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Food Chemistry 76 (2002) 307–310

Food Chemistry

www.elsevier.com/locate/foodchem

GC–MS analysis of essential oil of some commercial Fennel teas

A.R. Bilia[a], G. Flamini[b], V. Taglioli[a,*], I. Morelli[b], F.F. Vincieri[a]

[a]*Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Florence, via Gino Capponi 9 I-50121 Florence, Italy*

[b]*Department of Bioorganic and Biopharmaceutical Chemistry, Faculty of Pharmacy, University of Pisa, via Bonanno 33 I-56100 Pisa, Italy*

Received 31 October 2000; received in revised form 2 July 2001; accepted 2 July 2001

Abstract

Fennel teas were prepared by classical infusion or microwave decoction of unbroken and crushed fruits, three pre-packaged teabags and two instant teas. Their volatile constituents were obtained by extraction with *n*-hexane and analysed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC–MS), using two columns with stationary phases of different polarity. Of the constituents 85–95% were identified on the basis of their GC retention times and their mass spectra in relation to authentic compounds. No volatile constituents were detected in one sample of instant tea. Conventional teas from crushed fruits and teas prepared from the other instant tea showed the highest levels of volatile constituents. Anethole (30–90%) and/or anisaldehyde (0.7–51%) were the main constituents of all the samples. Methychavicol (0.8–4.1%), eugenol (1.5–11.3%) and fenchone (0.5–47%) were detected in most samples. Carvone (2.1–6.1%) was presenting only some teabags and camphor (2.3–2.6%) in others. The volatile constituents of only one instant tea included limonene (1.4%) and α-terpineol (0.4%).

Keywords: Fennel; Teas; Essential oil; GC/MS analysis

1. Introduction

Many herbal drugs, whose efficacy has been attributed to in the volatile constituents are used as teas, i.e. chamomile, peppermint leaf, aniseed and fennel (Czygan, 1989). Although very little is yet known about the qualitative and quantitative compositions of the volatiles of them, children and newborns consume these teas very often, so the analysis of their constituents represents a very important step in the evaluation of efficacy and safety.

In this study, the evaluation of the volatile constituents of some teas from sweet fennel is reported. Sweet fennel consists of the dry, whole cremocarps and mericarps of *Foeniculum vulgare* sub. *vulgare*, var. dulce (Miller) Thellung, as reported in the European Pharmacopoeia (1997) monograph. The essential oil consists mainly of anethole (80%), containing not more than 10% methylchavicol and not more than 7.5% fenchone (Brand, 1993). Other minor constituents include α-pinene, limonene, β-pinene, α-myrcene and *p*-cymene (Brand, 1993; Tóth, 1967; Trenkle, 1972).

Fennel and its herbal drug preparations are used for dyspeptic complaints such as mild, spasmodic gastrointestinal complaints, bloating and flatulence. Fennel is also used for catarrh of the upper respiratory tract (Forster, Niklas, & Lutz, 1980; Merkes, 1980; Weiß, 1991; Brand, 1993; Czygan, 1989; European Pharmacopoeia, 1997; Madaus, 1976; Reynolds, 1993; Tóth, 1967; Trenkle, 1972). The essential oil is currently considered to be responsible for the pharmacological properties of fennel, but it cannot be given as such to children and newborns, due to risk of laringospasms, dysponeas and states of agitation (Dersch, Loew, Meyer, & Schilcher, 1993; Kommentar).

This herbal drug is in general marketed as pre-packaged teabags containing unbroken and/or crushed fruit or powdered drug. However, the use of unbroken fruit to prepare infusions does not seem to be correct due to its intracellular localization (Trenkle, 1972), while crushed or powdered fruit gradually loses the volatile constituents during aging (Czygan, 1989).

* Corresponding author. Tel.: +39-055-2757288; fax: +39-055-240776.

E-mail address: vania.taglioli@unifi.it (V. Taglioli).

0308-8146/02/$ - see front matter
PII: S0308-8146(01)00277-1

2. Materials and methods

2.1. Materials

Aboca S.p.A. (Sansepolcro, Arezzo, Italy) generously provided commercial samples of fruits of *Foeniculum vulgare*, subsp. *vulgare* var. dulce (Miller) Thellung. The drug (lot 59710, 11/97) contained 27.7 ml/kg of essential oil. Herbal preparations A–C (teabags) and F and G (freeze-dried powders) were purchased from local pharmacies and grocery stores. Reference samples of *trans*-anethole, *p*-anisaldehyde, limonene, fenchone, camphor, methylchavicol, α-terpineol, carvone, and eugenol were purchased from Sigma Chemicals (Sigma-Aldrich Co., Milan, Italy). Two internal standards, butyl cellosolve and cellosolve acetate, were also purchased from Sigma Chemicals.

2.2. Methods

Traditional teas were prepared from fruits by infusion of 2.5 g of both unbroken and freshly crushed drug in 150 ml of incipiently boiling water (purified by a Milli-Q_{plus} system from Millipore, Milford, MA, USA) and left for 20 min in a covered cup with occasional stirring (Tóth, 1967).

The suspensions were then rapidly filtered through cotton wool. The aqueous filtrate was thrice extracted with *n*-hexane (100 ml, HPLC grade from Merck, Darmstadt, Germany). The organic layers were collected and evaporated to dryness in vacuum (20 °C, 120 mmHg) to evaluate the volatile oil content by GC (Section 2.3).

Teas were also prepared from 2.5 g of both unbroken and crushed fruit in a covered cup, containing 150 ml of water, by heating for 2 min in a microwave oven (Samsung, Korea, 600 W). The suspensions were rapidly filtered and processed, as described earlier for the traditional teas.

Furthermore, traditional fennel teas were obtained by infusion of teabags in 150 ml of water for 20 min. The bags were removed and the solutions processed, as described earlier for the traditional teas prepared from fruits. Fennel teas were also prepared by heating (in a microwave) a teabag in 150 ml of water, according the procedure described earlier for unbroken and crushed fruit.

Finally, teas were also obtained by dissolving two commercial freeze-dried powders in warm (30 °C) water (150 ml), using 7 g and 1 g of the powders, respectively, according to the suggestions on the label (one tablespoon and one teaspoon in a cup of warm water). These teas were also processed, as described earlier for the traditional infusion, by extraction with *n*-hexane.

All hexane solutions were dried with anhydrous Na_2SO_4 (Merck, Darmstadt, Germany) and, after filtration, they were concentrated under nitrogen at room temperature to obtain the essential oil.

2.3. GC and GC–MS analysis

Gas-chromatographic analysis was performed on a HP-5890 Series II instrument, with dual FID, a split ratio 1:30 and using two different columns, an HP-WAX and an HP-5 capillary (30 m×0.25 mm, 0.25 μm film thickness). The temperature program was 10 min at 60 °C, then to 220 °C at 5 °C/min. The carrier gas was nitrogen (0.5 ml/min); the injector and detector temperatures were 250 °C; injection of 10 μl (10% hexane solutions). The identification of the components was achieved, for both the columns, by comparison of their relative retention times with those of pure authentic samples, with respect to two internal standards (butyl cellosolve and cellosolve acetate). GC/EIMS (Electron Impact Ionization Mass Spectrometry) analyses were also performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m×0.25 mm, 0.25 μm film thickness) and a Varian Saturn 2000 ion-trap mass detector. Analytical conditions: injector, and transfer, line temperatures 220 and 240 °C, respectively; oven temperature was programmed from 60 to 240 °C at 3 °C/min; carrier gas was helium at 1 ml/min; injection volume was 0.2 μl (10% hexane solution); split ratio was 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples comparing their Kovats indices, and on computer matching against commercial (Adams, 1995; NIST 98) and home-made library mass spectra built up from pure substances and components of known oils and MS literature data (Adams, 1995; Davies, 1990; Jennings, & Shibamoto, 1980; Massada, 1976; Stenhagen, Abrahamsson, & McLafferty, 1974; Swigar, & Silverstein, 1981).

Moreover, all the molecular masses of the identified substances were confirmed by GC/CIMS (Chemical Ionization Mass Spectrometry), using MeOH as CI reagent gas, operating under the same conditions as described for GC/EIMS analyses. All constituents were identified by comparison with internal standards and on MS comparison with file spectra.

In Table 1 the constituents of samples A–F are listed and the total contents of essential oil (E. O.) are reported. The RI values were those calculated from our analyses with respect to a series of *n*-alkanes (Kovats Indices) and were very similar to the KI reported in the literature (Adams, 1995), with a few units (2–3 at most) margin.

3. Results and discussion

Fennel teas were prepared, both by infusion with occasional stirring (Forster, 1983) and by heating in a microwave oven, using about 2.5 g of both unbroken and crushed fruits in 150 ml of water. Commercial fruits containing 27.7 ml/kg of essential oil, according to the European Pharmacopoeia (1997), were used in this

Table 1
Qualitative and quantitative evaluation of constituents of volatile oil from fennel teas

Samples[a]	Limonene	Fenchone	Camphor	α-Terpineol	Methylchavicol	Carvone	Anisal-dehyde	Anethole	Eugenol	Amount E.O.[b] (mg) (per cup of tea)
A 0 c	–	8.9	–	–	2.3	3.9	25.4	55.4	3.3	2.2
A 0 m	–	10.2	–	–	2.1	6.1	21.6	48.3	4.2	1.8
A 30 c	–	–	–	–	–	–	48.3	40.3	11.3	2.0
A 30 m	–	–	–	–	–	–	40.2	33.2	5.6	1.5
B 0 c	–	10.9	–	–	2.7	2.1	23.3	53.6	2.2	2.8
B 0 m	–	11.5	–	–	2.7	3.1	18.9	58.2	3.2	2.7
B 30 c	–	12.5	–	–	–	–	29.4	58.0	–	2.7
B 30 m	–	11.3	–	–	4.1	–	22.0	59.3	3.3	2.6
C 0 c	–	30.8	2.4	–	0.9	–	1.0	59.7	–	1.4
C 0 m	–	41.5	2.4	–	1.0	–	0.7	52.4	–	1.3
C 30 c	–	35.6	2.3	–	1.1	–	1.7	58.3	–	1.3
C 30 m	–	47.3	2.6	–	0.8	–	1.6	46.3	–	1.3
Dc	–	0.5	–	–	2.4	–	9.3	85.3	1.5	11.4
Dm	–	–	–	–	–	–	50.7	29.6	5.8	6.5
Ec	–	–	–	–	–	–	29.5	59.3	5.8	3.9
Em	–	–	–	–	–	–	39.3	45.2	10.5	3.0
F	0.4	3.7	–	1.4	1.2	–	2.7	90	–	10.0
Apolar[c]	1035	1094	1149	1197	1200	1249	1275	1290	1356	
Polar[d]	1198	1402	1522	1698	1668	1741	2051	1831	2178	

[a] Compounds are listed in order of their elution from an HP-5 apolar column. Relative percentage of the identified volatile based on the FID chromatographic areas.
[b] E.O., essential oil.
[c] Retention Indices on an HP-5 apolar column.
[d] Retention Indices on an HP-WAX polar column.

investigation. Conventional teas, teas obtained by heating in a microwave oven using commercial teabags and teas obtained by dissolution in water of commercial freeze-dried powders were examined. The qualitative and quantitative compositions of the teas prepared using pre-packaged teabags 30 days after opening were also investigated to assess changes in volatile constituents over time.

All teas, after cooling, were extracted with *n*-hexane and the evaporated extracts analysed as such.

Teas obtained from teabags A–C were designated by the same capital letters. The letters D and E indicate crushed and unbroken fruits, respectively. The numbers, 0 and 30, indicate days of storage after opening. The lower case letters "c" (i.e. conventional infusion) or "m" (i.e. microwave heating) indicate the mode of preparation. Two freeze-dried powders were denominated F and G. The results are reported in Table 1. Teas of Product G did not lead to volatile constituents in the *n*-hexane extract, so they are omitted.

All the samples showed the presence of anethole as the dominant constituent (from 90.0 to 45.2%) with the exception of A30c, A30m, C30m and Dm.

Anisaldehyde was another important constituent (from 18.9 to 48.3%) of almost all samples. However, it was contained only in traces in teas from Product F (2.7%) and from Product C (from 0.7 to 1.7%). The better samples were characterized by the presence of a high content of fenchone (from 30.8 to 47.3%). Fenchone was also present but in lower amounts in all teas from Product B (from 10.9 to 12.5%), as well as in A0c (8.9%) and A0m (10.2%), Dc (0.5%) and F (3.7%).

Carvone was only present in teas from teabags A and B, subjected to both conventional infusion (3.9% in A and 2.1% in B) and microwave heating (6.1% in A and 3.1% in B) but it was not found in any other samples.

Other minor constituents of the fruits were eugenol (from 10.5% in unbroken fruits on microwave heating to 1.5% in crushed fruits in conventional infusion) and methylchavicol that was present only in Dc. This compound was also present in most of the teas from Products A–C (from 0.8 to 4.1%), while eugenol was also present in teas from products A and B. Finally, one instant tea, F, also showed the presence of limonene (0.4%) and α-terpineol (1.4%).

α-Pinene, β-pinene, α-myrcene and *para*-cymene, reported by other authors in the herbal drug, were not detected (Brand, 1993; Tóth, 1967; Trenkle, 1972).

Only the tea from conventional preparation of crushed fruits and one instant tea (Product F) showed a volatile consituent content of more than 10 mg. If crushed fruits were submitted to microwave heating the content was about a half of this, and one third in the

teas from unbroken fruits. All teabags showed a content less than 3.0 mg.

The compositions of the volatiles of teas were, in general, very different from those obtained by hydrodistillation of fruits, with the exception of teas obtained from Product F. The conventional teas obtained by infusion of crushed fruits, as suggested by literature data (Forster, 1983), showed a qualitative and quantitative profile of essential oil very different from the other preparations. Generally, these contained high levels of anisaldehyde, considered as a degradation product of anethole. In general, microwave heating led to a degradation of volatile constituents, and all teabags (containing unbroken fruits, crushed fruits or powdered material) did not reach a volatile constituent content similar to that of conventional teas. Furthermore, the presence of high levels of fenchone suggests the presence of bitter fennel or, perhaps for the powdered material (green-grey coloured) the presence of other parts of *Foeniculum vulgare* or other plants, in general implying that the quality of Products A–C was not high.

Finally, the earlier results show that only one tea (from a freeze-dried powder) showed a qualitative and quantitative profile very similar to that of freshly crushed fruits conventionally treated. These data suggest the prefferred use of instant teas over traditional preparations. However, the other instant tea did not contain any volatiles, so the mode of preparation of instant products could be crucial.

Finally, teabags examined 30 days after opening in general showed a loss of essential oil ranging from 10 to 4%.

In these samples a decrease of anethole content and an increase of anisaldehyde content considered the (degradation product of the former) were also evident.

The results, exemplify numerous problems in evaluating the quality of herbal drugs and the consequential necessity of laying down rules to assure their efficacy and safety.

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