Alqalam Journal of Medical and Applied Sciences. 2026;9(1):159-163
https://doi.org/10.54361/ajmas.269126

Original article

Comparative Analysis of Total Polyphenol Content in Olive Oil and Leaf Extracts from Frantoio and White Olive Cultivars in Libya

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Abstract
This research has been done to compare the total phenolic content (TPC) of olive oil and olive leaf extracts obtained from two Libyan olive cultivars, Tarhuna White and Frantoio. Phenolics were measured by the Folin-Ciocalteu colorimetric assay, and the results were converted to gallic acid equivalents per kilogram of oil (mg GAE/kg) and per gram of dry leaf extract (mg GAE/g). The findings showed very significant differences between the TPC of the leaf extracts and their respective oils, with the leaf extracts from both cultivars being much higher. The leaf extract of the White olive had the highest TPC (2.35 mg GAE/g), and the Frantoio leaf extract came next (1.86 mg GAE/g). On the other hand, the oils had considerably lower phenolic contents, with the White oil containing 1.21 mg GAE/kg and the Frantoio oil 1.07 mg GAE/kg. The variations demonstrate the effect of different cultivar types on phenolic content, which might be due to different genes and enzymes involved in phenolic biosynthesis pathways. The results show that the choice of cultivar is not only important for olive oil quality but also for the recovery of phenolic compounds from the olive by-products. In general, olive leaves can be considered a good source of natural antioxidants for use in the nutraceutical industry. Further studies should be directed to chromatographic profiling of individual phenolic compounds, besides their evaluation
Keywords. Phenolic Compounds, White Olive Oil, White Olive Leaf, Frantoio Olive Oil.

Introduction

While the olive tree is a noble edible crop, it has also gained recognition as a source of many uses for its oil and leaves, which are both nutritive and therapeutic, because they contain bioactive compounds (mainly antioxidants and anti-inflammatory agents), which can adjust oxidative stress; oxidative stress is the main cause of chronic diseases like diabetes, cancer, cardiovascular diseases, and neurodegenerative diseases [1-3]. The most significant contributors to these valuable effects are the phenolic compounds found in the leaves and high-phenolic olive oils, namely oleocanthal and oleacein; specifically, the phenolic compounds in these products may have a positive effect on vascular functions, decrease LDL (low-density lipoprotein) cholesterol levels, reduce systemic inflammation, and even protect cellular DNA from damage, thus, ultimately, increasing the role of olive oil as the source of disease prevention [2,4].

Polyphenols in olive cultivars vary based on the process used to produce the olive oil, the growing environment of the tree, and the genetic characteristics of the tree itself [5, 6]. Specifically, the Frantoio cultivar originated in Italy in the early 1900s and was later introduced into Libya. The Frantoio is prized for its oil, which contains a number of lignans, secoiridoids, and phenolic alcohols, including tyrosol and hydroxytyrosol, which contribute to the oil's content of polyphenols [7, 8]. Conversely, the white olive cultivar, which is native to Tarhuna, Libya, is different than other olive cultivars because it has a unique genotype. This cultivar is distinguished by its pale-colored drupes, and while there is some information available regarding its phytochemical profile, much about this profile remains unexplored.

This research project investigated the total polyphenol content (TPC) of methanolic extracts prepared from leaves and oil of Frantoio and White olive cultivars by utilizing the Folin-Ciocalteau assay. A secondary goal of the project was to determine if there were any statistical differences between cultivars, and between leaf extracts and oil extracts of the same cultivar.

Materials and Methods

Sample Collection

During the same ripening period in the 2024–2025 season, olive leaves and mature fruits from Frantoio and White (Tarhuna, Libya) cultivars were gathered from groves in Tarhuna (northwest Libya). In accordance with the International Olive Council (IOC) protocol, fruits with a maturation index of 2-4 were hand-picked (COI-OH-Doc.-1–2011-Eng, 2019). Fruits were processed right away in order to extract the oil. The leaves were cleaned, allowed to air dry for two weeks at room temperature in the dark, then ground into a powder and kept at 4 °C until they were analyzed.

Received*: 11-11-2025 -* ***Accepted****: 14-01-2026 -* ***Published****: 21-01-2026*

Preparation of Olive Leaf Extracts

Two grams of powdered leaves were mixed with 300 mL of 80% methanol and 20% water. Samples were extracted in a Soxhlet apparatus for four hours after being sonicated (50 kHz, 30 min, 50 °C) using an Argo lab DU-32S, Carpi, MO, Italy. At 40 °C and reduced pressure, the solvent was evaporated in a rotary evaporator (Heidolph, Laborota 4003, Heidolph Scientific Products GmbH, Schwabach, Germany). Prior to analysis, aqueous extracts were kept at 4 °C.

Determination of TPC

TPC was measured using the Folin–Ciocalteu procedure [9]. TPC was measured using the Folin–Ciocalteu procedure [9]. In brief, 0.5 mL of extract was combined with 2.5 mL of 10% Folin–Ciocalteu reagent. Left to stand for 5 minutes. Afterwards, 2 mL of 20% sodium carbonate solution was added. The mixture was incubated in darkness for 30 minutes, at ambient temperature. Absorbance was recorded at 760 nm (Jenway 7300 UV-Vis, UK). A blank was prepared with solvent only.

Calibration and Expression of Results

The calibration curve for gallic acid (20–120 mg/L) was used. Total phenolic content values were calculated from the regression equation (y = 0.0095x + 0.0232, R2 = 0.9981, where y is the absorbance value and x are the concentration of the solution) and expressed as milligrams of gallic acid equivalents per gram of fresh leaf weight (mg GAE/g FW) for leaf extracts and per kilogram of oil (mg GAE/kg oil) for oil samples.

Statistical Analysis

The results represent the mean value ± standard errors of the mean (SEM). The difference between the total TPC of Frantoio and White cultivars was evaluated by the Student's t-test. A p-value of less than 0.05 was used to indicate statistical significance. GraphPad Prism v.8.0 was the software utilized to run statistical analyses.

Results

The analysis of Total Phenolic Compound (TPC) content revealed distinct patterns across the Frantoio and white olive cultivars, as well as between their leaf extracts and olive oil. The results are summarized as follows (Table 1).

Table 1. Quality Parameters of FOO and WOO

Parameter	FOO ± SD	WOO ± SD
FFA (% oleic acid)	0.41	0.42
PV (meq O_2/kg)	3.18	3.58
chlorophylls (mg/kg)	10.46	11.33
carotenoids (mg/kg)	23.6	22
K_{232}	0.019	0.020
K_{270}	0.043	0.044

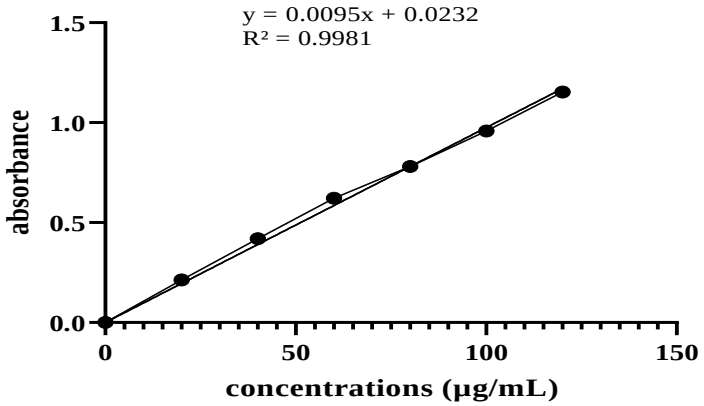


Figure 1. Calibration curve for Folin-Ciocalteu assay

UV Spectrophotometric Indices (K232 and K270)

The specific extinction coefficients K232 and K270 were determined to evaluate the oxidative status of both olive oil samples. While the FOO displayed similar values of 0.019 and 0.043, the WOO displayed K232 and K270 values of 0.020 and 0.044, respectively. Excellent oxidative quality is confirmed by the fact that all measured values are well below the IOC limits [10] for extra virgin olive oil (K232 ≤ 2.50 and K270 ≤ 0.22).

Received*: 11-11-2025 -* ***Accepted****: 14-01-2026 -* ***Published****: 21-01-2026*

These findings show that both oils were extracted and stored under ideal conditions, with little production of primary and secondary oxidation products.

Organoleptic Characteristics of Olive Oil Samples

Based on the CIO/T.20/Doc. No. 15 of the International Olive Council (IOC), a descriptive sensory evaluation was performed. A trained panel assessed two monovarietal virgin olive oil samples: White olive oil (WOO) and Frantoio olive oil (FOO). The radar sensory profiles are illustrated in (Figures 2 and 3). Neither of the two oils revealed any negative sensory characteristics. However, a number of differences in the good characteristics were detected. FOO was characterized by a stronger bitterness (7.8) while WOO was characterized by a higher fruity intensity (7.0) and pungency (5.5). Their biochemical profiles, where polyphenols are the main contributors to bitterness and pungency, are in agreement with these sensory differences. Considering its total phenolic content is comparatively higher, WOO's dominance of fruity notes is indicative of a more aromatic profile. These results demonstrate that cultivar has a significant impact on sensory qualities, with FOO exhibiting a strong bitter quality and WOO exhibiting a more balanced fruity–pungent profile.

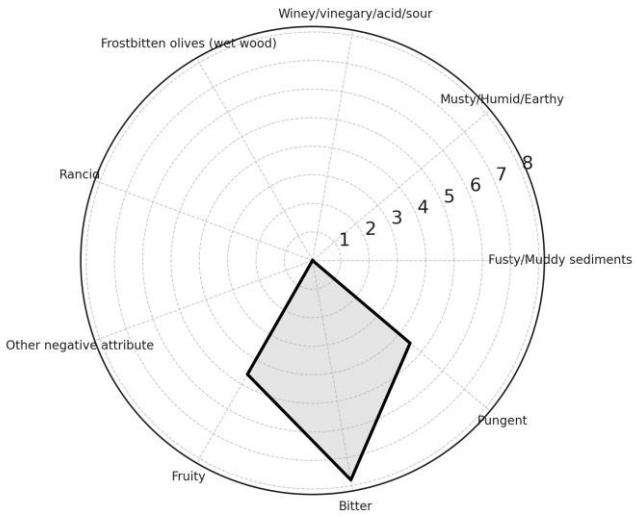


Figure 2. Organoleptic analysis of Frantoio olive oil (FOO)

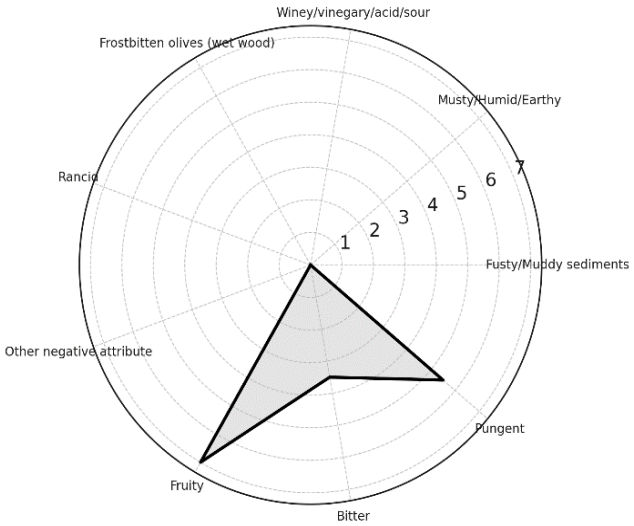
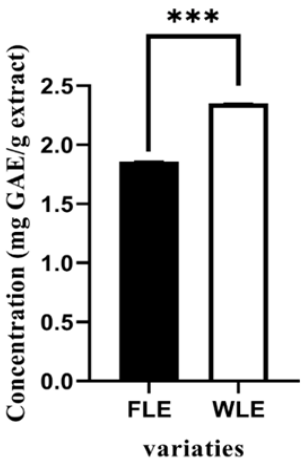
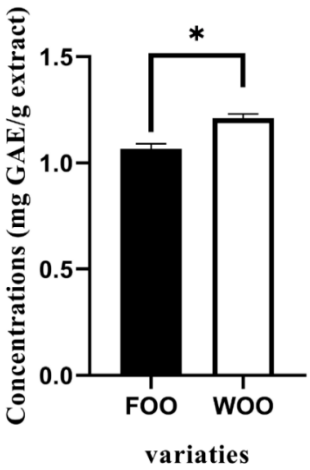


Figure 3. Organoleptic analysis of White olive oil (WOO)

TPC in olive leaf extracts and olive oil samples

TPC was expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g). White leaf extract (WLE) displayed a higher value of 2.35 mg GAE/g than Frantoio leaf extract (FLE), which had an average TPC of 1.86 mg GAE/g (Figure 4.). The mean TPC in the olive oil samples was 1.07 mg GAE/g for Frantoio olive oil (FOO) and 1.21 mg GAE/g for White olive oil (WOO) (Figure 4.). These results show that the leaf extracts have higher levels of phenolic compounds than their corresponding oils, with the White leaf extract having the highest total phenolic content.





Received*: 11-11-2025 -* ***Accepted****: 14-01-2026 -* ***Published****: 21-01-2026*

Figure 4. Total phenolic concentration (mg GAE/g extract) in Frantoio (FLE) and White (WLE) olive leaf extracts.

Figure 5. Total phenolic concentration (mg GAE/kg oil) in Frantoio olive oil (FOO) and White olive oil (WOO)

Discussion

This study showed that, in comparison to the Frantoio cultivar, the White olive cultivar accumulates more total phenolic compounds (TPC) in its leaves. These variations most likely represent genetic and metabolic variance in the control of the biosynthetic pathways that produce phenols. The phenylpropanoid and secoiridoid pathways are the two interrelated metabolic pathways that provide the majority of the phenolic compounds found in olives [11]. Basic phenolic acids, flavonoids, and phenolic alcohols like hydroxytyrosol and tyrosol are produced by the phenylpropanoid pathway and act as building blocks for more intricate secoiridoids. Oleuropein is one of the many secoiridoids found in leaves, which are known for their strong antioxidant and protective properties [12]. Cultivar differences in TPC are more likely to be due to changes in pathway efficiency than the presence of distinct compounds because these metabolites rely on the coordinated flux of both phenylpropanoid and isoprenoid precursors. In contrast to fruits, where phenolic accumulation is more ephemeral and developmentally controlled, leaves serve as phenolic factories, sustaining high and constitutive expression of biosynthetic genes [13]. Therefore, the higher TPC in White olive leaves indicates that this cultivar has a higher baseline metabolic activity than Frantoio, which is genetically programmed.

FOO exhibits a stronger bitterness, and the WOO expresses a more pronounced fruity and pungent profile; the sensory analysis validates the biochemical results. Since phenolic compounds, especially derivatives of oleuropein, directly contribute to bitterness and pungency, these patterns are consistent with the measured levels of polyphenols. WOO's increased pungency and aromatic intensity can be explained by its higher total phenolic content. These combined chemical and sensory results support earlier findings in the literature by confirming that cultivar genotype has a significant impact on the organoleptic properties of olive oil. Panel testing and phenolic profiling must be combined for cultivar characterization and quality evaluation.

The chemical and sensory aspects of the study are also evidenced by the UV spectrophotometric readings. The very low K232 and K270 values in both cultivars confirm high freshness and stability. WOO's oxidative indices were nearly as good as FOO's, even though it had a higher total phenolic content and more fruity-pungent sensory characteristics. This means that both oils are packed with enough antioxidants to prevent a significant accumulation of conjugated dienes or trienes. Together with the sensory and biochemical data, these UV parameters indicate that under the conditions of this study, the differences between the cultivars have a greater influence on flavor characteristics than the oxidative behavior.

Conclusion

In conclusion, White olive leaves have a higher phenolic content than Frantoio leaves, which may be attributed to the genetic and enzymatic differences that intensify secoiridoid biosynthesis, heighten precursor supply via the MEP pathway, and possibly open phenylpropanoid flux. The present data emphasize how essential metabolite regulation at the level of the specific cultivar is for the determination of phenolic profiles. To corroborate these hypotheses and pinpoint the genetic factors responsible for the variations in cultivars, more transcriptomic and proteomic studies will be imperative. The evidence we provide is that the choice of cultivar not only determines the potential of phenolic compound recovery from olive by-products but also the oil quality. Future research should primarily focus on the chromatographic profiling of specific phenolics coupled with the assessment of their biological activity and bioavailability, both in vitro and in vivo.

Conflict of interest. Nil

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Received: *11-11-2025* - ***Accepted***: *14-01-2026* - ***Published***: *21-01-2026*

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Received*: 11-11-2025 -* ***Accepted****: 14-01-2026 -* ***Published****: 21-01-2026*