Influence of Different Temperatures and Times on Antiradical Properties of Zataria multiflora Boiss. and Cinnamon zeylanicum Essential Oils by Using DPPH Method

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Abstract

The oxidation of fats and oils has a key role in the reduction of the nutritional and organoleptic properties of foodstuffs. Nowadays, there is a tendency to create to use natural preservatives, such as essential oils, for antioxidant, antiradical and antimicrobial properties in foodstuffs. In this study, the effect of thermal processing on the antiradical activities of Zataria multiflora (ZMEO) and Cinnamon zeylanicum (CZEO) essential oils is checked. Antiradical activities were measured with a 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH•) assay at 3 temperatures (100, 140 and 180 °C) and at 3 different time intervals (1, 2 and 3 h). The EC50 of ZMEO and CZEO were 4026.67 ± 2.2 and 2605.01 ± 15.57 ppm, respectively, at 25 °C. The 2 essences showed various reactions and characteristics at different temperatures (100, 140 and 180 °C) and time ranges (1, 2 and 3 h). Maximum and minimum antiradical properties were observed for ZMEO at respectively, 140 and 180 °C after 1 h heating.

Keywords: Antiradical, Zataria multiflora Boiss, Cinnamon zeylanicum, essential oil, DPPH•

Introduction

Concerns about the disadvantages of some chemical preservatives, and negative consumer reactions to chemical preservatives, have increased interest in more natural additives for the maintenance or extension of product shelf life. Particular interest has focused on the potential applications of essential plant oils. Zataria multiflora Boiss. is a member of the Lamiaceae family that is found naturally in Iran, Pakistan and Afghanistan [1,2]. This plant, with the folk name of Avishan Shirazi in Iran, has been used as an anesthetic, an antiseptic and an antispasmodic [2], and is widely used as a flavor compound in an extensive range of foodstuffs in Iran. The main constituents of Zataria multiflora essential oil (ZMEO) are phenolic compounds, such as carvacrol and thymol [3].

Cinnamon belongs to the Lauraceae family, and many species of cinnamon produce a volatile oil on distillation. The most important cinnamon oils are from C. zeylanicum, C. cassia and C. camphora [4]. Cinnamon is a common flavoring compound, widely used in foods. In addition to its flavouring role, cinnamon has exhibited health benefits, such as antimicrobial activity, control of glucose intolerance and diabetes, inhibition of the proliferation of various cancer cell lines, and treatment of the common cold [5].

The DPPH• has been widely used as a tool to estimate the free radical scavenging activity of antioxidants. Antioxidants, on interaction with DPPH•, either transfer electrons or hydrogen atoms to
DPPH•, thus neutralizing the free radical character [6]. According to Locatelli et al. [7], DPPH• is a resistant radical of organic nitrogen that can be used for antiradical activity determination. This is signified by a typical deep purple color and a maximum absorbance in the range of 515 - 520 nm. The DPPH• method is a simple technique, and needs only a UV-Vis spectrophotometer to perform in the presence of a hydrogen/electron donor (a free radical scavenging antioxidant); the absorption intensity is decreased, and the radical solution is discolored, according to the number of electrons captured. The results of a DPPH• assay are often expressed by the EC50 parameter, defined as the concentration of substrate that brings about a 50 % loss of the DPPH•. Antiradical activity is expressed as an inhibition percentage (I %) and calculated using the following equation;

\[
\text{Inhibition percentage (I %) } = \left( \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100
\]  

(1)

Abs_{control} is the absorbance of the control compound (containing all reagents except the test compound), and Abs_{sample} is the absorbance of the test compound [7].

The aim of this study was the evaluation of the antiradical properties of ZMEO and CZEO at different temperatures and times by using the DPPH• method.

Materials and methods

Air-dried aerial parts of Zataria multiflora Boiss. and the bark of Cinnamon zeylanicum were used to steam distillation by using Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulfate and, after filtration, were stored at 4 °C for testing and analyzing. Ethyl acetate was purchased from Merck Chemical Co (Darmstadt, Germany), and DPPH• was supplied from Sigma Aldrich Ltd (United States).

In determination of the effects of temperature, 3 ml of essential oils were dropped into a thermal resistant bottle and then heated at 100, 140 and 180 °C over 1, 2 and 3 h by using an oven (Memert, Germany). Two milliliters of different concentrations of essential oils (500, 1000 and 1500 ppm) were added to 1 ml of solution of DPPH• in ethyl acetate (0.2 mM) and allowed to stand for 30 min in the dark at room temperature for any reaction to take place [8]. Absorbance values were read at 517 nm, by using a Spectrophotometer (Scinko, South Korea). Different sample concentrations were used to obtain calibration curves and to find the EC50 values (EC50: concentration required to obtain a 50 % radical scavenging activity) [7].

Experimental data were analyzed for variance (ANOVA) and significant differences (LSD test) by using the SPSS software. Data were expressed as means of triplicate analyses ± SD. Differences were significant at P < 0.05.

Results and discussion

A comparison of the antiradical properties of both essential oils at 25, 100, 140 and 180 °C and 1, 2 and 3 h is shown at Figures 1 - 3. This study shows that the EC50 of ZMEO and CZEO were 4026.7 ± 2.2 and 2605.0 ± 15.6 ppm at 25 °C, respectively.
Figure 1 Antiradical activities of ZMEO and CZEO after 1 h heating at 25, 100, 140 and 180 °C.

*, P < 0.05 vs. ZMEO
#
*, P < 0.05 vs. 25 and 140 °C

Figure 2 Antiradical activities of ZMEO and CZEO after 2 h heating at 100, 140 and 180 °C.

*, P < 0.05 vs. 25, 100, and 180 °C
A higher DPPH radical-scavenging activity is associated with a lower EC_{50} value. The antiradical property of CZEO was significantly higher than ZMEO at room temperature (P < 0.05) (Figure 1). After 1 h heating at 100, 140 and 180 °C, the antiradical properties of CZEO declined. The antiradical properties of ZMEO during heating at 100 and 180 °C increased (P < 0.05), but at 140 °C, had the lowest antiradical properties among all of the samples.

After 2 h heating (Figure 2), the antiradical properties of CZEO declined, and the largest reduction was at 140 °C (P < 0.05). In ZMEO, the antiradical properties increased, and the largest increase was at 140 °C. Among all of the samples, ZMEO at 140 °C showed the maximum amount of antiradical properties (P < 0.05).

After 3 h heating, the antiradical properties of ZMEO increased in all samples, compared with the sample at 25 °C, and the highest antiradical properties belonged to 100 °C (P < 0.05). The antiradical properties of CZEO at 100 and 140 °C declined, and the highest antiradical properties were shown at 180 °C (P < 0.05). Among all of the samples, ZMEO at 180 °C showed the maximum and, at 140 °C, exhibited the minimum amount of antiradical properties after 3 h heating.

Carvacrol (26.08 %), p-cymene (20.34 %), thymol (17.23 %) and linalool (10.09 %) were the most abundant components, and comprised 73.74 % of the oil in Zataria multiflora essential oil [8]. Moosavy et al. [9] observed that the main components of ZMEO were carvacrol (71.1), γ-terpinene (7.34 %), α-pinene (4.26 %) and eucalyptol (3.37 %). Similarly, according to the study of Jayaprakasha et al. [10], the main components of CZEO were cinnamaldehyde (47.78 %), methyl eugenol (6.75 %), δ-cadinene (4.68 %) and γ-cadinene (3.13 %). The composition of the essential oil of plants can change extensively depending upon the geographical conditions, variety, age of the plant, and the methods of drying and extraction of the oil [11,12].

Shahsavari et al. [8] evaluated the EC_{50} of ZMEO, and found it was at 2220 ± 40 ppm. The chemical composition of essential oils in Zataria multiflora Boiss. from different parts of Iran and their radical scavenging were evaluated. Thymol was the most abundant compound among all of the constituents. Other compounds were reported; thymol, p-cymene, carvacrol, linalool and γ-terpinene. The study was done on five ecotypes of Zataria multiflora. In the DPPH assay, all samples exhibited a remarkable activity, with a higher degree of EC_{50} = 19.7 ± 0.7 ppm, almost similar to BHT (18.1 ± 0.4 ppm) [13]. The EC_{50} of Artemisia dracunculus and Matricaria chamomilla reported 3190 ± 130 and 5630
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± 200 ppm, respectively [14]. Fazel et al. [15] determined the antiradical properties of Thymus vulgaris L. and Satureja hortensis L. essential oils, and estimated the EC50 of essential oils to be 5800 and 8900 ppm, respectively. According to these results, the antiradical properties of ZMEO and CZEO were comparable with other natural antiradicals. Tomaino et al. [16] studied the effect of thermal processing on some essential spice oils at 80, 100, 120 and 180 °C. Heating basil, cinnamon, cloves, oregano and thyme oils (up to 180 °C) did not affect their chemical composition, and their antioxidant activities were measured by the DPPH• method. Conversely, when heated at 180 °C, nutmeg oil showed a significantly higher antiradical activity, with a significant loss of α-pinene and β-pinene, and an evident increase in safrole and myristicin contents. It was observed that the higher antiradical capacity of the nutmeg oil might be related to a heating induced increase in the content of these 2 components. Kelen and Tepe [17] evaluated the antiradical properties of Salvia aucheri and Salvia aramiensis, and reported the EC50 of them to be at 18.8 ± 1.2 and 12.2 ± 1.0 ppm, respectively. The Mosla chinensis Maxim oil (EC50 = 1230.4 ± 12.5 ppm) and its methanol extract (EC50 = 1482.5 ± 10.9 ppm) showed moderate DPPH radical scavenging activity, and were lower than that of the synthetic antioxidant BHT (EC50 = 181.2 ± 7.5 ppm) [18]. The EC50 of L.Petroselinum crispum essential oil was determined to be 80210 ppm; that showed the weak antiradical property of this essential oil [19]. In another study, the EC50 of Bidens pilosa was evaluated to be 47.5 ppm [20]. The heating process can decrease or increase some antiradical components of essential oils. These changes in antiradical activities depend on the kind of essential oils and their components and the amount of functional compounds. These results agreed with the study of Rababah et al. [21], who reported that the differences in antioxidant activities of plant extracts could be due to the different structures of plant extracts from phenolic acids and flavonoid compounds, as well as their derivatives. The antioxidant activities of phenolic acids and their derivatives, such as esters, depend on the number of hydroxyl groups in the molecules. It seems that the antiradical activity of ZMEO is mostly related to the presence of the phenolic compounds, such as flavonoids and phenolic acids [22]. The antioxidant activity of phenolic compounds is reported due to their redox properties, which can adsorb and neutralize free radicals, quench singlet and triplet oxygen, or decompose peroxides [6,23]. Cinnamon bark extract showed good free radical scavenging capacity. The EC50 value of Cinnamomum verum essential oil was found to be 4.21 ppm and that of BHA 5.79 ppm [24]. The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports [25,27]. Eugenol, cinnamaldehyde, cinnamic acid and cineol were responsible for the antiradical activity in cinnamon [24].

Conclusions

In conclusion, thermal processing decreased the antiradical properties of CZEO, and increased the antiradical properties of ZMEO at 100, 140 and 180 °C after 1, 2 and 3 h. We have demonstrated that the essential oils of Zataria multiflora Boiss. and Cinnamon zeylanicum exhibit good properties as free radical scavengers. This fact can support their use in controlling lipid oxidation during food processing, such as frying, baking and freezing. Further studies on GC/MS analysis of heated essential oils and other antioxidant assay conditions are necessary for a better understanding of the factors influencing their antioxidant activity.

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