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Effect of Low Temperatures on Phenolic Compounds, Flavonoid Content and Antioxidant Activity of Egyptian Olive Oils

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Abstract

This work studied the low temperatures on the chemical properties of two types of local Egyptian olive oils, one of them from Marsa Matrouh in Western desert (OO_1) and the other from AL-Arishin the eastern desert (OO_2). The parameters for edible olive oil are established by European Union regulations and by the International Olive Council. This paper presents the influence of gentile heating on the content of saturated and unsaturated fatty acids. The results showed that, heat treatment causes low decrease in the nutritional quality of the types of olive oile specially in that were heated at 80°C for 15 min. The content of unsaturated fatty acids decreased significantly from 92.15% in OO_1 before heating to 79.32% at 80°C for 15 min. while no significant change was found at 60°C of OO_1. Dihomo-γ-linolenic acid [DGLA, 20:3 (ω−6)] detected in OO_1 type after heating treatment where, which has antithrombotic effects. Also, these results indicate that the version OO_1 have a higher anti-inflammatory and antimicrobial activities. Also the higher antioxidant activity which not affected by low temperatures prevent the oils from deterioration. Also, the chemical analysis showed that the nutritional quality of OO_1 was higher than OO_2 due to a higher content of flavonoids which do not affected by heat.

Keywords: Olive oil; Phenolic compounds; Fflavonoids; Antioxidant activity

Introduction

Vegetable oils are regarded as the healthier choice relative to animal fats in view of their unsaturated fatty acid and cholesterol-free content. Extra Virgin Olive Oil (EVOO) is one of the most important ingredients of the Mediterranean diet. While originally limited to the Mediterranean regions, olive oil remains as source of external fat, *Olea europaea* L. Olive oil is the vegetable oil obtained from the fruit of the olive tree by mechanical extraction (**IOOC 2008**). The composition of olive oil is primarily triacyl glycerols and secondarily free fatty acids, mono- and diacyl glycerols, and an array of lipids such as hydrocarbons, sterols, aliphatic alcohols, tocopherols, and pigments. Some of these compounds contribute to the unique character of the oil [1]. The phenolic content was significantly highest in the unheated EVOO and decreased constantly by increasing thermal stress. The temperature very highly significantly influenced phenolic content, whereas the duration of heating showed a minor effect [2]. Several olive oil grades are commercially available. Its classification based on sensorial attributes and chemical parameters that give a general overview of its quality and authenticity. These characteristics, namely the Free Fatty Acids (FFA) are regulated by several organizations, including the European Commission itself, the International Olive Oil Council (IOC). Olive oil possesses a highly distinctive taste and flavor due to specific volatile organic compounds, namely aliphatic and aromatic hydrocarbons, aliphatic and triterpenic alcohols, furan and thiophene derivatives [3]. Oleic acid is the most abundant fatty acid in olive oil that is claimed to affect the increase the level of High-Density Lipoprotein (HDL) and to reduce levels of Low-Density Lipoprotein (LDL) in the blood plasma. For this reason, it is considered that oleic acid could prevent the occurrence of certain cardiovascular diseases, which are still one of the major causes of death. The high concentration of phenolic compounds in olive oil contributes significantly toward its antimicrobial activity [4-6]. Olive oil, in particular virgin olive oils with a high content in certain phenolic compounds, can inhibit the growth of pathogenic bacteria. Virgin Olive Oil (VOO) has nutritional and sensory characteristics that make it unique and a basic component of the Mediterranean diet. Its importance due to its richness in polyphenols which, act as natural antioxidants and may contribute to the prevention of several human disease [1]. Olive Oil (OO) constitutes the basis of the Mediterranean diet, and it seems that its biophenols, such as hydroxytyrosol (HT) may scavenge free radicals, attracting distinct attention due to their beneficial effects in many pathological conditions, such as cancer. To the best of our knowledge, this is the first study in which the functional properties of an olive oil Total Polyphenolic Fraction (TPF) and pure hydroxytyrosol were examined in order to determine their antioxidant effects at a cellular level in endothelial cells and myoblasts [7-9]. Direct evidence for the protective role of olive oil against cancer has been recently published [10]. Testing methods are needed to address the real cooking effect on olive oil composition and under different processing conditions, in comparison with other vegetable oils; such that, has good thermal resistance in general. High temperature and prolonged timed used on repeated frying; the oils progressively degraded by a complex series of chemical reactions including oxidation, hydrolysis, and polymerization. These reactions, however, are not equivalent for all the vegetable oils, and there is a

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Table 1: Phytochemical screening of OO_1 at 60°C, 80°C.

Compounds		60 °C			80°C		
	OO_1	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
Phenolic compounds	+++	+++	++	++	++	++	+
Flavonoids	+++	+++	+++	+++	++	++	+
Terpenoids	+++	+++	++	++	+++	+	+
Steroids	+++	+++	++	+	++	++	+
Saponins	-	-	-	-	-	-	-
Alkaloids	+++	+++	++	++	++	++	+

Highly positive '+++', Moderate '++', Negative '-'

Table 2: Phytochemical screening of OO_2 at 60°C, 80°C.

Compounds		60 °C			80°C		
	OO_1	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
Phenolic compounds	+++	+++	++	++	++	+	+
Flavonoids	+++	+++	+++	+++	+++	++	++
Terpenoids	+++	+++	++	+	+++	+	+
Steroids	+++	+++	+	+	++	++	+
Saponins	-	-	-	-	-	-	-
Alkaloids	+++	+++	++	++	++	++	+

Highly positive '+++', Moderate '++', Negative '-'

Table 3: Fatty acids composition % of OO_1 at 60°C, 80°C.

Compounds		60 °C			80°C		
	OO_1	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
Palmitic acid(C16:0)	0.80± 0.11	1.11± 0.11	2.83± 0.12	2.98± 0.14	3.80± 0.11	3.88± 0.11	11.95± 0.00
Heptadecanoic acid (C17:0)	4.52± 0.10	4.32± 0.11	4.22± 0.11	4.22± 0.14	4.12± 0.14	3.92± 0.10	3.51± 0.00
Heptadecenoic acid (C17:1)	0.40± 0.12	0.40± 0.13	0.36± 0.11	0.34± 0.12	0.29± 0.21	0.26± 0.10	0.22± 0.10
Stearic acid(C18:0)	1.85± 0.12	2.15± 0.11	2.95± 0.11	3.05± 0.12	3.95± 0.10	3.99± 0.00	4.54± 0.12
Oleic acid(C18:1)	52.53± 0.10	52.13± 0.11	51.91± 0.11	51.53± 0.00	50.51± 0.15	50.13± 0.12	48.99± 0.10
Linoleic acid(C18:2)	15.55± 0.10	15.15± 0.10	14.95± 0.11	14.15± 0.21	13.65± 0.11	13.55± 0.11	12.85± 0.1
Linoleinic acid(C18:3)	22.73± 0.10	21.13± 0.00	21.22± 0.10	20.73± 0.11	20.33± 0.11	20.13± 0.12	16.32± 0.11
Dihomo-γ-linolenic acid (C20:3)	-	0.23± 0.10	0.34± 0.21	0.37± 0.11	0.44± 0.13	0.47± 0.10	0.54± 0.11
Arachidoinc acid (C20:4)	0.94± 0.10	0.86± 0.00	0.74± 0.11	0.71± 0.11	0.64± 0.10	0.54± 0.10	0.40± 0.18
Total saturated fatty acids	7.17± 0.11	7.58± 0.11	10.00± 0.00	10.25± 0.11	11.87± 0.11	11.79± 0.12	20.00± 0.11
Total unsaturated fatty acids	92.15± 0.11	89.90± 0.11	89.52± 0.11	87.89± 0.12	85.86± 0.10	85.42± 0.11	79.32± 0.11
Ratio unsaturated/saturated fatty acids	12.85± 0.11	11.86± 0.11	12.85± 0.11	8.95± 0.10	7.23± 0.11	7.24± 0.11	3.96± 0.11

means ± DS, n= 3. Values are significantly different from each other (p< 0.05)

particular concern regarding olive oil since its bioactive attributes might be lost during this process, despite being highly resistant to thermal oxidation. The most common frying methods are deep-frying, being the food immersed in hot oil, and pan-frying when the food is cooked in a pan with little amounts of oil [11].

Materials and Methods

Chemicals

All solvents and chemicals were from Sigma-Aldrich (St. Louis, MO, USA). The international standards use different methods and parameters to evaluate the quality of the oils according to their use as food. Physicochemical parameters were considered about the requirements of standards.

Phytochemical Screening

Phytochemical screening for flavonoids, alkaloids, saponins and terpenoids were done following standard methods as described by Harborne and Sofowora [12,13].

Experimental Design

One hundred-gram samples of OO_1 and OO_2 were placed in steel containers and heated to either 60 or 80°C. These temperatures were chosen based on the fasting temperature of olive oil (60–80°C) and of the temperature that can be reached during fasting cooking (100°C). The samples were held hat each temperature for 5, 10 and15 min., giving a total of 12 trials. After heating, the oil was cooled to room temperature and analyzed within two hours.

Table 4: Fatty acids composition % of OO_2 at 60°C, 80°C.

Compounds		60 °C			80°C		
	OO_1	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
Palmitic acid(C16:0)	1.20± 0.10	1.91± 0.00	2.91± 0.32	4.99± 0.11	11.80± 0.11	13.98± 0.11	16.99± 0.00
Heptadecanoic acid (C17:0)	6.12± 0.10	6.37± 0.00	6.72± 0.12	7.43± 0.11	7.92± 0.11	8.12± 0.10	8.21± 0.00
Heptadecenoic acid (C17:1)	0.33± 0.11	0.31± 0.13	0.31± 0.12	0.30± 0.12	0.28± 0.21	0.25± 0.10	0.21± 0.10
Stearic acid(C18:0)	2.15± 0.22	2.23± 0.00	3.01± 0.11	3.15± 0.12	4.15± 0.10	4.19± 0.00	4.59± 0.1
Oleic acid(C18:1)	45.51± 0.10	44.93± 0.11	43.92± 0.11	43.53± 0.00	42.55± 0.15	42.13± 0.12	41.79± 0.10
Linoleic acid(C18:2)	13.59± 0.10	13.45± 0.10	13.15± 0.12	12.95± 0.21	12.65± 0.12	10.34± 0.11	10.85± 0.1
Linoleinic acid(C18:3)	20.71± 0.10	18.13± 0.13	17.92± 0.10	17.73± 0.11	17.23± 0.12	15.15± 0.12	15.32± 0.11
Arachidoinc acid (C20:4)	0.73± 0.10	0.72± 0.00	0.71± 0.11	0.70± 0.11	0.54± 0.10	0.51± 0.11	0.39± 0.11
Total saturated fatty acids	9.47± 0.11	10.51± 0.11	12.64± 0.11	15.57± 0.11	23.87± 0.15	26.29± 0.16	29.79± 0.11
Total unsaturated fatty acids	80.87± 0.11	77.54± 0.11	76.04± 0.12	75.21± 0.11	60.60± 0.11	68.38± 0.11	68.56± 0.11
Ratio unsaturated/saturated fatty acids	8.59± 0.11	7.37± 0.10	6.01± 0.11	4.83± 0.13	2.53± 0.11	2.60± 0.12	2.30± 0.11

means ± DS, n = 3. Values are significantly different from each other ($p < 0.05$)

Table 5: HPLC of Phenolic compounds of OO_1 at 60°C, 80°C.

Compounds		60°C			80°C		
	OO_1	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
Caffic acid	25.21	16.42	12.22	14.03	22.58	12.2	5.01
Catechol	22.4	18.2	26.12	25.52	21.2	18.6	4.12
Ellagic	18.44	8.4	8.3	8.22	12.42	7.41	4.52
Gallic	11.04	9.77	8.17	4.35	9.77	9.77	9.77
Quercetin	15.82	12.82	12.32	11.5	15.12	5.66	7.82
Total	92.91	65.61	67.13	63.77	81.09	53.64	31.24

Table 6: HPLC of Phenolic compounds of OO_2 at 60°C, 80°C.

Compounds		60°C			80°C		
	OO_2	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
Caffic acid	24.65	19.15	10.82	4.66	24.65	7.01	6.54
Catechol	28.2	25.32	7.88	4.54	28.2	15.14	8.44
Ellagic	14.66	12.61	11.23	8.12	14.66	4.25	5.01
Gallic	12.65	9.65	9.54	8.5	11.61	9.76	18.44
Quercetin	9.01	11.01	11.25	8.41	9.01	7.41	7.22
Total	89.17	77.74	50.72	34.23	88.13	28.43	32.4

Gas Chromatography- Mass Spectrum Analysis (GC-MS) analysis

The chemical composition of your samples was performed using Trace GC1310-IQS mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30m x 0.25mm x 0.25µm film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C /min to 230°C hold for 2 min. increased to the final temperature 290°C by 30°C /min and hold for 2 min. The injector and MS transfer line temperatures were kept at 250, 260°C respectively; Helium was used as a carrier gas at a constant flow rate of 1ml/min. The solvent delay was three min. and diluted samples of 1µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200°C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

High-Performance Liquid Chromatography –Mass Spectrum

HPLC-MS technique is often used for separation, identification and quantitation of flavonoids and phenolic acids in plants. The HPLC-MS system (Agilent 1100) is composed of a quaternary pump, a photodiode array detector, a UV/Vis detector, and a single quadruple MS detector with ion source (ESI).

Phenolic contents

Phenolic contents were separated from within 60 min by employing a gradient mobile phase of water/acetonitrile/glacial acetic acid (980/20/5, v/v/v, pH 2.68) and acetonitrile/glacial acetic acid (1000/5, v/v) with flow rate at 3mL/min and detection at 325nm.

Table 7: HPLC of Flavonoid compounds of OO_1 at 60°C, 80°C.

Compounds		60°C			80°C		
	OO_1	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
Myrectin	22.32	21.12	21.02	**21**	18.12	15.33	12.4
Apigenin	11.05	18.15	20.85	**22.35**	15.05	17.05	19.66
Luteolin	8.62	9.62	12.62	**18.62**	11.62	12.62	15.7
Oleuropein	20.3	18.32	18.22	**17.35**	17.28	15.33	12.43

Table 8: HPLC of Flavonoid compounds of OO_2 at 60°C, 80°C.

Compounds		60°C			80°C		
	OO_2	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
Myrectin	18.2	15.34	14.23	14.21	13.34	12.23	10.33
Apigenin	17.45	7.45	7.41	6.41	13.15	6.41	4.45
Luteolin	7.14	7.14	6.54	6.14	6.11	5.24	4.15
Oleuropein	29.3	21.13	20.03	18.43	20.11	19.23	15.23

Flavonoids contents

Phenolic contents were separated from within 60 min by employing contents were separated from within 60 min by employing a gradient solvent system of 0.1% formic acid solution with flow rate at 1.0mL/min, detection at 280nm and identification by ESI -MS, were separated within 70 min.

Antioxidant Activity (DPPH assay) oil

The DPPH assay measures the radical scavenging activity of vegetable oil. It was conducted in UV/Vis Spectrometer model Lambda 2, Perkin Elmer (Waltham, MA, USA), using the method proposed by kalantzakis et al. [14], modified as follows. Firstly, the oil was diluted with ethyl acetate (1:10, v/v). Secondly, 500µL of diluted oilwere added to 2mL of a 10-4 M DPPH solution, previously prepared with ethyl acetate and, thirdly, the absorbance of the mixture was measured immediately at 515nm (t0) and after 30 minutes of incubation (t30). The results were calculated with the following formula: % inhibition = [(T0 – T30)/T0] x 100 and they were expressed as % inhibition.

Results and Discussion

Phytochemical Screening

Phytochemical screening showed that the phenolic compounds were decreased for both OO_1 and OO_2 while the flavonoids contents do not affect by heating. These results indicated that, the olive oils after treatment with low temperatures have high antioxidant activity due to flavonoids compounds which do not affect by heat (Tables 1 & 2). The results showed that the presence of terpenoids, steroids and phenolic compounds in both which, their concentrations were decreased by heating at low temperatures. The contents of flavonoids not affect by heating.

The effect of heat treatment on the content of free fatty acids

Fatty Acids (FAs) are among the most important parameters to establish the edibility of vegetable oil; in particular, oleic acid content. The high Saturated Fatty Acid (SFA) content determines 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 oxidation ability of the vegetable oil due to the presence of double bonds. However, mono-unsaturated fatty acids (MUFAs) are lower the bad cholesterol in the blood and represented as essential fatty acids (EFAs) which, it must have to be taken with the diet. Saturated fatty acids namely, Palmitic acid (16:0), Heptadecanoic acid (C17:0) and Stearic acid (C18:0) were calculated as percentage content increased with increasing temperature. The minimum content (0.80%) and (1.20%) were found in the OO_1 and OO_2 respectively, before thermal treatment and the maximum (11.95% and 16.99%) of both OO_1 and OO_2 were found at 80°C for 15 min (Tables 3 & 4). Linoleic acid (18:2) showed a decreasing trend from 22.73% in the original OO_1 to 20.73% and 16.32% after 15 min heating at 60°C and 80°C respectively (Table 2). On the other hand, there is a significant decrease in linolenic acid content in OO_2 from 20.71% to 17.92% and 15.32% after 15 min heating at 60°C and 80°C respectively. These observations confirmed the fact that the fatty acid degradation rate increases with the number of double bonds in the molecule [2]. The results also confirmed that, different types of Egyptian olive oils OO_1 and OO_2 have higher content of active antioxidant compounds, which prevent or decrees the unsaturated fatty acids against deterioration during gentile heating. Also, the results showed that after heating Dihomo-γ-linolenic acid (DGLA) was detected in OO_1 type where, it is a 20-carbon ω−6 fatty acid. In physiological literature, it is given the name 20:3 (ω−6) contains three *cis* double bonds. DGLA is an extremely uncommon fatty acid, found only in trace amounts in animal products, which have antithrombotic effects. In addition, these results indicate that the version of olive oil OO_1 has higher anti-inflammatory and antimicrobial activities [15].

Phenolic compounds content

Olive oil contains many biologically active components, which exert antioxidant activity, differently from other edible vegetable oils. This implies that olive oil contains many minor bioactive compounds such as phenols whose content was found to decrease with heating. The phenolic content was significantly highest in the unheated of both olive oils OO_1 and OO_2 and decreased constantly with the increasing temperature. After 15 min. of heating, the phenolic content of OO_1 decreased from 92.91 to 63.77, and 31.24%, respectively at 60 and 80°C (Table 5). On the other, the phenolic content of OO_2 decreased

Table 9: Average of % inhibition of DPPH anti-oxidant assay OO_1.

Conc. (mg/mL)		60 °C			80 °C		
	OO_1	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
1.2	77.38	87.32	85.67	93.56	89.43	91.36	81.64
0.8	69.1	71.28	70.19	78.37	73.51	75.14	69.13
0.4	51.07	56.49	53.54	62.81	60.3	59.69	52.48
0.2	26.41	35.11	30.28	40.35	37.92	38.32	26.31
0.1	15.16	17.27	14.35	19.1	18.76	20.45	14.27
IC_{50} (mg/dl)	0.417	0.332	0.372	0.272	0.299	0.291	0.402
	±0.006	±0.003	±0.004	±0.001	±0.003	±0.002	±0.004

Table 10: Average of % inhibition of DPPH anti-oxidant assay OO_2.

Conc. (mg/mL)		60 °C			80 °C		
	OO_2	5 min.	10 min.	15 min.	15 min.	10 min.	5 min.
1.2	65.34	75.64	71.56	61.34	76.38	68.62	73.36
0.8	43.11	63.93	62.38	40.81	65.18	47.29	64.82
0.4	28.67	49.33	41.3	19.25	52.27	30.37	45.52
0.2	11.29	21.17	17.06	7.32	23.31	14.28	20.67
0.1	4.03	10.28	6.19	2.18	10.4	5.46	11.05
IC_{50} (mg/dl)	0.844	0.481	0.567	0.948	0.451	0.761	0.505
	±0.009	±0.006	±0.007	±0.010	±0.006	0.008	±0.007

from 89.17 to 34.23, and 32.4%, respectively at 60 and 80°C (Table 6).

Flavonoids content

Total flavonoid content for all the two types of local Egyptian olive oils OO_1 & OO_2 was evaluated and had been represented in (Tables 7 & 8). Results revealed that, OO_1 possessed the highest flavonoid content when it was heated at 60°C for 15 min. The most important flavonoid is Apigenin (22.35) compared to other version OO_2. The total flavonoid content decreased in the following order for all the other variants: OO_2 at (80°C) < OO_2 at (60°C)< OO_2 at (60°C).

Antioxidant activity

Fast food as one of the most popular culinary methods globally. Organoleptic and sensorial properties of fried food products, such as juicy taste, nice flavor, crispy texture and brownish color are largely desired and relished by consumers. Deterioration of natural antioxidant such as phenolic compounds was observed when virgin olive oil is heated [16]. In the present study, the antioxidant activity of original olive oils OO_1 & OO_2 or the ability of antioxidants to retain antioxidant activity after heat treatment was tested at two different temperatures, 60°C and 80°C. Heating causes changes in the physical and chemical characteristics of the oils leads to the degradation in the oil quality, with the formation of more saturated compounds such as hydro peroxides, monomers and high-molecular-weight compounds along with less proportion of unsaturated fats. Lipid peroxidation may be initially prevented by antioxidants. In this study due to the higher content of flavonoids which, dose not affected by low temperatures especially, when were heated at 60°C. After 15 min. of heating the antioxidant activity t of OO_1 was shown to possess greater antioxidant capacity (93.56) at concentration 1.2 mg/mL with IC_{50} (0.272±0.001) as compared with its version when was heated at 80°C (81.64, IC_{50} 0.402±0.004) (Table 9). Also, the highest antioxidant activity due to the presence of Apigenin, which, represented as more potent cytotoxic activity against most of tumor cell lines because of their highest antioxidant activity and prevent the formation of free radicals [17-20]. On the other hand, the antioxidant activity of the second version of olive oil OO_2 was less than OO_1 as (71.56, IC_{50} 0.567±0.007) and (76.38, IC_{50} 0.451±0.006) at 60°C and 80°C after 15 min. respectively (Table 10).

Conclusion

In conclusion, olive oil is safe to cook with heating at low temperatures and will not destroy the health benefits or turn olive oil unhealthy. You can feel confident using olive oil in all of your recipes especially when heating at 60°C. The antioxidant activity of both two versions of olive oils OO_1 & OO_2 were increased due to higher contents of phenolic and flavonoids compounds.

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