COMPARATIVE STUDY OF DIFFERENT ROSEMARY ESSENTIAL OIL

Socaci Sonia A., Maria Tofana, Carmen Socaciu, D. Varban, Sevastita Muste

University of Agricultural Sciences and Veterinary Medicine, Faculty of Agriculture, 3-5 Mănăștur Street, 400372 Cluj-Napoca, Romania, tel. 0264596384, e-mail: soniasocaci@gmail.com

Key words: essential oil, rosemary, GC-MS, fingerprint

Abstract: The present study includes researches regarding the composition of different varieties of rosemary essential oil. The essential oils were extracted from the plant material using the hydrodistillation technique. The analyses of the composition of essential oils were carried out by using a GC-MS system. The chemical constituents of the essential oil were separated and identified using the GC-MS NIST libraries.

Abreviations: E.O. – essential oil; HD – hydrodistillation; GC-MS – Gas Chromatography coupled with Mass Spectrometry

INTRODUCTION

Rosemary (Rosmarinus officinalis L.) is an important herb on the European market, used fresh or dried, or as oil or oleoresin, its culinary and medicinal properties being widely known. Rosemary it is an aromatic evergreen shrub that grows in the Mediterranean region. The plant belongs to the Labitae family, grows up to 2m height, and has dark-green lavender-like leaves and a long flowering season (from April to August).

The rosemary essential oil is used as a seasoning for food stuffs, such as meat, salami, sauces (Maria Lo Presti et al., 2005), but due to its chemical active constituents properties it is also used as an antioxidant (for food preserving), antibacterial and antifungal agent against some spoilage organisms such as pseudomonas fluorescens or brochothrix thermoplasta (S.A. Rezzoug et al., 2005).

In the recent years, there is a constant demand to improve the quality of essential oils, because consumers demand this quality in their food, pharmaceutical or perfumery products. The therapeutic and odoriferous properties of the essential oils are directly correlated with their qualitative and quantitative composition. The variability of the qualitative and quantitative composition of the essential oil is due to intrinsic features (e.g. genetics, plant age) and also to extrinsic factors such as climate, cultivation conditions, extraction methods, etc. (Maria Lo Presti et al., 2005). In order to establish if the essential oil was adulterated or not, we need some methods that are able to separate and identify each constituent of the essential oil. One of these methods is gas-chromatography coupled with mass spectrometry (GC-MS) (R. Oprean et al., 1998).

The rosemary essential oil composition has been investigated and reported in literature. The studies include countries mainly from the Mediterranean region, Balkans, South-Eastern regions (H.E. Katerinopoulos et al., 2005). Taking in consideration the facts above mentioned, our research group decided to investigate the rosemary essential oil from our greenhouse, in order to establish its chromatographic fingerprint.
MATERIALS AND METHODS

The researches were carried out in the Food Quality and Safety Testing Laboratory (FQSTL) from the University of Agricultural Science and Veterinary Medicine, Cluj-Napoca. The objective of our study was to show if there were any differences between the E.Os composition of the rosemary samples.

Plant material. The four samples of rosemary leaves were received from the Phytotechnology department of our university. The samples were codified as follows: V₁ – the plants were planted at a distance of 50 x 50 cm between them; V₂ - the plants were planted at a distance of 60 x 60 cm between them; V₃ - the plants were planted at a distance of 70 x 70 cm between them; V₄ - the plants were planted at a distance of 100 x 100 cm between them. The leaves were air-dried, in a cool dark place. The moisture content for the dry plant material was 9.47%.

Essential oil extraction. The E.Os were extracted by HD as follows: 50g of ground dried leaves and 750 ml distilled water were placed in the distillation flask. The distillation time was 3 hours since the distillation begins. At the end of extraction the obtained E.O. was collected and measured. The volume of E.O. isolated from sample V₁ was 0.45 ml; from sample V₂ 1.1 ml E.O.; from sample V₃ 1.4 ml E.O.; from sample V₄ 1.4 ml E.O. After extraction the E.Os were stored in refrigerator until chemical analysis. A 2% E.O. in hexane solution was prepared from each sample of E.O. in order to be analyzed by GC-MS.

Chemical analysis. The E.Os were analyzed by GC-MS. The analyses were carried out on a Shimadzu GCMS QP-2010 model gas chromatograph – mass spectrometer equipped with an AOC-20i series autosampler. The method used for the separation and identification of E.O. constituents was that described by Maria Lo Presti et al., 2005 but with some modifications. Column: AT-5, 30m x 0.25mm, ID 0.25µm film thickness (Alltech, USA). GC temperature program: 50.0°C (2 min) to 250.0°C (10 min) at 3°C/min. Injection temperature: 250.0°C. Injection volume: 1.0µL. Pressure: 37.1 kPa. Linear velocity: 32.4 cm/s. Split ratio: 50:1. Carrier gas: helium. Detector: MS Ion source temperature: 250.0°C. Interface temperature: 250.0°C. MS mode: EI. Detector voltage: 0.1 kV. Mass range: 40-400u. Scan speed: 769u/s.

RESULTS AND DISCUSSIONS

The volumes of E.Os extracted from the samples V₁-V₄ were measured and it can be observed that the quantity of E.Os depends by the distance that was left between the plants. The optimal distance between the plants was established to be 70 x 70 cm, due to the fact that for the V₃ the quantity of E.O. extracted was bigger (1.4 ml) than those extracted from samples V₁ (0.45 ml) and V₂ (1.1 ml), but equal to that from sample V₄ (1.4 ml).

The compositions of E.Os, extracted from samples V₁ – V₄, were studied and analyzed by GC-MS. The chromatograms for each E.O. are presented in figures 1 – 4.

The chemical components of E.Os were identified using the GC-MS soft libraries NIST147 and NIST27. The most commonly reported main constituents of rosemary E.O. are camphene, p-cymene, myrcene, α and β-pinene, camphor, borneol, eucalyptol (1,8-cineol), bornyl acetate, while others like terpinen-4-ol, α-terpineol, (E)-carophyllene, 3-octanol, geranyl acetate, linalyl acetate, are referred to as secondary compounds (Maria Lo Presti et al., 2005). Table 1 shows the constituents that we were able to separate and identify and their concentrations (%).
Figure 1. V₁ E.O. GC-MS chromatogram. Peak identification: 2 – α-pinene; 3 – camphene; 4 – β-pinene; 5 – 3-octanone; 6 – β-myrcene; 7 – 3-octanol; 8 – α-phellandrene; 9 – α-cymene; 10 – limonene; 11 – eucalyptol; 12 – sabinene hydrate; 16 – camphor; 17 – isoborneol; 20 – D-verbenone; 21 – bornyl acetate.

Figure 2. V₂ E.O. GC-MS chromatogram. Peak identification: 1 – α-pinene; 2 – camphene; 3 – β-pinene; 4 – 3-octanone; 5 – β-myrcene; 6 – α-phellandrene; 8 – limonene; 9 – eucalyptol; 10 – sabinene hydrate; 13 – camphor; 16 – D-verbenone; 17 – bornyl acetate.
Figure 3. V₁ E.O. GC-MS chromatogram. Peak identification: 1 – α-pinene; 2 – camphene; 3 – β-pinene; 4 – 3-octanone; 5 – β-myrcene; 6 – α-phellandrene; 7 – limonene; 8 – eucalyptol; 12 – camphor; 16 – bornyl acetate.

Figure 4. V₄ E.O. GC-MS chromatogram. Peak identification: 1 – α-pinene; 2 – camphene; 3 – β-pinene; 4 – 3-octanone; 5 – β-myrcene; 6 – α-phellandrene; 7 – limonene; 8 – eucalyptol; 10 – camphor; 14 – bornyl acetate.

Table 1. Composition of E.Os. of V₁ – V₄ Rosemary samples

<table>
<thead>
<tr>
<th>N°</th>
<th>Compound</th>
<th>Retention time (min)</th>
<th>Concentration (%)</th>
<th>V₁</th>
<th>V₂</th>
<th>V₃</th>
<th>V₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>α-pinene</td>
<td>9.154</td>
<td></td>
<td>30.746</td>
<td>39.302</td>
<td>32.702</td>
<td>35.414</td>
</tr>
<tr>
<td>4.</td>
<td>3-octanone</td>
<td>11.387</td>
<td></td>
<td>4.452</td>
<td>3.912</td>
<td>3.458</td>
<td>2.895</td>
</tr>
<tr>
<td>5.</td>
<td>β-myrcene</td>
<td>11.610</td>
<td></td>
<td>4.144</td>
<td>6.191</td>
<td>6.443</td>
<td>6.842</td>
</tr>
<tr>
<td>6.</td>
<td>α-phellandrene</td>
<td>12.171</td>
<td></td>
<td>0.290</td>
<td>0.904</td>
<td>0.894</td>
<td>1.053</td>
</tr>
<tr>
<td>7.</td>
<td>o-cymene</td>
<td>13.094</td>
<td></td>
<td>1.153</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Limonene</td>
<td>13.318</td>
<td></td>
<td>1.995</td>
<td>2.214</td>
<td>2.229</td>
<td>2.175</td>
</tr>
</tbody>
</table>
The major component found in all E.Os samples was α-pinene (30.746 – 39.302 %), followed by eucalyptol (21.204 – 22.977%) and camphor (14.081 – 21.604%). From qualitative point of view, in all cases, the composition of E.O. was almost the same. For the E.O. extracted from sample V1 we were able to identify the o-cymene. Its presence wasn’t found in the other E.Os. Also, the sabinene hydrate was identified only in the V1 – V2 samples. According to O.Y. Celiktas et al. (2007) there is an influence of the climate on the content of eucalyptol. The E.Os extracted from rosemary that grows in a very hot climate have a higher content in eucalyptol (50-61%) than those extracted from rosemary that grows in moderately hot (15-35%) or cool (12-13%) climate. It can be observed that the eucalyptol content found in our E.O. samples correspond to a moderately hot climate as that of Romania. α-Pinene is mentioned as a major component of rosemary E.O. in most literature reports and that is also the case of our E.Os. But there are literature reports that mention that α-pinene was not found in the composition of rosemary E.O. (H.E. Katerinopoulos et al., 2005).

The composition of E.O. isolated from V3 rosemary sample show to have the highest amounts of limonene, eucalyptol, and bornyl acetate.

CONCLUSIONS

The E.O. composition is a very complex one also because it is influenced by different factors such as climate, extraction methods, etc. In order to obtain a high fidelity fingerprint of the E.O. it is necessary an optimization of the separation method but also of the extraction technique. Due to high temperatures that we need during the HD process it is possible that some of the E.O. components are degraded. The next step in our researches is to run a comparative study on the composition of E.Os isolated by different extraction methods, but also on the composition of the E.Os isolated from rosemary in different vegetative phases (before flowering, during the flowering period and after the flowering period).

BIBLIOGRAPHY