Comparison of Phenolic Compounds in Olive Leaves by Different Drying and Storage Methods

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Abstract: Oleuropein, a bitter substance that exists in olive leaves, can be hydrolyzed to hydroxytyrosol. These are the main phenolic compounds, and they have beneficial properties to human bodies. In this study, we established a simple and new method to determine oleuropein and hydroxytyrosol quickly by HPLC. HPLC conditions were set as follows: water (A) acetonitrile (B) as mobile phase, gradient elution orders: 90%A–10%B for 0–10 min, 80%A–20%B for 14–30 min, and then change to 90%A–10%B for 30–33 min; detection wavelength: 280 nm. Compared with other detection methods, the method simplified the elution procedure and shortened the time. Additionally, we provided a better drying method and preservation of olive leaves in tea drinking production that were air-dried at room temperature of 25 °C.

Keywords: olive leaves; oleuropein; hydroxytyrosol; HPLC analysis; dry method; storage temperature

1. Introduction

The olive (\textit{Olea europaea} L.) is one of the most important economic trees in Mediterranean countries. Since the olive trees were introduced into China from Albania in 1956, they have rapidly become an emerging economic tree species. Recent advances have seen 80,000 hectares of olive trees being widely cultivated in several regions such as Gansu, Sichuan, Yunnan, and Hubei Province (China) by the end of 2017 [1,2].

The leaves were traditionally regarded as important by-products in olive oil production because they account for 10% of total weight of olive trees [3]. Accumulating evidence has demonstrated that the olive leaves are potential resources of various antioxidants, such as oleuropein and hydroxytyrosol. The leaf extracts are often mixed into oil to increase the flavor and resist deterioration [4,5]. In addition, the leaf extract is also commonly used as a safe natural additive in cosmetics, functional foods, and medicines [6]. Furthermore, due to the abundant antioxidant ingredients, Olive leaves have been used to develop novel beverages, such as olive leaf tea.

Oleuropein, hydroxytyrosol, flavonoids, and other phenolic compounds (rutin, caffeic acid and tyrosol) can contribute to many health promoting effects due to their free radical scavenging activity [7]. Oleuropein, the major component in olive fruits and leaves, exhibits strong preventive effects against oxidation [8,9]. Oleuropein consists of a molecular of hydroxytyrosol and elenolic acid glucoside [10]. It was well investigated that oleuropein possessed many beneficial pharmacological properties, such as antioxidant, anti-inflammatory, anti-microbial, anti-viral, neuro-protective effects, as well as anti-ageing [11], and it is widely used as a functional component in the food industry.

However, oleuropein is unstable and could degrade into hydroxytyrosol in fruits and leaves under certain conditions [12–14]. One of the products, hydroxytyrosol, also belongs...
to a strong anti-oxidative compound that has similar biological properties to oleuropein. The process of oleuropein degradation is also called de-astringent, and the hydroxytyrosol content is often evaluated as oleuropein degradation rate \[15\]. Therefore, a simple method to quickly detect these two components is necessary.

Additionally, processing methods significantly affect the nutrition and ingredients of many plant materials. For example, drying methods, storage conditions and processing had a varying degree of effect on the phenolic components of olive leaves after collection. Hence, it is an essential quality guarantee and cost-effectiveness issue to optimize these processes before commercial applications of olive leaves. Several common methods, such as vacuum drying, microwave, oven, and infrared heating were applied to the drying process. The content of most plant phenolic components was sensitive to the drying methods and preservation conditions. Therefore, it is of vital importance to optimize the dry conditions of olive leaves in food and animal feeding industries.

In this study, we established a simple chromatographic method to simultaneously detect oleuropein and hydroxytyrosol contents in olive leaves. Then, we investigated the effects of different drying methods on oleuropein, hydroxytyrosol and other phenolic compounds content in olive leaves in the drying process. Moreover, we also evaluated the effects of storage temperature and time on the content of oleuropein and hydroxytyrosol in dried leaf powder.

2. Materials and Methods

2.1. Reagents and Olive Leaves Sample

Hydroxytyrosol (≥98%), tyrosol (≥98%) and oleuropein (≥98%), which are used as standards, were obtained from Chengdu Biopurity Phytochemicals Ltd. China. Folin-Ciocalteau, rutin (≥99%), gallic acid (≥99%), HPLC grade acetonitrile and methanol were from Sigma-Aldrich (Shanghai, China).

Fresh olive leaves (Manzanilla variety) were harvested randomly from pruned branches and collected from Jintang, Sichuan province in October 2019. Collected leaves were divided into four parts, then dried in the oven at the temperature of 45 °C and 70 °C (DO45 and DO70) for 2 days. The other two parts were either air-dried at room temperature of 25 °C (ADRT) under ventilation for 10 days or dried in freezing vacuum dryer (DFVD) for 2 days. All groups were dried to constant weight. The olive leaves dried with different methods were powered and refined by a 60-mesh filter, then delivered to three sealed container and stored at the temperature of −20 °C, 4 °C, 25 °C in the dark, respectively.

2.2. Sample Preparation of Olive Leaves

A quantity of 0.1 g of dried leaf powder was mixed with 5 mL 78% v/v methanol-water solution, and incubated at 37 °C for 20 min for extraction. Then the extract was centrifuged at 4000 r/min for 4 min. The supernatant was collected for subsequent determination and analysis.

2.3. Determination of Chemical Composition

2.3.1. Total Phenols Content of Olive Leaves

The total phenols content (TPC) determination of olive leaves under different drying methods was carried out as previously with some adjustments \[16\]. The experiment procedure was established in line with the Folin-Ciocalteau method. In brief, the olive leaf extracts (0.1 mL) were reacted with the 0.25 mL Folin-Ciocalteau for 2 min. Then we added 1 mL 7% (w/v) sodium carbonate solution and mixed uniformly. The mixture solution was diluted to 10 mL with ultra-pure water. The reaction solution in tubes was incubated at 25 °C for 1.5 h. The absorbance at 760 nm was measured by a micro-plate reader (Spectra max M2, Molecular Devices, Sunnyvale, CA, USA). The total phenols content was calculated with milligrams of Gallic acid equivalent (GAE) per gram dry weight of the sample (mg/g) as per the following equation: \[y = 0.5944x + 0.0376, R^2 = 0.9915\].
2.3.2. Total Flavonoids Content of Olive Leaves

Total flavonoids content (TFC) of the extract was estimated by NaNO$_2$-Al(NO$_3$)$_3$ method that was previously described by [17,18] and with some modifications. Briefly, an 0.2 mL sample was diluted with 0.8 mL ethanol (70% w/v). The diluted sample solution was added with 0.2 mL NaNO$_2$ (5% w/v) and Al(NO$_3$)$_3$ (10% w/v), then mixed to react for 5 min. Then we added 1 mL NaOH (4% w/v). The solution was adjusted to 3 mL with ethanol (70% w/v), and shaken to react for 15 min. The absorbance was measured at 510 nm. The TFC was expressed in terms of milligrams of rutin equivalent (RE) per gram dry weight of the sample (mg RE/g) with the following formula: $y = 0.4172x - 0.0014$, $R^2 = 0.9995$.

2.4. HPLC Analysis of Phenolic Compounds

In our study, we have established a simple chromatographic method to analyze the two main phenols compounds. The extract was performed according to the method described in Section 2.2 and filtered through 0.22 µm filter. The sample analysis was conducted on the Agilent series 1260 HPLC instrument (Agilent Technologies, Shanghai, China) with a G1311B quaternary pump, a G1329B auto-sampler, a G1316A thermo-stated column compartment and equipped with a G1315D diode array detector (DAD). The chromatographic separation was performed on a reverse phase column ZORBAX SB-C18 (150 mm × 4.6 mm, 5.0 µm, Agilent). For hydroxytyrosol (HT), tyrosol (TYR), and oleuropein (OLE) determination, the mobile phase consisted of water (A) and acetonitrile (B) with a gradient elution of 90%A–10%B for 0–10 min, 80%A–20%B for 14–30 min, and then changed to 90%A–10%B for 30–33 min. The column temperature was set to 30 °C and the flow rate of mobile phase was 0.8 mL/min. The injection volume was 10 µL, and DAD recorded the ultraviolet light absorption value at 254, 260, and 280 nm. The other phenolic compounds analysis was performed as reported previously [16].

The data analyzed with the Agilent OpenLAB CDS ChemStation Edition software (revision C.01.05, Agilent Technologies, Shanghai, China). The extraction yield was calculated by the following equation: extraction yield (mg/g dw) = ([C × V]/W)/1000 where C is the concentration of analysis in each solvent calculated by calibration curves Table 1, V is the volume of the extract (mL), and W is the dried weight (dw) of sample (g).

**Table 1.** Analysis of phenolic compounds derived from olive leaves.

| Compound       | Linear Calibration Range (mg/mL) | Retention Time (min) | Calibration Equation          | Regression Coefficient ($R^2$) | LOD (µg/mL) | LOQ (µg/mL) |
|----------------|----------------------------------|----------------------|--------------------------------|-------------------------------|-------------|-------------|
| Hydroxytyrosol | $1.25 \times 10^{-3}$–0.16       | 4.8                  | $y = 9292.4x + 0.16$           | 0.9999                        | 1.25        | 4.47        |
| Tyrosol        | $2.81 \times 10^{-3}$–0.18       | 8.3                  | $y = 6797.1x + 6.21$           | 0.9999                        | 2.81        | 7.41        |
| Oleuropein     | 0.01–2.72                        | 29.3                 | $y = 4252.1x + 39.22$          | 0.9999                        | 5.32        | 3.38        |

y: peak area; x: concentration as mg/mL; LOD: limit of detection; LOQ: limit of quantitation.

Precision: In order to evaluate the reproducibility and stability of the detection method, the precision experiment was conducted as previously [19] with some adjustments. All calibration points were obtained in duplicate injection to set the confidence intervals. According to the chromatographic conditions, the mixed standards solution was continuously injected six times ($n = 6$) to obtained the precision data.

Recovery: Blank solution (methanol), appropriate standards, and olive leaf extract sample were analyzed under the analytical method conditions. The recovery rates were measured by spiking the olive leaf extract (0.6 mL) with a suitable volume of mixed phenolic standard stock solution. The mixed samples were continuously injected and analyzed five times ($n = 5$).

Repeatability: The olive leaves samples were prepared and analyzed in HPLC under the 280 nm for repeatability experiments. The sample detection was performed continuously, and each experiment was replicated five trials ($n = 5$).
2.5. Identified Reaction Monitoring Based on Quantitation of HPLC Analysis Condition

The standard stock solutions of HT, TYR and OLE were dissolved with methanol at the final concentration of 160 µg/mL, 180 µg/mL, 2723.84 µg/mL, respectively, and stored at 4 °C. HT working solutions at 1.25 µg/mL, 2.50 µg/mL, 5.00 µg/mL, 10.00 µg/mL, 20.00 µg/mL, 40.00 µg/mL, 80.00 µg/mL, and 160.00 µg/mL were prepared by dilution of the stock solutions with methanol. TYR working solutions at 22.81 µg/mL, 5.63 µg/mL, 11.25 µg/mL, 22.50 µg/mL, 45.00 µg/mL, 90.00 µg/mL, and 180.00 µg/mL were prepared by dilution of the stock solutions with methanol. OLE working solutions at 10.64 µg/mL, 21.28 µg/mL, 42.56 µg/mL, 85.12 µg/mL, 170.24 µg/mL, 340.48 µg/mL, 680.96 µg/mL, 1361.92 µg/mL, and 2723.84 µg/mL by dilution of the stock solutions with methanol. The working standard solutions were used to prepare matrix-matched standards and spike samples in the validation studies.

2.6. HPLC Analysis of Olive Samples under Different Drying and Storage Condition

The fresh olive leaves were separated into four parts by using the four different drying methods. Then the materials were analyzed by HPLC of the contents of hydroxytyrosol, tyrosol and oleuropein at the 1st, 3rd, 5th, 7th and 9th week.

2.7. Statistical Analysis

The results were expressed as means ± standard deviation (SD) of replicate solvent extractions and triplicate of assays. One-way analysis of variance (ANOVA) was used to compare the means. The means were separated by Least Significant Difference (LSD). All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 19.0. The statistical graphs were drawn with GraphPad Prism software (version 6.0, San Diego, CA, USA).

3. Results and Discussion

3.1. Evaluation of HPLC Detection Method

Up to now, many researchers have developed a series of HPLC methods to determine the phenolic compounds. Different types of combined mobile phase, such as methanol, acetonitrile, isopropanol, phosphoric acid, formic acid or acetic acid, and sodium acetate, were applied in terms of various chromatographic columns [20–24]. In this study, we established a simple chromatographic method with water and acetonitrile with short detection time.

The target phenolic compounds were qualitatively and quantitatively analyzed in this study. In Figure 1, we chose the 254, 260 and 280 nm wavelengths that were commonly used to detect these compounds. The peak of the three compounds were completely detected in standard sample. However, in olive extract sample, the content of HT may be extremely low, and it can only be detected at 280 nm (Figure 1B). Thus, we chose 280 nm for subsequent experiments (Figure 1A).

![Figure 1. HPLC chromatograms of standard sample at 280 (A) and olive leaves at 254, 260 and 280 nm (B). 1. Oleuropein; 2. Hydroxytyrosol; 3. Tyrosol.](image-url)
As shown in Table 1, the retention times of HT, TYR and OLE standards were 4.8, 8.3 and 29.3 min, respectively. However, the TYR in olive sample failed to be detected. This might be due to the low content which exceeded the detection limit in the olive sample. The linear calibration ranges, calibration equations, regression coefficients, limit of detections (LOD), and quantitation (LOQ) of three compounds applied with this chromatographic method were shown in Table 1 in detail. The lowest detectable limitation for OLE of this method was lower than the study from Japón-Luján, which showed LOD values of 11.46 and 11.04 µg/mL for OLE using ultrasonic or microwave assisted extraction with HPLC tandem MS detection method in olive leaves [25,26]. The LOD value for HT was 1.25 µg/mL which was higher than other reported methods (LOD values for HT were 0.14 to 0.4 µg/mL) [27,28]. These results might be due to different plant materials, extraction methods, quantification conditions, and apparatus.

3.1.1. Precision

The Table 2 showed that the peak areas were extremely similar and the relative standard deviations (RSD) of HT, TYR and OLE were 0.822, 0.811 and 0.766%, respectively, indicated that the established method has high precision and strong repeatability.

Table 2. Precision and repeatability analyses of phenolic compounds in standard and olive leaf sample at 280 nm.

| Sample          | Compound    | 1       | 2       | 3       | 4       | 5       | 6       | RSD/% |
|-----------------|-------------|---------|---------|---------|---------|---------|---------|-------|
| Standard sample | Hydroxytyrosol | 1492.12 | 1485.62 | 1487.43 | 1489.81 | 1513.50 | 1510.78 | 0.822 |
|                 | Tyrosol     | 1230.29 | 1234.90 | 1232.04 | 1235.02 | 1253.48 | 1250.83 | 0.811 |
|                 | Oleuropein  | 11,622.70 | 11,665.50 | 11,653.60 | 11,679.30 | 11,834.80 | 11,814.50 | 0.766 |
| Olive leaf sample | Hydroxytyrosol | 41.81 | 42.73 | 44.07 | 44.63 | 43.43 | nd | 2.556 |
|                 | Tyrosol     | nd | nd | nd | nd | nd | nd | nd |
|                 | Oleuropein  | 6352.98 | 6493.57 | 6383.46 | 6026.10 | 6372.61 | nd | 2.786 |

“nd” means “not detected”.

3.1.2. Recovery

The recovery data were calculated by peak area under the 280 nm. The results were summarized in Table 3. The recovery rates of HT, TYR and OLE were in the range of 102.79–109.60, 97.14–102.28 and 89.43–95.24%, respectively. Similar recovery rates analyzed by HPLC were reported from an olive pomace extraction (recovery rates were 93–98%) [29]. The RSD values of HT, TYR and OLE were 2.208, 1.742 and 2.322%, respectively. The results indicated that using the new chromatographic method could obtain a stable, accurate, and reliable recycling result.

Table 3. Recovery of phenolic compounds standard sample at 280 nm.

| Standard Compound | 1       | 2       | 3       | 4       | 5       | 6       | RSD/% |
|-------------------|---------|---------|---------|---------|---------|---------|-------|
| Hydroxytyrosol    | 109.60  | 106.46  | 104.96  | 107.63  | 105.43  | 102.79  | 2.208 |
| Tyrosol           | 101.07  | 100.78  | 99.54   | 102.28  | 100.12  | 97.14   | 1.742 |
| Oleuropein        | 92.41   | 92.84   | 95.24   | 89.86   | 92.56   | 89.43   | 2.322 |

3.1.3. Repeatability

The results were showed in Table 2. The peak area values of HT and OLE are in the range of 41.82–44.63 mAU and 6026.10–6493.57 mAU, respectively. However, the tyrosol in olive leaves did not be observed, may be the content was below the detection limit. The RSD values of HT and OLE were 2.556 and 2.786%, respectively, which were better than the RSD value from a study employing dispersive liquid–liquid micro extraction coupled with HPLC analysis (RSD for OLE and HT were 4.12–5.63%) [29].
### 3.2. Effects of Different Drying Methods on Phenolic Compound Contents

The olive leaves samples were extracted and determined according to the Section 2.2, respectively. The results showed that the TPC contents of DO70, DO45, ADRT and DFVD were 17.58 ± 1.14, 40.09 ± 1.91, 51.48 ± 1.58 and 51.07 ± 1.60 mg GAE/g, respectively (Figure 2A), indicated that plant materials processing with high temperature significantly reduced the total phenolic content. Similar results were reported in a green coffee roasting study [30]. The TFC contents of DO70, DO45, ADRT and DFVD were 2.91 ± 0.41, 16.12 ± 0.39 and 28.59 ± 3.20 and 25.59 ± 1.58 mg GE/g, respectively (Figure 2B). In other aqueous methanol extract of olive leaves, similar TFC content of 15.55 mg GE/g was determined [31]. The TPC contents of ADRT and DFVD had insignificant differences (p > 0.05), but obviously differed from DO70 and DO45 (Figure 2A) (p < 0.05). As shown in Figure 2B, the TFC contents of four groups of leaf powder exhibited significant differences (p < 0.05) with a trend that relates higher temperature to lower contents.

| Compound             | DO70            | DO45            | ADRT            | DFVD            |
|----------------------|-----------------|-----------------|-----------------|-----------------|
| Hydroxytyrosol       | 109.60 ± 0.39   | 106.46 ± 0.53   | 104.96 ± 0.45   | 107.63 ± 0.58   |
| Oleuropein           | 92.41 ± 0.39    | 92.84 ± 0.45    | 95.24 ± 0.39    | 89.86 ± 0.45    |
| Tyrosol              | 101.07 ± 0.38   | 100.78 ± 0.47   | 99.54 ± 0.38    | 102.28 ± 0.47   |

The contents of extracted ingredients from plants were often related to the material processing and extraction methods [18]. The polyphenol compounds containing multiple hydroxyl groups were unstable and more easily oxidized during the material processing, so there was an obvious difference in composition and content of active ingredients under different processing procedure or temperature [32], especially OLE and HT. Therefore, we investigated the OLE and HT contents of olive leaves materials under four drying methods. As can be seen in Figure 3A, the OLE contents of DO70, DO45, ADRT and DFVD were 1.32 ± 0.11, 40.75 ± 1.02, 82.72 ± 0.71, and 69.00 ± 0.61 mg/g dw, respectively. Similarly, 23 and 43.2 mg/g of OLE contents in olive leaves were shown in other studies with different extraction and drying methods [25,33]. However, these contents were lower than a previous study which reported that the content ranged from 87.2 to 136.1 mg/g dw in 73 olive leaf samples [34]. In Figure 3B, the HT contents of DO45, ADRT and DFVD were 0.15 ± 0.02, 0.36 ± 0.01 and 0.27 ± 0.01 mg/g dw, respectively. The HT content was lower than a hydrolyzed phenolic extract of olive leaves (0.2 g/100 g), which might be due to the different processing method [35].

![Figure 2](image-url)  
**Figure 2.** Contents of TPC (A) and TFC (B) in olive leaves after drying in an oven at 70 and 45 °C, room temperature (25 °C) and freeze dryer, respectively. Values are the mean of triplicate experiments, and the error bar indicates the standard error of duplicate data. Significance data were obtained by analysis of variance (ANOVA). Different lowercase letters (a, b, c and d) on the top of columns represent significant difference (p < 0.05).
Figure 3. Contents of oleuropein (A) and hydroxytyrosol (B) in olive leaves after drying in an oven at 70 and 45 °C, room temperature (25 °C) and freeze dryer, respectively. Values are the mean of triplicate experiments and the error bar indicate the standard error of duplicate data. “nd” means “not detected”. Significance data were obtained by analysis of variance (ANOVA). Different lowercase letters (a, b, c and d) on the top of columns represent significant difference (p < 0.05).

In total, the OLE and HT contents in the experiments showed the same trend as TFC. Materials of ADRT contain the highest levels of OLE and HT. Secondly, the DO45 was followed by DFVD. The sample of DO70 contained a low OLE content and undetectable HT content. For the olive leaves treated with high temperature, the contents of OLE and HT decreased rapidly with increasing temperature. Normally, OLE could be hydrolyzed to HT. However, HT content did not increase with the OLE degradation under high temperature conditions, indicating that HT was sensitive to high temperature and might degrade quickly at higher temperatures. The findings showed that the OLE and HT contents were significantly different under the four common drying methods (i.e., different drying temperatures). The best drying method was air-dried at room temperature, followed by freeze-drying, and then drying at 45 °C. Air-dried at room temperature is the best drying method for olive leaves, because it maximizes OLE and HT contents in olive leaves.

Drying was the first procedure for olive leaves to apply in the process industry. As illustrated above, the bioactive components in olive leaves were influenced by different dehydrating methods. ADRT was the best method with the advantage that it is appropriate, simple, and low-cost. The olive leaves with different drying methods showed different contents of TP, TF, OLE, and HT. When treated with high temperature, the four main components were extremely reduced. Similar results were reflected in other phenolic compounds that are displayed in Table 4 (standard curve shown in Table 5). Low content compounds, such as rutin, luteolin-7-O-lucoside, and apigenin-7-O-glucoside, were also tested. The content of these compounds, except for luteolin, showed a similar trend to OLE and HT. Leaves dried at room temperature contained high phenolic compounds. Thus, high temperature was not suitable for olive leaves because it may cause a loss of bioactive components. Olive leaves should be protected from high temperatures during the drying process.

Table 4. Contents of phenolic compounds (mg/g dw) affected by different drying methods.

| Drying Method | DO70       | DO45       | ADRT       | DFVD       |
|---------------|------------|------------|------------|------------|
| Rutin         | 0.041 ± 0.003 d | 0.587 ± 0.007 b | 0.628 ± 0.009 a | 0.538 ± 0.027 c |
| Luteolin-7-O-lucoside | 0.57 ± 0.04 d | 4.13 ± 0.03 c | 6.27 ± 0.06 a | 4.41 ± 0.23 b |
| Apigenin-7-O-glucoside | 0.56 ± 0.03 c | 1.05 ± 0.01 a | 1.10 ± 0.01 a | 0.92 ± 0.05 b |
| Luteolin      | 0.080 ± 0.011 c | 0.100 ± 0.006 b | 0.083 ± 0.005 c | 0.215 ± 0.007 a |
| Apigenin      | nd         | nd         | nd         | nd         |

“nd” means “not detected”, different letter means different significance.
3.3. Effects of Different Storage Temperatures and Time on the OLE and HT Contents

Based on the results of the leaves treated with four drying methods, we found that the group dried at 25 °C had the highest contents of TP, TF, OLE and HT. The group in which leaves were air-dried in room temperature (ADRT) was selected for subsequent experiments. The ADRT leaf powder was stored at −20 °C, 4 °C and 25 °C for 9 weeks, and the OLE and HT contents were measured consecutively by HPLC. As can be seen in Table 6, there were no significant differences in the OLE and HT contents at three storage temperatures. As the HPLC chromatogram results show in Table 6, the OLE content decreased significantly from the beginning of the experiment to one week later. At −20, 4 and 25 °C, the OLE content was reduced from 82.72 to 78.28, 82.72 to 77.43 and 82.72 to 77.58 mg/g dw, respectively, in first week. However, from the 3rd to 9th week, it exhibited only a slight fluctuation. In general, the OLE contents maintained a stable state in long-term storage.

Table 6. Contents of oleuropein and hydroxytyrosol in olive leaf powder after storage at −20, 4 and 25 °C for different time periods.

| Storage Time (weeks) | Oleuropein (mg/g dw) | Hydroxytyrosol (mg/g dw) |
|----------------------|----------------------|--------------------------|
|                      | 25 °C | 4 °C | −20 °C | 25 °C | 4 °C | −20 °C |
| 0                    | 82.72 ± 0.54 Ab       | 82.72 ± 0.54 Ab          | 82.72 ± 0.54 Ab          | 0.36 ± 0.02 Ab            | 0.36 ± 0.02 Ab            | 0.36 ± 0.02 Ab            |
| 1                    | 77.58 ± 0.63 Ab       | 77.43 ± 1.47 Ab          | 78.28 ± 0.59 Ab          | 0.45 ± 0.04 Ab            | 0.41 ± 0.01 Ba            | 0.45 ± 0.02 Ab            |
| 3                    | 75.93 ± 1.34 Ab       | 73.91 ± 0.65 Bc          | 75.21 ± 1.02 Ab          | 0.24 ± 0.02 Bc            | 0.29 ± 0.03 Bc            | 0.33 ± 0.02 Ab            |
| 5                    | 74.61 ± 0.97 Ab       | 75.40 ± 0.87 Bc          | 73.90 ± 0.87 Bc          | 0.24 ± 0.03 Ac            | 0.28 ± 0.01 Ac            | 0.26 ± 0.03 Ac            |
| 7                    | 74.48 ± 0.90 Ab       | 74.94 ± 0.85 Ab          | 76.02 ± 0.45 Ab          | 0.22 ± 0.02 Bc            | 0.28 ± 0.01 Ac            | 0.26 ± 0.01 Ac            |
| 9                    | 74.91 ± 0.87 Ab       | 74.51 ± 0.76 Ab          | 76.17 ± 0.83 Ab          | 0.25 ± 0.04 Ac            | 0.27 ± 0.02 Ac            | 0.28 ± 0.03 Ac            |

Note: Different capital letters mean significant difference of oleuropein and hydroxytyrosol content stored under 25 °C, 4 °C and −20 °C within the same time; different lowercase letters mean significant difference of oleuropein and hydroxytyrosol content stored for different time under the same temperature.

The HT content showed a significant increasing trend in the 1st week, which might be due to the ability for OLE to be converted to HT by β-glucosidase in the storage process. Then, there was a downward trend during the following 2 weeks. The HT contents were reduced from 0.45 to 0.33, 0.41 to 0.29, and 0.45 to 0.24 mg/g dw, respectively, at −20, 4 and 25 °C. Then, the HT content remained stable fluctuation after the 3rd week. In summary, the contents of OLE and HT in the powdered dry olive leaves were affected significantly in the early stage, then generally became unchanged under these three common storage conditions.

Therefore, we suggest that olive leaves are air-dried or dehydrated at room temperature before being made into food or quality additives. Olive leaves air-dried at room temperature preserve the leaf greenness of leaves, and their luminosity is enhanced. Considering industrial processing costs, material preservation and the nutritional contents and olive tea drinking habits, we recommend that fresh leaves are stored at room temperature for drying, and it is advisable to store dried olive leaves or olive tea at 4 °C.
4. Conclusions

In this study, a simple method was established for determining the contents of OLE and HT in olive leaves by HPLC. The results showed that OLE and HT could be completely detected within 33 min. The determination results had good repeatability, high accuracy, and good recovery under the chromatographic conditions.

The most suitable drying method for olive leaves was air-drying at room temperature. It attributed high phenolic compounds content. Moreover, it is recommended to prevent the high temperature baking olive leaves during the drying process. For storage conditions, there were insignificant differences between the OLE and HT contents stored at −20, 4 and 25 °C for several weeks. However, in industrial processing, the advisable storage condition for fresh or dried olive leaves could be 25 °C, because it is convenient, economical, and suitable for cosmetic applications.

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