

Determining Odor-Active Compounds in a Commercial Sample of *Cinnamomum cassia* Essential Oil Using GC-MS and GC-O

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Abstract

The volatiles of a commercial sample of *Cinnamomum cassia* (Nees and T. Nees) J. Presl. essential oil were analyzed by gas chromatography-mass spectrometry (GC-MS). The identification of the components was confirmed by Kovats retention index and their quantities were established using internal standard. These analyses had led to the identification of 72 chemicals and quantification of 41 of them. The majority of volatiles identified belongs to oxygenated compounds (e.g., aldehydes) while non oxygenated terpenes represent about 18% of the oil. The odor quality of cassia essential oil was assessed by Gas Chromatography-Olfactometry (GC-O). Among the 26 components identified with GC-O, AEDA (Aroma Extract Dilution Analysis) has allowed to establish a number of components with high dilution factor (strongly odorous) such as cinnamaldehyde, 3-phenylpropanal, guaiacol and 2-phenylethanol.

Keywords: *Cinnamomum cassia*; GC-O; AEDA; Essential oil

Abbreviations: GC: Gas Chromatography; MS: Mass spectrometry; O: Olfactometry; AEDA: Aroma Extract Dilution Analysis; EO: Essential Oil; F.I.D./FID: Flame Ionization Detector; FD: Flavor Dilution (factor); VCF: Volatile Compounds in Food; FEMA: Flavor and Extract Manufacturers Association (ingredients generally recognized as safe under conditions of intended use as flavors from the FEMA expert panel are listed); RI: Retention Index.

Introduction

Cinnamomum cassia (Nees and T. Nees) J. Presl, Lauraceae family, is one of the oldest known spices. The volatile oil from leaf and bark and the oleoresin from bark are used in soaps, perfumes, spice essences, food and beverages. In traditional medicine it is widely used to treat dyspepsia, gastritis, blood circulation disturbance and inflammatory diseases [1]. The essential oil (EO) obtained from cassia is widely used in cosmetics and foods especially for its antioxidant, antifungal and antibacterial properties [2,3]. Beside its use in fragrances, cassia is used as a flavoring agent in foods for its spicy, sweet and warm aromatic notes both in savory (ham and meat) and sweet food (e.g., beverages such as cola), although with some limits provided by law (Regulation EC N°1334/2008).

The need of analytical methods for quality assessment is directly related to the economic importance of cassia and his derivatives as raw materials in the food industry. This point is crucial if cassia end-use is to make flavors.

In this study a commercial-grade cassia essential oil was analyzed with GC-MS and GC-O was used to investigate the olfactory profile [4]. In literature, several sample of different origin and grade of Cassia EO, from laboratory scale to commercial, have been screened with significant variability on reported results [5]. Moreover, quantitative analyses of constituents in cassia oil are not always available as GC percentages are more often reported.

Literature papers tend agree on cassia oil major component being cinnamic aldehyde, ranging from 50% to 93%, o-methoxy cinnamaldehyde (0.1-25.4%), cinnamyl acetate (0.3-7.6%), benzaldehyde (0.3-2.9%) [5,6]. In literature, the number of compounds identified with GC-olfactometric analysis in food product usually

varies with the nature of the raw material.

Literature data [7] for spices such as black pepper shows that important odorants are about 14, mainly terpenes and oxygenated compounds. For some spices, the odor is well represented by that of the main component of the volatile fraction. An example is cinnamon (*Cinnamomum zeylanicum*, *C. aromaticum*, *C. burmanii*) whose odor is mainly characterized by the high content in cinnamic aldehyde [7].

Generally the concentration of cinnamic aldehyde determines the flavor quality of cassia too, low levels being known to represent material of low quality [8,9] for these reasons, other aromatic compounds in the oil have not been examined in depth so there is a lack of odor profile characterization of cassia oil in literature.

Despite the considerable amount of cinnamic aldehyde, cassia oil have a more complex odor profile with spicy, warm and sweet notes with a woody and earthy background.

Gas chromatography-olfactometry (GC-O) can help achieve this goal since GC-O allows to determinate the contribution of single constituents to the overall flavor of a product. GC-O enables the assessment of odor-active components in complex mixtures, through the specific correlation with the chromatographic peaks of interest; this is possible because two detectors, one of them being the human olfactory system, perceive the eluted substances simultaneously.

Different GC-O methods are available such as dilution, time-

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intensity, detection frequency and posterior intensity [10,11]. Dilutions methods are the most used methods and they are based on successive dilutions of an aroma extract. AEDA-Aroma Extraction Dilution Analysis-is one of the most used GC-O methods because it permits to identify the most important aroma compounds [12,13].

In AEDA the odorants are separated by gas chromatography on a capillary column (Figure 1). To determine the retention times of the aroma substances, the carrier gas stream, after leaving the capillary column, goes to sniffing port for detection by panelist (GC-O). The sensory assessment of a single GC run is not very meaningful because the perception of aroma substances in the carrier gas stream depends on limited quantities, e.g., the degree of concentration of the volatile fraction, and the amount of sample separated by gas chromatography [7]. These limitations are overcome by the stepwise dilution of the volatile fraction with solvent (Figure 2), followed by the gas chromatographic/olfactometric analysis of each dilution. The dilution process is repeated until no more aroma substance can be detected by the panelist.

Our GC-O equipment splits the flux into sniffing port (so human nose as detector) and analytical detector F.I.D. In order to get a precise identification of the peaks, a GC-MS analysis of the EO was run as well. GC-MS has been proven to be a powerful and suitable tool for the determination of volatile compounds because of its high separation efficiency and sensitive detection [14]. In order to avoid errors due to peaks overlapping, an equipment with double column was used.

Materials and Methods

Essential oil and reagents

A commercial grade cassia (*Cinnamomum cassia* (Nees and T.

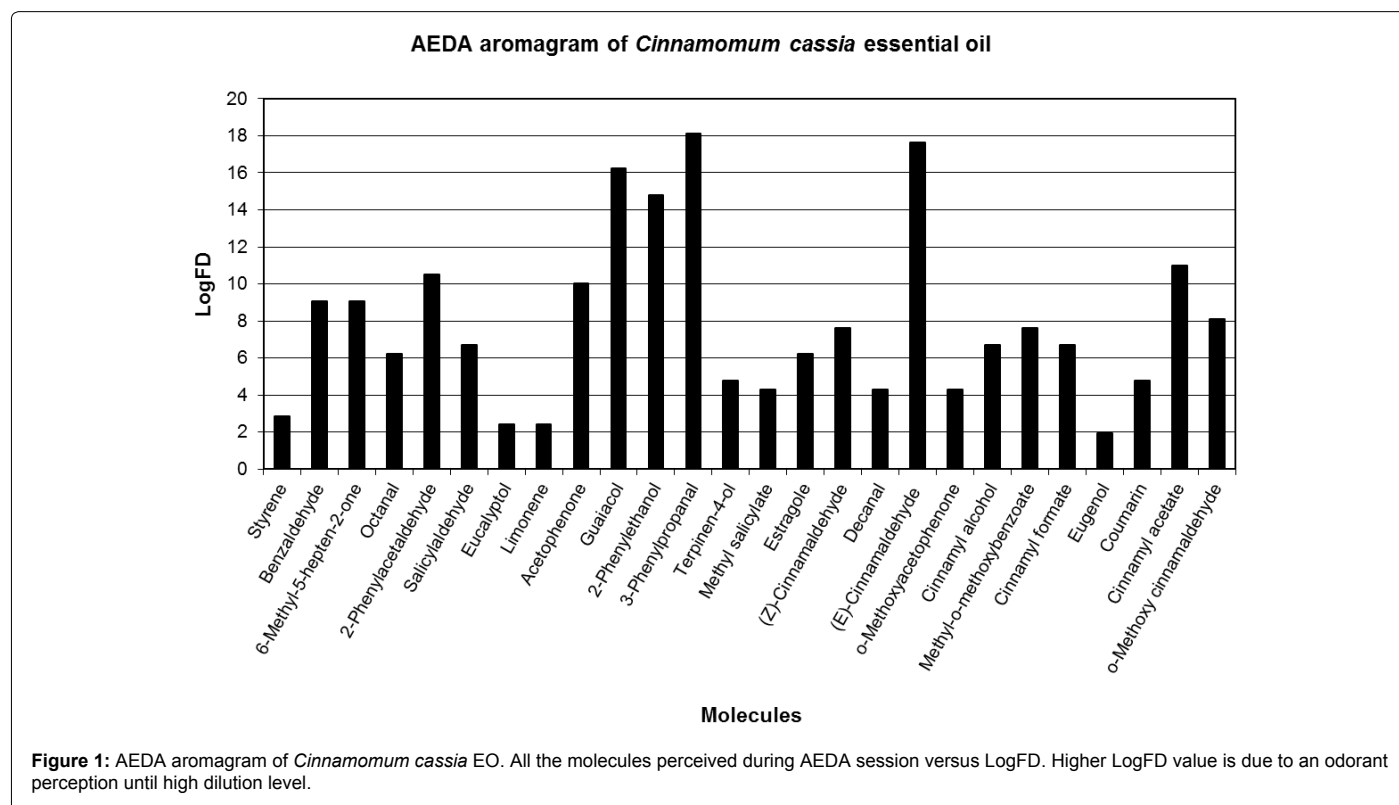
Nees) J. Presl.) essential oil obtained by steam distillation from cassia bark, leaves and twigs was used for all tests. Ethyl alcohol (96°, food grade) was used in the experiments.

Analysis of volatile composition

The identification of volatile aroma compound was performed on a gas-chromatograph Thermo Trace coupled with a Thermo ISQ mass-spectrometer. The GC-MS system was equipped with a DB1 (30 m, 0.25 mm i.d., 0.25 μ m film thickness) capillary column (Agilent JandW). The starting temperature of the column was 50°C, which was held for 3 min, then increased 5°C/min to 280°C, where was held for 10 min. The constant column flow was 1,5 ml/min, using helium as carrier gas, the injector was in split mode at 280°C. Mass spectrometer parameters were as follow: ionization mode EI at 70 eV, source temperature 250°C, scan range m/z 33 to 350, scan mass 1 s. The components were identified by comparing their spectra with those present in the Wiley and NIST spectra collection and in an authentic chemicals spectra library.

The mass spectra identifications were confirmed using Kovats Indices (n-alkanes) on DB1 and DB1701 columns (Figure 3). To calculate the Kovats Indices a GC analysis was performed using an Agilent 5890 gas-chromatograph equipped with an auto sampler. The two capillary columns (Agilent JandW) DB1 and DB1701 (30 m, 0.25 mm i.d., 0.25 μ m film thickness) were assembled on the same injector. Helium (flow 1.5 ml/min) was used as carrier gas, the injector was in split mode (ratio 1:50) and two F.I.D. detector were used. The injector and detectors temperatures were 280°C and 285°C respectively. The column temperature was initially maintained at 50°C for 3 min before increasing to 280°C at a rate of 5°C/min and held for 10 min.

The Kovats Indices were compared to those of authentic chemicals elute in the same conditions. For quantitative analysis, the response factor relative to the internal standard (benzyl benzoate) was previously



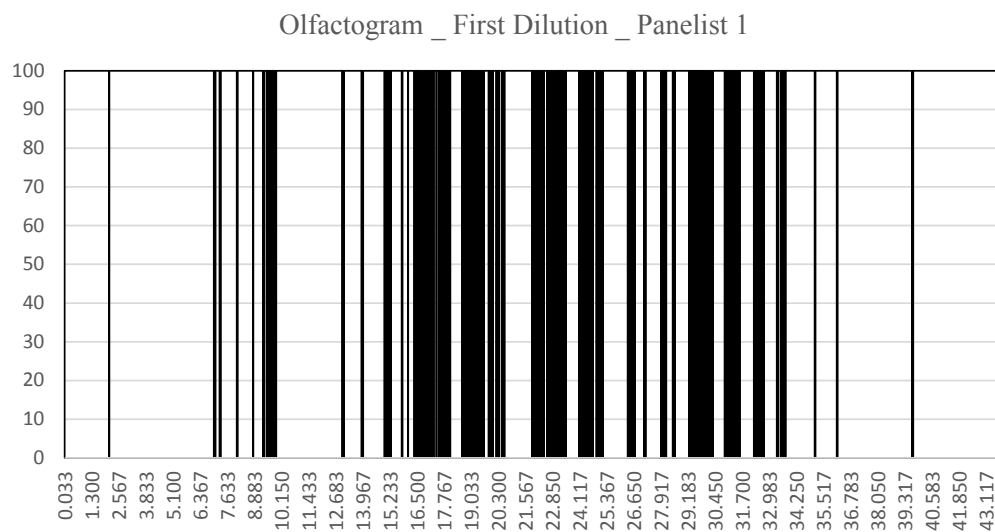


Figure 2: Olfactogram of first dilution of *Cinnamomum cassia* obtained by Panelist1: retention time (X Axis) versus signal from GC-O joystick. When the panelist push the joystick, the instrument records a 100 signal that lasts until the panelist release the joystick.

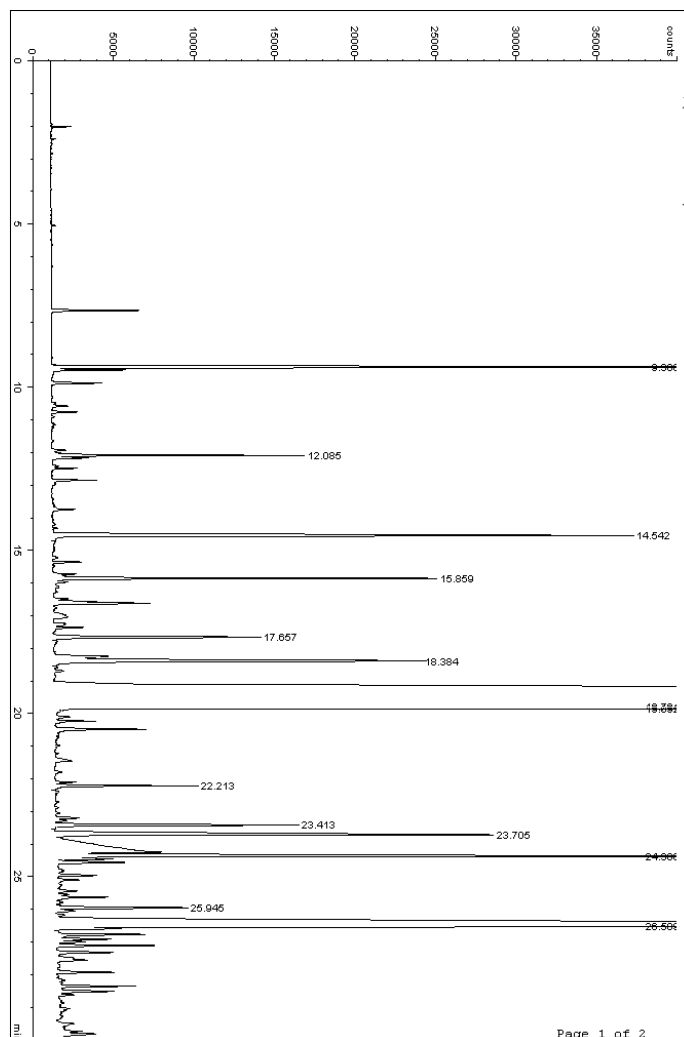


Figure 3: Chromatogram (FID) *Cinnamomum cassia* essential oil (FID obtained by elution from column DB1).

determined and stored in an inland library for each molecule identified. The choice of benzyl benzoate as internal standard has been made after a preliminary evaluation confirmed negligible quantities of this component in our sample. The percentage of every component was then calculated using the response factors.

Response factor (K) is calculated (for each molecule, on both capillary column) using the following:

$$K=W(x) \times A(IS)/W(IS) \times A(x)$$

Where: W(x)=weight (g) of molecule "x"; W(IS)=weight (g) of internal standard (Benzyl benzoate); A (IS)=Peak area of internal standard; A (x)=peak area of molecule "x".

GC-O analysis

Cassia essential oil was analyzed with GC-O using an Agilent 5890 gas chromatograph equipped with DB-1 (30 m, 0.25 mm i.d., 0.25 μm film thickness) capillary column (Agilent J and W).

Chromatographic conditions were 50°C held for 3 min, 5°C/min to 220°C, 15°C/min to 280°C held for 10 min. The first temperature ramp was the same used for the determination of Kovats indices. Helium was used as carrier gas (flux 1.5 ml/min). The injector was in splitless mode, column flow was divided (70:30) by a splitter between the sniffing port and a flame ionization detector (F.I.D.).

A tailor-made software (Simplicius® from ABREG) was used to allow the recording of the odor intensity from the mechanical input of the panelists and to store the recorded signals that were further transformed into FD values or Log(FD).

GC-O analysis was performed using aroma extraction dilution analysis (AEDA) method. In order to find the most important odor-active compounds, which have the lowest odor thresholds, the amount of sample was reduced by ten dilution (w/v) of essential oil in ethyl alcohol (96°, food grade) factor dilution was 3. The sniffing started from the third dilution (1:27) due to peak overlapping (e.g., cinnamic aldehyde) and to overall too strong smell intensity of main eluting peak. According to AEDA method, samples were evaluated by the panelists in increasing dilution order and the impact of an odor-active component was measured as the last dilution value (FD).

The sniffing of cassia essential oil was carried-out by four trained assessors (1 male and 3 female, age 25-50 years), not smokers and without anosmia [15].

Results

In total 72 components were identified through GC-MS analysis and confirmed by Kovats indices. Out of them 41 were quantified using internal standard and response factor while 31 were found in traces (<0.01%).

The majority of volatiles identified belongs to oxygenated compounds (e.g., aldehydes) while non oxygenated terpenes represent about 18% of the oil. In the analyzed cassia oil sample, cinnamic aldehyde is confirmed as the major component (about 78.4%), followed by o-methoxy cinnamaldehyde (7.40%), cinnamyl acetate (1.70%) and benzaldehyde (1.13%) (Table 1).

Different publications on volatiles components of cassia oil have also been reviewed by VCF [16]; 69 components have been listed, with only 25% have been associated with a content range. In our analyses, we are able to quantify as much as 55% of the overall molecules including an high numbers of components usually found in traces such as eucalyptol (0.01%), 3- phenylpropanol (0.01%), 2,4-decadienal (0.01%), cinnamyl formate (0.01%).

Due to safety concerns related to coumarine presence in flavor and fragrance, it was important to detect its content in cassia oil. In our sample, coumarine content reached 0.88%, consistent with the previously reported 0-12.2% [5]. Phenylacetaldehyde was not found in quantitative data in literature but in our sample it was present in small quantity (0.01%); this compound has a relevant odor impression since it was been perceived for many dilution in GC-O analysis. The odor active components determined in our cassia oil sample were 26. The odorants were identified by comparing their mass spectra, retention index, Kovats index and odor description with those of authentic standards.

The data in Table 2 show that most intense odor active compounds were aldehydes: 3- phenylpropanal and cinnamic aldehyde were perceived until last dilution. Some compounds present in smaller quantity had stronger olfactory impression (e.g., guaiacol) than other compounds present in higher quantities (e.g., o-methoxy cinnamaldehyde, benzaldehyde). The odor quality was obtained by olfactory evaluation and description perceived during GC-O session. Among perceived odorants there were some compounds that have a typical spicy connotation: cinnamic aldehyde, methoxy cinnamic aldehyde, cinnamyl acetate, eugenol and cinnamyl alcohol.

Compound	FEMA	Kovats RI DB1a	Kovats RI DB1701 ^a	Kovats RI DB1 ^b	Kovats RI DB1701 ^b	Quantitative data (%)
Ethanol	2419	500	554	513	550	0.02
Hexanal	2557	777	883	773	878	Traces
Styrene	3233	876	960	875	963	0.08
Benzaldehyde	2127	930	1085	927	1080	1.11
α-Pinene	2902	933	951	933	949	0.07
Camphene	2229	946	972	946	970	0.06c
Phenol	3223	964	1220	960	1218	Traces
6-Methyl-5-hepten-2-one	2707	966	1085	966	1084	0.03
β-Pinene	2903	972	1001	969	997	0.02
Benzofuran	-	974	-	-	-	Traces
(E,E)-2,4-Heptadienal	3164	977	1144	980	1144	Traces
2-Pentylfuran	3317	980	1034	980	1034	Traces
Octanal	2797	981	1090	981	1084	Traces
α-Phellandrene	2856	994	1033	994	1033	Traces

Benzyl alcohol	2137	1003	1207	1001	1202	0.02
2-Phenylacetaldehyde	2874	1004	1185	1004	1184	Traces
Salicylaldehyde	3004	1009	1171	1009	1176	0.41
p-Cymene	2356	1012	1075	1009	1072	0.03
Eucalyptol	2465	1019	1077	1016	1072	0.01
Limonene	2633	1022	1058	1020	1057	0.02
β -Ocimene	3539	1033	1085	1029	1085	Traces
Acetophenone	2009	1038	1200	1034	1203	0.05
m-Tolualdehyde	3068	1038	-	1038	-	Traces
γ -Terpinene	3559	1052	1085	1050	1083	Traces
Octanol	2800	1057	1183	1057	1184	Traces
Guaiacol	2532	1061	1236	1061	1231	0.04
Methyl benzoate	2683	1070	1204	1069	1204	Traces
α ,p-Dimethylstyrene	3144	1074	1156	1073	1153	Traces
Terpinolene	3046	1078	1119	1079	1116	Traces
6-Methyl-3,5-heptadien-2-one	3363	1078	1238	1079	1238	Traces
2-Phenylethanol	2858	1084	1280	1084	1275	0.86
Fenchol	2480	1098	1227	1098	1227	Traces
Unknown	-	1104	-	-	-	0.01c
Unknown	-	1108	-	-	-	0.04c
Veratrole	3799	1110	1271	1111	1267	Traces
Camphor	2230	1115	1271	1115	1267	Traces
3-Phenylpropional	2887	1125	1314	1125	1311	0.62
Pinocarvone	-	1138	-	1136	-	Traces
2-Phenylethyl formate	2864	1145	1302	1145	1302	Traces
Unknown	-	1148	-	-	-	0.06c
Borneol	2157	1149	1290	1147	1283	0.11
p-Methylacetophenone	2677	1151	1324	1155	1324	Traces
p-Mentha-1,8-dien-4-ol	-	1162	-	1155	-	Traces
Terpinen-4-ol	2248	1164	1271	1160	1273	0.02
Estragole	2411	1169	1302	1172	1291	0.03
Methyl salicylate	2745	1169	1302	1166	1302	0.06
α -Terpineol	3045	1173	1290	1170	1293	0.03
(Z)-Cinnamaldehyde	2286	1181	1377	1181	1369	0.31
Decanal	2362	1183	1291	1183	1291	Traces
3-Phenylpropanol	2885	1199	1396	1197	1395	0.05
Anisaldehyde	2670	1203	1415	1210	1427	0.86
Carvone	2249	1214	1373	1214	1377	0.02
(E)-Cinnamaldehyde	2286	1238	1462	1228	1463	78.20
o-Methoxyacetophenone	4163	1263	-	1263	-	Traces
Anethole	2086	1266	1396	1260	1393	0.05
Vitispirane	-	1264	-	1267	-	Traces
Cinnamic alcohol	2294	1275	1505	1270	1506	0.15
(E,E)-2,4-Decadienal	3135	1291	1428	1291	1430	0.01
Methyl o-methoxybenzoate	2717	1307	1502	1306	1502	Traces
3-Phenylpropionic acid	2889	1307	1581	1312	1581	0.11
Cinnamyl formate	2299	1319	1495	1319	1499	0.01
Eugenol	2467	1331	1512	1326	1506	0.11
Unknown	-	1335	-	-	-	0.22c
Methyl cinnamate	2698	1352	1514	1348	1519	Traces
α -Copaene	-	1377	1405	1377	1404	0.33c
Coumarin	2381	1388	1692	1388	1686	0.88
β -Elemene	-	1392	1435	1385	1436	Traces
Cinnamic acid	2288	1405	1702	1405	1697	0.74
Cinnamyl acetate	2293	1411	1593	1405	1586	1.70

α -Bergamotene		1434	1463	1434	1461	Traces
α -Curcumene		1465	1532	1464	1530	Traces
o-Methoxycinnamaldehyde	3181	1487	1756	1492	1752	7.40

^aObserved Kovats RI; ^bAuthentic chemicals analysis Kovats RI; ^c% of FID area calculated without using response factor

Table 1: Identifications and quantitative data of commercial sample of Cinnamon Cassia essential oil.

Molecule	Odor description	Mean dilution	Log FD
Styrene	Resinous, balsamic, pungent, plastic, ethereal	1.75	2.86
Benzaldehyde	Almonds, sweet cherry	5	9.07
6-Methyl-5-hepten-2-one	Oily, green, herbaceous	5	9.07
Octanal	Oily, fruity, sweet, citrus	3.5	6.20
2-Phenylacetaldehyde	Floral, hyacinth, rose, honey	5.75	10.50
Salicylaldehyde	Pungent, spicy, phenolic, almond, medicinal	3.75	6.68
Eucalyptol	Strong camphor, fresh	1.5	2.39
Limonene	Fresh, sweet, orange	1.5	2.39
Acetophenone	Sweet, pungent, cherry, sour	5.5	10.02
Guaiacol	Strong, sweet, smoky, vanilla, phenolic, medicinal	8.75	16.22
2-Phenylethanol	Flowers, rose	8	14.79
3-Phenylpropanal	Green, floral, hyacinth, balsamic, melon	9.75	18.13
Terpinen-4-ol	Fresh, green, lemon, fresh, spicy and musky with woody notes	2.75	4.77
Methyl salicylate	Warm, sweet, evergreen	2.5	4.29
Estragole	Sweet, herbaceous, anise-fennel, basil, licorice	3.5	6.20
(Z)-Cinnamaldehyde	Spicy, cinnamon, cassia, sweet, warm	4.25	7.63
Decanal	Strong, pungent, sweet, waxy, orange peel	2.5	4.29
(E)-Cinnamaldehyde	Spicy, cinnamon, cassia, sweet, warm	9.5	17.65
o-Methoxyacetophenone	Sweet, phenolic, evergreen, chemistry, medicine, musky, earthy	2.5	4.29
Cinnamyl alcohol	Balsamic, sweet with floral notes and spicy	3.75	6.68
Methyl-o-methoxybenzoate	Herbaceous, musk, anis, fruity, winey	4.25	7.63
Cinnamyl formate	Balsamic, herbaceous, fruity, apple, slightly bitter	3.75	6.68
Eugenol	Strong, spicy, dry, pungent, smoky, clove	1.25	1.91
Coumarin	Sweet, dried fruit, hay, tobacco	2.75	4.77
Cinnamyl acetate	Sweet, balsamic, floral, spicy	6	10.97
o-Methoxy cinnamaldehyde	Sweet, warm, spicy, cassia	4.5	8.11

Table 2: AEDA results. Mean dilution indicates the last dilution until the molecule had been identified. Odor description indicates the odor perception of panelist.

The identification of a large number of odorants, some of which found in trace and others such as cinnamaldehyde and methoxy cinnamaldehyde that made up to approximately 90% of the oil, confirm AEDA as a powerful technique to analyze the olfactory profile of complex mixtures [17-19].

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