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A kinetic study of essential oil components distillation for the recovery of carvacrol rich fractions

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ABSTRACT

The distillation kinetics of the different essential oil components from four Lamiaceae herbs was investigated. *Origanum vulgare* L. ssp. *hirtum*, *Origanum onites* L., *Origanum dictamnus* L., and *Satureja thymbra* L. (harvests of 2013–2015), that present similar composition of essential oil were distilled in a laboratory-scale steam distiller. The recovery of the essential oil (EO) and its components was adequately described by non-steady state diffusion kinetics. The results clearly evidenced that the components present different distillation rates, thus resulting in variation of the EO composition during the process. The distillation rate constants decreased with the increase of the individual components boiling points, therefore, the heavy volatiles, such as carvacrol, could be recovered separately after the distillation of the most volatile compounds. This hypothesis was experimentally confirmed for *O. vulgare* and *S. thymbra*; the separate fractions were obtained from 40 and 50 min respectively up to the end of distillations. GC–MS analyses of the fractions revealed very high carvacrol content that amounted up to 92.5% for *O. vulgare*. The procedure could be applicable in industrial practice in order to recover fractions with pre-designed quality characteristics.

1. Introduction

Medicinal and aromatic plants have been used for the treatment of various diseases all over the world since antiquity. The essential oil (EO) constitutes a valuable fraction of these plants, and extensive scientific studies concern its recovery and determination of biological activity (Gilling et al., 2014; Raut and Karuppayil, 2014; Langeveld et al., 2014; Lang and Buchbauer, 2012; Vimalanathan and Hudson, 2012; Pilau et al., 2011; Baser, 2008). Due to their pleasant odor, EOs from various medicinal and aromatic plants are used in cosmetics and food products, as fragrance ingredients and flavor enhancers, respectively. Moreover, EOs may be potent food preservatives (Prakash et al., 2015; Rasooli and Rasooli, 2013; Burt, 2004; Atarés and Chiralt, 2016; Otoni et al., 2016; Van Long et al., 2016; Palou et al., 2015).

Among the aromatic plants, most of the largest Lamiaceae genera contain the monoterpenoid phenol carvacrol, which amounts up to 95% in the EO of specific species. Carvacrol has been recognized as an important compound with biological antimicrobial and antiviral activity (Gilling et al., 2014; Orhan et al., 2012; Lai et al., 2012; Guarda et al., 2011; Pilau et al., 2011; Baser, 2008). The activity of the compound against bacteria includes membrane disruption, inhibition of ATPase activity, leakage of cell ions, fluidization of membrane lipids and reduction of proton motive force (Langeveld et al., 2014). Carvacrol-based additives for livestock feed are already commercially available for reducing or assisting conventional antibiotics in poultry, swine, ruminants and aquaculture production. Furthermore, the compound has been approved by the European Union as a flavoring substance for food products (Regulation No 872/2012 and 1334/2008), while the EOs from the Lamiaceae plants that contain carvacrol can be used by the food industry as natural flavoring preparations (defined by EU Regulation 1334/2008).

The distillation rate of carvacrol and other EO components from a plant material depends, among others, on the tissue structure. The epidermal glandular trichomes in Lamiaceae are divided to peltate and capitate, with different morphology (Huang et al., 2008) and different composition of volatiles (Schmiderer et al., 2008). For the capitate trichomes, there is some evidence that their secretion consists mainly of a complex mixture of carbohydrates, lipids, and proteins (Turner et al., 2000). Baâtour (2012) observed by light microscopy, under the cuticule of the head cells of the peltate trichomes, three types of EO secretion: dark red droplets, clear lipid droplets and secretion of lucid appearance. The dark red color of the secretion might suggest that carvacrol is located in these specific oil glands. Furthermore the individual components of EO are primary located in the special secretory structures

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either on the surface of the plant or within the plant tissues (Svoboda et al., 2000). Consequently, the diffusion of some components from the interior of the plant tissue to the surface may affect their distillation rate. Moreover, the vapor pressure of the different volatile components is another main factor affecting their distillation.

The production of EOs with a high carvacrol content could enhance their quality and commercial value, as well as their applicability as feed and food additives. However, a substantial part of the global production of EOs is performed by small and medium enterprises that focus on the yield and the cost of production, and not on high quality in terms of carvacrol content. The co-distilling compounds might not present the same distillation kinetics and consequently the composition of the EO being produced, could markedly change during the process.

The current work focused on the kinetic study of the distillation of individual EO components. Herbs of the Lamiaceae family, with considerable carvacrol content were examined, with the aim to investigate if the separation of carvacrol-rich fractions is attainable, even by using conventional distillation equipment. This would allow the application in the production line of small and medium enterprises.

2. Material and methods

2.1. Plant samples

Air dried herbs (leaves and flowers) of *O. onites* L. (harvested in July) and *O. dictamnus* L. (harvested in June) were purchased from Alexopoulos Alexandros and Co (Athens, Greece), and the local market of Crete, respectively. *O. vulgare* L. ssp. *hirtum* (harvested in June), and *S. thymbra* L. (May 2013, May 2014 and July 2015 harvest) were provided by the Agricultural Research Centre of Northern Greece (member of the Hellenic Agricultural Organization-DEMETER). The plant species and varieties were defined by the providers.

2.2. Steam distillations

The steam distillation was performed in a pilot scale distiller of 10 L useful capacity (Chalkos, Greece) with inner perforated grid to hold the plant material above the boiling water. Dry herbs (500 g), with moisture content varying from 7.9% (*O. dictamnus)* to 9.1% (*S. thymbra 2014*), wet basis, were subjected to distillation for a period up to 5 h with simultaneous determination of the essential oil (EO) volume. The process was terminated when the volume of the EO was practically constant (recovery of EO less than 0.2% v/w of dry herb for a period of 30 min). The steam supply rate varied in the range of $5.7 \pm 1.4\ mL\ min^{-1}\ kg^{-1}$. The EO was being received in a graduated glass collector and the volume was recorded versus distillation time. The yield was expressed as the EO volume (mL) obtained by 100 g dry herb per time unit (min). During the distillation process, 2 μL samples of EO were withdrawn with a Transferpette S digital automatic micropipette (0.2–10 μL) (Brand, Wertheim, Germany), equipped with nanocap™ tips, from the upper oil phase of the glass collector, periodically, diluted in 10 mL of hexane, and subjected to GC–MS analysis.

Distillation experiments for each plant material were performed in duplicate. A series of additional duplicate distillation experiments was performed for *O. vulgare* (ssp. *hirtum*) and *S. thymbra* (2015 harvest) and in each case two fractions of EO were recovered. Fraction F1 was collected up to 40 min for *O. vulgare* and up to 50 min for *S. thymbra*, followed by F2 that was collected up to the end of distillations. The specific fractionation time for each herb was defined according to the results obtained from the data analysis of the previous experiments.

2.3. GC–MS analysis

GC–MS analysis was performed by using an HP 6890 GC system (plus +) coupled to an HP 5973 mass selective detector (Hewlett Packard, Palo Alto, CA, USA), and equipped with an HP-5 MS column (30 m × 20 μm × 0.25 μm, Hewlett Packard, Palo Alto, CA, USA). The oven temperature was started at 50 °C, increased to 100 °C at $10\ °C\ min^{-1}$ rate, then to 220 °C at $15\ °C\ min^{-1}$ rate and hold at 220 °C for 7 min. Helium was used as a carrier gas at $1\ mL\ min^{-1}$ column flow, inlet temperature 220 °C and split 20:1. The mass range was 40–400 and compounds were identified by comparison of their mass spectra with the data of NIST and Wiley mass spectral libraries, as well as the spectral database of Adams (2007). The retention times of compounds were converted to the respective Kovats Retention Indices (RI) by analyzing a standard C7–C30 alkanes mixture, with the above-mentioned gas-chromatographic method, and using the equation:

$$RI = 100 \times \left[n + (N-n) \frac{t_{r(compound)} - t_{r(n)}}{t_{r\,(N)} - t_{r(n)}} \right] \quad (1)$$

where: RI: Kovats retention index

n: the number of carbon atoms in the smaller n-alkane

N: the number of carbon atoms in the larger n-alkane

t_r: the retention time

The quantification of the compounds was performed by the area normalization method according to the formula:

$$C_i = \frac{A_i}{\sum A_i} \times 100 \quad (2)$$

whereC_i : the % content of analyte in the EO

A_i : the area of individual compound in the chromatogram

ΣA_i : the sum of all the peak areas in the chromatogram

2.4. Statistical analysis

One way ANOVA analysis was performed to test the statistical differences between the results obtained, using STATISTICA 7.0 (StatSoft Inc., Tulsa, OK, USA). In case that values were significantly different, Duncan's Test was further applied. Correlation coefficients were determined using Excel® 2013 (Microsoft Corporation).

3. Results and discussion

3.1. Distillation kinetics of EOs

The yields of EOs versus distillation time are presented in Fig. 1A, while the respective rates (r_y, (mL EO/100 g dry herb) min^{-1}) during the distillation are depicted in Fig. 1B. The yield of EO increased very fast at the beginning of distillation and the herbs presented high variation of rates, while over time the rates decreased and the variations were minimized.

Milojević et al. (2008) proposed the kinetic model of Eq. (3), based on non-steady state diffusion for the hydrodistillations of comminuted ripe juniper berries. The linearized form (Eq. (4)) can be used to calculate the parameters of the kinetic model.

$$\frac{q_o - q}{q_o - bq_o} = e^{-kt} \text{ or } \frac{q_o - q}{q_o} = (1-b)e^{-kt} \quad (3)$$

$$ln\left(\frac{q_o - q}{q_o}\right) = \ln(1-b) - kt \quad (4)$$

Where q_o:EO content initially present in the herbs (equal to the total EO yield at the end of distillation, mL/100 g)

q :EO yield at any moment of steam distillation

b :fast distillation coefficient

k :distillation rate constant (min^{-1})

In this model, b (fast distillation coefficient) represents the portion of the EO, probably located on the external surface of the plant particles that is removed during an initial, short period of distillation (theoretically at t = 0). It can be characterized by a rapid increase in the oil yield at the very beginning of the process. On the other hand k