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

16
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24
25 **Abstract**

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

44
45 **Keywords**

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

49 **Introduction**

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

77 **Materials and Methods**

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

80 considered in relation to the requirements of standards.

81

82 **Regulations for Olive Oil as a Food**

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

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

92

93 **Quality Standards for a Vegetable Oil as a BioFuel**

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

101

102 **Experimental Design**

103 One hundred-gram samples of EVOO were placed in steel containers and heated to either
104 180, 210, or 240 °C. These three temperatures were chosen on the basis of the frying
105 temperature of olive oil (180–210 °C) and of the temperature that can be reached during
106 deep frying (240 °C). The samples were held at each temperature for 15, 30, 60, and
107 120 min, giving a total of 12 trials. After heating, the oil was cooled to room temperature
108 and analysed within 2 h. The experiment on the heated extra virgin olive oil (H-EVOO)
109 was conducted in triplicate.

110

111 **Chemicals**

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

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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-diphenyl-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–Ciocalteu colourimetric method was applied and results were expressed as mg/kg of gallic acid [11].

148 *p*-Anisidine Value

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

154

155 *Totox Index*

156 It was calculated as 2PV + *p*-AnV.

157

158 *Fatty Acid Methyl Esters*

159 The olive oil methylation was carried out using the CON- SLEG, annex XB method A
160 [5]. FAME analysis was conducted as reported in a previous study and results are
161 expressed as % m/m [14].

162

163 *Acid Value*

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

166

167 *Iodine Value*

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

173

174 *Oil Stability Index*

175 The oil (3 g) was weighed in a glass tube and the analysing instrument (Rancimat model
176 679, Metrohm, Switzerland) was set with: 10 L/h air flow, 60 mL deionized
177 water, 110 °C temperature, 1 cm/h chart speed. Results are expressed as h of resistance to
178 oxidation.

179

180 *Kinematic Viscosity*

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

182 [13]. Analysis was conducted at 30-40-50 °C and results are expressed as mm²/s.

183

184 *Higher Heating Value*

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

188

189 *Density*

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

192

193 *Cetane Number*

194 CN was estimated from FAME profile using the equation:

195

$$196 \quad CN = \sum_{i=1}^n x_i CN_i$$

197

198 where: x_i is the mass fraction of the individual FAME and CN_i is the experimental CN of
199 the individual methyl ester [16].

200

201 **Statistical Analysis**

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

209

210 **Results and Discussion**

211

212 **Refractive Index**

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

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Free Acidity

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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).

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Peroxide Value, p-Anisidine Value, Totox

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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).

243

Spectrophotometric Indices

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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

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

260

261 **Antiradical Activity**

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

268

269 **Phenolic content**

270 The phenolic content was significantly highest in the unheated EVOO (2511 mg/kg) and
271 decreased constantly with the increase in thermal stress. After 2 h of heating the phenolic
272 content decreased 63.44, 70.61, and 80.17%,
273 respectively, at 180, 210, and 240 °C (Table 1). The temperature very highly
274 significantly influenced phenolic content ($p < 0.001$), whereas duration of heating
275 showed a minor effect ($p < 0.05$), (Table 2).

276

277 **Fatty Acid Methyl Esters**

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

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

284 presence of the double bonds. However, mono-unsaturated
285 fatty acids (MUFAs) are recognized to lower the bad cholesterol in the blood and
286 essential fatty acids (EFAs) have to be taken with the diet.
287 Palmitic acid (16:0) calculated as percentage content increased with increasing
288 temperature and with duration of heating. The minimum content (15.19%) was found in
289 the EVOO before thermal treatment and the maximum (18.71%) was found in the olive
290 oil heated at 240 °C for 120 min, i.e. when the oil was most stressed (Table 3). The same
291 trend was found in the SFA content: 18.37% in the olive oil before thermal treatment and
292 22.09% at 240 °C for 120 min (Table 4). Linoleic acid (18:2) showed a decreasing trend
293 from 18.45% in the unheated EVOO to 13.22% after 120 min heating at 240 °C (Table
294 3). Poly-unsaturated fatty acid (PUFA) content showed a decreasing trend in accordance
295 with linoleic acid, the major unsaturated fatty acid (Table 4). FAME composition is very
296 important for both food use and biofuel production. Linolenic acid (18:3, 0.38–0.74%)
297 always was below the maximum stated by the EN 14214 (12%) for a bio-diesel [8].
298 Large part of the FAMES were influenced in very highly significant level by the
299 combined effect of temperature and duration of heating (Table 5).

300

301 **Acid Value**

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

312

313 **Iodine Value**

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

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

324

325 **Oil Stability Index**

326 The studied olive oil presented the maximum resistance to oxidation before the thermal
327 treatment (18.10 h, Table 6). The OSI decreased with the increase in temperature ($p <$
328 0.001) and with duration of heating exposure ($p < 0.001$). Temperature time ($p < 0.001$)
329 very highly significantly influenced OSI (Table 7). After 2 h of treatment at 240 °C the
330 OSI was 1.33 h, only this sample was below the minimum (6 h) indicated by the DIN
331 51605 [7] for a rapeseed oil to be used for biodiesel production. The sample heated for
332 120 min at 210 °C presented a critical value,
333 6.63 h: a border line condition according to the International Standard. OSI was
334 influenced by temperature, time and their combination in a very highly significant level
335 (Table 7).

336

337 **Kinematic Viscosity**

338 Heating promotes vegetable oil polymerisation and produces high molar mass
339 compounds which determines the KV increase and lower the suitability of the heated
340 olive oil for biodiesel production. The DIN 51605 [7] for a rapeseed oil indicates
341 $36 \text{ mm}^2/\text{s}$ as a maximum value at $40 \text{ }^\circ\text{C}$. EVOO exceeded this value before thermal
342 stressing ($46.04 \text{ mm}^2/\text{s}$). KV was significantly influenced by heating and increased with
343 the increase in time and temperature (Table 6). The maximum KV determined at $40 \text{ }^\circ\text{C}$
344 was found after 120 min heating at $240 \text{ }^\circ\text{C}$: $70.82 \text{ mm}^2/\text{s}$, i.e. almost two times the
345 maximum stated by the DIN 51605. The KV at the three studied temperatures was
346 influenced in a very highly significant level by the combined effect of temperature and
347 duration of heating (Table 7).

348

349 **Higher Heating Value**

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

352 melting point. The higher the SFA content, the higher the KV, the higher the HHV. The
353 DIN 51605/2012 [7] requires at least 36.0 MJ/kg as from the combustion of a rapeseed
354 oil. In the H-EVOO the HHV was influenced by temperature ($p < 0.05$), by duration of
355 heating ($p < 0.01$) and by the combination of the two variables ($p < 0.001$), (Table 7).
356 Each sample showed a HHV value higher than 36 MJ/kg and the maximum (40.30
357 MJ/kg) was found in the most heat-stressed olive oil (Table 6). All the HHV calculated
358 on EVOO and H-EVOOs were higher than HHV found in peanut oil from Algeria
359 [12] and in tomato seed oil after cold break or hot break treatments [20]. HHV was very
360 highly significantly influenced by the combination of temperature and time of heating
361 (Table 7).

362

363 **Density**

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

373

374 **Cetane Number**

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

386 increase and the presence of oligomers of FAMES, aldehydes, and hydroperoxides as
387 products of the oxidation.

388

389 **Conclusion**

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

398

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403

404 **References**

- 405 1. World olive oil figures (2016). IOC. Accessed on 25 August 2016.
406 <http://www.internationaloliveoil.org/estaticos/view/131-world-olive-oil-figures>
- 407 2. Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ (2003) Use of
408 antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis
409 of randomised trials. *Lancet* 361:2017–2023
- 410 3. Pillon NJ, Soulière L, Vella RE, Croze M, Caré BR, Soula HA, Doutheau A,
411 Lagarde M, Soulage CO (2010) Quantitative structure–activity relationship for
412 4-hydroxy-2-alkenal induced cytotoxicity in L6 muscle cells. *Chem Biol Interact*
413 188:171–180. doi:10.1016/j.cbi.2010.06.015
- 414 4. Macario A, Giordano G, Onida B, Cocina D, Tagarelli A, Giuffrè AM
415 (2010) Biodiesel production process by homogeneous/heterogeneous catalytic
416 system using an acid–base catalyst. *Appl Catal A* 378:160–168. doi:10.1016/j.
417 apcata.2010.02.016
- 418 5. Consleg (2015). Consolidated text. Characteristics of olive oil. 1991R2568—
419 EN—01.01.2015—027.001. Annexes II, III, IX, X-B, XVI

- 420 6. IOC, 2015. Trade standard applying to olive oils and olive- pomace oils.
421 COI/T.15/NC No 3/Rev. 9 Jun 2015
- 422 7. DIN 51605:2012. Crude degummed rapeseed oil specification. Fuels for
423 vegetable oil compatible combustion engines—Fuel from rapeseed oil
- 424 8. EN 14214:2014. European standard—automotive fuels—fatty acid methyl esters
425 (FAME) for diesel engines—requirements and test methods. (February 2014)
- 426 9. ASTM D6751:2012. American biodiesel quality standard. Standard specification
427 for biodiesel fuel blend stock (B100) for middle distillate fuels
- 428 10. AOAC methods. (Official methods of analysis of the association of official
429 analytical chemists, 17th Edition, 2000)
- 430 11. Kalantzakis G, Blekas G, Pegklidou K, Boskou D (2006) Stability and radical-
431 scavenging activity of heated olive oil and other vegetable oils. *Eur J Lipid Sci*
432 *Technol* 108:329–335. doi:10.1002/ejlt.20
- 433 12. Giuffrè AM, Tellah S, Capocasale M, Zappia C, Latati M, Badiani M, Ounane
434 SM (2016) Seed oil from ten Algerian pea- nut landraces for edible use and
435 biodiesel production. *J Oleo Sci* 65:9–20. doi:10.5650/jos.ess15199
- 436 13. NGD -1979 (Norme Grassi e Derivati—III edizione). Norme italiane per il
437 controllo dei grassi e derivati. Stazione Speri- mentale per le industrie degli oli e
438 dei grassi (Milano, Italy)
- 439 14. Louadj L, Giuffrè AM (2010) Analytical characteristics of olive oil produced
440 with three different processes in Algeria. *Riv Ital Sostanze Gr* 87:186–195
- 441 15. Demirbas A (2008) Relationships derived from physical properties of vegetable
442 oil and biodiesel fuels. *Fuel* 87:1743–1748. doi:10.1016/j.fuel.2007.08.007
- 443 16. Viola E, Zimbardi F, Valerio V (2011) Graphical method to select vegetable oils
444 as potential feedstock for biodiesel production. *Eur J Lipid Sci Technol*
445 113:1541–1549
- 446 17. Codex Standard for olive oils and olive pomace oils. Codex Stan 33-1981 (Rev.
447 2-2003)
- 448 18. Issaoui M, Flamini G, Ellouze Hajaij M, Cioni PL, Hammami M (2011)
449 Oxidative evolution of virgin and flavored olive oils under thermo-oxidation
450 processes. *J Am Oil Chem Soc* 88:1339–1350. doi:10.1007/s11746-011-1800-5
- 451 19. Maggio RM, Valli E, Bendini A, Gómez-Caravaca AM, Gallina Toschi T,
452 Cerretani L (2011) A spectroscopic and chemometric study of virgin olive oils
453 subjected to thermal stress. *Food Chem* 127:216–221

- 454 20. Giuffrè AM, Capocasale M, Zappia C, Sicari C, Pellicanò TM, Poiana M,
455 Panzera G (2016) Tomato seed oil for biodiesel pro- duction. *Eur J Lipid Sci*
456 *Technol* 118:640–650. doi:10.1002/ ejlt.201500002
- 457 21. Karmakar A, Karmakar S, Mukherjee S (2010) Properties of various plants and
458 animals feedstocks for biodiesel production. *Biores Technol* 101:7201–7210.
- 459 22. Wadumesthrige K, Smith JC, Wilson JR, Salley SO, Simon Ng KY (2008)
460 Investigation of the parameters affecting the cetane number of biodiesel. *J Am*
461 *Oil Chem Soc* 85:1073–1081. doi:10.1007/s11746-008-1290-2
- 462 23. Ladommatos N, Parsi M, Knowles A (1996) The effect of fuel cetane improver
463 on diesel pollutant emissions. *Fuel* 75:8–14
- 464 24. Atabani AE, Silitonga AS, Ong HC, Mahlia TMI, Masjuki HH, Badruddin IA,
465 Fayaz H (2013) Non-edible vegetable oils: a criti- cal evaluation of oil extraction,
466 fatty acid compositions, biodiesel production, characteristics, engine performance
467 and emissions production. *Renew Sust Energ Rev* 18:211–245. doi:10.1016/j.
468 rser.2012.10.013
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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	ΔK	AA (%)	Phenols (mg/kg)	p-AnV	TOTOX
180	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
	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.		**	***	***	***	***	***	***	***	***	***

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

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

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* $p < 0.05$

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** $p < 0.01$

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*** $p < 0.001$

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486 **Table 2** Signification levels of the studied effects (temperature, time and temperature
 487 time) on the physicochemical properties of the olive oil for edible use
 488

Property	Temperature	Time	Temperature × time
490 Refractive index	n.s.	n.s.	n.s.
491 Free acidity (%)	n.s.	n.s.	n.s.
492 Peroxide value (mEq O ₂ / kg oil)	**	**	***
493 <i>p</i> -AnV	**	***	***
494 TOTOX index	*	***	***
495 K232	n.s.	n.s.	***
496 K266	***	***	n.s.
497 K270	***	***	n.s.
498 K274	***	***	n.s.
499 ΔK	n.s.	***	***
500 Antiradical activity (% inhibition)	*	**	***
501 Total phenols (mg/kg)	***	*	***

502

503 Two-way ANOVA

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

505 * $p < 0.05$

506 ** $p < 0.01$

507 *** $p < 0.001$

508

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

510

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
180	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
	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
240	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
	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.	***	***	***	*	***	***	***

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

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

513 * $p < 0.05$

514 *** $p < 0.001$

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527**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
180	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
	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
210	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
	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
240	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
	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.		***	***	***	***	***	***	***	***	***	***

528

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

529

*** $p < 0.001$

530

531

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 *** $p < 0.001$

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

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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
180	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
	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
210	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
	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
240	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
	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.		*	***	***	***	***	***	***	***	***

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

541 * $p < 0.05$

542 *** $p < 0.001$

543

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 /100g l)	***	***	***
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/m ³) 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