Thermal Stability of Chlorophyll Pigments in Virgin Olive Oil*

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Abstract: The aim of the present study was to determine the changes in the chlorophyll content and the K_{232} and K_{270} specific extinction coefficient values in virgin olive oil (VOO) subjected to high temperatures for 24 h. VOO, was obtained from Kahramanmaraş city, Turkey, and subjected to 150, 160, 170, 180. 190 and 200ºC for 2, 4, 6, 8, 10, 12 and 24 h, and the chlorophyll content and the K_{232} and K_{270} specific extinction coefficient values were determined. The chlorophyll content of VOO decreased significantly as the treatment temperature and the extent of heat treatment increased (P<0.05) Especially, the temperature above 180ºC, the chlorophyll content decreased below 3% after 24 h. The K_{232} and K_{270} specific extinction coefficient values of VOO increased significantly with increasing the treatment temperature and time (P<0.05). The values of K_{232} and K_{270} specific extinction coefficients reached to 9.12 and 5.28 (1%, 1 cm), respectively, at 200ºC after 24 h. However, the rates of the increase in these values did not change with respect to different temperature and time. A significant inverse relationship was found between the chlorophyll content and the K_{232} and K_{270} specific extinction coefficient values (P<0.05). This study showed that chlorophyll, as one of the major quality criteria for olive oils, is heat-liable, and decomposes at high temperatures and long treatment times. In addition, increases in the conjugated diene and triene contents of VOO indicate the accelerated oxidation reactions at high temperatures; therefore VOO is no longer classified as "virgin olive oil" according to the official standards.
Keywords: Virgin olive oil, chlorophyll, conjugated diene and triene, thermal stability

Natürel Zeytin Yağındaki Klorofil Renk Maddelerinin Isısal Kararlılığı

Özet: Bu çalışmanın amacı, yüksek sıcaklıklara maruz bırakılan natürel zeytinyağında klorofil ve konjuge bağların miktarlarındaki değişimi 24 saat süresince belirlemektir. Kahramanmaraş, Türkiye'den temin edilen natürel zeytinyağı, 150, 160, 170, 180. 190 ve 200ºC sıcaklıklarda 2, 4, 6, 8, 10, 12 ve 24 saat bekletilmiş ve natürel zeytinyağının klorofil miktarı ve 232 ve 270 nm'deki absorbans değerleri ölçülmüştür. Natürel zeytinyağının klorofil miktarı sıcaklık ve süre arttıkça istatistiksel olarak önemli oranda düşüş göstermiştir (P<0,05). Özelikle, 180ºC üzeri sıcaklıklarda 24 saat işlem gören sızma zeytinyağında klorofil miktarı %3'ün altına düşmüştür. Natürel zeytinyağının K_{232} ve K_{270} absorbans değerleri, sıcaklık ve sürenin artışına paralel istatistiksel olarak önemli oranda artmıştır (P<0,05). 200ºC sıcaklıkta ve 24 saat sonunda natürel zeytinyağının K_{232} absorbans değeri 9,12 (1%, 1cm) ve K_{270} absorbans değeri 5,28 (1%, 1 cm)'ye ulaşmıştır. Ancak, farklı sıcaklık ve süreler sonunda K_{232} ve K_{270} absorbans değerlerindeki artış hızı hemen hemen sabit kalmıştır. Lineer regresyon analizinde, klorofil miktarındaki düşüş ile K_{232} ve K_{270} absorbans değerlerindeki artış arasında istatistiksel olarak önemli negatif bir ilişki bulunmuştur (P<0,05).Sonuç olarak, natürel zeytinyağının kalite göstergelerinden olan klorofilin ısıya karşı duyarlı olduğu ve uzun süre sıcaklık uygulamalarından olumsuz etkilendiği görülmüştür. Ayrıca, yüksek sıcaklıkla meydana gelen oksidasyon reaksiyonları zeytinyağında konjuge bağların artışına neden olmuştur. Bulunan K_{232} ve K_{270} absorbans değerleri, resmi standartlarda verilen değerleri aştığı için natürel zeytinyağı vasfını yitirmiştir.
Anahtar kelimeler: Natürel zeytinyağı, klorofil, konjuge bağ, ısı kararlığı

INTRODUCTION

Virgin olive oil (VOO) is obtained from the olive fruits using solely mechanical or other physical means, which do not alter the oil quality in any way. It has not undergone any sort of chemical treatments (use of solvents or re-esterification methods etc.), other than washing, decanting, centrifuging, and filtering. This creates authenticity for the VOO, and distinguishes it from other edible vegetable oils in terms of aroma, taste, color, nutritional properties and oxidative stability (Morello *et al.*, 2004; Angerosa *et al.*, 2006; Tsimidou, 2006).

In the last few years, interest in VOO, traditionally consumed in the Mediterranean area, has been extended to other countries (Northern Europe, USA, Japan, etc.) due its highly appreciated organoleptic and nutritional characteristics by the consumers. The healthy benefits of VOO arise from its chemical composition and have traditionally been attributed to the high content of monounsaturated acids (oleic acid) and minor components such as phenolics, tocopherols and carotenoids (Angerosa, 2002; Boskou *et al.*, 2006; Perez-Jimenez *et al.*, 2007). The positive influence of VOO on health include an improvement in blood lipid profile by lowering the total and LDL (Low Density Lipoproteins)-cholesterol levels while significantly raising the level of HDL (High density Lipoproteins)-cholesterol in the blood stream, reducing degenerative

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and coronary heart diseases, diabetes, certain cancer risks such as breast, prostate and colon cancers, and certain malignant tumors (endometrium, digestive tract, skin tumors) (Anon, 2004; Ozyilkan *et al.*, 2005; Visioli *et al.*, 2006; Perez-Jimenez *et al.*, 2007).

The color of VOO is an important characteristic of quality, and plays a key role as a factor of acceptability among consumers. In fact, many consumers particularly appreciate a deep green color in oil, like in VVO. The green-yellowish color of VOO is due to various pigments, i.e. chlorophylls, pheophytins and carotenoids, distinguishing it from other vegetable oils (Cichelli and Pertesana, 2004; Del Giovine and Fabietti, 2005; Boskou *et al.*, 2006). However, the oxidative stability of VOO is greatly affected by the presence of these compounds and their derivatives.

VOO provides a rich source of natural antioxidants. These include carotenoids, tocopherols and phenolic compounds which have been reported to play a key role in preventing lipid oxidation by different mechanisms (Aparicio *et al.*, 1999; Cichelli and Pertesana, 2004; Morello *et al.*, 2004). On the other hand, chlorophylls and the pheophytins in presence of the light act as catalysts in the formation of singlet state oxygen, which reacts with unsaturated fatty acids and produces fatty acid hydroperoxides. Decomposition of these hydroperoxides initiates a free-radical type of autoxidation. This photooxidation results in a change in color and, because of the formation of hydroperoxide decomposition products, develops undesirable odor and flavor constituents (Chen and Liu, 1998; Endo *et al.*, 1984; Rahmani and Csallany, 1998). Thus, prevention of oxidation reactions in VOO is of great importance to ensure palatability, economy, and nutritional value.

The objective of the present study was to evaluate the changes in the chlorophyll and conjugated diene and triene contents in the VOO with temperature and the extent of heat treatment. The results of the study will help us understand the fate of chlorophyll during the oxidation reactions.

MATERIAL and METHODS

Material

VOO from the olives in 2012-2013 harvest seasons was obtained from a local olive oil plant (Demirkol Ltd., Kahramanmaraş). The characteristic of the olive oil are as follows: free acidity, 0.49% (as oleic acid); peroxide value, 5.22 $meqO_2kg^{-1}$; K_{232} and K_{270} extinction coefficients, 1.89 and 0.15, respectively; total phenolic content, 65.2 mg tannic acid kg^{-1}, and chlorophyll content, 200 mgL^{-1}.

Sample Preparation

The oil samples (25 ml each) were transferred into 50 ml serum bottles. The serum bottles were sealed airtight with Teflon-coated rubber seals and aluminum caps and heated to 150, 160, 170, 180, 190 and 200ºC under dark condition in a forced air oven. All samples were prepared in duplicate. Chlorophyll and conjugated diene and triene contents were measured periodically every 2-h intervals until the time at which chlorophyll content decreased to 1 mgL^{-1}.

Determination of Chlorophyll content in VOO

Chlorophyll content in oil samples was determined by reading the absorbance at 630, 670 and 710 nm in a 10 mm spectrophotometer cell against air as described by Pokorny*et al*. (1995) and expressed as milligrams of pheophytin "a" per kilogram of oil. The method is suitable for the determination of quantities of chlorophylls higher than 1 $mgkg^{-1}$. The following equation was used for the determination of total chlorophyll content in VOO:

$$C = 345.3 \times (A_{670} - 0.5 \times A_{630} - 0.5 \times A_{710})L^{-1}$$

where; C is the content of chlorophylls (mg pheophytin "a"kg^{-1} oil), A is the absorbance at the respective wavelength (nm), and L is the thickness of the spectrophotometer cell (mm).

Determination of Conjugated Diene and Triene Contents

Content of conjugated dienes as absorbance at 232 nm (A_{232}) and content of conjugated trienes as absorbance at 270 nm (A_{270}), were determined by dissolving weighed-out samples in isooctane (0.1%) and reading the sample absorbance at 232 nm (A_{232}) and 270 nm (A_{270}), using a UV/VIS double-beam scanning spectrophotometer (Shimadzu, Japan) (Anon, 2001).

Statistical Analyses

For the statistical design, a completely randomized experimental design was applied. The data in each analysis were subjected to a two-way ANOVA (SPSS 13.0 for Windows (SPSS Inc., 2004) with "temperature" and "the extent of heat treatment" as independent variables. The significant differences among the means are determined using Duncan's Multiple Range test. Differences were considered statistically significant when the probability was greater than 95% (P<0.05).

RESULT and DISCUSSION

Changes in Chlorophyll Content

Chlorophyll is responsible for the greenish coloration of certain olive oils. Those pigments are also important in olive oil stability. The thermal stability of chlorophyll in the VOO samples was determined for 0, 2, 4, 6, 8, 10, 12, and 24 hours at 150, 160, 170, 180, 190 and 200ºC. The changes in the chlorophyll content at different temperature over time are shown in Figure 1. Two-way ANOVA results indicated that the interaction of temperature and the extent of heat treatment was significant (P<0.05).

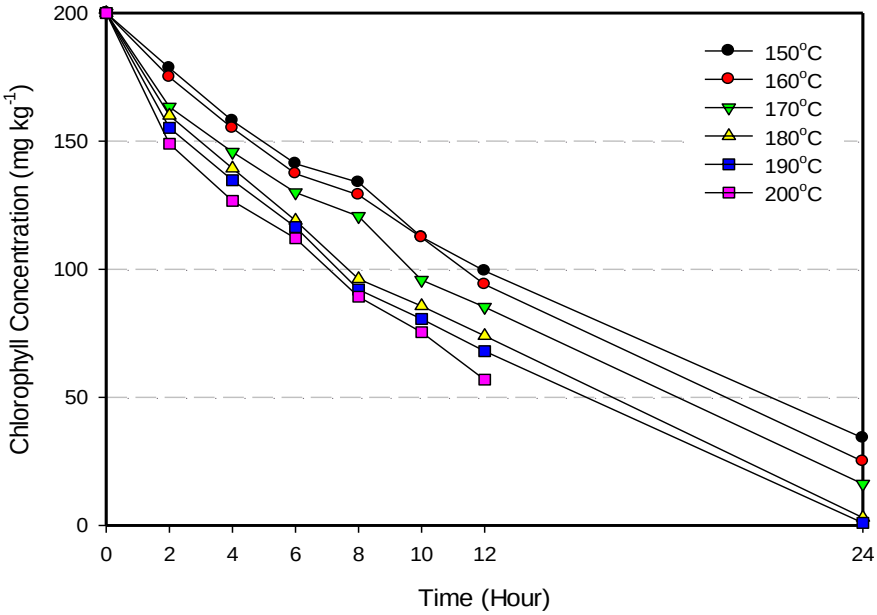

Figure 1. Effect of temperature on thermal stability of chlorophyll in VOO

The initial chlorophyll content of VOO was 200±0.57 mgkg^{-1}. It was found that the higher the treatment temperature, the lower the chlorophyll content in the VOO sample (P<0.05). A 10ºC increase in the temperature caused 2 to 15% decrease up to 12 h, and 27 to 67% decrease up to 24 h in the chlorophyll content (Table 1). No chlorophyll was detected at 200ºC after 24 h heat treatment.

Table 1. Changes in the chlorophyll content (mgkg^{-1}) of VOO

	Extent of Heat Treatment (h)							
Temperature (°C)	0	2	4	6	8	10	12	24
150	200.0 $^{a\,H}$	178.6 $^{d\,G}$	158.0 $^{d\,F}$	141.2 $^{d\,E}$	133.9 $^{e\,D}$	112.6 $^{e\,C}$	99.4 $^{f\,B}$	34.1 $^{d\,A}$
160	200.0 $^{a,\,H}$	175.0 $^{d\,G}$	155.1 $^{d\,F}$	137.3 $^{d\,E}$	129.0 $^{d\,D}$	112.4 $^{e\,C}$	94.1 $^{e\,B}$	25.0 $^{c\,A}$
170	200.0 $^{a\,H}$	163.4 $^{bc\,G}$	145.8 $^{bc\,F}$	130.0 $^{c\,E}$	120.7 $^{c\,D}$	95.8 $^{d\,C}$	85.2 $^{d\,B}$	16.2 $^{b\,A}$
180	200.0 $^{a\,H}$	160.0 $^{b\,G}$	139.4 $^{b\,F}$	119.0 $^{b\,E}$	96.2 $^{b\,D}$	85.6 $^{c\,C}$	74.0 $^{c\,B}$	3.0 $^{a\,A}$
190	200.0 $^{a\,H}$	155.2 $^{b\,G}$	134.8 $^{b\,F}$	116.5 $^{ab\,E}$	92.1 $^{ab\,D}$	80.6 $^{b\,C}$	68.0 $^{b\,B}$	1.0 $^{a\,A}$
200	200.0 $^{a\,G}$	149.0 $^{a\,F}$	126.7 $^{a\,E}$	112.1 $^{a\,D}$	89.2 $^{a\,C}$	75.4 $^{a\,B}$	56.9 $^{a\,A}$	n.d.

Means followed by different letters within each column (series "a-f") and each row (series "A-H") are significantly different (P<0.05), n.d.: not detected

The extent of heat treatment accelerated decomposition of chlorophyll in the VOO sample significantly (P<0.05). The chlorophyll content decreased up to 71.6 and 99.5% of the initial value at the end of 12- and 24-h treatments, respectively. Especially high treatment temperatures (180, 190 and 200ºC) were found to have more detrimental effect on the stability of chlorophyll in VOO.

The findings indicate that chlorophyll is heat-labile and severely affected by the extent of heat treatment. These results are in good agreement with Ayadi and Grati-Kamun (2009), Malheiro *et al.* (2009) and Jaber *et al.* (2012) who reported more than 90% chlorophyll loss in olive oil samples.

Changes in Conjugated Diene (K_{232}) and Triene (K_{270}) Contents

The ultraviolet spectrophotometric analysis at 232 and 270 nm is simple and useful parameters for assessing the state of olive oil oxidation, being its values expressed as specific extinction coefficients. K_{232} is a measure of the primary oxidation products, conjugated dienes, which are formed by a shift in one of the double bonds. K_{270} is the indicative of conjugated trienes (the primary oxidation products) and secondary oxidation products such as aldehydes and ketons (Kiritsakis *et al.*, 2002). The maximum values permitted for K_{232} and K_{270} are respectively 2.50 and 0.20 for extra VOO, and 2.60 and 0.25 for VOO, respectively (Annexes II and IX in European Community Regulation EEC/2568/91).

The conjugated diene content (K_{232} value) of the VOO sample was determined for 0, 2, 4, 6, 8, 10, 12, and 24 hours at 150, 160, 170, 180, 190 and 200ºC. Figure 2 shows the changes in K_{232} specific extinction coefficient at different temperature over time. It was found that the interaction of temperature and the extent of heat treatment significantly affected the K_{232} specific extinction coefficient (P<0.05).

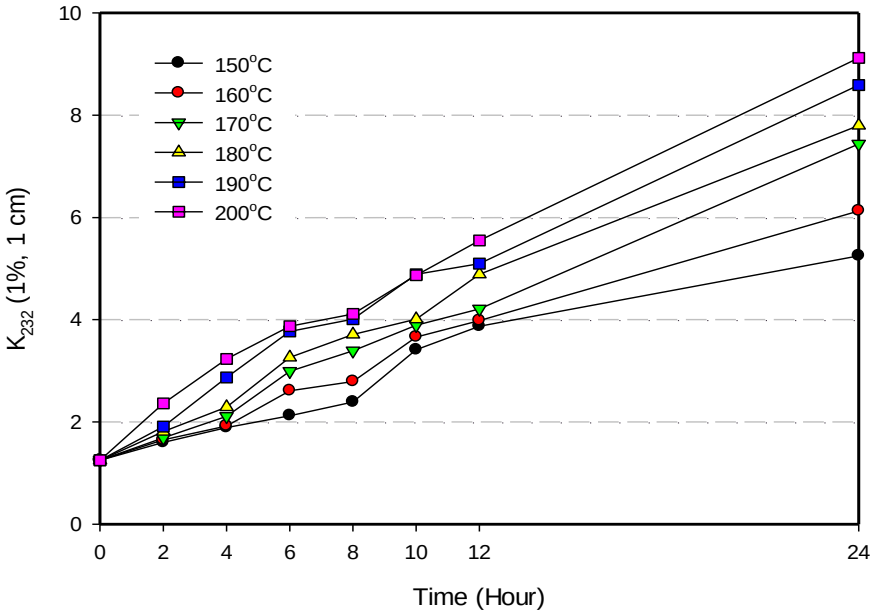

Figure 2. Effect of temperature on the K_{232} specific extinction coefficient of VOO

The initial K_{232} specific extinction coefficient of VOO was 1.25±0.13 (1%, 1 cm). The increase in the temperature increased the K_{232}specific extinction coefficient significantly (P<0.05), and reached up to 9.12 (1%, 1 cm) at 200ºC after 24 h of heat treatment (Table 2). Every 10ºC increase from 150 to 200ºC resulted in 4 to 7 times increase in the K_{232} specific extinction coefficient, indicating that higher temperatures had more effective on the formation of conjugated dienes in VOO.

Table 2. Changes in the K_{232} specific extinction coefficient of VOO

	Extent of heat treatment (h)							
Temperature (°C)	0	2	4	6	8	10	12	24
150	1.25 ^{aA}	1.60 ^{aB}	1.89 ^{aC}	2.12 ^{aD}	2.39 ^{aE}	3.41 ^{aF}	3.87 ^{aG}	5.25 ^{aH}
160	1.25 ^{aA}	1.65 ^{abB}	1.92 ^{aC}	2.61 ^{bD}	2.79 ^{bE}	3.66 ^{bF}	3.98 ^{bG}	6.13 ^{bH}
170	1.25 ^{aA}	1.69 ^{bB}	2.11 ^{bC}	2.99 ^{cD}	3.39 ^{cE}	3.89 ^{cF}	4.21 ^{cG}	7.44 ^{cH}
180	1.25 ^{aA}	1.81 ^{cA}	2.29 ^{cC}	3.26 ^{dD}	3.71 ^{dE}	4.01 ^{dF}	4.89 ^{dG}	7.80 ^{dH}
190	1.25 ^{aA}	1.91 ^{dB}	2.87 ^{dC}	3.77 ^{eD}	4.01 ^{eE}	4.89 ^{eF}	5.10 ^{eG}	8.59 ^{eH}
200	1.25 ^{aA}	2.36 ^{eB}	3.23 ^{eC}	3.87 ^{fD}	4.11 ^{fE}	4.87 ^{eF}	5.55 ^{fG}	9.12 ^{fH}

Means followed by different letters within each column (series "a-f") and each row (series "A-H") are significantly different (P<0.05).

It was found that the higher the extent of heat treatment, the higher the K_{232} specific extinction coefficient in the VOO sample (P<0.05). When the extent of heat treatment increased from 0 to 24 h the K_{232} specific extinction coefficient increased around 1.6±0.2 times. However, the rate of the increase in K_{232} specific extinction coefficient did not change with the 2-h increment in the heat treatment (P<0.05), indicating that the formation of conjugated dienes in VOO occurred almost same rate.

The conjugated triene content (K_{270}) of the VOO sample was determined for 0, 2, 4, 6, 8, 10, 12, and 24 hours at 150, 160, 170, 180, 190 and 200ºC. The changes in K_{270} specific extinction coefficient at different temperature over time are illustrated in Figure 3. The result of two-way analysis of variance (ANOVA) indicated that the interaction of temperature and the extent of heat treatment significantly affected the K_{270} specific extinction coefficient (P<0.05).

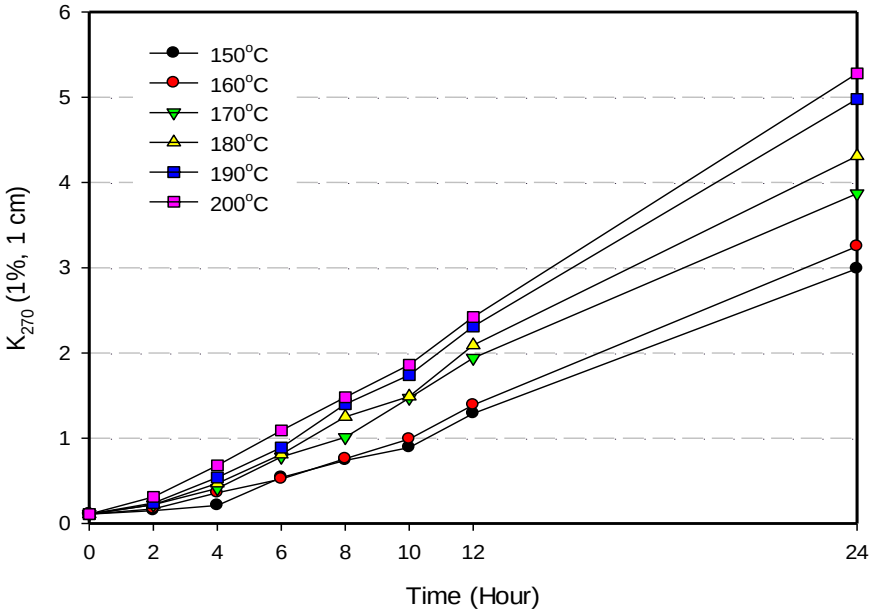

Figure 3. Effect of temperature on the K_{270} specific extinction coefficient of VOO

The initial K_{270} specific extinction coefficient of VOO was 0.11±0.03 (1%, 1 cm). It was found that the greater the temperature, the greater was the increase in the K_{270} specific extinction coefficient. The K_{270} specific extinction coefficient significantly increased when the temperature increased (P<0.05), and reached up to 5.28 (1%, 1 cm) at 200ºC after 24 h of heat treatment (Table 3). Every 10ºC increase from 150 to 200ºC accelerated the K_{270} specific extinction coefficient about 27 to 48 times, indicating that higher temperatures had more effective on the formation of conjugated trienes in VOO.

It was observed that when the extent of heat treatment was increased, the K_{270} specific extinction coefficient in the VOO sample was increased significantly (P<0.05). When the extent of heat treatment increased from 0 to 24 h the K_{270} specific extinction coefficient increased around 2.2±0.5 times. However, the rate of the increase in K_{270} specific extinction coefficient did not change with the 2-h increment in the heat treatment (P<0.05), indicating that the formation of conjugated trienes in VOO occurred almost same rate.

These experimental results are in agreement with the expectation because the formation of conjugated -C=C-C=C-C=C- double bond system is improbable even in the case of linolenic acid oxidation. The –C=C-C=C-C=O systems are more probable, and could interfere with the measurement at 270 nm. The value of K_{232} and K_{270} specific extinction coefficients in this study exceeded the limits established by European Community Regulation (Annexes II and IX in EEC/2568/91) for VOO.

Table 3. Changes in the K_{270} specific extinction coefficient of VOO

	Extent of heat treatment (h)							
Temperature (°C)	0	2	4	6	8	10	12	24
150	0.11 ^{aA}	0.15 ^{aA}	0.21 ^{aA}	0.54 ^{aB}	0.74 ^{aC}	0.89 ^{aD}	1.29 ^{aE}	2.99 ^{aF}
160	0.11 ^{aA}	0.17 ^{aA}	0.36 ^{bB}	0.52 $^{a.C}$	0.76 ^{aD}	0.99 ^{aE}	1.39 ^{aF}	3.25 ^{bG}
170	0.11 ^{aA}	0.22 ^{bA}	0.41 ^{bB}	0.78 ^{bC}	1.01 ^{bD}	1.47 ^{bE}	1.94 ^{bF}	3.87 ^{cG}
180	0.11 ^{aA}	0.22 ^{bA}	0.47 ^{bcB}	0.81 ^{bcC}	1.25 ^{cD}	1.49 ^{bE}	2.09 ^{cF}	4.31 ^{dG}
190	0.11 ^{aA}	0.24 ^{bB}	0.54 ^{cC}	0.89 ^{cD}	1.40 ^{dE}	1.74 ^{cF}	2.31 ^{dG}	4.98 ^{eH}
200	0.11 ^{aA}	0.31 ^{cB}	0.68 ^{dC}	1.09 ^{dD}	1.48 ^{dE}	1.86 ^{dF}	2.42 ^{dG}	5.28 ^{fH}

Means followed by different letters within each column (series “a-f”) and each row (series “A-H”) are significantly different (P<0.05).

These results are in good agreement with the previous studies in which different storage or treatment temperature and time accelerated the value of K_{232} and K_{270} specific extinction coefficients in VOO (Caponio *et*

al., 2005; Allouche *et al.,* 2007; Bester *et al.,* 2008; Ayadi and Grati-Kamun, 2009; Mahmoud *et al.,* 2009; Malheiro *et al.,* 2009; Farhoosh *et al.,* 2012).

Correlation of Chlorophyll Content with Specific Extinction Coefficients

The relationship between the changes in the chlorophyll and conjugated diene (K_{232}) and triene (K_{270}) contents with temperature and the extent of heat treatment was studied by regression analysis. The relationship was found to be significant (P<0.05), and chlorophyll content inversely related to K_{232} and K_{270} specific extinction coefficients.

The coefficient of determination (R^2) was higher than 0.95 for K_{232} specific extinction coefficient and 0.88 for K_{270} specific extinction coefficient, indicating a good correlation between the chlorophyll content and the K_{232} and K_{270} specific extinction coefficients (Table 4). Our findings are in agreement with those of Malheiro *et al.* (2009).

Table 4. Linear regression analysis between chlorophyll content and K_{232} and K_{270} specific extinction coefficients

		K_{232}		K_{270}	
		Equation	R^2	Equation	R^2
Chlorophyll content	150	y= –37.46x+234.21	0.96	y= –51.72x+176.96	0.91
	160	y= –33.75x+229.67	0.98	y= –50.19x+175.84	0.92
	170	y= –27.95x+213.85	0.96	y= –43.23x+172.65	0.93
	180	y= –28.29x+212.29	0.96	y= –41.17x+164.97	0.89
	190	y= –25.96x+211.15	0.95	y= –35.57x+160.32	0.88
	200	y= –32.74x+233.66	0.98	y= –54.92x+177.99	0.90

CONCLUSION

This study evaluated the changes in chlorophyll content and oxidative stability of virgin olive oil at the temperatures between 150 and 200ºC during 24 hours.

During heat treatment, a decrease in the chlorophyll content and an increase in the UV absorbance values (K_{232} and K_{270}) took place in VOO, as the measure of oxidative degradation of VOO. Significant differences were found among the oil samples treated at different temperature and time at 5% significance level.

The thermo-oxidative stability of VOO was higher at high temperature and for long treatment time. Chlorophyll content decreased around 99.5% at 200ºC after 24 h of heat treatment. K_{232} and K_{270} specific extinction coefficients of VOO exceeded the maximum allowable limits of the European Community Regulation at the experimental conditions used, being not considered as VOO anymore. These results showed that VOO is not suitable for high temperature processes.

Linear regression analysis was able to predict VOO degradation in terms of chlorophyll content and K_{232} and K_{270} specific extinction coefficients. The coefficient of determination (R^2) ranged between 0.88 and 0.98, indicating that determination of chlorophyll content and K_{232} and K_{270} specific extinction coefficients was assigned as the useful markers of oxidative status of VOO.

REFERENCES

Allouche, Y., Jimenez, A., Gaforio, J.J., Uceda, M., Beltran, G. 2007. How Heating Affects Extra Virgin Olive Oil Quality Indexes and Chemical Composition. Journal of Agricultural and Food Chemistry, 55:9646–9654.

Angerosa, F. 2002. Influence of Volatile Compounds on Virgin Olive Oil Quality Evaluated by Analytical Approaches and Sensor Panels. European Journal of Lipid Science and Technology, 104:639–660.

Angerosa, F., Campestre, C., Giansante, L. 2006. Analysis and Authentication. In: Olive Oil: Chemistry and Technology (Editor: Boskou, D.). AOCS Press, IL. pp. 113–172.

Anonymous, 2001. Spectrophotometric Investigation in the Ultraviolet. COI/T20/Doc. no. 19/Rev.1. International Olive Oil Council, Spain.

Anonymous, 2004. International Conference on the Health Effect of Virgin Olive Oil. European Journal of Clinical Investigation, 35:421–424.

Aparicio, R., Roda, L., Albi, M.A., Gutiérrez, F. 1999. Effect of Various Compounds on Virgin Olive Oil Stability Measured by Rancimat. Journal of Agricultural and Food Chemistry, 47:4150–4155.

Ayadi, M.A., Grati-Kamun, N.H. 2009. Physico-Chemical Change and Heat Stability of Extra Virgin Olive Oils Flavored by Selected Tunisian Aromatic Plants. Food Chemistry and Toxicology, (47): 2613–2619.

Bester, E., Butinar, B., Bucar-Miklavcic, M., Golob, T. 2008. Chemical Changes in Extra Virgin Olive Oils from Slovenian Istra after Thermal Treatment. Food Chemistry, 108:446–454.

Boskou, D. 2006. Characteristics of the Olive Tree and Olive Fruit. In: Olive Oil: Chemistry and Technology (Editor: Boskou, D.). AOCS Press, IL. pp. 13–19.

Boskou, D., Blekas, G., Tsimidou, M. 2006. Olive Oil Composition. In: Olive Oil: Chemistry and Technology (Editor: Boskou, D.). AOCS Press, IL. pp. 41–72.

Caponio, F., Bilancia, M.T., Pasqualone, A., Sikorska, E., Gomes, T. 2005. Influence of the Exposure to Light on Extra Virgin Olive Oil Quality during Storage. European Food Research and Technology, 221:92–98.

Chen, B.H., Liu, M.H. 1998. Relationship between Chlorophyll a and β-Carotene in a Lipid-Containing Model System during Illumination. Food Chemistry, 63:207–213.

Cichelli, A., Pertesana, G.P. 2004. High-Performance Liquid Chromatographic Analysis of Chlorophylls, Pheophytins and Carotenoids in Virgin Olive Oils: Chemometric Approach to Variety Classification. Journal of Chromatography A, 1046:141–146.

Del Giovine, L., Fabietti, F. 2005. Copper Chlorophyll in Olive Oils: Identification and Determination by LIF Capillary Electrophoresis. Journal of Food Control, 16:267–272.

Endo, Y., Usuki, R., Kaneda, T. 1984. Prooxident Activities of Chlorophylls and Their Decomposition Products on the Photooxidation of Methyl Linoleate. Journal of the American Oil Chemists' Society, 61:781–784.

Farhoosh, R., Khodaparast, M.H.H., Sharif, A., Rafiee, S.A. 2012. Olive Oil Oxidation: Rejection Points in terms of Polar, Conjugated Diene, and Carbonyl Value. Food Chemistry, 131: 1385–1390.

Jaber, H., Ayadi, M., Makni, J., Rigane, G., Sayadi, S., Bouaziz, M. 2012. Stabilization of Refined Olive Oil by Enrichment with Chlorophyll Pigments Extracted from Chemlali Olive Leaves. European Journal of Lipid Science and Technology, 114:1274–1283.

Kiritsakis, A., Kanavouras, A., Kritsakis, K., 2002. Chemical Analysis, Quality Control and Packaging Issues of Olive Oil. European Journal of Lipid Science and Technology, 104:628–638.

Mahmoud, E.A.E-.M., Dostalova, J., Pokorny,J., Lukesova, D., Dolezal M. 2009. Oxidation of Olive Oils during Microwave and Conventional Heating for Fast Food Preparation. Czech Journal of Food Sciences, 27:S173–S177.

Malheiro, R., Oliveira, I., Vilas-Boas, M., Falcão, S., Bento, A., Pereira, J.A. 2009. Effect of Microwave Heating with Different Exposure Times on Physical and Chemical Parameters of Olive Oil. Food and Chemical Toxicology, 47:92–97.

Morello, J.-R., Motilva, M.-J., Tovar, M.-J., Romero, M.-P. 2004. Changes in Commercial Virgin Olive Oil (cv Arbequina) during Storage, with Special Emphasis on the Phenolic Fraction. Food Chemistry, 85:357–364.

Ozyilkan, O., Colak, D., Akcali, Z., Basturk, B. 2005. Olive: Fruit of Peace against Cancer. Asian Pacific Journal of Cancer Prevention**,** 6:77–82.

Perez-Jimenez, F., Ruano, J., Perez-Martinez, P., Lopez-Segura, F., Lopez-Miranda, J. 2007. The Influence of Olive Oil on Human Health: Not a Question of Fat Alone. Molecular Nutrition Food Research, 51:1199–1208.

Pokorny, J., Kalinova, L., Dysseler, P. 1995. Determination of Chlorophyll Pigments in Crude Vegetable Oils. Pure & Applied Chemistry, 67:1781–1787.

Rahmani, M., Csallany, A.S. 1998. Role of Minor Constituents in the Photooxidation of Virgin Olive Oil. Journal of the American Oil Chemists' Society, 75:837–843.

Tsimidou, M.Z. 2006. Olive Oil Quality. In: Olive Oil: Chemistry and Technology (Editor: Boskou, D.). AOCS Press, IL. pp. 93–111.

Visioli, F., Bogani, P., Galli, P., 2006. Healthful Properties of Olive Oil Minor Components. In: Olive Oil: Chemistry and Technology (Editor: Boskou, D.). AOCS Press, IL. pp. 173–190.