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Extraction of natural product by using techniques assisted by microwaves

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Abstract— The new methods as accelerated steam distillation assisted by microwave (ASDAM) is a combination of microwave heating and steam distillation, performed at atmospheric pressure at very short extraction time. Isolation and concentration of volatile compounds are performed by a single stage. (ASDAM) has been compared with (ASDAM) with cryogrinding of seeds (CG) and a conventional technique, hydrodistillation assisted by microwave (HDAM) [1,2,3], hydro-distillation (HD) for the extraction of essential oil from aromatic herb as caraway and cumin seeds. The essential oils extracted by (ASDAM) for 1 min were quantitatively (yield) and qualitatively (aromatic profile) no similar to those obtained by ASDAM-CG (1 min) and HD (for 3 h). The accelerated microwaves extraction with cryogrinding inhibit numerous enzymatic reactions as hydrolysis of oils.
Microwave radiations constitute the adequate mean for the extraction operations from the yields and high content in major component majority point view, and allow to minimise considerably the energy consumption, but especially heating time too, which is one of essential parameters of artifacts formation.
The ASDAM and ASDAM-CG are a green techniques and yields an essential oil with higher amounts of more valuable oxygenated compounds comparable to the biosynthesis compounds, and allows substantial savings of costs, in terms of time, energy and plant material

Keywords: **microwave, steam distillation, caraway, cumin, cryogrinding, GC-MS,**

I. Introduction

Cuminum cyminum L. is one of the most widely used spices. Crushed cumin seeds are used as a condiment in a variety of dishes. Cumin seeds contain volatile oil (2-5%) that imparts the characteristic aroma to the seeds. The proximate composition of the seeds indicates that they contain fixed oil (approx.10%), protein, cellulose, sugar and other mineral elements, and the physicochemical properties of the volatile oil have already been reported. Cumin seeds possess an aromatic odour and have a spicy and bitter taste. They are used as an essential ingredient inmixed soups, sausages, pickles, cheese and meat dishes, and for seasoning breads, cakes and candies. Cumin has appreciable amounts of essential amino-acids like lysine and threonine. Volatile oil of cumin is employed advantageously, instead of the seeds, in many types of flavouring compounds. The essential oil present in cumin seeds prevents butter from deterioration and improves its acid value. It has an anti-hydrolytic effect and is better than conventional synthetic antioxidants [3]. Cumin is widely used in ayurvedic medicine for the treatment of dyspepsia.

In recent years, microwave-assisted extraction (MAE) has attracted growing interest as it allows rapid extractions of solutes from plant material, with extraction efficiency comparable to that of the classical techniques. In particular, numerous applications of this recent techniques deal with the extraction of oils from herbs and spices samples.
Caraway (*Carum carvi* L.) is a biennial and in cultivated form is also an annual herb of Apiaceae family native to Europe and west Asia [4]. The dried fruit, commonly called seeds, contain 2-8% essential oil, with carvone and limonene as the principal components, in addition to trace amounts of other constituents (acetaldehyde, furfural, carveol, pinene, thujone, camphene, phelandrene .....etc.). Caraway seeds also contain lipids (13-21%), nitrogen compounds (25-35%), fiber (13-19%) and water (9-13%) [5].
Caraway seed is used in meat, food and distillery industries due to its pleasant flavor and intense taste. Its antibacterial and fungicidal properties are important in pharmaceutical applications and also in human and veterinary medicine[6]. Caraway is considered a feedstuff that increases milk production, improves taste and digestibility and reduces flatulence of cattle. Caraway essential oil is used as a natural inhibitor of sprouting, mainly in stored potatoes [7]. It has also antiseptic, pain sedative, antispasmodic, depletive and antioxidant properties [8,9].

The MAE has been widely used for sample preparation to replace other extraction methods such as Soxhlet, sonication, supercritical fluid extraction. In addition, it considerably reduces extraction time [10,11], energy consumption and enhances the efficiency of the extraction [12]. Indeed, microwaves interact selectively with the free water molecules present in the gland and vascular systems, this leads to localized heating, and the temperature increases rapidly near or above the boiling point of water. Thus, such systems undergo a dramatic expansion, with subsequent rupture of their walls, allowing the essential oil to flow towards free water [13].
The goal of the present investigation was to study the chemical composition of volatile oil of caraway and cumin seed, extracted by Steam distillation and hydrodistillation assisted by microwave.

2-Methodology

-Plant Material

Mature Caraway and Carum carvi seeds were purchased from a herbal market in Semmar, a little town situated in the East of Algiers. These samples was reported to be imported respectively from respectively from Morocco and Syria. The samples were directly stored at 4 °C. The initial moisture of these seeds was 8.0 and 6.5 %. Seeds material (60 g) was milled in an electric heavy-duty grinder for 20 s to 180-250 mm average size (Ika Werke standard model Germany) at a speed of 20,000 rpm, and subjected immediately to oil extraction.
The cryogenic grinding was carried out by adding about 80 ml of the liquid nitrogen at -196 °C to seeds (60 g) and subjected immediately to grinding in the same conditions as the classical grinding.

-HD, HDAM and SDAM Apparatus and Procedure

The hydrodistillation assisted by microwave (HDAM) was carried out in a microwave laboratory oven as described in [14], at atmospheric pressure with grinded seeds.
The samples treated above was also submitted to SDAM using a simple column (l= 40 cm, φ= 3 cm, h=15 cm) supporting the grinded seeds according to the figure 1 coupled to an Clevenger apparatus [15] and extracted with 300 ml water (until no more essential oil was obtained). The power used for the two techniques is 600 W with a non-focused radiation.
The volatile oils were collected, dried over anhydrous sodium sulphate and stored at −4 °C until used.

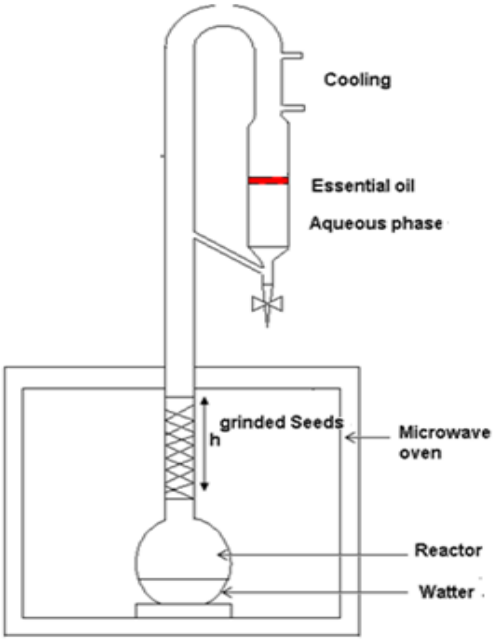

Fig. 1: Steam distillation assisted by microwave, seeds inside of oven apparatus SDAM

GC-MS analysis

GC analysis was performed on a HP 6890 standard model using the following conditions: fused-silica-capillary column with a non polar stationary phase HP5-MS (60 m, 0.25 mm i.d, 0.25 µm film, 5% biphenyl, 95 % dimethylpolysiloxane), detector used FID, carrier gas Helium (0.03 MPa, flow rate 0.5 mL min^{-1}), injector and detector temperature are respectively regulated at 280 and 300 °C. The splitless injection mode was used; injection volume for all samples 1µL (1 % in hexane); the oven temperature was programmed at 60 °C for 10 min, then progressed from 60 to 250°C at 4°C min^{-1} and was held at 250 °C for 8 min.

GC-MS analysis

The volatile oil samples were injected in a Thermoquest Trace GC chromatograph connected to a Finnigan PolarisQ mass-selective detector, with electronic impact. The temperature interface of the mass spectrometer was fixed to 280 °C; the solvent delay time was 4 min. The source temperature was 230 °C. The instrument was operated in electron-impact (EI) mode (ion trap) with an electron energy of 70 eV, and scanned in the 30-550 m/z range.
The fused-silica HP-5 MS capillary column (30 m, 0.25 mm ID, film thickness of 0.25 lm) was directly coupled to the MS. The carrier gas was helium, with a flow rate of 1.2 ml/mn. Oven temperature was programmed (60, C for 8 min, then 60-

250°C at 5 °C/min) and subsequently, held isothermal for 10 min. Injector port: 250 °C, detector: 280 °C. The splitless mode was used. Volume injected: 1 µl of 1% solution (diluted in hexane): HP 5972 recording at 70 eV; scan time 1.5 s; mass range 40–300 amu. Software adopted to handle mass spectra and chromatograms was a Chem Station. The components of the oil was identified by comparison of their mass spectra with those in the Wiley 275 GC–MS library and other authors [16]. Retention indices of the components were determined relative to the retention times of a series of n-alkanes (relative to C6-C28 on the HP5 column).

GC-GC-MS analysis

GC analysis was performed on a HP 7890 standard model using the following conditions: fused-silica-capillary column1 (GC oven) with a non polar stationary phase HP5-MS (40 m, 0.25 mm i.d, 0.25 µm film, 5% biphenyl, 95 % dimethylpolysiloxane) and column2 (secondary oven) RXI-17 (1 m (0.79+0.21) m, 0.1 mm i.d, 0.1 µm film) detector used TOF, carrier gas Helium (0.03 MPa, flow rate 0.5 mL min^{-1}), injector and detector temperature are respectively regulated at 280 and 300 °C. The splitless injection mode was used; injection volume for all samples 1µL (1 % in hexane); the Primary (GC) oven temperature was programmed at 60 °C for 10 min, then progressed from 60 to 250°C (T1) at 5°C min^{-1} and was held at 250 °C for 10 min. The secondary oven temperature programmed at 100 °C for 10 mn, then progressed from 100°C (T2) to 290°C at 5°C min^{-1} and was held at 320 °C for 10 min. The cryogenic fluid used for the diminution of temperature T1 to T2 is Liquid nitrogen at -196°C.The temperature interface of the mass spectrometer was fixed to 280°C. The source temperature was 230 °C. The instrument was operated in electron-impact (EI) mode (ion trap) with an electron energy of 70 eV, and scanned in the 5-800 m/z range.

Results and discussion

In agreement with previous workers [17], the most representative components of Carum carvi volatile oils were limonene (HDAM-CG: 48.06%, SDAM-CG: 41.70%), carvone (32,98%) (HDAM-CG:32.89%, SDAM-CG: 55.83%) and myrcene (7,96 %) (HDAM-CG: 7.96%, SDAM-CG: 0.31%) followed by other minor compounds as, linalool acetate (HDAM-CG: 1,53%), α-terpinen-7-al (HDAM-CG: 1.23%, SDAM-CG: tr), carvenone (HDAM-CG: 1.20%, SDAM-CG: tr) and p-cymene (1,11%) (HDAM-CG: 1.10%, SDAM-CG: 0.15%).

Table 1 : Chemical composition of from Carum carvi volatile oil isolated by HDAM-CG

N°	Compounds	LRI	LRI*	%
1	α-Pinene	939	939	0,02
2	β-Pinene	979	977	0,25
3	Myrcene	990	1000	7,96
4	p-Cymene	1024	1023	1,11
5	Limonene	1029	1028	48,06
6	1.8-Cineol	1031	1032	1,71
7	Z-β -Ocimene	1037	1040	0,07
8	E-β-Ocimene	1050	1047	0,27
9	γ-Terpinene	1059	1054	0,63
10	Cis-limonene oxide	1136	1136	0,00
11	Terpinen-4-ol	1177	1173	0,00
12	Cis-dehydro carvone	1192	1196	0,06
13	Trans-dihydrocarvone	1200	1203	0,27
14	Carvone	1243	1254	32,98
15	Carvenone	1258	1258	1,20
16	Linalool acetate	1257	1260	1,53
17	Cis-carvone oxide	1263	1263	1,13
18	α-terpinen-7-al	1285	1283	1,23
19	γ-terpinen -7- al	1291	1291	0,98
20	α-Cubebene	1348	1351	0,08
21	Copaene	1376	1376	0,05
22	β-Bourbonene	1388	1370	0,02
23	Z-Caryophyllene	1408	1402	0,21
24	D Germacrene	1485	1480	0,05
25	Aciphyllene	1501	1488	0,09
26	trans sesquisabinene	1579	1577	0,03
27	Caryophyllene oxide	1583	1576	0,03
Monoterpene hydrocarbons				58.37
Sesquitepene hydrocarbons				0.52
Oxygenated compounds				41,12

t : trace < 0.01%, * : Lineare retention index of work

Table 2 : Chemical composition of from Carum carvi volatile oil isolated by SDAM-CG

N°	Compounds	KIref	KI	%
1	β- Pinene	979	980	tr
2	Myrcéne	990	983	0.31
3	p-Cymene	1024	1020	0.15
4	Limonene	1029	1029	41.70
5	Z –β- Ocimene	1037	1040	tr
6	E -Z- Ocimene	1050	1053	tr
7	γ-Terpinene	1059	1059	0.13
8	Cis- limonene oxide	1136	1136	0.10
9	Trans-dehydro carvone	1200	1195	0.22
10	Carvone	1243	1231	55.83
11	Carvenone	1258	1260	tr
12	Cis-carvone oxide	1263	1265	tr
13	α-Terpinen-7- al	1285	1288	tr
14	copaéne	1376	1371	0.09
15	β-Bourbonene	1388	1392	0.41
16	D Germacrene	1485	1486	0.24
17	Aciphyllene	1501	1500	0.14
18	Trans-sesquisabinene hydrate	1579	1575	0.31
19	Caryophyllene oxide	1583	1587	0.38
Monoterpene hydrocarbons				42.70
Sesquitepene hydrocarbons				1.19
Oxygenated compounds				56.15

t : trace < 0.01%, * : Lineare retention index of work

Substantially higher amounts of oxygenated (HDAM-CG: 41,12%, SDAM-CG: 56.15%) and monoterpene hydrocarbons (HDAM-CG: 58,37%, SDAM-CG: 42.60%) and lower amounts of sesquiterpenes hydrocarbons (HDAM-CG: 0,52%, SDAM-CG: 1.19%) are present in Cuminum ciminum volatile oil (Tables 3 and 4) in comparison with other works having used the hydrodistillation [15]. Monoterpenes hydrocarbons are less valuable than oxygenated compounds in terms of their contribution to the fragrance of the essential oil.
Conversely, the oxygenated compounds are highly odoriferous and, hence, the most valuable. The greater proportion of oxygenated compounds in the SDAM-CG.

Table 3 : Chemical composition of from Cuminum ciminum (C) volatile oil isolated by HDAM-CG

N°	Compounds	LRI	LRI	%
1	Tricyclene	926	926	0,47
2	α-Thujene	930	934	1,34
3	Sabinene	975	973	0,77
4	β-Pinene	979	981	13,78
5	cis-m-Mentha-2,5-diene	987	988	1,38
6	p-Cymene	1024	1024	18,95
7	β-Phellandrene	1029		tr
8	γ-Terpinene	1059	1059	14,70
9	p-Mentha-2,4(8)-diene	1088	1085	0,09
10	δ-Camphenone	1096	1097	0,07
11	cis-Verbénol	1141	1144	0,31
12	Pentyl-Cyclohexa-1,3-diene	1160	1166	tr
13	Unknown	1167	1168	0,43
14	Terpinen-4-ol	1184	1183	0,37
15	n-Dodecane	1195	1200	8,44
16	Citronellol	1225	1227	0,09
17	Ascaridole	1237	1235	0,12
18	Cuminaldehyde	1241	1257	20,50
19	p-Cymen-7-ol	1291	1292	3,46
20	2-Caren-10-al	1292	1297	7,03
21	p-Mentha-1,4-dien-7-ol	1327	1331	0,17
22	α-Copaene	1276	1363	0,09
23	Daucene	1381	1381	0,57
24	α-Thujaplicin	1411	1418	0,15
25	β-Duprezianene	1422	1425	0,25
26	α-trans-Bergamontene	1434	1433	0,33
27	Unknown sesquitepene	1451	1449	0,98
28	cis-Maurola-4(14) ,5-diene	1466	1465	0,15
29	γ-Muurolene	1479	1478	tr
30	ar-Curcumene	1480	1481	0,75
31	Unknown sesquitepene	1506	1507	tr
32	iso-Italicene epoxide	1515	1511	0,74
33	10 epi-Italicene ether	1516	1520	0,35
34	Carotol	1594	1592	0,44
35	iso- Longifolan-7-α-ol	1619	1612	0,90
36	(E)- Amyl-Cinnamaldehyde	1668	1671	0,2
37	5-neo-Cedranol	1685	-	tr
38	Unknown	1757	1756	1,4
Monoterpene hydrocarbons				51.48
Sesquitepene hydrocarbons				2.46
Oxygenated compounds				43,49

t : trace < 0.01%, * : Lineare retention index of work

volatile oils is probably due to the diminution of thermal and hydrolytic effects, compared with conventional hydrodistillation which uses a large quantity of water and is relatively more energy consuming. In addition, no trace of acid was detected in our volatile oils for this method. This fraction are generally formed under the effect (Hydrolysis) of the temperature (from the seed during various stages of extraction and separation [12].

The GC-GC-MS allowed to resolve some co-elution especially for the major pics as limonene and carvone (Carum Carvi). In fact, when some compounds have retention time closes the major components co-elute the minor constituents

GC and GC-MS analysis allowed the detection of 38 and 32 peaks (HDAM-CG and SDAM-CG respectively) in Cuminum ciminum (C) volatile oil (Table 2), and the identification of 34 compounds. It could be shown that the HDAM-CG extraction provides also the high mass molecular components as the 5-neo-Cedranol.

In agreement with previous workers [18], the most representative components were cuminaldehyde (HDAM-CG: 20.5%, SDAM-CG: 21.68%), p-cymene (HDAM-CG: 18,95%, SDAM-CG: 20.18), β-pinene (HDAM-CG: 13,78%, SDAM-CG: 32.65%), γ-Terpinene (HDAM-CG: 14.70%, SDAM-CG: 18.23%), 2-Caren-10-al (7,03%) (HDAM-CG: 7.03%, SDAM-CG: 2.41%) and p-Cymen-7-ol (3,46%) (HDAM-CG: 3.46%, SDAM-CG: 0%). Other significant compounds were α-thujene (HDAM-CG: 1.34%, SDAM-CG: 0.33%), iso-Longifolan-7-α-ol (HDAM-CG: 0,90%), sabinene (HDAM-CG: 0,77%, SDAM-CG: 0.08), ar-curcumene (HDAM-CG:0,75%), α-trans-bergamotene (HDAM-CG:0,33%), cis-verbenol (HDAM-CG:0,31%) and Carotol (HDAM-CG:0.44%).

Previous studies showed that cuminaldehyde have a pronounced antibacterial [19, 20], antiviral [21], fungicide [22], larvicide [23], pesticide [21] activities.

In addition, we note that the monoterpene hydrocarbons (51.4%) (HDAM-CG: 51,40%, SDAM-CG: 74.51%)and oxygenated compounds (43,40%) (HDAM-CG: 43.40%, SDAM-CG: 25.02%)were in important proportions. Moderate amount of sesquiterpene hydrocarbons (2.46%) (HDAM-CG: 2.46%, SDAM-CG: 0.09%) was isolated (Table 2).

Whereas not trace of acids (artifact) was noted in our volatile oils, what confirms the the efficiency of this technique.

Tables 2 and 3 showed that the chemical composition of *cumin* seeds oil vary considerably according to the grinding used.

Table 4 : Chemical composition of from Cuminum ciminum volatile oil isolated by SDAM-CG

N°	Compounds	KIref	KI	%
1	β- Pinene	979	980	tr
2	Myrcéne	990	983	0.31
3	p-Cymene	1024	1020	0.15
4	Limonene	1029	1029	41.70
5	Z –β- Ocimene	1037	1040	tr
6	E -Z- Ocimene	1050	1053	tr
7	γ-Terpinene	1059	1059	0.13
8	Cis- limonene oxide	1136	1136	0.10
9	Trans-dehydro carvone	1200	1195	0.22
10	Carvone	1243	1231	55.83
11	Carvenone	1258	1260	tr
12	Cis-carvone oxide	1263	1265	tr
13	α-Terpinen-7- al	1285	1288	tr
14	copaéne	1376	1371	0.09
15	β-Bourbonene	1388	1392	0.41
16	D Germacrene	1485	1486	0.24
17	Aciphyllene	1501	1500	0.14
18	Trans-sesquisabinene hydrate	1579	1575	0.31
19	Caryophyllene oxide	1583	1587	0.38
Monoterpene hydrocarbons				42.70
Sesquitepene hydrocarbons				1.19
Oxygenated compounds				56.15

t : trace < 0.01%, * : Lineare retention index of work

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