EFFECT OF DRYING METHOD ON SOME BIOACTIVE PROPERTIES OF OLIVE LEAVES (VAR. SERRANA) EXTRACTS

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Abstract: Olive leaves phenolic compounds have excellent bioactive properties that may be affected by drying. Thus, the aim of this work was to study the influence of the drying technique on some bioactive properties of olive leaves extracts. Drying affected the bioactive properties of the olive leaves extracts, as well as its composition after in vitro digestion. Hot air drying at 120 °C was the best method to preserve the antioxidant potential of olive leaves giving extracts with high content of oleuropein and verbascoside. Among polyphenols studied, tyrosol was the most stable regardless of the drying method.

Keywords: antioxidant capacity, polyphenols, HPLC

INTRODUCTION

Olive oil has an important place in the Mediterranean diet (Ryan et al. 2001). Olive leaves are considered as byproduct of olive oil industry. However, bioactive compounds have been found in olive leaves and branches (Japón-Luján and Luque de Castro 2007) and their extraction could represent an interesting way to increase in value this byproduct. Usually, drying of raw material precedes extraction processes, which reduces moisture content and avoids the interference of water. Nevertheless, drying process may affect the matrix structure and the bioactive components (Boudhrioua et al. 2009; Erbay and Icier 2009). Among other properties, antioxidant capacity is used as reference to identify the bioactive potential of extracts. In addition, it should be also relevant to explore how the digestion affects the bioavailability of extracts, which is essential in the design of new food ingredients. Thus, the main goal of this work was to study the effect of different drying alternatives on some bioactive properties of olive leaves extracts, such as antioxidant capacity and bioavailability after digestion.

MATERIALS AND METHODS

Raw material and Drying experiments

Olive leaves (Olea europea, var. Serrana) were collected in a farm located in Segorbe (Castellón, Spain) and stored at 4 °C until drying. The initial moisture content was determined by AOAC n° 934.01 (AOAC 1997). The air drying experiments were carried out using two different alternatives: Hot air drying (HAD) and freeze drying (FD). In HAD experiments, two temperatures were tested: 70 (HAD-70) and 120 °C (HAD-120). FD experiments were conducted at initial temperature -48±2 °C, pressure 1.4 10^-1 mbar and drying time of 24 hours. In all cases, dehydration was stopped when samples lost 40 % of the initial weight. After drying, olive leaves were milled until obtaining a fine powder.

Extraction experiments

3.75 g of olive leaves powder and 30 mL of solvent (ethanol-water 80:20, v/v) were placed in a hermetic container. The container was protected from light and stirred (170 rpm) at 22±1 °C for 24 h. The final extract was centrifuged (10 min at 5000 rpm) and
filtered (nylon filters of 0.45 µm). At least, 3 extraction replicates were made for each drying condition tested.

In vitro digestion

All the extracts were subjected to a gastric/intestinal in vitro digestion process. The in vitro method used was a slightly modified version of previously described methods (Miller et al. 1981; Garret et al. 1999). Firstly, the extract sample (10 g) was diluted with distilled water (1:8, p/v) and subjected to gastric digestion (pH 2.0, pepsin 160 mg/mL, stirring 120 rpm, 2 hours at 37 ºC). Finally, extract was subjected to intestinal digestion (pH 7, pancreatin 4 mg/mL, bile 25 mg/mL, stirring 120 rpm, 2 hours at 37 ºC). During the in vitro digestion the samples were protected from light.

Total phenolic content measurement

The phenolic content was determined, before and after digestion, by the Folin-Ciocalteu method (Singleton et al. 1999) and expressed as g gallic acid (GAE)/g dry matter. At least, 3 replicates were made for each olive leaves extract.

Antioxidant capacity measurement

The antioxidant capacity of extracts was measured with two different methods: FRAP and TEAC. FRAP method was used according to the procedure described by Pulido et al. (2000), while TEAC method was performed as previously described by Laporta et al. (2007). In both FRAP and TEAC, the antioxidant capacity was expressed as g trolox/g dry matter. In both methods, at least, 3 replicates were made for each extract, before and after the in vitro digestion.

HPLC-DAD separation-detection-quantification

The extracts were analyzed and quantified using a Merck Lichrospher 100RP-18 (5 µm, 250 x 4 mm) column. Separation was carried out using a linear gradient method using 2.5 % acetic acid (A) and acetonitrile (B), starting the sequence with 10 % B and programming the gradient to obtain 20 % B at 10 min, 40 % B at 35 min, 100 % B at 40 min, 100 % B at 45 min, 10 % B at 46 min and 10 % B at 50 min. The flow-rate was 1 mL/min and the chromatograms monitored at 240, 280 and 330 nm. The results of quantitative analysis were expressed as units of area.

RESULTS AND DISCUSSION

As can be observed in Table 1, drying process had a significant (p<0.05) influence on the total phenolic content and antioxidant capacity. HAD-120 involved a low degradation of phenols allowing to obtain olive leaves extracts with higher antioxidant capacity than extracts prepared with other dried materials (HAD-70 and FD). Short drying times at high temperatures seem to preserve the phenol content better than long drying processes at mild temperatures (García-Pérez et al. 2010). Thus, the degradation of phenolic compounds during drying could be explained from the drying time/temperature combination. Although freeze drying is a proper treatment to preserve the quality of dried products, in this case, the results showed that FD was the worst technique to preserve the olive leaves phenolic compounds. Comparing FD with HAD-70 and HAD-120, the total phenolic content of the extract was reduced by 23.5% and 47 % (Table 1), respectively.

### Table 1. Effect of drying (D) and in vitro digestion on the total phenolic content (PC, g GAE/g dry matter) and the antioxidant capacity (AC, g trolox/g dry matter).

<table>
<thead>
<tr>
<th>D</th>
<th>Initial extract</th>
<th>Digested extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAD-70</td>
<td>83.2±1.4</td>
<td>102.5±0.7</td>
</tr>
<tr>
<td>HAD-120</td>
<td>104.6±2.1</td>
<td>122.3±1.6</td>
</tr>
<tr>
<td>FD</td>
<td>62.5±0.5</td>
<td>79.8±3.6</td>
</tr>
<tr>
<td>TEAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAD-70</td>
<td>4.1±0.1</td>
<td>1.6±0.1</td>
</tr>
<tr>
<td>HAD-120</td>
<td>2.8±0.1</td>
<td>1.6±0.1</td>
</tr>
<tr>
<td>FD</td>
<td>3.1±0.1</td>
<td>2.4±0.2</td>
</tr>
<tr>
<td>AC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAD-70</td>
<td>58.3±3.8</td>
<td>42.5±0.7</td>
</tr>
<tr>
<td>HAD-120</td>
<td>30.9±0.1</td>
<td>25.6±1.5</td>
</tr>
<tr>
<td>FD</td>
<td>8.3±0.1</td>
<td>6.9±0.3</td>
</tr>
</tbody>
</table>

The phenolic content decreased significantly (p<0.05) after in vitro digestion. However, the results of antioxidant capacity resulted confused, since the observed trend was different depending on the method used (TEAC or FRAP). The antioxidant activity measured by TEAC method was reduced after digestion between 22 % (HAD-70) and 61 % (HAD-120). It should be expected a decrease of the antioxidant activity of the extracts after digestion due to the reduction of phenolic content, such as is showed by the TEAC method. However, the results from FRAP method resulted contradictory showing an increase of the antioxidant activity after digestion (Table 1). This fact could be only explained considering that these methods are based on different chemical principles, which involve a different sensibility to evaluate changes of extract composition after digestion. In order to clarify this issue, the identification of the most representative phenolic components is necessary (Fig 1).

The initial extract for HAD-120 dried samples showed the highest values of oleuropein, verbascoside and hydroxytyrosol. Thus, it could be stated that this method was the best to obtain an extract whith high content of bioactive compounds. The air drying temperature (70 and 120 ºC) only influenced significantly (p<0.05) on verbascoside and hydroxytyrosol content but not for oleuropein content. Digestion involved a significant (p<0.05) reduction of oleuropein and verbascoside for all the dried samples. However, digestion also increased the content of hydroxytyrosol for HAD-70 and FD extracts. Hydroxytyrosol is considered a product...
resulting from the degradation of oleuropein and verbascoside. Therefore, the reduction of oleuropein and verbascoside content should lead to an improvement of hydroxytyrosol, as is observed in Figure 1. Finally, during drying and digestion, tyrosol was the most stable polyphenol.

CONCLUSIONS
Hot air drying at 120 °C was considered the best drying method to preserve olive leaves phenols providing extracts with high antioxidant potential and high contents of oleuropein and verbascoside. On the other hand, in vitro digestion affected significantly the extracts composition modifying their antioxidant activity and reducing total phenolic content. Tyrosol was the most resistant polyphenol to drying and digestion.

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