Effect of drying temperature on the oleuropein content of olive (Olea europea L.) leaves

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Olive leaves were dried between 25°C and 150°C or were freeze-dried, the effects of drying temperature on oleuropein contents of olive (Olea europea L.) leaves were estimated. A specific drying temperature region from 65 to 80°C was revealed a declining region of oleuropein contents in the leaves. When olive leaves were treated with boiling water, the oleuropein contents were restored. Enzyme activities might be related to this declining and inactivated with boiling water.

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Popular medicine and phytotherapy use olive leaves to treat and prevent hypertension. Oleuropein, a bitter-tasting secoiridoid glycoside present in olive leaves, are suggested to support hypotensive activity[1]. Oleuropein is also known for its antioxidant activity[2], and its hydrolysis leads to antimicrobial compounds[3, 4]. Recently NISHIBE et al.[5] reported that oleuropein showed a inhibitory effect on TNF-α production linked to allergic reaction.

GONZALEZ et al.[6] reported that oleuropein changes seasonally and the content of this compound harvested during summer is lower than that of winter. SAVOURNIN et al.[7] estimated the content of oleuropein with new developed HPLC method and when olive leaves dried with microwave, the content of oleuropein is higher than that of previously reports. These reports suggest that further studies of drying method of olive leaves are necessary.

The present study showed the effect of drying temperature on the contents of oleuropein of olive leaves and a specific drying temperature region occurs a decline of oleuropein in the leaves.

Materials and Methods

1. Materials

Oleuropein was purchased from Funakoshi. One-year-old olive trees, the cultivar Nevadillo blanco and Manzanillo were purchased from Nihon olive Co. Ltd. These trees were planted in a garden of Tama Biochemical Co. Ltd. (Isehara, Kanagawa) and a few of the flesh leaves were sampled.

2. Identification of oleuropein in the olive leaves

Leaf samples were immediately dried at various temperature or were immediately frozen in −80°C and subsequently freeze-dried. When the leaves dried at 25°C or freeze-dried, the drying time was 72h, dried at 50, 60 or 65°C, the time was 18h, and at more than 70°C, the time was 3h. These dried leaves were less than 1.0% weight loss on drying at 105°C for 3h.

If necessary, fresh olive leaves were cut in half, half parts of leaves were soaked into boiled water for 2 min and dried at 80°C for 3h (BW treatment) and other half parts were dried at 80°C for 3h immediately (no treatment).

Dried leaves were cut into small pieces (approximately 2 ~ 4 ml) and used for quantitative analyses. One hundred mg of dry olive leaf pieces was extracted with 50ml of 80% ethanol at 60°C for 2h, filled up to 100ml precisely. Ten μl of the 100 ml filled-up solution was sampled with an automatic sampler (JASCO AS−650) and subjected to analytical HPLC using a JASCO PU−1580 pump equipped with a 20μl loop injector. A UV−visible detector (JASCO UV−1570) connected in series served to monitor the column eluent. Separation was achieved on a 250mm ×4.6mm i.d. Inertsil ODS−3 column (GL Science Inc.) at 35°C with gradient elusion. An elusion gradient program employed water : acetonitrile : acetic acid (85 : 15 : 0.1 v/v/v) as solvent A and water : acetonitrile : acetic acid

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(65:35:0.1 v/v/v) as solvent B, and a flow rate of 1 ml/min was used. A stepwise linear gradient commencing with 0% solvent B was employed. This was increased to 50% at 25 min, followed by further increase to 100% at 40 min, with a 5 min isocratic run followed by return to initial conditions at 50 min. Oleuropein was quantified at 280 nm.

Results and Discussion

First, the leaves of a Nevadillo blanco were sampled in February and dried with different temperatures. As shown in Fig. 1, the oleuropein contents of the leaves made a specific decline region between 65 and 80°C. Similar result was also obtained in a Manzanillo.

Dried at 80°C, the content of oleuropein in Nevadillo blanco and Manzanillo were 0.109 mmol/g dried leaves and 0.111 mmol/g, respectively. At 150°C, the content of oleuropein in Nevadillo blanco and Manzanillo were 0.117 mmol/g and 0.128 mmol/g, respectively. Whereas, dried at 110°C, the content of oleuropein in Nevadillo blanco and Manzanillo were 0.322 mmol/g and 0.250 mmol/g, respectively (Table 1).

These data suggested the existence of a specific declined region of oleuropein in drying temperature.

Leaves of the privet tree, Ligustrum obtusifolium, contain a large amount of oleuropein which is stably kept in a compartment, when the leaf tissue is destroyed by herbivores, enzymes start activate oleuropein into a very strong protein denaturant. Since enzyme activities might be related to the specific declined oleuropein contents, fresh olive leaves sampled in April were cut in half, half parts of leaves were soaked into boiled water for 2 mins and dried at 80°C for 3 h (BW treatment) and other half parts were dried at 80°C for 3 h immediately (no treatment).

In the BW treatment, the content of oleuropein in Nevadillo blanco and Manzanillo were 0.268 mmol/g dried leaves and 0.420 mmol/g, respectively. Whereas, in the no treatment, the content of oleuropein in Nevadillo blanco and Manzanillo were 0.076 mmol/g and 0.139 mmol/g, respectively (Table 2). The content ratio of no treatment to BW treatment was 0.283 in Nevadillo blanco, 0.330 in Manzanillo. By the BW treatment, the oleuropein contents of both cultivars were higher than that by no treatment. As a control, Nevadillo blanco was dried at 110°C in both treatment. The content of oleuropein in the BW treatment was 0.381 mmol/g, the no treatment was 0.353 mmol/g and the content ratio was 0.927. In drying at 110°C, a significant difference was not observed between BW

**Table 1** The effect of drying temperature on the contents of oleuropein of dried olive leaves

<table>
<thead>
<tr>
<th>Drying Temperature (°C)</th>
<th>Nevadillo blanco</th>
<th>Manzanillo</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>0.109 ± 0.026</td>
<td>0.111 ± 0.004</td>
</tr>
<tr>
<td>110</td>
<td>0.322 ± 0.115</td>
<td>0.250 ± 0.006</td>
</tr>
<tr>
<td>150</td>
<td>0.117 ± 0.035</td>
<td>0.128 ± 0.011</td>
</tr>
</tbody>
</table>

* the mean and standard deviation (n = 3)

**Table 2** The effect of boiled water treatment on the contents of oleuropein of dried olive leaves

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Drying Temperature (°C)</th>
<th>no treatment (mmol/g dried leaves)</th>
<th>BW treatment (mmol/g dried leaves)</th>
<th>The ratio of no treatment to BW treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nevadillo blanco</td>
<td>80</td>
<td>0.076 ± 0.007*</td>
<td>0.268 ± 0.024</td>
<td>0.283</td>
</tr>
<tr>
<td></td>
<td>110</td>
<td>0.353 ± 0.065</td>
<td>0.381 ± 0.052</td>
<td>0.927</td>
</tr>
<tr>
<td>Manzanillo</td>
<td>80</td>
<td>0.139 ± 0.043</td>
<td>0.420 ± 0.043</td>
<td>0.330</td>
</tr>
</tbody>
</table>

* the mean and standard deviation (n = 3)
and no treatment. These data suggested that enzyme might be inactivated by BW treatment.

It has not been clear the existence of the enzymes related to the degradation of oleuropein in the olive leaves. As described above, however, in the leaves of privet tree, oleuropein is localized stably in compartments, the compartments are broken by a kind of stress and oleuropein is degraded by the activating enzymes localized in organelles separated from oleuropein. It also might be occur in the olive leaves. Drying between 65°C and 80°C, the compartments are broken and oleuropein is degraded by the activating enzymes. More than 80°C, the compartments are broken however the degrading enzymes are inactivated and oleuropein is not degraded.

References