# OLIVE OIL AS A POTENTIAL SOURCE OF ANTIOXIDANT AND ANTICANCER AGENTS

Saad Ahmed Saad **Halla**[1], Mohamed Mohamed Ahmed **El-Nikeety**[1], Samah Said Mahmoud **Allam**[2]and Asmaa **Gamal**[2*]

[1]*Food Science & Technology Department Faculty of Agriculture, Cairo University, Giza, Egypt*

[2]*Oils and Fats Researches Department, Food Technology Researches Institute, Agriculture Researches Center, Giza, Egypt*

Email :asmaagamal2009@yahoo.com

***Abstract***

*The aim of this study was to evaluate the variations in quality parameters of olive oil extracted from two different olives varieties(Coratina and Picual) were followed at three different stages of maturity: free fatty acids %, peroxide value, $K_{232}$, $K_{270}$ and $\Delta K$ were decreased during maturation course. Fatty acids composition of olive oil samples were analyzed by gas chromatography (GC).Oleic acid was the major fatty acid and represented the monounsaturated fatty acid in both oil varieties followed by palmitic acid which represented the major saturated fatty acid. The total polyphenols level in early maturation stages was higher than late maturation stages for both varieties. Coratina olive oil had higher phenols content than Picual olive oil. Salycilic and oleuropein were the major identified phenolic compounds in both olive oils varieties followed by hydroxytyrosoland they were declined through maturation process. The antioxidant activity was evaluated by 2-diphenyl-1-picrylhydrazyl (DPPH) in olive oils and olive oils phenolic extracts for both varieties. Olive oils and their phenols extracts had a higher antioxidant activity at the early stage of ripeness and this activity decreased with ripening progress. Phenols extracted from both olive oils exhibited an anticancer effect against four human carcinoma cell lines; intestinal, liver, prostate and colon cell lines.*

**Keywords:** olive oil, quality parameters, phenolic compounds, antioxidant activity, anticancer effect.

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## 1. INTRODUCTION

Virgin olive oil is a valuable vegetable oil extracted from fresh and healthy olive fruits (*Oleaeuropaea* L.) by mechanical processes (pressing and centrifuging) and without heat, solvents or any preliminary refining. It is practically the only vegetable oil that can be consumed directly in its raw state as well as it contains important nutritional elements (fatty acids, vitamins, sterols, etc.). Virgin olive oil (VOO) is one of the main components of the Mediterranean diet. It is highly appreciated all over the world for its taste and aroma, as well as for its nutritional properties. Different cultivars, pedoclimatic conditions of the orchards, and varying agricultural practices, together with olive ripeness and olive oil extraction techniques, result in a great diversity of olive oil chemical(López-Cortés *et al*., 2013).

The Mediterranean diet is associated with a lower incidence of atherosclerosis, cardiovascular disease, neurodegenerative diseases and certain types of cancer. The apparent health benefits have been partially ascribed to the dietary consumption of virgin olive oil by Mediterranean populations. Many researches have focused on the biologically active phenolic compounds naturally present in virgin olive oils to aid in explaining reduced mortality and morbidity experienced by people consuming a traditional Mediterranean diet. Studies (human, animal, *in vivo* and *in vitro*) have demonstrated that olive oil phenolic compounds have positive effects on certain physiological parameters, such as plasma lipoproteins, oxidative damage, inflammatory markers, platelet and cellular function, antimicrobial activity and bone health (Cicerale *et al*., 2010).

Ripening stages play important key roles in oil quality and have a major effect on these minor

components and also affect the fatty acids composition and oxidative stability of olive oil. The present work was carried out to monitoring the effect of ripening stages of Coratina and Picual olive varieties on their oils quality characteristics, separation and identification of their phenolic compounds and determining the effect of these phenolic compounds as antioxidant and anti-cancer agents.

## 2. MATERIALS AND METHODS

**2.1. Olive samples** of two different olive varieties (Coratina and Picual) were collected from El-Monsorya, Giza Governorate, Egypt at three different ripening stages and subjected immediately to extraction process with an experimental mill that reproduced the industrial process at laboratory scale. The apparatus consists of the following elements: hammer crusher, kneader and horizontal centrifugate unit (decanter). Oil samples were filtered with cotton wool and stored in dark glass bottles at -20 ºC till analyses.

**2.2. Quality characteristics**: peroxide value (PV) and free fatty acids % (FFAs) were evaluated following the methodology proposed byA.O.A.C. (2012). UV spectrophotometric indices ($K_{232}$, $K_{270}$, and $\Delta K$) were measured according to the EEC 2568/ (1991) Regulation methods.

**2.3. Fatty acids** analysis and determination were carried out by preparation of methyl ester followed by the identification of methyl esters using an Agilent 6890 series gas chromatograph apparatus equipped with a DB23 (60 m X 0.32 (Stefanoudaki *et al.*, 1999).

**2.4. Total phenols content** were determined according to the method described by Gutfinger (1981), and results were expressed as mg of caffeic acid per kg of oil.

**2.5. Phenolic compounds** of oils samples were identified and determined using HPLC following the method described by Goupy *et al.* (1999).

**2.6. Antioxidant activity** of both olive oils varieties was analyzed using the stable 2, 2-diphenyl-1-picryl-hydrazil (DPPH) following the procedure described by Malheiro *et al.* (2012), while the antioxidant activity of both olive oils phenolic extracts was determined using the stable DPPH with the procedure described by Blois (2002).

**2.7. Potential cytotoxicity** of olive oil phenolic compounds on human cancer cell lines was carried out using Sulphorhodamine-B (SRB) assay following the method reported by Skehan *et al.* (1990).

## 3. RESULTS AND DISCUSSION

### 3.1. Quality parameters

FFAs %, PV and UV absorbances$K_{232}$, $K_{270}$ and $\Delta K$ are used as traditional criterion in which the International Olive Council, IOC (2015) classified olive oils into various commercial categories.

During ripening process FFAs % and PV (as primary oxidation products) were slightly increased with ripening progress for both Coratina and Picual olive oils and results in Table 1 revealed that in spite of FFAs % and PVs increments throughout the three ripening stages, Coratina and Picual olive oils till within the limits established by IOC (2015) for extra virgin olive oil (EVOO); FFAs% $\leq$ 0.80% and peroxide value $\leq$ 20.00 meq.$O_2$/kg (Keceli, 2013).

Olives at a later stage of maturity give oils with higher levels of FFAs % and PV since they undergo an increase in lypolitic and lipoxygenase enzymes activity in fruits (Ben Youssef *et al.*, 2010).

$K_{232}$ values; UV absorbance at 232 (as an indicator for primary oxidation; hydroperoxides and peroxides) increased during olive ripening for both varieties as shown in Table (1). Simultaneous with the formation of hydroperoxides, conjugated dienes are produced as a result of double bond displacements in polyunsaturated fatty acids and this leads to an increase in absorption at 232 nm in oxidized oils (Wanasundara *et al.*, 1995).

**Table 1. PhysiochemicalpropertiesofCoratina and Picualolive oils during ripening stages.**

| Physical and chemical properties | Coratina | | | Picual | | |
|---|---|---|---|---|---|---|
| | I | II | III | I | II | III |
| Free fatty acids (% as oleic acid) | 0.135 | 0.143 | 0.150 | 0.125 | 0.178 | 0.190 |
| Peroxide value (meqO2/kg oil) | 4.06 | 5.17 | 5.48 | 3.84 | 6.63 | 7.38 |
| $K_{232}$ | 1.33 | 1.51 | 1.60 | 1.49 | 1.62 | 1.83 |
| $K_{270}$ | 0.045 | 0.063 | 0.072 | 0.042 | 0.086 | 0.089 |
| ΔK | -0.0065 | -0.0040 | -0.0020 | -0.0025 | -0.0015 | -0.0010 |

I, II, III: ripening stages.

$K_{270}$ was considered as an indicator for secondary oxidation products (aldehydes and ketones). $K_{270}$ values of Coratina and Picual olive oils were slightly increased during the three ripening stages as shown in Table (1) (Bengana *et al*., 2013). In spite of the increment in $K_{232}$, $K_{270}$ and ΔK (Table, 1), they were lower than the maximum limits established by IOC (2015) for EVOO; $K_{232} \leq$ 2.50, $K_{270} \leq 0.22$ and $\Delta K \leq 0.01$.

### 3.2. Fatty acids composition

Table (2) illustrated the changes in fatty acids composition of both Coratina and Picual olive oils varieties during the three ripening stages.

Oleic acid was the major fatty acid and represented the monounsaturated fatty acids in both oil varieties which gradually decreased with the maturation process of Picual olives, while in Coratina olive oil it decreased at the second stage and raise again at the third stage of ripeness, this variability may due to genetic factors and environmental conditions (Yorulmaz *et al*., 2013),followed by palmitic acid which represented the major saturated fatty acid, linoleic acid, stearic acid and linolenic acid and the rest of fatty acids were found in traces in both Coratina and Picual olive oils except for palmioleic acid which was increased in Picual olive during ripening process at the second and third stages of ripeness (Keceli, 2013).

Table (2) also showed that fatty acids profile of both olive oils varieties was within the range stated by the IOC (2015) except for Picual olive oil at the second stage of ripeness which exceeded the IOC limits of linolenic acid (≤ 1.0 %).

### 3.3. Total phenols content

Phenols content is an important factor when evaluating olive oil quality, given that the natural phenols improve its resistance toward oxidation, nutritional properties and its flavor, and is varying depending on several factors; cultivars, agronomic trails and fruit maturation (Dag *et al*., 2011).

Coratina olive oil had higher phenols content than Picual olive oil and both of them decreased with the maturation process (Table,3), (Bengana *et al.*, 2013).

### 3.4. Phenolic compounds

Series of metabolic processes, chemical and enzymatic reactions, take place during fruits ripening, resulting in the production of free phenols and inducing variations in the phenolic profile of several compounds. These changes have an effect on the quality, sensory properties, oxidative stability and/or the nutritional value of the olive oil obtained (Vekiari *et al*., 2010).Ten phenolic compounds with different retention times were detected in both olive oils varieties (Coratina and Picual): hydroxytyrosol, protocatchuic, chlorogenic, caffeic acid, vanillic acid, ferulic acid, salycilic, oleuropein, coumarin and cinnamic acid (Table 3).

Salycilic and oleuropein phenolic compounds were the major phenolic compounds identified in both olive oils varieties.

Oleuropein, the phenol mainly responsible for the bitterness attribute in olive oils was found in higher concentration (5 folds) in Coratina olive oil than Picual olive oil. Also salycilic was found with almost 5 folds in Coratina olive oil than Picual olive oil, whileferulic acid and protocatchuic were presened as minor phenolic compounds in both Coratina and Picualvarieties throughout the three ripening stages (Bengana *et al*., 2013).

**Table 2. The relative percentage of fatty acids of Coratina and Picualolive oils during ripening stages.**

| Relative percentage of fatty acids | Coratina | | | Picual | | |
|---|---|---|---|---|---|---|
| | I | II | III | I | II | III |
| Palmitic (C16:0) | 14.38 | 15.19 | 13.81 | 14.43 | 16.26 | 16.06 |
| Palmitoleic (C16:1) | 0.46 | 0.72 | 0.44 | 0.77 | 2.44 | 2.30 |
| Maragic (C17:0) | 0.04 | 0.04 | 0.05 | 0.04 | 0.04 | 0.05 |
| Margoleic(C17:1) | 0.06 | 0.06 | 0.07 | 0.07 | 0.09 | 0.10 |
| Stearic (C18:0) | 2.33 | 2.09 | 2.13 | 2.17 | 2.47 | 3.38 |
| Oleic (C18:1) | 71.09 | 68.16 | 70.72 | 70.38 | 67.88 | 65.96 |
| Linoleic (C18:2) | 9.70 | 11.83 | 10.57 | 10.13 | 8.97 | 10.49 |
| Linolenic (C18:3) | 0.86 | 0.96 | 1.00 | 0.94 | 1.13 | 1.00 |
| Arachidic (C20:0) | 0.51 | 0.44 | 0.51 | 0.48 | 0.38 | 0.37 |
| Gadoleic (C20:1) | 0.45 | 0.41 | 0.53 | 0.45 | 0.24 | 0.21 |
| Behenic (C22:0) | 0.12 | 0.10 | 0.17 | 0.14 | 0.10 | 0.08 |

I, II, III: ripening stages.

**Table 3. Phenolic compounds of Coratina and Picual olive oils during ripening stages.**

| Phenoles (mg/kg) | Coratina | | | Picual | | |
|---|---|---|---|---|---|---|
| | I | II | III | I | II | III |
| Hydroxytyrosol | 45.77 | 37.49 | 31.61 | 27.82 | 19.19 | 13.98 |
| Protocatchuic | 7.97 | 6.98 | 5.64 | 2.91 | 2.81 | 2.71 |
| Chlorogenic | 17.86 | 14.40 | 8.79 | 13.45 | 6.00 | 5.60 |
| Caffeic acid | 46.58 | 13.63 | 8.52 | 10.93 | 9.64 | 6.74 |
| Vanillic acid | 6.55 | 6.37 | 3.36 | 4.23 | 3.59 | 3.54 |
| Ferulic acid | 1.13 | 2.42 | 2.27 | 0.86 | 0.91 | 0.44 |
| Salycilic | 353.75 | 236.38 | 227.21 | 70.36 | 23.19 | 11.09 |
| Oleuropein | 265.30 | 226.25 | 107.51 | 62.84 | 37.65 | 36.26 |
| Coumarin | 33.01 | 18.99 | 8.35 | 15.31 | 6.20 | 6.46 |
| Cinnamic acid | 64.63 | 29.51 | 28.31 | 8.19 | 4.51 | 1.56 |
| Total phenols content (mg/kg as caffeic acid) | 1686.19 | 776.11 | 590.46 | 448.47 | 163.64 | 128.68 |

I, II, III: ripening stages.

**Table 4. Antioxidant activity of Coratina and Picualolive oilsand their phenols extracts during ripening stages.**

| **Olive oils varieties** | | **Antioxidant activity of olive oils %** | **Antioxidant activity of olive oil phenols %** |
|---|---|---|---|
| **Coratina** | **I** | 94.11 | 97.45 |
| | **II** | 93.20 | 94.61 |
| | **III** | 92.42 | 91.21 |
| **Picual** | **I** | 87.58 | 94.30 |
| | **II** | 69.28 | 51.84 |
| | **III** | 53.50 | 40.79 |

I, II, III: ripening stages.

Hydroxytyrosol, the one with the highest antioxidant activity, was greater in early harvest samples of both Coratina and Picual olive oils and gradually decreased during maturation at the second and third stages of ripeness (Jiménez *et al*., 2013).

Results in Table (3) also revealed that all phenolic compounds decreased during maturation process in both olive oils varieties and this may be correlated with the increased activity of the hydrolytic enzymes with maturation (Ryan *et al*., 2002).

Meanwhile Baccouri *et al*. (2008) stated that phenolic compounds progressively increased until they reached a maximum at the reddish and black pigmentation stages, after which it decreased.

### 3.5. Antioxidant activity of olive oils

Natural antioxidant compounds: phenolic compounds, chlorophylls, carotenoids and tocopherols, that are mainly responsible for the oxidative stability of olive oils, were gradually decreased during ripening process (Table, 1).

Variation in antioxidant activity (%) between Coratina and Picual olive oils during maturation process (Table 4) could be attributed to the variation in their content of the natural antioxidant compounds.

Coratina olive oil had a higher antioxidant activity than Picual olive oil and during ripening process it was declined for both extra virgin olive oils as a result of the declined of their antioxidant compounds especially the polyphenols compounds.

Janakat *et al*. (2013) pointed to the correlation between total phenolic compounds and antioxidant activity of olive oil.

### 3.6. Antioxidant activity

Antioxidant activity of olive oil phenols extracts during ripening process was varied depending on olive cultivar; Coratina olive oil had a higher antioxidant activity than Picual olive oil at the three ripening stages, and that may be due to its higher content of total phenols (Table 1). The antioxidant activity correlated positively with total phenols content and not only depends on the phenols concentration, but also on the specific chemical structure of each phenolic compound (Gambacorta *et al*., 2012) and that was very obvious with Coratina olive oil; despite of its sharp decrease in its phenols content (almost more than 60 % lower, Table 3) during ripening process, the antioxidant activity of its phenols did not decrease with the same pattern (only 6 % decreased, Table, 4).

Antioxidant activity (%) of phenols extracts gradually decreased with ripening progress for both olives varieties.

### 3.7. Cytotoxicity effect of olive oils phenols extract

Agents that can eliminate abnormal clones by induction of apoptosis rather than merely slowing down their proliferation may have chemo preventive potential. Apoptosis is a specialized process of cell death that is part of the normal development of organs and tissue maintenance, but may also occur as a response to various environmental stimuli, indicating toxicity.

Phenolic compounds extracted from Coratina and Picual olive oils at the first stage of ripeness, where the oils had the highest phenols content, were evaluated at the National Cancer Institute, Cairo, Egypt, for their cytotoxicity activities in vitro disease oriented antitumor screening using sulphorhodamine B (SRB) assay including five human tumor cell lines representing different cancer types (intestinal, liver, prostate, colon and breast).Materials that caused less than 50 % survival fraction ($IC_{50}$, the dose of phenols which reduces survival fraction to 50 %) were considered as anticancer agents for the organ it were tested for (Samah and Eid, 2009).

Results in Table (5) revealed that phenolic compounds extracted from Coratina and Picual extra virgin olive oils exhibited an efficient cytotoxicity against four human tumor cell lines representing intestinal tumor cell lines, liver, prostate and colon carcinoma cell lines (Anter *et al*., 2014) and the ability of phenols extracted from Picual olive oil to inhibit 50 % of the tumor was higher than the ability of phenols extracted from Coratina olive oil.

**Table 5. Cytotoxic activity (IC50) of phenols extracted from Coratina and Picual olive oils***

| Tumor cells | Coratina | Picual |
|---|---|---|
| **Intestinal** | 3.23 | 2.93 |
| **Liver** | 4.13 | 3.98 |
| **Prostate** | 4.13 | 3.83 |
| **Colon** | 41.30 | 23.80 |
| **Breast** | Negative effect | Negative effect |

$IC_{50}$: the dose of phenols that reduce survival fraction to 50 %.

*:Phenols were extracted from Coratina and Picual olive oils at the first stage of ripening.

On the other hand, phenols extracted from both olive oils (Coratina and Picual) were proven to have no cytotoxic effect against breast carcinoma cell line under our experimental conditions contrary to the results found by Ahmed *et al*. (2010), who detected the anticancer effect of olive phenols against breast tumor cell line.

## 4. CONCLUSION

As the maturation process continues, a number of changes, both physical and chemical, occurred in olives fruits. They include decrease in phenolic compounds, which were responsible for the sensory attribute and stability of olive oil and these compounds were considered as antioxidant and anticancer agent. The chemical data discussed in this work can be considered useful for providing information about the evolution of some major and minor compounds in relation to the olive ripening degree. Chemical parameters studied (free fatty acids, peroxide value and spectrophotometric absorption $K_{232}$and $K_{270}$) remained within estimated limits of the IOC throughout the maturity process.

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