

This work was supported by the Agence Universitaire de la Francophonie (AUF) through the "*Eugen Ionescu*" postdoctoral scholarship 2010/2011 and financed by the Romanian Government.

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