Effects of High Temperatures and Duration of Heating on Olive Oil Properties for Food Use and Biodiesel Production

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Abstract

Heating deteriorates the physicochemical proper- ties of a vegetable oil for both edible and biofuel uses. The parameters for edible olive oil are established by European Union regulations and by the International Olive Council. The properties of a vegetable oil to be used as a source for biodiesel production are indicated by the German DIN 51605 for rapeseed oil. Biofuel properties are described by the European EN 14214 and the North American ASTM 6751 standards for biodiesel. It is useful to know how temperature and heating duration influence the physicochemical properties of olive oil. Free acidity, refractive index and myristic acid were not significantly influenced by temperature and heating duration. K232, K266, K270, K274, *p*-anisidine value, totox index, kinematic viscosity (at 30, 40, 50 °C), estimated higher heating value, relative density, and cetane number increased during olive oil heating. The biological properties: iodine value, oxidative stability index, antiradical (2,2-diphenyl-1-picrylhydrazyl radical, DPPH·) activity, and phenol content, decreased when time and temperature increased. Fatty acid methyl esters were highly influenced by the applied variables. Almost all the fatty acid methyl esters, except myristic, stearic, and arachidic acid esters, were influenced by the combined effect of temperature and time in a very highly significant level. These results show how temperature and duration of heating influence extra virgin olive oil degradation for both edible use and biodiesel production.

Keywords

Biodiesel properties · Fatty acid methyl esters · International standard · Mono-alkyl esters · Renewable energy · Vegetable oil quality standard

Introduction

Extra virgin olive oil (EVOO) is one of the most important ingredient of the Mediterranean diet. In the crop year 2013–2014, Italy produced 464,000 tonnes of olive oil, and Europe produced 2,482,700 tonnes [1]. Frying is one of the most common cooking systems for fast foods, and a high quantity of olive oil used for frying remains as a waste after its use and has to be disposed of. During frying, the high temperature causes changes in the physicochemical parameters of olive oil. This has important consequences for both human health and for a possible use of the heated olive oil for biodiesel production. From the point of view of human health, an increase in free acidity causes difficulty in digesting the olive oil. An increase in oxidative products due to oxidative stress is considered to contribute to the atherosclerosis [2]; in addition, lipid aldehydes (produced by oil oxidation) were found to have a deleterious effect by inducing apoptosis and necrosis [3]. From the point of view of its use as a biodiesel feedstock, it is important to note that there is a deep worldwide uncertainty about the price and supply of crude oil. Hence, non-oil-producing countries are studying different options to reduce their dependence on crude oil and thus reduce the costs imports. Biofuel production from vegetable oils is one of the possibilities under study. If biofuel is produced from an edible vegetable oil, this will cause its price to increases due to the competition in the demand between edible and biofuel purposes. As a consequence, a used vegetable oil which would otherwise be waste, is preferred for biodiesel production. The cost of rectifying the heated oil depends on its physicochemical properties, which are related to the thermal stress applied. Biodiesel possesses similar physical and chemical characteristics of petro-diesel and does not contain polluting sulphuric compounds [4].

The aim of this work is to study the effects of high temperature and duration of heating on the physicochemical parameters of olive oil both for edible use and for biodiesel production. The variation of the edible properties and of the bio-fuel aptitude are studied and quantified during heating.

Materials and Methods

The international standards use different methods and parameters to evaluate the quality of the oils according their use as food or fuel. Physicochemical parameters were

considered in relation to the requirements of standards.

Regulations for Olive Oil as a Food

The European Union and the International Olive Council (IOC) state the physicochemical parameters for an EVOO.

The European regulation [5] contains the most recent European statements for an EVOO for edible use, after a long series from 1991 when the first European regulation was established. The IOC regulation [6] lists the most recent regulations for an edible olive oil accepted by a wide number of State members such in North Africa (Morocco, Algeria, Egypt, Libya, and Tunisia), South America (Argentina and Uruguay), the Middle East (Jordan, Iran, Iraq, Israel, Lebanon, and Turkey), and Europe (Italy, Spain, Greece, Albania, Montenegro, etc.).

Quality Standards for a Vegetable Oil as a BioFuel

The German standard DIN 51605:2012 [7] lists the physicochemical properties for a rapeseed oil to be used as a source for bio-fuel production. This standard can be used to compare the physicochemical properties of a vegetable oil with the those of rapeseed oil. The European Standard EN 14214:2014 [8] contains the requirements and the test methods for bio-fuel to be used in Europe. The American Society for Testing and Materials published the standard specification for biodiesel fuel in North America (ASTM 6751) [9].

Experimental Design

One hundred-gram samples of EVOO were placed in steel containers and heated to either 180, 210, or 240 °C. These three temperatures were chosen on the basis of the frying temperature of olive oil (180–210 °C) and of the temperature that can be reached during deep frying (240 °C). The samples were held hat each temperature for 15, 30, 60, and 120 min, giving a total of 12 trials. After heating, the oil was cooled to room temperature and ana- lysed within 2 h. The experiment on the heated extra virgin olive oil (H-EVOO) was conducted in triplicate.

Chemicals

All solvents were sourced from Panreac (Barcelona, Spain), FAMEs were from Sigma-Aldrich (St. Louis, MO, USA).

Physicochemical Analyses on Olive Oil

Refractive Index

An Abbe refractometer was used at 20 °C as suggested by the Association of Analytical Communities (AOAC, 2000) [10].

Free Acidity

The oil/ethyl ether/ethylic alcohol solution was titrated by a 0.1 N sodium hydroxide/water solution. Results are expressed as g oleic acid/100 g (CONSLEG, 2015—Annex II) [5].

Peroxide Value

The oil-chloroform-acetic acid solution was titrated by a 0.01 N sodium tiosulphate/water solution Results are expressed as mEq O2/kg (CONSLEG, 2015—Annex III) [5].

Spectrophotometric Indexes

The oil/cyclohexane solution (1%, w/v) was read in a double ray spectrophotometer Perkin Elmer model Lambda 2. The indexes are expressed as extinction coefficients (K) at different wavelengths and ΔK at 270 nm (CONSLEG, 2015—Annex IX) [5].

Antiradical Activity

DPPH is a stable free radical which is reduced to DPPH-H when antioxidant compounds present in EVOO react with DPPH. This chemical reaction is accompanied by a change of colour from purple to yellow.

A mixture containing olive oil/ethyl acetate/2,2-diphe- nyl-1-picrylhydrazyl radical (DPPH·) was read at 515 nm (30 min) using an Agilent model 8453 spectrophotometer, Santa Clara, CA, USA). Results are expressed as % of inhibition [11, 12].

Phenolic Content

The Folin–Ciocalteau colourimetric method was applied and results were expressed as mg/kg of gallic acid [11].

p -Anisidine Value

The assay measures aldehydes (principally 2-alkenals) as secondary products of oil oxidation by reacting them with p-anisidine; the increase in absorbance at 350 nm estimates the amount of these aldehydes and is used to calculate the p-AnV. Olive oil was dissolved in iso-octane and after reacting with p-anisidine in acetic acid the mixture was read in a spectrophotmeter (Perkin Elmer, model Lambda 2) [13].

Totox Index

It was calculated as 2PV + p-AnV.

Fatty Acid Methyl Esters

The olive oil methylation was carried out using the CON- SLEG, annex XB method A [5]. FAME analysis was conducted as reported in a previous study and results are expressed as % m/m [14].

Acid Value

The olive oil was analysed using the AOAC 969.17 method, by titration with a KOH solution. Results are expressed as mg KOH/g [10]

Iodine Value

The olive oil (1 g) was dissolved in a (1:1, v/v) cyclohexane/acetic acid solution. The mixture was titrated with a 0.1 N sodium thiosulphate solution after adding 20 mL of Wijs reagent, 20 mL of a 100 g/L KI aqueous solution, 150 mL of deionised water and fve drops of a 3% aqueous starch solution. Results are expressed as g I2/100 g (CONSLEG, 2015, Annex XVI) [5].

Oil Stability Index

The oil (3 g) was weighed in a glass tube and the analysing instrument (Rancimat model 679, Metrohm, Switzerland) was set with: 10 L/h air fow, 60 mL deionized water, 110 °C temperature, 1 cm/h chart speed. Results are expressed as h of resistance to oxidation.

Kinematic Viscosity

An Ubbelohde viscometer was used. The Norme Grassi e Derivati method was applied

[13]. Analysis was conducted at 30-40-50 °C and results are expressed as mm2/s.

Higher Heating Value

Demirbas [15] estimated the HHV for a vegetable oil with the equation HHV 0.0317 kV (mm2/s) 38.053. A positive relation between KV and HHV (R 0.9435) was found. The HHV estimation was on the basis of KV measured at 40 °C.

Density

A pycnometer was used as suggested by the AOAC 920.212 method. Results are expressed as kg/m3 [10].

Cetane Number

CN was estimated from FAME profile using the equation:

$$\mathrm{CN} = \sum_{i=1}^{n} x_i \, \mathrm{CN}_i$$

where: xi is the mass fraction of the individual FAME and CNi is the experimental CN of the individual methyl ester [16].

Statistical Analysis

Means of three replicates (separately prepared) were calculated by Microsoft Excel software (2010 version). One way ANOVA was conducted at $p < 0.05$ using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA) to determine the statistical differences and the Tukey's test was used. Two-way ANOVA for analysis of the variance was conducted at $p < 0.05$ using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA) to determine the effect of temperature, duration of heating exposure and the interaction between temperature and duration of heating exposure.

Results and Discussion

Refractive Index

This value is given by the ratio between the speed of light in vacuum and that determined in the studied olive oils. The RI showed only a slight increase when EVOO was heated, although this change was found not to be significantly

Free Acidity

FA ranged from 0.53 to 0.62%. The highest values were found after 120 min of heating (Table 1). In all cases FA was found to be below the 0.80% stated by the European Union Regulation [5] and by IOC [6] for an EVOO. Temperature, duration of heating, and their combination did not influence the free acidity even after olive oil heating at 240 °C for 2 h (Table 1).

Peroxide Value, p-Anisidine Value, Totox

PV gives the actual state of oxidation of a vegetable oil. p-AnV predicts the secondary step of oxidation evolution. Totox index includes both PV and p-AnV for more complete information of the vegetable oil oxidation. Air (oxy- gen), high temperature, and light determine and accelerate the oxidative alteration of a vegetable oil. In the first step hydroperoxides are produced, but due to their chemical instability they begin to decompose and tend to be trans- formed into aldehydes and ketones, which are more stable volatile products (second step) [18]. This explains why the highest PVs were found when the oil was heated at 180 °C (the lowest studied temperature), in fact at this temperature the hydroperoxides are less transformed into aldehydes. The contrary happened when the temperature increased and the hydroperoxides were quickly transformed into aldehydes (Table 1). The heating duration caused an increase in the PV at each temperature. The present European [5] and IOC [6] regulations state 20 mEq/kg of oil as the maximum for an EVOO. In EVOO was found 3.32 as PV which was 7.28 (i.e. 2.19 times more) after 2 h heating at 180 °C. Temperature and duration of heating high significantly influenced the PV and their combination showed a very high influence (Table 2). Duration of heating and the combination temperature time very highly significantly influenced p-AnV and Totox index (Table 2).

Spectrophotometric Indices

Spectrophotometric analysis in the ultraviolet gives additional information about the oxidative state of an oil. Conjugated dienes and trienes, which are formed during heating, are detected at 232 and 270 nm, respectively. Primary and secondary products of oxidation also influence the absorbance at these two wavelengths [19].

Spectrophotometric indices of unheated EVOO were below the maximum indicated by the EU [5] and IOC [6]. At all the three applied temperatures, K232 increased constantly

for 1 h and decreased after 2 h of heating. This was mostly evident at 210 °C (Table 1). Only the absorbance reading after 15 min of heating (K232 2.33) did not exceed the maximum indicated by the international regulations (2.50). At the same time, K270 was below the legal limit (0.22) only in the unheated EVOO and increased constantly during heating with a higher rate compared to the rate found at 232 nm. This is probably due to the high quantity of secondary products formed during heating, also because in an olive oil the linolenic acid content is low and consequently the influence of the conjugated trienes in the K270 value is low. The conjugated trienes were very highly influenced by the temperature and by the time of heating, whereas the conjugated dienes were very highly influenced by the combination of these two factors (Table 2).

Antiradical Activity

The AA was highest in the unheated EVOO (81.58%) and decreased constantly during olive oil heating in parallel with the decrease in phenolic content (Table 1). The lowest values were found at 210 and 240 °C when the AA became less than half after 2 h, 35.87 and 37.28, respectively. The applied variable influenced the DPPH as follows: temperature ($p < 0.05$), duration of heating ($p < 0.01$) temperature × time ($p < 0.001$) (Table 2).

Phenolic content

The phenolic content was significantly highest in the unheated EVOO (2511 mg/kg) and decreased constantly with the increase in thermal stress. After 2 h of heating the phenolic content decreased 63.44, 70.61, and 80.17%,

respectively, at 180, 210, and 240 °C (Table 1). The temperature very highly significantly influenced phenolic con- tent ($p < 0.001$), whereas duration of heating showed a minor effect ($p < 0.05$), (Table 2).

Fatty Acid Methyl Esters

FAMEs are among the most important parameters to establish the edibility of a vegetable oil; in particular, oleic acid content and the mono-unsaturated/poly-unsaturated ratio are considered.

The high saturated fatty acid (SFA) content deter- mines vegetable oil solidification at low temperatures and increases the cholesterol content in the blood. The high unsaturated fatty acid (UFA) content determines an increase in oxidability of the vegetable oil for the

presence of the double bonds. However, mono-unsaturated fatty acids (MUFAs) are recognized to lower the bad cholesterol in the blood and essential fatty acids (EFAs) have to be taken with the diet.

Palmitic acid (16:0) calculated as percentage content increased with increasing temperature and with duration of heating. The minimum content (15.19%) was found in the EVOO before thermal treatment and the maximum (18.71%) was found in the olive oil heated at 240 °C for 120 min, i.e. when the oil was most stressed (Table 3). The same trend was found in the SFA content: 18.37% in the olive oil before thermal treatment and 22.09% at 240 °C for 120 min (Table 4). Linoleic acid (18:2) showed a decreasing trend from 18.45% in the unheated EVOO to 13.22% after 120 min heating at 240 °C (Table 3). Poly- unsaturated fatty acid (PUFA) content showed a decreasing trend in accordance with linoleic acid, the major unsaturated fatty acid (Table 4). FAME composition is very important for both food use and biofuel production. Linolenic acid (18:3, 0.38–0.74%) always was below the maximum stated by the EN 14214 (12%) for a bio-die sel [8]. Large part of the FAMEs were influenced in very highly significant level by the combined effect of temperature and duration of heating (Table 5).

Acid Value

A high AV implies the necessity to de-acidify the oil before the biodiesel production, with the consequence of a loss of oil and an increase in production costs. The presence of free fatty acids reduces the quantity of bio-diesel produced. For this reason, a vegetable oil with a low acidity is appreciated. The oil heating caused a slight increase in AV although all values were below the 2 mg KOH/g of oil indicated as a maximum limit by the DIN51605 [7]. The EVOO showed 1.05 mg KOH/g of oil as AV and until 1.23 mg KOH/g of oil when the olive oil was heated at 210 °C for 120 min (Table 6). The two-way ANOVA analysis demonstrated a not significant effect of the two variables, temperature and time, and their combination on AV (Table 7).

Iodine Value

The IV of a vegetable oil is very similar to the IV of the same vegetable oil after methylation. The DIN 51609 states 125 g I2/100 g as the maximum IV for a rapeseed oil to be used as a source for biodiesel production. The oil heating caused a constant decrease in IV of the studied oils. In the unheated EVOO was found 92.24 as IV,

whereas after 2 h heating at 210 °C the IV was 60.48 (Table 6). The IV is a measure of the total unsaturation of a vegetable oil and the IV decreasing trend found in the heated olive oils was in accordance with the UFA decreasing trend (Table 4). It is important to note that the MUFA percentage slightly increased during heating whereas PUFA percentage decreased with greater evidence; therefore, the UFA decrease was mainly due to the PUFA content (Table 4).

Oil Stability Index

The studied olive oil presented the maximum resistance to oxidation before the thermal treatment (18.10 h, Table 6). The OSI decreased with the increase in temperature ($p < 0.001$) and with duration of heating exposure ($p < 0.001$). Temperature time ($p < 0.001$) very highly significantly influenced OSI (Table 7). After 2 h of treatment at 240 °C the OSI was 1.33 h, only this sample was below the minimum (6 h) indicated by the DIN 51605 [7] for a rapeseed oil to be used for biodiesel production. The sample heated for 120 min at 210 °C presented a critical value,

6.63 h: a border line condition according to the International Standard. OSI was influenced by temperature, time and their combination in a very highly significant level (Table 7).

Kinematic Viscosity

Heating promotes vegetable oil polymerisation and produces high molar mass compounds which determines the KV increase and lower the suitability of the heated olive oil for biodiesel production. The DIN 51605 [7] for a rapeseed oil indicates 36 mm^2/s as a maximum value at 40 °C. EVOO exceeded this value before thermal stressing (46.04 mm^2/s). KV was significantly influenced by heating and increased with the increase in time and temperature (Table 6). The maximum KV determined at 40 °C was found after 120 min heating at 240 °C: 70.82 mm^2/s, i.e. almost two times the maximum stated by the DIN 51605. The KV at the three studied temperatures was influenced in a very highly significant level by the combined effect of temperature and duration of heating (Table 7).

Higher Heating Value

HHV of a vegetable oil is the quantity of heat produced by its complete combustion. In this work, HHV was estimated from the KV which is related to the SFAs and their

melting point. The higher the SFA content, the higher the KV, the higher the HHV. The DIN 51605/2012 [7] requires at least 36.0 MJ/kg as from the combustion of a rapeseed oil. In the H-EVOO the HHV was influenced by temperature ($p < 0.05$), by duration of heating ($p < 0.01$) and by the combination of the two variables ($p < 0.001$), (Table 7). Each sample showed a HHV value higher than 36 MJ/kg and the maximum (40.30 MJ/kg) was found in the most heat-stressed olive oil (Table 6). All the HHV calculated on EVOO and H-EVOOs were higher than HHV found in peanut oil from Algeria [12] and in tomato seed oil after cold break or hot break treatments [20]. HHV was very highly significantly influenced by the combination of temperature and time of heating (Table 7).

Density

The density of a bio-fuel is related to the density of the vegetable oil used as a source for its production. The potential energy increases with the increase in density [21]. D of EVOO was 913.70 kg/m3. This value increased with duration of heating and the maximum (922.03 kg/ m3) was found in the olive oil after 120 min heating at 210 °C (Table 6). The DIN 51605/2012 [7] indicates a range from 910 to 925 kg/m3. All samples were found to be within this range. It can be predicted that the maxi- mum limit will be exceeded if the temperature and/or duration of heating increase. Density was highly influenced by temperature and by time of heating ($p < 0.01$), the combination of these two factors produced a very highly significantly effect ($p < 0.001$) (Table 7).

Cetane Number

The CN is probably the most important parameter used to judge the performance of a bio-fuel. The higher the CN the lower the ignition delay of an engine [22]. The higher the CN the lower the NOx emissions [23]. The EN 14214:2012 [8] indicates at least 51 as CN for a biodiesel. The CN of a biofuel depends essentially from the fatty acid profile of oil from which was obtained. In the studied samples the CN increased with duration of heating and ranged from 61.12 in the EVOO to 63.50 in the olive oil after 120 min heat- ing at 240 °C (Table 6). Temperature ($p < 0.05$) duration of heating ($p < 0.01$) and their combination ($p < 0.001$) influenced this parameter (Table 7). The CN values found in the heated olive oils were similar to Simmondsia chinensis (jojoba) CN 63.5 [24], higher than 56.88–58.64 found in raw peanut oil [12], and higher than 52.51–54.71 found in raw tomato seed oil [20]. Wadumesthrige et al. [22] found a positive relation between the CN

increase and the presence of oligomers of FAMEs, aldehydes, and hydroperoxides as products of the oxidation.

Conclusion

The high heat treatment, the duration of heating and their combination were proved to significantly and differently influence the physicochemical parameters of extra virgin olive oil. Only free acidity, acid value, myristic acid, stearic acid, and arachidic acid were not significantly influenced by either of the applied variables. After 2 h of heating at 240 °C the extra virgin olive oil had deteriorated, neverthe- less it maintained a free acidity and a peroxide value below the maximum stated for an extra virgin olive oil. This ther- mal stress allows the heated oil to be used as a source for bio-diesel production, exploiting what would, otherwise be a waste product with its associated disposed costs.

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Table 1 Physicochemical properties of thermal stressed olive oil for food use

Temp. (°C)	Time (min)	RI	FA (%)	PV (meqO_2/kg)	K232	K266	K270	K274	Δ*K*	AA (%)	Phenols (mg/kg)	*p*-AnV	TOTOX
	0	1.467 a	0.53 b	3.32 de	1.68 i	0.14 h	0.14 h	0.13 i	0.01 f	81.58 a	2511 a	5.55 g	18.91 g
180	15	1.469 a	0.56 ab	4.48 cd	2.33 g	0.68 efg	0.69 ef	0.65 efg	0.03 e	80.79 a	1684 b	17.80 f	27.16 f
	30	1.469 a	0.56 ab	4.90 c	2.72 defg	0.84 de	0.86 de	0.81 cde	0.04 de	78.54 a	1771 b	17.12 f	26.92 f
	60	1.469 a	0.56 ab	6.22 ab	3.52 a	1.00 cd	1.02 cd	0.97 c	0.04 de	74.13 ab	1589 bc	24.84 e	37.28 e
	120	1.469 a	0.56 ab	7.28 a	3.30 abc	1.70 a	1.70 a	1.49 a	0.10 a	53.79 cd	918 de	45.64 c	60.20 c
210	15	1.469 a	0.56 ab	2.37 e	2.51 fg	0.48 g	0.48 g	0.43 h	0.03 e	74.77 ab	1390 c	28.29 de	33.04 ef
	30	1.469 a	0.57 ab	3.30 de	2.96 cde	0.55 fg	0.55 fg	0.48 gh	0.03 de	73.59 ab	1128 d	28.06 de	34.65 e
	60	1.469 a	0.57 ab	3.12 e	3.64 a	0.74 ef	0.75 e	0.65 efg	0.06 c	75.68 ab	1082 d	32.81 d	39.05 e
	120	1.469 a	0.62 a	5.29 bc	2.82 def	1.38 b	1.39 b	1.17 b	0.11 a	35.87 e	738 ef	70.10 b	80.68 b
240	15	1.469 a	0.53 b	3.39 de	3.48 ab	0.73 ef	0.73 ef	0.63 fg	0.05 cd	74.27 ab	720 ef	29.08 de	36.06 e
	30	1.469 a	0.55 b	2.91 e	3.09 bcd	0.86 de	0.87de	0.75 def	0.06 c	64.84 bc	654 f	30.01 de	35.84 e
	60	1.469 a	0.55 b	3.16 e	2.66 efg	1.07 b	1.07 c	0.91 cd	0.08 b	51.64 d	500 f	44.21 c	50.53 d
	120	1.469 a	0.59 ab	6.30 ab	2.51 fg	1.68 a	1.64 a	1.40 a	0.10 a	37.28 e	498 f	75.99 a	88.60 a
Sign.		n.s.		**	***	***	***	***	***	***	***	***	***

One-way ANOVA: means in the same column followed by a different letter are significantly different according to Tukey's test

n.s. not significant, $p > 0.05$

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table 2 Signification levels of the studied effects (temperature, time and temperature time) on the physicochemical properties of the olive oil for edible use

Property	Temperature	Time	Temperature × time
Refractive index	n.s.	n.s.	n.s.
Free acidity (%)	n.s.	n.s.	n.s.
Peroxide value (mEq O_2/kg oil)	**	**	***
p-AnV	**	***	***
TOTOX index	*	***	***
K232	n.s.	n.s.	***
K266	***	***	n.s.
K270	***	***	n.s.
K274	***	***	n.s.
ΔK	n.s.	***	***
Antiradical activity (% inhibition)	*	**	***
Total phenols (mg/kg)	***	*	***

Two-way ANOVA

n.s. not significant, $p > 0.05$

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table 3 Fatty acid methyl esters (% m/m)

Temp. (°C)	Time (min)	14:0	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	24:0
	0	0.01 a	15.19 h	1.34 a	0.04 gh	0.06 ef	2.60 a	60.76 f	18.45 a	0.72 ab	0.36 b	0.22 c	0.09 ab	0.17 a
180	15	0.01 a	15.88 g	1.25 b	0.12 c	0.09 abc	2.68 a	60.84 f	17.43 b	0.74 a	0.44 a	0.26 b	0.10 a	0.17 a
	30	0.02 a	16.44 de	1.24 b	0.15 b	0.11 a	2.62 a	61.38 e	16.47 cd	0.71 ab	0.40 ab	0.23 bc	0.10 ab	0.15 a
	60	0.01 a	16.21 efg	1.26 b	0.16 ab	0.08 bc	2.67 a	61.69 de	16.30 c	0.72 ab	0.42 ab	0.25 b	0.10 a	0.12 b
	120	0.02 a	17.14 b	1.28 b	0.17 a	0.10 ab	2.67 a	61.62 e	15.60 fg	0.62 def	0.39 ab	0.23 bc	0.09 ab	0.08 c
210	15	0.01 a	16.03 fg	1.28 b	0.09 d	0.08 cd	2.61 a	62.13 bcd	16.20 cd	0.72 ab	0.41 ab	0.23 bc	0.10 a	0.12 b
	30	0.01 a	16.04 fg	1.09 e	0.03 h	0.05 f	2.67 a	62.22 bc	16.32 cd	0.72 ab	0.43 a	0.24 bc	0.09 ab	0.08 cd
	60	0.01 a	16.15 efg	1.10 de	0.07 ef	0.06 ef	2.67 a	62.26 bc	16.14 d	0.69 abc	0.44 a	0.24 bc	0.10 a	0.07 cd
	120	0.02 a	16.88 bc	1.15 cd	0.05 gh	0.06 def	2.73 a	62.59 ab	15.12 h	0.57 f	0.43 a	0.25 bc	0.10 a	0.05 d
240	15	0.01 a	16.38 def	1.13 cde	0.04 h	0.06 def	2.63 a	62.20 bc	16.06 de	0.67 bcd	0.41 ab	0.24 bc	0.09 ab	0.08 c
	30	0.01 a	16.69 cd	1.15 cd	0.04 h	0.07 cdef	2.65 a	62.11 cd	15.82 ef	0.65 cde	0.41 ab	0.24 bc	0.09 ab	0.08 c
	60	0.02 a	17.10 b	1.18 c	0.06 fg	0.06 def	2.67 a	62.16 bcd	15.35 gh	0.59 ef	0.41 ab	0.26 b	0.08 bc	0.06 cd
	120	0.02 a	18.71 a	1.24 b	0.08 de	0.07 cde	2.76 a	62.74 a	13.22 i	0.38 g	0.39 ab	0.30 a	0.06 c	0.01 e
Sign.		n.s.	***	***	***	***	n.s.	***	***	***	*	***	***	***

One-way ANOVA: means in the same column followed by a different letter are significantly different according to Tukey's test

n.s. not significant, $p > 0.05$

* $p < 0.05$

*** $p < 0.001$

Table 4 FAMEs discriminated by types and relations between them

Temp. (°C)	Time (min)	SFA	UFA	MUFA	PUFA	UFA/SFA	MUFA/PUFA	SFA/PUFA	18:1/18:2	18:1/16:0	18:2ω6/18:3ω3
	0	18.37 g	81.63 a	62.42 f	19.20 a	4.44 a	3.25 h	0.96 g	3.29 i	4.00 a	25.76 bc
180	15	19.34 f	80.66 b	62.46 f	18.20 b	4.17 b	3.43 g	1.06 f	3.49 h	3.83 bc	23.50 de
	30	20.11 cd	79.89 de	62.76 ef	17.13 c	3.97 de	3.66 f	1.17 d	3.73 g	3.73 cde	23.34 de
	60	19.64 ef	80.36 bc	63.32 cd	17.04 cd	4.09 bc	3.71 ef	1.15 de	3.78 fg	3.81 bcd	22.66 e
	120	20.65 b	79.35 f	63.14 de	16.21 fg	3.84 f	3.89 c	1.27 b	3.95 d	3.60 f	24.98 bcd
210	15	19.34 f	80.67 b	63.73 bc	16.94 cd	4.17 b	3.76 e	1.14 de	3.84 f	3.88 b	22.62 e
	30	19.34 f	80.66 b	63.60 bcd	17.06 c	4.17 b	3.73 ef	1.13 e	3.81 fg	3.88 b	22.63 e
	60	19.50 f	80.50 b	63.65 bc	16.84 cd	4.13 b	3.78 de	1.16 de	3.86 ef	3.85 b	23.42 de
	120	20.26 bcd	79.74 def	64.04 ab	15.70 h	3.93 def	4.08 b	1.29 b	4.14 b	3.71 de	26.51 b
240	15	19.63 ef	80.37 bc	63.64 bc	16.74 de	4.10 bc	3.80 de	1.17 d	3.87 def	3.80 bcd	24.03 cde
	30	19.95 de	80.05cd	63.57 cd	16.48 ef	4.01 cd	3.86 cd	1.21 c	3.93 de	3.72 de	24.51 bcde
	60	20.40 bc	79.60 ef	63.65 bc	15.95 gh	3.90 ef	3.99 b	1.28 b	4.05 c	3.64 ef	26.08 bc
	120	22.09 a	77.91 g	64.30 a	13.61 i	3.53 g	4.73 a	1.62 a	4.75 a	3.35 g	34.60 a
Sign.		***	***	***	***	***	***	***	***	***	***

One-way ANOVA: means in the same column followed by a different letter are significantly different according to Tukey's test

*** $p < 0.001$

532 **Table 5** Two-way ANOVA

533

Fatty acid	Temperature	Time	Temperature × time
Myristic (14:0)	n.s.	n.s.	n.s.
Palmitic (16:0)	*	*	***
Palmitoleic (16:1)	n.s.	n.s.	***
Heptadecanoic (17:0)	**	n.s.	***
Heptadecenoic (17:1)	**	n.s.	***
Stearic (18:0)	n.s.	n.s.	n.s.
Oleic (18:1)	***	n.s.	**
Linoleic (18:2)	*	*	***
Linolenic (18:3)	*	*	***
Arachidic (20:0)	n.s.	n.s.	n.s.
Eicosenoic (20:1)	n.s.	n.s.	***
Behenic (22:0)	n.s.	n.s.	***
Lignoceric (24:0)	***	**	***
SFA	n.s.	*	***
UFA	n.s.	*	***
MUFA	**	n.s.	***
PUFA	*	*	***
PUFA/MUFA	*	*	***
UFA/SFA	*	*	***
SFA/PUFA	n.s.	*	***
Oleic/linoleic	*	*	***
Oleic/palmitic	*	*	***
Linoleic/linolenic	n.s.	n.s.	***

534 *n.s.* not significant, $p > 0.05$

535 * $p < 0.05$

536 ** $p < 0.01$

537 *** $p < 0.001$

Table 6 Physicochemical properties of thermal stressed olive oil as a source for biodiesel production

Temp. (°C)	Time (min)	AV (mg KOH/g)	IV (g I_2/100 g of oil)	OSI (h)	KV (30 °C) (mm^2/s)	KV (40 °C) (mm^2/s)	KV (50 °C) (mm^2/s)	HHV (MJ/kg)	D (kg/m^3)	CN
	0	1.05 b	92.24 a	18.10 a	59.22 i	46.04 i	32.51 m	39.51 i	913.70 g	61.12 g
180	15	1.12 ab	86.20 bc	17.87 ab	59.34 i	45.90 i	36.32 l	39.51 i	913.77 g	61.12 f
	30	1.11 ab	85.74 bc	17.83 b	61.60 h	50.26 h	38.76 i	39.65 h	913.98 g	61.98 de
	60	1.12 ab	84.79 bc	16.10 c	70.89 e	53.82 f	45.40 f	39.76 f	914.87 f	61.96 de
	120	1.11 ab	84.22 c	8.03 g	73.85 c	56.16 d	48.39 d	39.83 d	915.85 de	62.41 b
210	15	1.12 ab	75.70 e	16.00 c	66.80 g	51.74 g	38.96 i	39.69 g	916.52 cd	61.89 e
	30	1.13 ab	72.14 f	13.87 d	67.34 g	53.02 f	40.92 h	39.73 f	917.15 c	61.89 e
	60	1.13 ab	69.79 f	13.00 e	72.27 d	55.14 e	47.06 e	39.80 e	920.14 b	61.98 de
	120	1.23 a	60.48 g	6.63 i	76.47 b	60.94 b	52.07 b	39.98 b	922.03 a	62.48 b
240	15	1.04 b	87.01 b	13.90 d	68.66 f	53.28 f	40.31 h	39.74 f	913.32 g	62.04 d
	30	1.08 b	86.77 b	11.63 f	68.98 f	54.73 e	42.86 g	39.79 e	915.06 ef	62.20 c
	60	1.06 b	85.68 bc	7.40 h	72.22 d	59.95 c	49.43 c	39.95 c	915.84 de	62.46 b
	120	1.17 ab	80.61 d	1.33 j	88.03 a	70.82 a	55.91 a	40.30 a	919.67 b	63.50 a
Sign.		*	***	***	***	***	***	***	***	***

One-way ANOVA: means in the same column followed by a different letter are significantly different according to Tukey's test

* $p < 0.05$

*** $p < 0.001$

544 **Table 7** Signification levels for the studied effects (temperature, time and temperature x time) on
545 the properties of the olive oil for biofuel production

546

Property	Temperature	Time	Temperature x time
Acid value (AV, mg KOH/g oil)	n.s.	n.s.	n.s.
Iodine value (IV, g I_2/100gl)	***	***	***
Oil stability index (OSI, h)	***	***	***
Kinematic viscosity (KV, mm^2/s at 30 °C)	*	**	***
Kinematic viscosity (KV, mm^2/s at 40 °C)	*	**	***
Kinematic viscosity (KV, mm^2/s at 50 °C)	***	***	***
Higher heating value (HHV, MJ/kg)	*	**	***
Density (D, kg/m3) at 15 °C	**	**	***
Cetane number (CN)	*	**	***

547 Two-way ANOVA
548 *n.s.* not significant, $p > 0.05$
549 * $p < 0.05$
550 ** $p < 0.01$
551 *** $p < 0.001$
552