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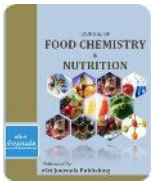

CHANGES IN CHEMICAL COMPOSITIONS OF OLIVE OIL UNDER DIFFERENT HEATING TEMPERATURES SIMILAR TO HOME COOKING

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ABSTRACT

Four olive oils with varying amounts of total phenols were exposed to four different heating conditions. Chemical parameters such as free fatty acid, peroxide values, UV absorbency, total phenols, individual phenols, α-tocopherol, squalene, oleocanthal, fatty acid profile and smoke point were measured before and after heating to evaluate the impact of heating conditions on the oils. We found olive oils have reasonably high smoke point that is suitable for typical home-cooking conditions and fresh olive oil with low FFA and high phenolics are important for the conservation of olive oil quality and health benefits. A larger degree of oxidation occurred as with increase of heating time and temperature, oils with high level of phenols produced less polar compounds than oils with lower levels of phenols, including refined oils. A significant amount of total phenols and individual phenols such as oleocanthal remained after heating at 121 °C for 10 and 20 minutes, most of squalene stay intact even after heating at 220 °C.

Keywords: Olive oil, quality parameters, phenolic compounds, heating conditions, oleocanthal.

INTRODUCTION

Extra virgin olive oil (EVOO), along with fruits, vegetables, and fish, is an important constituent of the diet in the Mediterranean area (Owen *et al.,* 2000). It has been widely associated with the prevention and reduction of numbers of diseases such as cancer (Alu'datt *et al.,* 2014), inflammatory and joint issues (Rosillo *et al.,* 2014), and cardiovascular and metabolic diseases (Bulotta *et al.,* 2014). In comparison with other vegetable oils, EVOO exhibits high resistance to oxidation (Allouche *et al.,* 2007). The appreciable oxidative stability of EVOO is mainly due to its characteristic composition of high monounsaturated fatty acids like oleic acid (C18:1) and the presence of significant amount of minor components such as phenolic compounds an d α-tocopherol that with strong antioxidative properties (Psomiadou and Tsimidou, 1999).

Organizations such as the International Olive Council (IOC), the Codex Alimentarius Commission (CAC), and the United States Department of Agriculture (USDA) have established standard limit on the quality parameters of free fatty acids (FFA), peroxide value (PV), ultraviolet absorbance (UV) and organoleptic characteristics (odor, taste and color) for olive oils in order to define different characteristics and grades of olive oil and olive-pomace oil. Among all the olive oil grades, EVOO is the most prized one with its top quality attributes producing from olive fruit only by mechanical means (Santos *et al.,* 2013).

Due to the unique organoleptic characteristics and health benefits, EVOO is widely consumed raw on toasts and salads, but more often being used in domestic cooking including sautéing, boiling, and microwaving heating (Brenes *et al.,* 2002). In the process of heating, a series of chemical reactions can occur which includes hydrolysis, oxidation, and polymerization leading which could lead to irreversible loss of nutritional components in olive oil (Wanasundara and Shahidi, 1998).

In the past, extensive studies have been conducted on how

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different heating conditions (e.g. heating temperature and time duration) affect the quality of olive oils (Brenes *et al.,* 2002; Casal *et al.,* 2010; Fullana *et al.,* 2004; Uriarte and Guillen, 2010). Isothermal breakdown kinetics of the artificial rancidification of olive oil have also been studied (Tomassetti *et al.,* 2013). However, many of these studies were done using heating conditions that would exceed those of used in home food preparation to accelerate the reaction rate of olive oil (e.g. frying at 180 °C for 1.5 hours to 25 hours or heating at 170 °C for periods of 3 hours) (Brenes *et al.,* 2002; Casal *et al.,* 2010). In addition, it was not clearly shown in literature how different oils with varying initial quality parameters would behave differently under the same heating conditions (Ramírez-Anaya *et al.,* 2015). To study this relationship, we used four different heating temperatures that were commonly used in home cooking with four olive oils with different amounts of total phenolics. Chemical parameters such as FFA, UV, PV, total phenols, individual phenols, α-tocopherol, squalene, oleocanthal, fatty acid profile and smoke point were measured before and after heating to evaluate the impact of heating conditions on the oils.

EXPERIMENTAL

Chemicals: All certified ACS grade chemicals and solvents used in the analyses of quality indices, total phenol content determination, and polar compounds analyses were obtained from Fisher Scientific (Pittsburgh, PA, USA). All HPLC grade solvents were also purchased from Fisher Scientific. Tyrosol, hydroxytyrosol, oleuropein, α-tocopherol acetate, squalene, 3, 5-dimethoxyphenol, and fatty acid methyl ester (FAME) standards were from Sigma-Aldrich (St Louis, MO, USA). Nanopure water was prepared with Milli-Q system (Millipore, Bedford, MA, USA).

Olive oil samples: Four olive oil samples were used in this project. High-phenolics EVOO sample (452 mg/kg caffeic acid equivalent total phenol content) was donated by Boundary Bend (Lara Victoria, Australia); medium-phenolics EVOO sample (309 mg/kg caffeic acid equivalent total phenol content) was made by 75% Koroneiki EVOO and 25% Miller's Blend EVOO (both were donated by California Olive Ranch, Chico, CA, USA); low-phenolics EVOO sample (140 mg/kg caffeic acid equivalent total phenol content) was from Corto Olive Co. (Lodi, CA, USA); refined extra light olive oil sample (12 mg/kg caffeic acid equivalent total phenol content) was purchased from supermarket. The initial fatty acid compositions and smoke points were listed in Table 1.

Table 1. Initial characteristics of each olive oil sample.

Initial Characteristics	Refined Olive Oil	Low Phenol EVOO	Med Phenol EVOO	High Phenol EVOO
Fatty Acid Composition (%)				
C16:0	10.81	13.60	14.76	11.24
C16:1	0.64	1.01	1.12	0.58
C17:0	0.08	0.11	0.11	0.07
C17:1	0.13	0.26	0.22	0.14
C18:0	3.18	2.13	2.27	2.08
C18:1+C18:2+C18:3	84.38	81.97	80.73	85.03
C20:0	0.41	0.43	0.42	0.38
C21:1	0.27	0.32	0.31	0.39
Smoke Point (°C)	240	215	208	205

Heating conditions: Four heating temperatures/ durations were adopted for the purpose of the project: heated at 121 °C for 10 min and 20 min respectively (in the range of a medium stove top heat); heated at 180 °C for 10 min (temperature typically used in the currently available published studies); heated at 220 °C for 10 min (around smoke point temperatures). The time count started when the desire temperature was achieved. A deep-fry thermometer (Polder, Boca Raton, FL, USA) was used to measure the heating temperature. A stove top with a stainless 9-inch open skillet (Cuisinart, East Windsor, NJ, USA) was used to heat the oil samples. Two 70 mL oil samples were measured and heated continuously under each heating condition, cooled separately, and combined together to get a total of 140 mL heated oil samples for all the chemical analyses. Chemical measurements were taken before and after heating.

Quality indices: The quality indices free fatty acid (FFA) (expressed as % oleic acid), peroxides value (PV)

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(expressed as milliequivalents of peroxide per 1,000 g test portion), and ultraviolet coefficients (UV) (K_{232} and K_{268} from absorption at 232 nm and 268 nm) were determined respectively following the AOCS official method Ca 5a-40, Cd 8b-90 and Ch 5-91. All the tests were done in duplicates.

Total phenol content: The total phenol content of each cooked oil sample were extracted from the oils based on the Gutfinger method (Gutfinger, 1981).

Polar compounds: The determination of polar compounds in frying oil was conducted following AOCS official method Cd 20-91.

Squalene: Squalene in oil samples were determined based on the method developed by Nenadis and Tsimidou (2002). An Agilent Infinity 1290 UHPLC system was used in this analysis. The stationary mobile phase was acetone/acetonitrile (40/60, v/v) and the flow rate was 0.5 mL/min on a 3.5 µm, 100 mm × 3.0 mm C18 column (Agilent Technologies, Santa Clara, CA, USA) kept at 30 °C. The injection was 5 µL. The diode array detector (DAD) was performed at 208 nm. A calibration curve of squalene standard was obtained for quantification.

Alpha-tocopherol: The method developed by Gimeno *et al.,* (2000) was used to determine α-tocopherol in oil samples. An aliquot of the overlay from oil sample preparation was directly injected to the UHPLC system with a C18 column (3.5 µm, 100 mm × 3.0 mm) which was kept at 45 °C. The mobile phase consisted of methanol and water (96/4, v/v) was used. The sample injection volume was 20 µL and the flow rate was 1.0 mL/min. The DAD was adopted at 292 nm. The peak area ratio of α-tocopherol and α-tocopherol acetate was calculated to determine the α-tocopherol content in oil sample.

Individual phenolics: Individual phenolics were extracted from oil samples and measured under UHPLC system based on the method provided by Mateos *et al.,* (2001).

A 5 µm, 250 mm × 4.6 mm C18 column (Agilent Technologies, Santa Clara, CA, USA) was used in this method. The column was maintained at 30 °C. The sample injection was 20 µL and the flow rate was 1.0 mL/min. In this analysis, the mobile phase A was water/acetic acid (97/3, v/v) and B was methanol/acetonitrile (50/50, v/v). The solvent gradient changed according to the following conditions: from 0 to 25 min, 95% A – 5% B to 70% A – 30% B; from 25 min to 35 min, to 65% A – 35% B; from 35 min to 40 min, to 60% A – 40% B; from 40 min to 50 min, to 30% A – 70% B; from 50 min to 55 min, to 100% B; the gradient then went back to 95% A – 5% B in 5 min. The DAD was performed at 280 nm (tyrosol and hydroxytyrosol) and 240 nm (oleuropein). The quantification was determined by using external calibration curves respectively.

Oleocanthal: Oleocanthal was extracted and measured according to Impellizzeri and Lin (2006) with several modifications. 1 g of oil sample was dissolved with 2 mL hexane in a 50 mL centrifuge tube and vortexed for 30 s. 5 mL of acetonitrile was then added and vortexed for another 30 s. The tubes were centrifuged at 4000 rpm (DuPont, Wilmington, DE, USA) for 5 min to separate the solvent from the oil phase, and the solvent extract was collected in another 50 mL centrifuge tube. The extraction process was repeated twice more and the acetonitrile phases were combined into one flask and evaporated under a rotary evaporator until dryness. 1 mL of methanol/water (1/1, v/v) was added to extract the oleocanthal portion while 1 mL of hexane was used to wash away hexane residue. The aqueous phase was further centrifuged and 20 µL of sample solution was used for HPLC analysis.

The same Agilent UHPLC system equipped with a 5 µm, 250 mm × 4.6 mm C18 column was employed. The flow rate was 1.0 mL/min. The mobile phase consisted of water (A) and acetonitrile (B) and followed the gradient as: 0 to 35 min, remained at 75% A and 25% B; changed to 20% A and 80% B and maintained at this ratio for 10 min; changed back to 75% A and 25% B and ran for another 10 min. The wavelength of 278 nm was used for DAD detection. A calibration curve of 3, 5-dimethoxyphenol was obtained for quantification of oleocanthal.

Fatty acid profile: The IOC official method (COI/T.20/Doc. no. 24-2001) was adopted for fatty acid profile analysis with modifications. 0.01 g oil sample was dissolved in 0.4 mL of toluene. 3 mL methanol and 0.6 mL methanol/HCl (80/20, v/v) was then added. The sample mixture was kept at 40 °C overnight in a heat stock for reaction. 1.5 mL of hexane and 1 mL of nanopure water was added and vortexed well. The methyl esters top layer was decanted after stratifying until two layers were completely separated. Anhydrous sodium sulfate was used to dry out water residue. The supernatant was then injected onto GC column.

A Varian 450-GC equipped with a FID was used in this analysis. The injection volume was 0.2 µL. The carrier

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gas (He) was at the flow rate of 1.5 mL/min. A 30 m × 0.25 mm × 0.10 µm DB-5HT capillary column (Agilent Technologies, Santa Clara, CA, USA) was used to achieve the separation of individual fatty acid compositions. The injector temperature was held at 40 °C at a spilt ratio of 150. The GC oven program was initially held at 80 °C for 10 min; then ramped at 7 °C/min to 230 °C and held for 5 min; finally ramped at 15 °C/min and held for 8 min. The FID temperature was 260 °C. The detector gas consisted of hydrogen (30 mL/min), air (300 mL/min), and helium make up gas (25 mL/min). A FAME mix was used as standards for identification.

Smoke point: Oil sample were sent to Eurofins Scientific Supplemental Analysis Center (Petaluma, CA, USA) for the measurement of smoke point.

Statistical Analysis: R (version 3.2.0, R Core Team, Vienna, Austria) was used to perform one-way and two-way ANOVA on different parameters with factors as olive oil type and/or heating conditions. Pairwise t-tests were also conducted to compare between different types of olive oil or heating conditions. F-statistics and t-statistics were calculated respectively among the measured variables at a 95% confidence level (p-value < 0.05). The significance of the differences of the means was determined at a 5% level.

RESULTS AND DISCUSSION

FFA: During refining process, FFA is removed; therefore refined olive oils have very low levels of free fatty acidity. Figure 1 and pairwise t-test both show that FFA did not change significantly in different heating conditions. None of the EVOO samples exceeded the upper limit of FFA set by IOC (0.8 % expressed as oleic acid). Our results of refined olive oil were consistent with some other researchers (Brenes *et al.,* 2002; Gertz, 2000) have found that FFA increased with heated oil, though additional experiments will be necessary to explain the decease seen in the EVOO samples.

PV: Figure 2 shows that PV increased the most for refined and low phenol oils after heating at 180 °C. A smaller increase in PV was found under heating at 180 °C for medium phenol EVOO and heating at 220 °C for all the oil samples. Peroxides are the primary oxidation products of autoxidation, at higher temperature, they decompose the into secondary oxidation products which would lower the observed PV as seen in the case of 220 °C (Cheikhousman *et al.,* 2005). Satue and coworkers also found that the antioxidant effectiveness of phenolic compounds in virgin olive oils can be significantly diminished if the initial PV is high, which might explain the rapid increase of PV in low phenol oil sample at 180 °C and slight change of PV in high phenol oil sample when it reached high heating temperatures (Satue *et al.,* 1995). The ANOVA analysis of PV also showed that oil sample, heating condition and the interaction had a significant effect on PV.

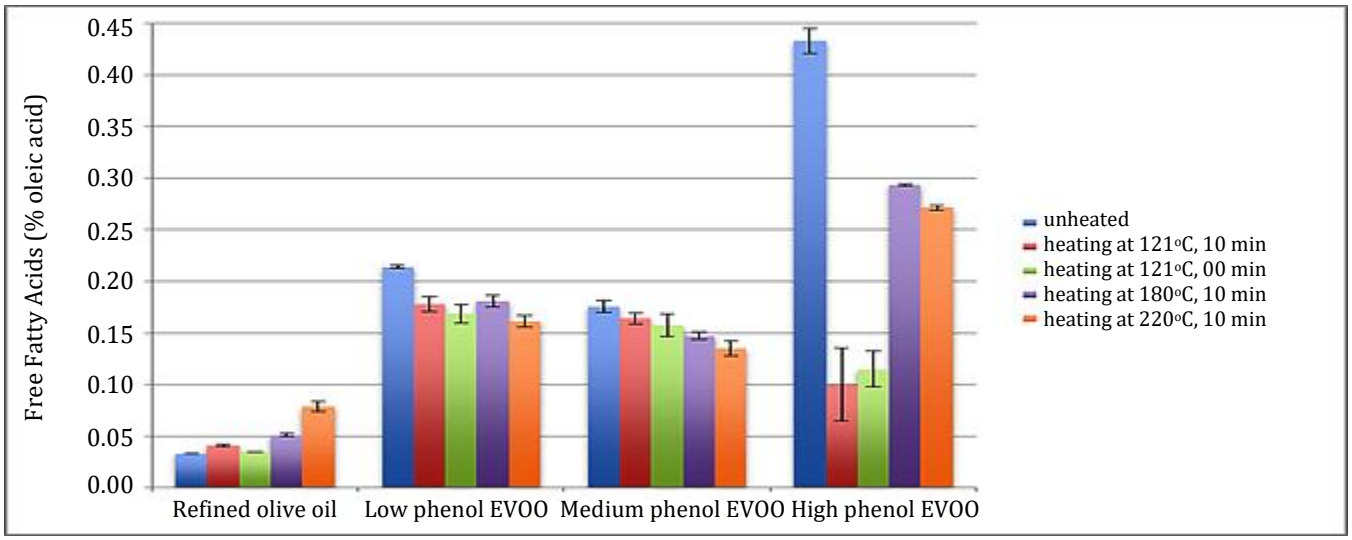

Figure 1. Changes of FFA (% oleic acid) under different heating conditions for each oil sample.

UV: As expected, both K_{232} and K_{268} values increased during heating process and all of the K_{232} values exceeded the upper limit of EVOO when the oil was heated at 180 °C and 220 °C (Figure S1, S2). Daskalaki and coworkers (Daskalaki *et al.,* 2009) found K_{232} exceeded the upper limit of 2.60 for both frying (180 °C) and boiling (100 °C) temperatures due to hydroperoxides decomposition while conjugating dienes steadily accumulated. And as

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measured by K_{270}, changes in the content of carbonyls recorded higher values at frying than at boiling due to greater hydroperoxides degradation at the higher temperature. Our results showed the largest increase in both values (K_{232} and K_{268}) when the oils were heated at 180 °C and 220 °C regardless of their initial phenol content, which was consistent with what was reported by Daskalaki *et al.,* (2009). Heating oil at 121 °C for 10 minutes had a very modest effect on both K_{232} and K_{268} values for all of the oils.

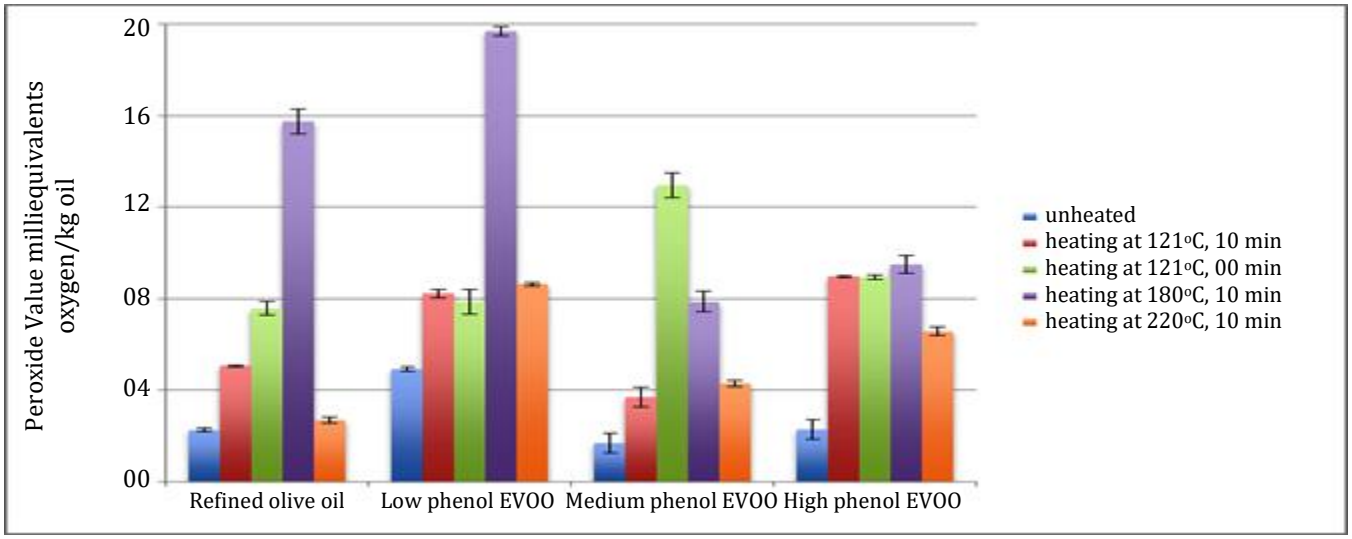

Figure 2. Changes of PV (milliequivalents oxygen/kg oil) under different heating conditions for each oil sample.

Phenols (individual and total): A reduction in individual phenolic concentration, especially hydroxytyrosol (from 9.83 mg/kg to 3.79 mg/kg), has been shown to occur in pan-frying temperatures of 180 °C for 30 min (Carrasco-Pancorbo *et al.,* 2007). As one of the phenolic compounds with the highest antioxidant activity in olive oil, the extensive loss of hydroxytyrosol is most likely due to its protection of the lipids from oxidation. Moreover, of the measured phenols, oleocanthal had the highest temperature tolerance while hydroxytyrosol had the lowest, which was in accordance with previous studies (Gomez-Alonso *et al.,* 2003). Another important phenolic compound, tyrosol showed a much smaller change with different heating methods among all the oil samples, which was in accordance with the study results from Brenes and coworkers (Brenes *et al.,* 2001). Total phenol content, expressed as mg per kg caffeic acid equivalent, was as high as 452.08 ± 0.57 mg/kg in high phenol EVOO sample and as low as 11.61 ± 0.59 mg/kg in refined olive oil before heating. Refined olive oil had very low levels of total and individual phenols due to the refining process. The loss of total phenol content was observed for all oils, 40.80% (184.45 ± 0.23 mg/kg) of loss in total phenol content was found in high phenol EVOO under heating at 121 °C for 10 min while 78.41% (354.48 ± 0.45 mg/kg) of total phenol loss was observed with heating at 220 °C for 10 min. Other oils followed a similar pattern where heating at 121 °C retained most of important phenolics (Table 2) while heating under higher temperature in air stream caused significant loss of total phenol content. Interestingly, oils that were heated at 121 °C for 10 mins and 20 mins had comparable amount of oleuropein, oleocanthal, and total phenols, showing that heating temperature of 121 °C is a reasonable temperature for home-cooking in without destroying these phenol compounds. The oils with the highest initial specific phenol and total phenolic contents ended with the highest contents after all heating conditions (Table 2).

Alpha-tocopherol: The small amount of α-tocopherol left after heating at 180 °C and 220 °C were not quantifiable, however there were quantifiable amounts after heating at 121 °C (Figure 3). Casal and coworkers observed that α-tocopherol degraded sharply to almost inexistent after 3-6 hours of frying at 170 °C (Casal *et al.,* 2010). Similar to phenolic content, EVOO samples had higher initial concentration of α-tocopherol ended up with more α-tocopherol after heating. In this case, medium phenol EVOO reserved most α-tocopherol as it had the highest α-tocopherol concentration (0.19 g/L) to start with. In the study conducted by Pellegrini et al. (Pellegrini *et al.,* 2001), they elucidated that polyphenols from EVOO were effective stabilizers of α-tocopherol during olive oil cooking, which was in alignment with our study.

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Table 2. Change of individual and total phenols under different heating conditions for each oil sample.

	Unheated	Heated at 121 °C, 10 min	Heated at 121 °C, 20 min	Heated at 180 °C, 10 min	Heated at 220 °C, 10 min
Hydroxytyrosol (mg/L)					
Refined Olive Oil	0.39 ± 0.02	0.15 ± 0.04	0.00	0.00	0.00
Low Phenol EVOO	6.42 ± 0.02	4.67 ± 0.06	3.63 ± 0.05	0.98 ± 0.01	0.40 ± 0.13
Med Phenol EVOO	18.02 ± 1.91	17.25 ± 0.23	3.74 ± 0.37	2.16 ± 0.03	0.90 ± 0.02
High Phenol EVOO	29.37 ± 1.09	20.85 ± 1.13	10.88 ± 0.19	0.55 ± 0.03	0.49 ± 0.05
Tyrosol (mg/L)					
Refined Olive Oil	0.65 ± 0.02	0.42 ± 0.02	0.00	0.00	0.00
Low Phenol EVOO	4.62 ± 0.14	2.94 ± 0.20	2.27 ± 0.06	1.11 ± 0.04	1.27 ± 0.32
Med Phenol EVOO	12.14 ± 0.26	10.10 ± 0.17	5.60 ± 0.53	4.52 ± 0.09	4.29 ± 0.03
High Phenol EVOO	36.86 ± 1.41	18.13 ± 0.29	8.70 ± 0.51	6.72 ± 0.01	5.22 ± 0.06
Oleuropein (mg/L)					
Refined Olive Oil	0.41 ± 0.01	0.00	0.00	0.00	0.00
Low Phenol EVOO	5.42 ± 0.16	1.97 ± 0.07	1.95 ± 0.09	1.39 ± 0.07	1.28 ± 0.01
Med Phenol EVOO	8.45 ± 1.92	5.94 ± 0.16	4.03 ± 0.18	3.78 ± 0.36	3.66 ± 0.49
High Phenol EVOO	4.02 ± 0.03	2.82 ± 0.33	2.16 ± 0.15	2.04 ± 0.03	0.64 ± 0.03
Oleocanthal (mg/L as 3, 5-dimethoxyphenol)					
Refined Olive Oil	0.00	0.00	0.00	0.00	0.00
Low Phenol EVOO	46.06 ± 0.20	36.49 ± 0.40	28.41 ± 4.89	21.77 ± 0.54	19.26 ± 0.15
Med Phenol EVOO	145.80 ± 8.30	142.13 ± 0.84	133.33 ± 1.63	125.99 ± 2.13	114.52 ± 0.54
High Phenol EVOO	389.16 ± 11.61	293.04 ± 3.11	251.76 ± 0.89	234.61 ± 1.04	228.56 ± 1.98
Total Phenols (mg/kg caffeic acid equivalent)					
Refined Olive Oil	11.61 ± 0.59	8.41 ± 0.60	5.23 ± 1.71	5.21 ± 0.57	3.21 ± 1.13
Low Phenol EVOO	139.77 ± 2.17	137.50 ± 0.10	136.96 ± 0.86	58.70 ± 4.59	42.11 ± 0.57
Med Phenol EVOO	309.18 ± 5.34	275.00 ± 13.05	132.35 ± 0.37	89.08 ± 2.46	64.03 ± 1.90
High Phenol EVOO	452.08 ± 0.57	267.63 ± 3.78	271.91 ± 2.65	93.66 ± 0.87	97.60 ± 0.36

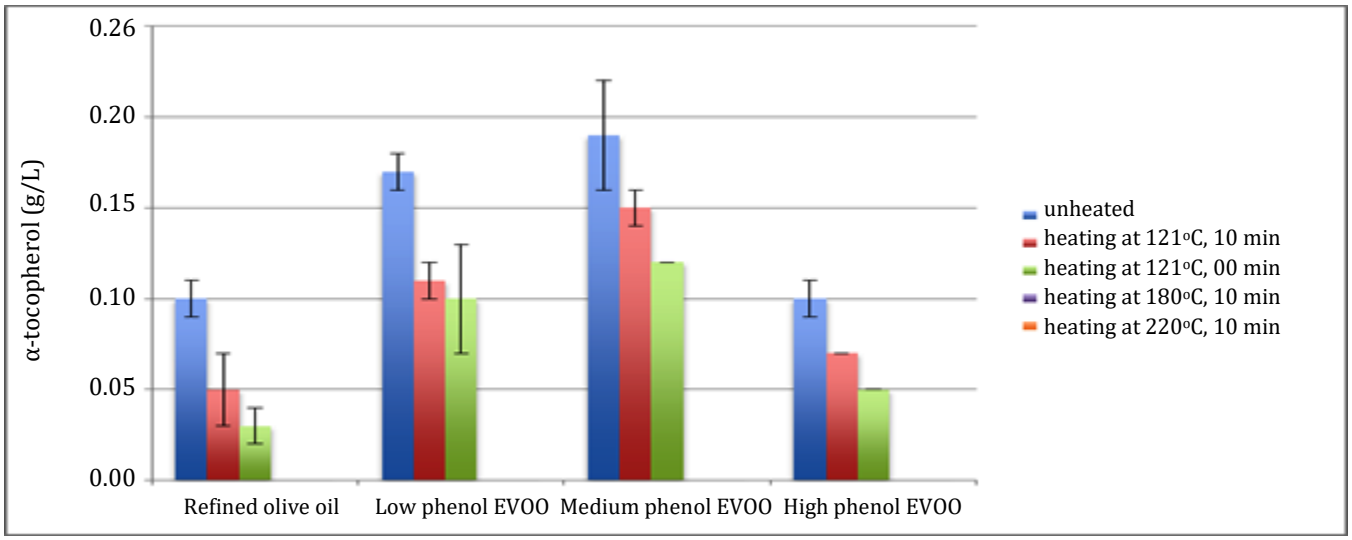

Figure 3. Changes of α-tocopherol (g/L) under different heating conditions for each oil sample.

Squalene: Squalene appeared to be relatively stable under all heating conditions, especially at 121 °C (Figure 4). Our results are consistent with Kalogeropoulos and coworkers' finding that the squalene content of the frying oils was reduced during frying, but it was higher in fried VOO (428 mg/100 g oil) when compared with

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cooked fats (5.9 mg/100 g fat) after frying at 175 ± 5 °C for 6 minutes (Kalogeropoulos and Andrikopoulos, 2004). Refined olive oil samples suffered the most loss when heating at 220 °C compared to other olive oil samples. EVOO rich in dominant antioxidants such as α-tocopherol and phenolic compounds might be responsible for the minor loss of squalene during heating as observed here.

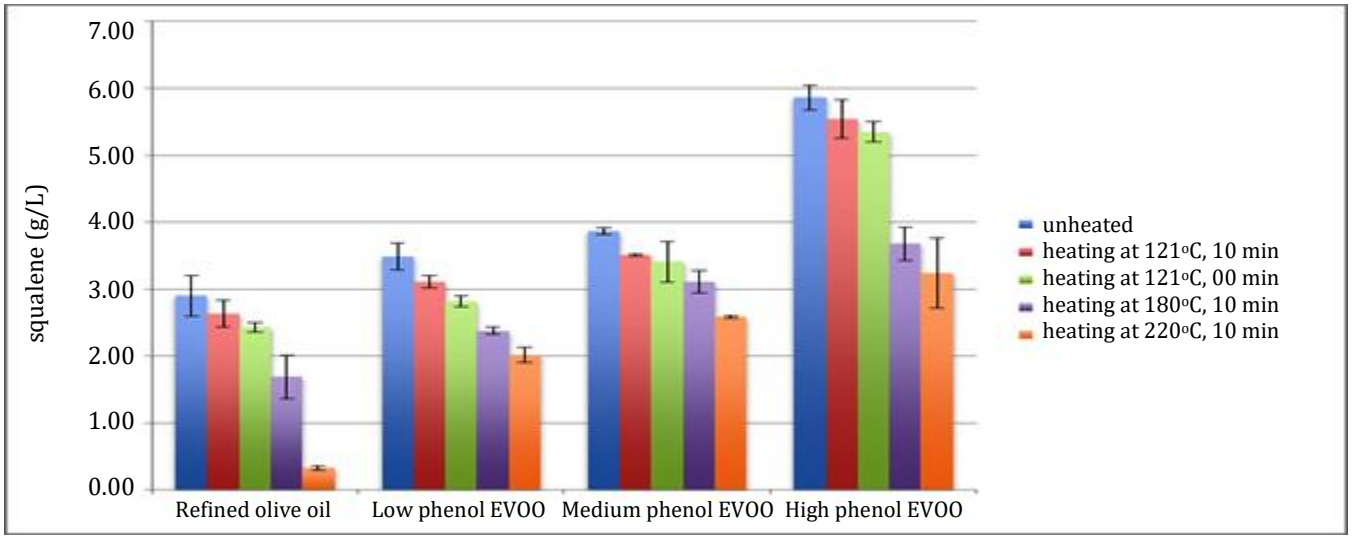

Figure 4. Changes of squalene (g/L) under different heating conditions for each oil sample.

Polar compounds: Figure 5 shows a trend of increasing polar compounds as heating temperature and time increase. Polar compounds have been found to rapidly increase in correlation with low antioxidant activity (Gomez-Alonso *et al.,* 2003); as the amount of antioxidants decreased, the reactions that formed polar compounds can compete for the reactive oxygen species. This phenomenon was especially pronounced in refined olive oil heated at 220 °C.

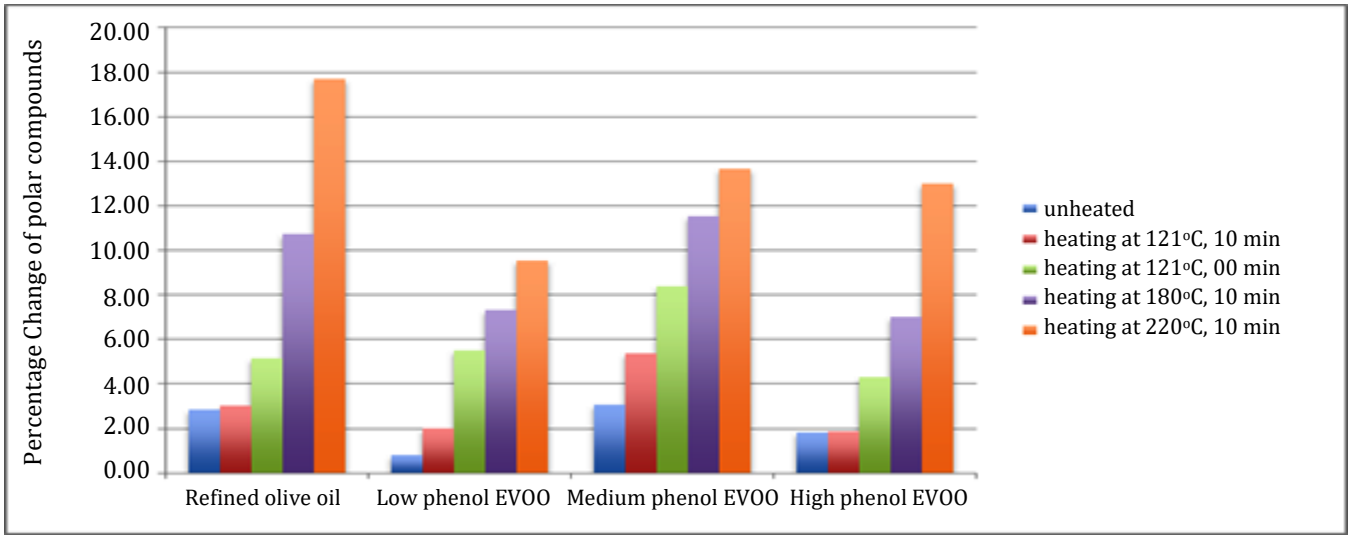

Figure 5. Percentage Change of polar compounds under different heating conditions for each oil sample.

Fatty acid profiles: Most fatty acids essentially remained the same for all oils after each heating condition. A decrease occurred in unsaturated fatty acids oleic acid (C18:1), linoleic acid (C18:2), and α-linolenic acid (C18:3) among all the samples (Figure S3), presumably by oxidation (Casal *et al.,* 2010), especially after heating at 220°C. As a result, a slight increase in the proportion of saturated fat including C16:0 and C18:0 occurred (data not shown). In general, the fatty acid profiles showed very little to no perturbation based on heating conditions for each oil sample.

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Smoke point: the smoke point is an indicator of chemical breakdown of the fat to glycerol and FFA (Katragadda *et al.,* 2010). High level of FFA decreases the smoke point of an oil (Morgan, 1942). This is consistent with our findings of the smoke points for refined, low, medium and high phenol oils were 240 °C, 215 °C, 208 °C and 205 °C, respectively, as the high phenol oil had a highest level of FFA.

CONCLUSIONS

Our results showed that initial total phenol content of an oil and heating temperatures that are associated with common home cooking influence how the chemical parameters of an oil change. Heating at 121 °C is preferable than heating at 180 °C and above to preserve the phenols and therefore the health benefits of olive oils. While total and individual phenols and antioxidants suffered loss after heating in all oils despite different initial total phenols, healthful compounds such as hydroxytyrosol, tyrosol, oleuropein, oleocanthal, and squalene appeared to be reasonably heat-resistant. Contrary to the common belief that extra virgin olive oils have low smoke point and should only be consumed in raw, our study showed that olive oils have reasonably high smoke point that is suitable for heating and fresh olive oil with low FFA and high phenolics are important for the conservation of olive oil quality and health. Future studies would include addition of food to see if the same trends are observed.

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