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Comparison of the Volatile Constituents in Cold-Pressed Bergamot Oil and a Volatile Oil Isolated by Vacuum Distillation

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The vacuum distillation of bergamot peels furnishes a high-quality essential oil that is totally bergapten-free. This oil was compared with that produced by distillation of cold-pressed oils and those commercially available. The oil obtained by vacuum distillation of the bergamot vegetable matrix shows a composition quite similar to that of the cold-pressed oil. It also displays qualitative characteristics that are superior with respect to those normally observed for essential oils isolated by distillation of cold-pressed oils. Oils isolated by the method presented here can constitute ideal candidates in producing foods, for example, Earl Grey tea, and cosmetic preparations.

KEYWORDS: Extraction; distillation; bergamot essential oils; bergapten; cosmetics; food; GC-MS analysis

INTRODUCTION

Bergamot (*Citrus bergamia* Risso et Poiteau) constitutes an agricultural production of Calabria, a region of Southern Italy. The cultivation of bergamot can become a leading economic resource of the zones of production in Southern Europe. The essential oil of bergamot is an important ingredient in many alimentary, cosmetic, and sanitary preparations (*1, 2*). Bergamot is also widely used in aromatherapy (*3, 4*). Bergamot oil is obtained by subjecting the peel of the fruit to cold-pressing procedures, or it is recovered by other different methodologies and contains bergapten. Bergapten is phototoxic (*5–9*), mutagenic (*10, 11*), and tumorigenic (*12*) . For these reasons, different methodologies have been proposed for also determining bergapten contents in biological fluids (*9, 13–18*). Recently (*19, 20*), bergapten has been reported to be the component responsible for serious problems caused in humans by regular consumption of Earl Grey tea, an alimentary preparation containing bergamot extracts. Thus, quality, trade valence, and uses of bergamot essential oils strictly depend on their contents of bergapten.

Bergapten is generally removed together with the other coumarin components of the oil by fractionated distillation under vacuum. This procedure causes loss of product and a considerable impoverishment of the composition of the thus-obtained oil (*21*) . The same troubles are encountered when bergapten is removed by treatment with sodium hydroxide, another procedure that produces bergamot oils with altered compositions (*21*).

We thought that a simplified distillation procedure for extracting the bergamot essential oils should be planned starting from following hypotheses: (i) direct use of the vegetable matrix, without any pretreatment; (ii) vacuum conditions previously settled and rapid heating both to facilitate the kinetics of the process and to avoid a prolonged exposure of the matrix components to hard operative conditions; and (iii) no addition of external water, since the only natural water content of the vegetable matrix could be enough to transform the process into a distillation of products realized by a steam distillation under reduced pressure.

This work discloses a vacuum distillation process that is directly applied to the vegetable matrix of bergamot. It is of importance to note that the methodology proposed here does not represent a purification of the bergamot oil obtained from the fruit pressing rather than a new method to easily produce essential oils directly from the bergamot matrix. The data reported in this paper were obtained by comparing the composition of the bergamot essential oil contained in commercial products with that of the oil obtained by our procedure. The purpose of this work is the obtainment of a high-quality commercial bergamot oil that maintains the peculiarities of the bergamot essential oil lacking in coumarins and psoralens. The improvement of the qualities of bergamot oil represents valuable progress in the agricultural production of Southern Italy.

MATERIALS AND METHODS

Materials. Bergamot (*C. bergamia*, Risso et Poiteau) was supplied by the "Consorzio del Bergamotto di Reggio Calabria" (Southern Italy). A sample of cold-pressed bergamot essential oil was provided by the Capua Co. (Campo Calabro, Reggio Calabria, Southern Italy). The

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Bergatrade Co. (Reggio Calabria, Southern Italy) supplied serviettes moisturized with bergamot oil. A commercial solution of distilled bergamot oil (Bergarital Co., Reggio Calabria, Southern Italy) was also analyzed. Tetradecane and standard samples of the bergamot essential oil components for the gas chromatography-mass spectrometry (GC-MS) analysis were purchased from Sigma-Aldrich (Milano, Italy) and from TCI Europe N.V. (Zwijndrecht, Belgium).

Extraction of the Essential Oils. *Vacuum Distillation.* In a typical experiment, a weighted amount of bergamot fruits was peeled, and the recovered peels were chopped. The pulp was collected in a flask and subjected to vacuum distillation by immersion in a previously heated oil bath (120 °C). The essential oil was separated from the aqueous phase produced during distillation and dried over Na_2SO_4. The procedure was then applied to five bergamot fruit samples (weights: 297, 208, 206, 246, and 283 g). The recovered amounts of bergamot oil were 0.69, 0.69, 0.83, 0.74, and 0.90 g, respectively. The obtained average amount of bergamot essential oil was 311 g for quintal of fruits. An aliquot of the final bergamot essential oil (0.01 mL) was diluted with diethyl ether (5 mL), and 1 μL of the ethereal solution was used for the qualitative GC-MS analysis.

Analysis of Cold-Pressed Bergamot Essential Oil. An aliquot of a commercial sample of cold-pressed bergamot essential oil (0.01 mL) was diluted with diethyl ether (5 mL). After filtration, 1 μL of the resulting solution was subjected to the qualitative GC-MS analysis. During storage at low temperature, a sample of cold-pressed oil (120 mL) furnished a solid residue (0.13 g), which was collected, dried, solubilized in chloroform, and then subjected to the qualitative GC-MS analysis.

Extraction of Bergamot Essential Oil from Moisturized Serviette. A commercial refreshing serviette soak with bergamot essential oil was immerged in diethyl ether (10 mL) and kept in contact with the solvent for 2 h. The ethereal layer was separated and dried over Na_2SO_4. After filtration, an aliquot (1 μL) of the organic solution was subjected to the qualitative GC-MS analysis.

Analysis of Bergamot Essential Oil Contained in Commercial Solutions. A commercial solution of bergamot essential oil (0.01 mL) was diluted with diethyl ether (5 mL) and dried over Na_2SO_4. After filtration, an aliquot (1 μL) of the resulting solution was subjected to the qualitative GC-MS analysis.

GC-MS Analysis Conditions. GC-MS analyses were performed using a 6890N Network GC System (Agilent Technologies Inc., Palo Alto, CA) equipped with a HP-5MS (30 m × 0.25 mm i.d., 5% dimethylsiloxane; film thickness, 0.25 μm) capillary column and with a 5973 Network MSD mass spectrometer, operated in electron impact ionization mode (70 eV). GC-MS analyses were carried out in split mode (split ratio, 1:50), using helium as the carrier gas (1 mL/min flow rate). The injection port was heated at 250 °C. The column was maintained at an initial temperature of 60 °C for 2 min and then programmed at 7 °C/min to a final temperature of 280 °C, where it was maintained for 10 min. Quantitative GC-MS analysis was carried out in splitless mode (splitless time, 1 min), by using tetradecane as the internal standard.

Qualitative GC-MS Analysis. All analytes were characterized by injecting samples of the oils enriched with an authentic sample of each standard compound and by observing the enhancement of the corresponding peak area.

Quantitative GC-MS Analysis. For the quantitative analysis, two stock solutions were prepared. Stock 1 was prepared using 50 μL of each liquid oil component and 50 mg of each solid oil component (camphene, *cis*-sabinene hydrate, dodecanal, 5,7-dimethoxycoumarin, and bergaptene), by diluting the resulting mixture to 100 mL with chloroform. Aliquots of the stock 1 were then used to prepare three solutions containing, respectively, 0.5 (solution A), 5 (solution B), and 50 μL (solution C) of each analyte per liter. Tetradecane was added to solutions A–C before dilution to obtain a final concentration of the standard of 500 μL per liter. Stock 2 was prepared using 50 μL of each oil component and 50 mg of each solid oil component by diluting the resulting mixture to 10 mL with chloroform. Aliquots of the stock 2 were then used to prepare three solutions containing, respectively, 0.5 (solution D), 0.83 (solution E), and 2.5 mL (solution F) of each analyte per liter. Tetradecane was added to solutions D–F as the internal standard before dilution to obtain a final concentration of the standard of 500 μL per liter. Stocks 1 and 2, containing tetradecane (500 μL per liter), were also used for the quantitative analysis. Three aliquots of the oil isolated by vacuum distillation of the fruit peels (73.9, 123.2, and 172.5 mg) and three aliquots of cold-pressed oil (74.2, 125.7, and 174 mg), containing tetradecane (5 μL), were diluted to 10 mL with chloroform and then subjected to the quantitative analysis. Quantitative data were obtained by comparing the analyte/tetradecane area ratios in the standard solutions with the corresponding ratios in the oil samples solutions.

GC-Flame Ionization Detection (FID) Analysis. Linear retention indices of all analytes were determined using a standard mixture of homologue hydrocarbons (C_8–C_{20}). Analyses were carried out by means of a GC-2010 system (Shimadzu, Japan), equipped with an Equity-5MS column (Supelco, United States) having the following dimensions: 30 m × 0.25 mm i.d.; film thickness, 0.25 μm. The oven temperature program was as follows: 50 °C at 3°/min to 250 °C, held 10 min. Sample injection was in split mode (split ratio, 1:50). Injector and FID temperatures were set at 280 °C. The carrier gas was helium (1 mL/min flow rate).

RESULTS AND DISCUSSION

The essential oil obtained by the vacuum distillation procedure is characterized by the presence of cyclic and acyclic monoterpenes, which can or cannot possess oxygen atoms in their structures (**Table 1**). The most important components are the open chain monoterpenes myrcene, linalool, and linalyl acetate (**Table 1**, entries 12, 15, and 19, respectively). (*Z*)-β-Ocimene, (*E*)-β-ocimene, nerol, neryl acetate, geranyl acetate, farnesene (**Table 1**, entries 13, 14, 16, 21, 22, and 37, respectively) are also present in the oil produced by vacuum distillation. The linalyl acetate and linalool are generally present in a 3.4 relative ratio. This value is a diagnostic index for the good quality of the obtained oils. The volatile fraction of oils obtained from vacuum distillation also contains oxygenated molecules, featuring aldehydic functions, such as neral, geranial, nonanal, and decanal (**Table 1**, entries 18, 20, 27, and 28, respectively). The chromatographic profile shows cyclic monoterpenes not containing oxygen, such as α-thujene, α-pinene, sabinene, β-pinene, α-terpinene, γ-terpinene, and terpinolene (**Table 1**, entries 1, 2, 4, 5, 7, 10, and 11, respectively). Limonene (**Table 1**, entry 9) represents the major component of the profile (45.21%). Finally, it is important to underline that cyclic oxygenated monoterpenes, for example, terpinen-4-ol and α-terpineol (**Table 1**, entries 31 and 32, respectively), are also present in the obtained essential oil. Low contents of alcoholic components and the absence of oxydated monoterpenes are characteristic of the oil isolated by vacuum distillation of the bergamot peels. In particular, *cis*- and *trans*-limonene oxide (**Table 1**, entries 33 and 34) are not detected, unlike distilled oils (*21*). For the oils analyzed, the percentages of each analyte are reported. The values were measured by GC-MS by using tetradecane as the internal standard.

Any single component of the oil was identified by using commercially available authentic samples of all of the analytes. Standards matched the retention time values and mass spectra of the corresponding components of the oil subjected to analysis. Four peaks in the chromatogram were tentatively attributed to bergamotene, germacrene D, bicyclogermacrene, and β-sesquiphellandrene (**Table 1**, entries 40, 41, 42, and 44, respectively), since standard samples of these analytes were not available. The attribution was made by interpretation of the corresponding experimental mass spectrum. Data already reported in the literature were also used to support the identification (*22*, *23*).

Table 1. Peak Identification and Weight Percents for the Components of Oils Obtained by Vacuum Distillation of the Bergamot Peels[a]

entry	compound	method of identification	LRI[f]	vacuum distillation oil[e]	cold-pressed oil[e]	distilled bergapten-free oils (ref *21*)	cold-pressed oils (ref *23*)
			cyclic hydrocarbon monoterpenes				
1	α-thujene	*b, c, i*	925	0.14	0.27	0.12	0.33
2	α-pinene	*b, c, j*	932	1.21	1.01	0.45	1.27
3	camphene	*b, c, j*	949	0.03	0.02	0.02	0.04
4	sabinene	*b, c, j*	972	0.91	0.91	4.24	1.16
5	β-pinene	*b, c, j*	978	5.54	5.20	not reported	7.04
6	α-phellandrene + octanal	*b, c, j*	1007	0.06	0.10	0.02	0.08
7	α-terpinene	*b, c, j*	1017	0.13	0.05	0.13	0.15
8	3-carene	*b, c, j*	1009	traces	traces	0.01	traces
9	limonene + *p*-cymene	*b, c, j*	1033	46.29	32.52	23.14[f]	42.66
10	γ-terpinene	*b, c, j*	1059	5.95	5.49	6.00	7.84
11	terpinolene	*b, c, j*	1086	0.27	0.18	0.31	0.32
			acyclic hydrocarbon monoterpenes				
12	myrcene	*b, c, j*	989	1.47	1.10	0.71	0.98
13	(*Z*)-β-ocimene	*b, c, j*	1035	0.07	0.05	0.12	0.02
14	(*E*)-β-ocimene	*b, c, j*	1046	0.26	0.21	0.28	0.21
			acyclic oxygenated hydrocarbon monoterpenes				
15	linalool	*b, c, j*	1102	7.81	14.41	36.85	7.52
16	nerol	*b, c, j*	1225	traces	0.17	0.13[g]	0.05
17	citronellal	*b, c, j*	1152	0.01	absent	traces	0.01
18	neral	*b, c, j*	1239	0.10	0.14	0.09	0.24
19	linalyl acetate	*b, c, j*	1251	26.31	31.01	22.76	27.32
20	geranial	*b, c, j*	1268	0.17	0.69	0.12[h]	0.36
21	neryl acetate	*b, c, j*	1358	0.14	0.28	0.40	0.39
22	geranyl acetate	*b, c, j*	1377	0.19	0.57	0.45	0.36
			esters				
23	octyl acetate	*b, c, j*	1210	0.13	0.14	0.01	0.12
24	nonyl acetate	*b, c, j*	1309	0.04	0.10	0.04	0.02
25	decyl acetate	*b, c, j*	1409	0.01	0.03	0.02	0.03
			aldehydes				
26	octanal + α-phellandrene	*b, c, j*	1007	0.06	0.10	0.02	0.08
27	nonanal	*b, c, j*	1105	traces	traces	0.03	0.03
28	decanal	*b, c, j*	1208	0.04	0.07	0.17	0.06
29	undecanal	*b, c, j*		absent	absent	0.01	0.01
30	dodecanal	*b, c, j*	1401	traces	absent	0.04	0.01
			cyclic oxygenated hydrocarbon monoterpenes				
31	terpinen-4-ol	*b, c, j*	1181	0.03	0.05	0.36	0.03
32	α-terpineol	*b, c, j*	1195	0.03	0.13	1.30	0.09
33	*cis*-limonene oxide	*b, c, j*		absent	absent	0.01	traces
34	*trans*-limonene oxide	*b, c, j*		absent	absent	0.02	traces
35	*cis*-sabinene hydrate	*b, c, j*	1070	0.08	0.06	0.01	0.04
36	bornyl acetate	*b, c, j*	1284	0.05	0.12	0.02	0.05
			sesquiterpenes				
37	*cis*-β-farnesene	*b, c, j*	1452	0.12	0.45	0.04	0.03
38	sesquithujene	*b, c, j*		absent	absent	not reported	0.02
39	caryophyllene	*b, c, j*	1420	0.33	0.55	0.25	0.33
40	bergamotene	*b, d*	1433			0.30	0.29
41	germacrene D	*b, d*	1482			0.03	0.05
42	bicyclogermacrene	*b, d*	1496			0.01	0.03
43	β-bisabolene	*b, c, k*	1508	0.32	0.47	0.36	0.43
44	β-sesquiphellandrene	*b, d*	1524			not reported	0.01
45	nerolidol	*b, c, j*		absent	0.02	traces	0.02
46	α-bisabolol	*b, c, j*		absent	0.01	traces	absent
			coumarins and psoralens				
47	5,7-dimethoxycoumarin	*b, c, j*		absent	0.82	absent	not reported
48	bergapten	*b, c, j*		absent	0.21	absent	not reported

[a] Comparison with the composition of a cold-pressed oil and with data reported in the literature. [b] Identification based on comparison of mass spectra. [c] Identification based on the injection of an authentic sample. [d] Analytes were not quantified since the respective authentic samples were not available. [e] The w/w percents were determined by the internal standard method and referred to the amount of each component contained in 100 g of oil. Traces indicate percents < 0.01. [f] Sum of limonene, β-phellandrene, and *p*-cymene. [g] Sum of nerol and citronellol. [h] Sum of geranial and perillaldehyde. [i] Authentic sample available in our laboratory, prepared according to the facile procedure already published (ref *27*). [j] Authentic samples of the analytes were purchased from Sigma-Aldrich. The absence of sesquithujene in the analyzed oils was verified by using a bergamot standard oil containing the analyte. [k] The authentic sample of the analyte was purchased from TCI Europe N.V. [l] LRI reported only for the analytes present in the oil isolated by vacuum distillation.

Limonene is the principal component of bergamot oils, and its content is strictly related to the fruit harvest period. Fruits harvested in different seasons (*21*) also show linalool percentage amounts that can vary between 5 and 11%. The presence of bergapten and other coumarins, responsible for undesired health effects, has determined the complete prohibition or severe limitations in the cosmetic use of bergamot oils containing these harmful substances (*24*). To take off bergapten and to reduce

the amounts of the other coumarins in cold-pressed bergamot oils, distillation is usually performed under vacuum or at atmospheric pressure. This procedure provokes a relevant variation of the oil composition. Distillation furnishes linalool-enriched oils: The percentage of this compound rises up to 37%, a value higher than those generally observed for cold-pressed oils (*21*, *25*, *26*). The relative content of linalool represents an important parameter for identifying bergamot oils produced by distillation (*21*). In distilled bergamot oils, variations of the alcoholic components coming from hydration of monoterpenes are generally observed; linalool, nerol, and terpinen-4-ol can therefore show increased concentrations.

Bergamot oil obtained by our procedure is totally bergapten-free. The absence of this undesirable component was verified by parallel analysis of an authentic sample of bergapten. Upon the experimental conditions applied for the GC-MS analysis of the bergamot oil, standard bergapten showed a retention time value of 24.10 min. The chromatogram of the essential oil isolated by vacuum distillation of bergamot peels was characterized by the absence of any peak attributable to bergapten. In the cold-pressed oil, bergapten is present, as confirmed by injecting a standard sample of the same coumarin derivative.

Another peak detectable at the retention time value of 23.05 min corresponds to 5,7-dimethoxycoumarin. The oil obtained by vacuum distillation does not contain 5,7-dimethoxycoumarin.

Also, the solid residue obtained during storage at low temperature of the cold-pressed oil was analyzed by GC-MS. Bergapten is the principal component of this residue. A minimal amount of 5,7-dimethoxycoumarin was also detected. The composition of the bergamot essential oil isolated by vaccum distillation of the fruit peels is similar to that of the cold-pressed essential oil. Chromatographic profiles of the oils obtained by the two procedures slightly differ only in the relative abundances of some components. Furthermore, the vacuum distillation procedure successfully furnished oils containing all of the other components in percentages quite similar to those featured by cold-pressed oils and fitting those already reported in the literature for the composition of cold-pressed oils (**Table 1**).

Moreover, bergamot oils produced by direct distillation of the vegetable matrix show high concentrations of limonene and low contents of linalool, peculiarities that are not featured (**Table 1**) by distilled oils (*21*). In fact, oils obtained by direct distillation of cold-pressed oils are characterized by high contents of alcoholic components, the most important of which is linalool, and by low amounts of limonene.

It is possible to identify commercial bergamot essential oils, probably distilled, by simply comparing them with a sample of bergamot oil produced by vacuum distillation of the fruit peels. For example, high relative concentrations of linalool (40.44%) can be detected for essential bergamot oils used to soak commercial refreshing serviettes. The oil isolated from these serviettes by solvent extraction shows a low content of limonene and a high relative amount of linalool. The 0.59 linalyl acetate/linalool ratio is indicative of a bergamot essential oil probably obtained by distillation.

The GC-MS analysis was extended to a sample of a solution of bergamot oil used as a commercially available sanitary preparation. In this case also, the linalool content is very high (29%), denoting that the bergamot oil used as an ingredient of the solution probably originates from distillation of a cold-pressed oil. In fact, the linalyl acetate/linalool ratio is 0.48, confirming that the analyzed bergamot oil is produced by further elaboration of a cold-pressed oil. Thus, distillation causes a sensible variation of the cold-pressed oil composition, also lowering the quality of the bergamot oil. Both of the commercial oils analyzed also contain a consistent relative amount of α-terpineol. A high α-terpineol content is another index useful for identifying distilled oils, as already well-established (*21*).

Bergamot oil isolated by vacuum distillation of the fruit peels shows remarkable similarities with a cold-pressed oil and can easily be distinguished by oils obtained from further distillation of cold-pressed oils. The methodology here disclosed also allows production of oils in which the linalyl acetate vs linalool ratio results are inverted with respect to the values observed for distilled oils. GC-MS analysis of the oil obtained by vacuum distillation of the vegetable matrix reveals bergapten and the other coumarins to be totally absent. Oils obtained by the described methodology could be ideal candidates for all of the commercial alimentary, cosmetic, and sanitary preparations. Bergapten-free oils obtained by the procedure here discussed might be used in safety as an ingredient by the food industry, for example, in the production of Earl Grey tea. Oil composition is in fact in accordance with the law provisions of the European community and western countries.

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