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Accelerated Microwave Extraction of Natural Product using the Cryogrinding

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***Abstract*—**Team distillation assisted by microwave extraction (SDAM) considered as accelerated technique extraction is a combination of microwave heating and steam distillation, performed at atmospheric pressure. SDAM has been compared with the same technique coupled with the cryogrinding of seeds (SDAM -CG). Isolation and concentration of volatile compounds are performed by a single stage for the extraction of essential oil from Cuminum cyminum seeds. The essential oils extracted by these two methods for 5 min were quantitatively (yield) and qualitatively (aromatic profile) no similar. These methods yield an essential oil with higher amounts of more valuable oxygenated compounds, and allow substantial savings of costs, in terms of time, energy and plant material. SDAM and SDAM-CG is a green technology and appears as a good alternative for the extraction of essential oils from aromatic plants.

***Keywords*—**Steam distillation, microwave extraction, Cuminum cyminum, chromatography, mass spectrometry

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 [1]. Cumin has appreciable amounts of essential amino-acids like lysine and threonine [2]. 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.

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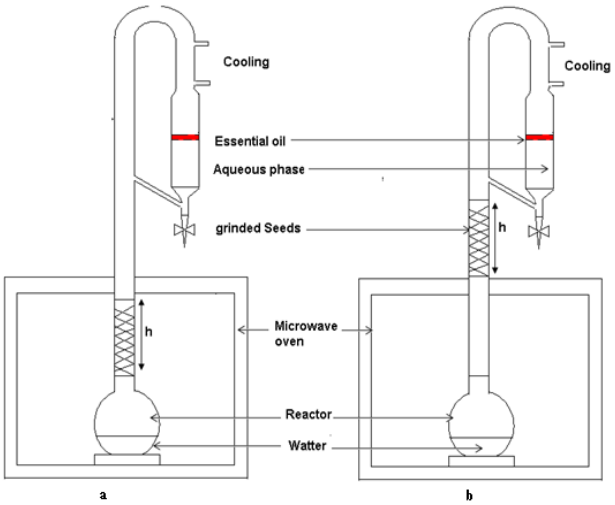
In particular, numerous applications of this recent techniques deal with the extraction of oils from herbs and spices samples. The MAE has been widely used for sample preparation to replace other extraction methods such as Soxhlet, sonication, supercritical fluid extraction

In addition, it considerably reduces extraction time [4], [5], energy consumption and enhances the efficiency of the ex traction [6]. 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 [7]. The goal of the present investigation was to study the chemical composition of essential oil of Cuminum cyminum L. seed, extracted by Steam distillation assisted by microwave realized at different extraction time in discontinuous process. The kinetic of this method has been investigated for determining the effective time of this process.

II. Results And Discussion

A. Plant materiel

Mature cumin seeds were purchased from a herbal market in Semmar, a little town situated in the East of Algiers. This sample was reported to be imported from Syria. The sample were directly stored at 4 °C. The initial moisture of these seeds was 7%. Seed material (50 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.



Figs. 1 a-Steam distillation assisted by microwave, seeds inside of oven apparatus SDAM1, b-Steam distillation assisted by microwave, seeds outside of oven apparatus SDAM2

The microwave extraction process was carried out in a microwave laboratory oven as described in [6], [8] (Fig. 1), at atmospheric pressure with grinded seeds. The power used is 500 W with a non-focused radiation.

The samples treated above was submitted to SDAM using a simple column (l= 40 cm, φ= 3 cm, h=15 cm) supporting the grinded seeds according to the Figs. 1 and 2 coupled to an Clevenger apparatus [9] and extracted with 300 ml water for 5 min (until no more essential oil was obtained). The plant material is the first method inside of the microwave apparatus SDAM 2 (Fig.1-a) and in the second method is placed out of oven (SDAM1) (Fig.1-b). The essential oil was collected, dried over anhydrous sodium sulphate and stored at −4 °C until used.

B. GC and 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 5°C min^{-1} and was held at 250 °C for 10 min. The essential oil samples were injected in a HP 6890 chromatograph connected to a Hewlett-Packard 5973 mass-selective detector. Oven temperature progression, column operating conditions, volume and injection mode, carrier gas conditions and injector temperature were similar to GC ones. 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 homologous n-alkanes series injected in GC and GC-MS in the same conditions as the essential oils, were used to calculate the retention indices. Peak area percentages were calculated by using the normalization method where the response factor for each component was supposed to be equal to one. The component identification used the comparison of the mass spectral fragmentation patterns with those stored in the database (Nist 2002, Wiley 7) and with the previously published spectra. The comparison of the linear retention indices (L.R.I) of the essential oil constituents compared with those of the published index data [10] confirmed the identification.

C. 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 min, 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.

The homologous n-alkanes series injected in GC, GC-MS and GC-GC-MS in the same conditions as the essential oils, were used to calculate the retention indices. Identification of the components was achieved from their linear retention indices LRI on HP5-MS. Peak area percentages were calculated by using the normalization method where the response factor for each component was supposed to be equal to one. The component identification used the comparison of the mass spectral fragmentation patterns with those stored in the database (Nist 2002, Wiley 7) and with the previously published spectra. The comparison of the linear retention indices (L.R.I) of the essential oil constituents compared with those of the published index data [10] confirmed the identification.

D. Extraction

The global yields obtained are respectively 3.5 and 1.9 % for SDAM1 (15 min) and SDAM2 (25 min) respectively (Fig. 3). These yields are comparable than those indicated by some authors (2 to 5 %) [11]. The extracted essential oils were of a yellow colour. All oils had a characteristic odor, especially those isolated in the first minutes of extraction.

The majority of volatile oil yield (differential yields) is essentially obtained in the first minutes of extraction (10 at 15 min) as shown in Fig; 2.

The Fig. 3 shows the variation of extraction yield according to time. We observe three phases in the process of microwave extraction, the first step (1) is represented by an increasing line OA and OB, which characterizes the first quantities extracted, located at the surface of vegetable particles representing respectively (approxymatively) 50 % and 90 % of the yield obtained into 1.5 min. This phase is followed by the curvilinear branchs AA' and BB' representing the intern diffusion of essential oil from the midst of particles towards the external middle involved by the intern warming of the water located in the plant cells and the water humection. In this stage (realized into 23.5 and 10 min for SDAM1 and SDAM2), the oil amount extracted represents nearly 50% (SDAM1) and 10% (SDAM2) of the remaining yield. The third part (3) corresponds to a horizontal line A'A'' and B'B'' which marks the end of extraction process. These curves (OAA'A'' and OBB'B'') are different to that obtained from the conventional extraction technique (hydrodistillation) [6,8] with a curvilinear curve tending towards a horizontal line.

Lucchessi et al. [6] found a different yield profile to that described here, using solvent-free microwave extraction of essential oil from aromatic herbs.

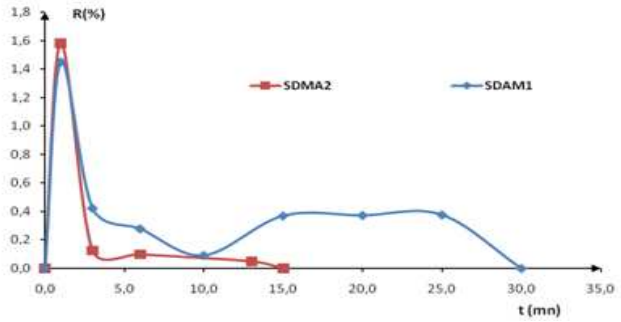


Fig. 2 Differential yields profile as a function of time extraction of volatile oil from cumin essential oil seeds extracted by SDAM1 and SDAM2

It is important to underline that the second phase of SDMA2 (BB') is very short compared to the SDAM1 (AA'). SDAM2 allowed to extract more the exogenic sides whereas SDMA1 the second phase (BB') controlled by diffusion and migration of the compounds (internal sides) dominates [8].

As is indicated in Figs. 2 and 3, we note that more of 50% (SDAM1) and 90% (SDAM2) of extraction yield it's obtained for 10 and 1.5 min respectively. From that, it's possible to reduce still the time extraction until these last values.To study the effect of cryogrinding (CG) on the yields of volatile oils we chose the SDAM2 technique using the same conditions and the same apparatus. The results (Figs. 4 and 5) showed increase of yields after 5 min of extraction process. Indeed, the CG permits a good diffusion of essential oil from the midst of particles towards the external middle.

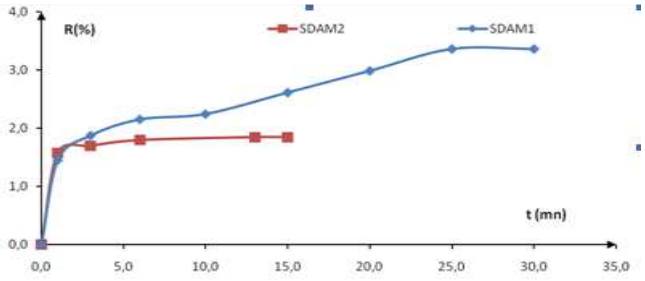


Fig. 3 Accumulated yields profile as a function of time extraction of volatile oil from cumin essential oil seeds extracted by SDAM1 and SDAM2

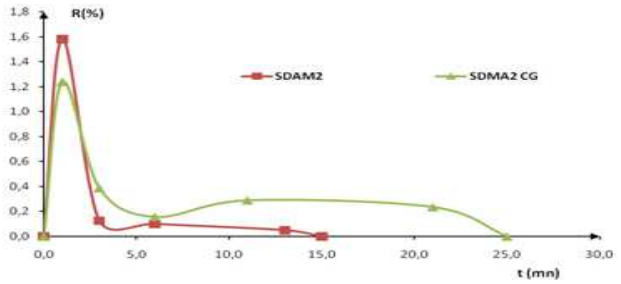


Fig. 4 Differential yields profile as a function of time extraction of volatile oil from cumin essential oil seeds extracted by SDAM2 and SDAM2-CG

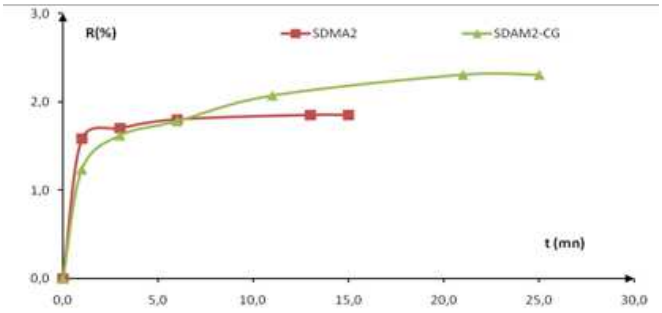


Fig. 5 Accumulated yields profile as a function of time extraction of volatile oil from cumin essential oil seeds extracted by SDAM2 and SDAM2-CG

E. Chemical composition of Cuminum cyminum L. volatile oil extracted by steam distillation assisted by microwaves using the cryogrinding SDAM2-CG

A total of 55 different compounds were identified by GC and GC-MS in the Cuminum cyminum L. volatile oil extracted by steam distillation assisted by microwaves using the cryogrinding SDAM2-CG (TABLE I). The lower number of compounds extracted by SDAM2-CG compared to the previously results (Extraction by hydrodistillation) [12] is probably related to the possible degradation of products by hydrolysis, oxidation and trans-esterification because a longer extraction time (2 h for HD against 15 min for SDAM2-CG), and a greater quantity of water.

TABLE I
CHEMICAL COMPOSITION OF CUMINUM CYMINUM L. VOLATILE OIL EXTRACTED BY STEAM DISTILLATION ASSISTED BY MICROWAVES USING THE CRYOGRINDING SDAM2-CG

	Compounds	(%)
1	Tricyclene	0.02
2	α-Thujene	0.15
3	α-Fenchene	0.36
5	Camphene	0.02
6	Isoamyl propionate	0.01
7	β-pinene	8.64
8	β-Mycrene	0.51
9	α-Phellandrene	0.15
10	p-Cymene	8.48
11	Limonene	0.44
12	γ-Terpinene	8.91
13	α-Terpinolene	0.05
14	Linalool	0.05
15	Nopinone	0.12
16	IsopuIegoI <neo->	0.07
17	Pinocarvone	0.13
18	Menthol	0.05
19	Terpinen-4-ol	0.65
20	Dihydro carvone <cis->	0.10
21	Cuminaldehyde	57.58
22	<E>-Anethole	0.15
23	2-Caren-10-al	8.31
24	Carvacrol	0.27
25	Cinnamaldehyde -<m-methyl->	0.05
26	p-Mentha-l,4-dien-7-ol	0.22
27	Daucene	0.03
28	β-Cubebene	0.19

29	(Z)-Zaryophyllene	0.01
30	α-trans-Bergamotene	0.02
31	γ-Elemene	0.06
32	β-Gurjunene	0.31
33	α Acoradiene	0.05
34	β-Acoradiene	0.02
35	γ-Himachalene	0.14
36	Aristolochene	0.24
37	(E,E)-α-Fernesene	0.07
38	Sesquiphellandrene	0.07
39	γ-Cuprenene	0.01
40	Z-Isoeugenol acetate	0.02
41	Caryophyllene oxide	0.02
42	Cinnamaldehyde <hydro->	0.03
43	Guaiol	0.02
44	Eudesmol <5-epi-7-epi-α>	0.17
45	Bisaboladien-4-ol <2, (7Z)->	0.68
46	β-Acorenol	0.05
47	Germacra-4(15),5,l0(14)-trien-l-a-ol	0.05
48	4-E-Methoxy cinnamic acid*	0.07
49	Elema-l,3-dien-8-ol <7-acetoxy->	0.10
50	α-Vetivone	0.15
51	Encecalin*	0.01
52	Farnesy lacetone (5E,9E)	0.99
53	Geranyl linalool (Z,Z)	0.03
54	Nootkatinol*	0.03
55	Labd-7,13-dien-15-ol*	0.02
56	Abietal	0.01

*Tentatively identified

The main components in SDAM2-CG oil were Cuminaldehyde (57.58%) and γ-terpinene (8.91%), β-pinene (8.64%), p-cymene (8.48%) and 2-Caren-10-al (8.31%) representing approximatively 92 % of the total oil. Whereas other minor components was isolated as terpinen-4-ol 0.65%, β-myrcene 0.51%, limonene 0.44% and <E>-anethole 0.15%.

In addition, The SDAM2-CG oil could be characterized by their greater richness in aldehydes (66%) and monoterpene hydrocarbons (27%) and sesquiterpene hydrocarbons (1 %). Moreover, the alcohols were found in amounts of a little over 1.2 %. Both the ketones and esters fractions were also present in percentages no more than 1% and 0.5 % respectively.

The results showed also a high percentage in antioxidant compound cuminaldehyde (57.58%) compared to the previously works (32.27%) [12].

III. Conclusion

The proposed methods of SDAM1, SDAM2 and SDME2-CG extraction are an original combination of microwave heating and steam distillation. They provide more valuable essential oils and allow substantial saving of energy. Additionally, these methods offer important advantages over traditional alternatives, namely: shorter extraction times, substantial savings of energy, and a reduced environmental burden (less CO2 rejected in the atmosphere). All these advantages make SDAM a good alternative for the extraction of volatile oil from aromatic plants.

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