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Effect of microencapsulation on chemical composition and antioxidant activity of cumin and fennel essential oils.

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ABSTRACT

This study aimed to evaluate the effect of some carrier materials, i.e. alginate, chitosan, carrageenan and carboxy methyl cellulose (CMC) on the chemical composition and antioxidant activity of cumin and fennel essential oils (EOs). GC and GC-MS analysis identified 22 compounds, representing 86.77% of the total cumin EO, and 16 compounds, representing 97.10 % of the total fennel EO. The major compounds in cumin and fennel EOs were P-mentha1, 4-diene-7-al, cumin aldehyde, P-mentha-1, 3-diene-7-al, γ-terpinene, p-cymene and estragole, limonene, fenchone, trans-anethole, respectively. Microencapsulation efficiency of cumin and fennel EOs in different carrier materials ranged between 92.3-97.0% and 93.4 to 98.1%, respectively. The encapsulated oils that loaded with alginate beads were higher than other carrier materials. The changes in the composition and antioxidant activity of encapsulated EOs were evaluated. Cumin EO encapsulated in CMC, Carrageenan and Alginate, comprised higher content of cumin aldehyde, P-cymene and γ-terpinene, respectively compared to their concentration in the initial EO. Also, the concentration of p-mentha-1, 3-diene-7-al in all encapsulated materials was higher than that in the initial EO. On contrary, P-mentha1, 4-diene-7-al was encapsulated in lower relative concentration than in the initial EO. In fennel EO, estragole showed decrease in encapsulated oil samples with carrageenan and CMC compared to in initial EO. Also, limonene was not detected or present in lower concentration in encapsulated oil samples compared to initial EO. While, the concentration of fenchone showed opposite trend. Also, Trans- anethole was encapsulated in lower relative concentration than in initial EO. The antioxidant activity and total phenolic contents of cumin and fennel EOs before and after encapsulation were evaluated. The antioxidant activities and total phenolic contents in cumin and fennel EOs were (74.50%, 276.15 µg/g) and (78.87%, 103.25 µg/g), respectively. The obtained results revealed that antioxidant activity and total phenolic contents in initial EOs were higher than those of encapsulated oil samples. The encapsulated oil samples after storage for 6 months exhibited slight decrease in antioxidant activity and total phenolic contents.

Keywords: Antioxidant activity, cumin oil, fennel oil, microencapsulation, essential oil, GC-MS.

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INTRODUCTION

Several spices are used to provide aroma, texture and color to food products. Furthermore, they act as preservative materials and provide nutritional and health benefits. The cumin seeds (*Cuminum cyminum* L.) are consumed as a flavouring agent in whole or grounded form all over the world. It has been found to possess various pharmacological activities such as antimicrobial, antidaibetic, antiepileptic antifertility, anticancer, antioxidant and Immunomodulatory (Kaur and Sharma, 2012). Fennel (*Foeniculum vulgare* Mill.) is a biennial medicinal plant belonging to the family Apiaceae (Umbelliferae). Fennel essential oil is used as flavouring agents in food products such as beverages, bread, pickles, pastries, and cheese; cosmetic and pharmaceutical products (Piccaglia and Marotti, 2001) and herbal drugs. Also, essential oils of fennel have hepatoprotective effects (Özbek *et al*., 2004), anti-inflammatory, analgesic and antioxidant activities (Choi and Hwang, 2004). They are also used as an appetite stimulant and to ease several stomach disorders (Kaur and Sharma 2012). Composition of essential oil depends upon internal, environmental and agricultural practices and factors affecting the plant such as genetics, and ecological conditions (Fuente et al., 2003; Telci et al., 2006).

The biological activity of EOs can be lost by volatilization or degradation of active compounds (Ayala-Zavala et al., 2008). The encapsulation of essential oil and flavour ingredients is among the most important applications in the food industry to entrap sensitive ingredients, such as volatile and labile flavours, into solid carriers to increase their protection, reduce evaporation, promote easier handling, and control their release during storage and application (Kim and Morr, 1996; Gouin, 2004; Bylaite et al., 2001; Baranauskieneù et al., 2006). Therefore, the aim of this study was to evaluate the effect of microencapsulation by using different carrier materials on chemical composition and antioxidant activity of cumin and fennel essential oils.

MATERIALS & METHODS

Materials

The plant materials of cumin seeds (*Cuminum cyminum)* and fennel (*Foeniculum vulgare* Mill) were obtained from production medicinal and aromatic plant untie at National Research Centre, Cairo, Egypt. Sodium alginate (Alg), chitosan low MW (Chi), carrageenan (Car), carboxy methyl cellulose (CMC), trisodium polyphosphate (TPP) ferric chloride ($FeCl_3$) and 2,2-diphenyl-1-picryhydrazyl (DPPH) were purchased from Sigma-Aldrich Co. (St. Louis, Mo, USA). Calcium chloride ($Ca\ Cl_2$) and potassium chloride (KCl) were purchased from Park Scientific Limited (U/K). Authentic volatile compounds and standard n-paraffin (C8-C22) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany). All other chemicals were of analytical grade and the solvents were purified and distilled before using.

Methods

Extraction of essential oil

One hundred gram of crushed dried plant materials were subjected to three-hours of hydro-distillation using Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulphate and filtered before analysis by GC and GC-MS.

Encapsulation of Essential Oils (EOs)

Microencapsulation of oil was conducted using emulsion extrusion technique described by Chan (2011). Sodium alginate, *K*- Carrageenan, Carboxy Methyl Cellulose and chitosan were dissolved in distilled water to produce polymer solutions with a concentration of 2 % w/v; the solutions were left standing for 3 h. to disengage bubble before use. Afterwards, polymer solution (100 ml) and EOs (1 ml) were homogenized into a 200 ml beaker with stirring at a speed of 300 rpm for 10 h. using magnetic stirrer. The oil was gradually added to the polymer solution during mixing until the desired oil loading was obtained. Fifty milliliters of alginate-oil emulsion, *K*- Carrageenan-oil emulsion, Carboxy Methyl Cellulose (CMC)-oil emulsion and chitosan-oil emulsion were then sprayed into a collecting water bath containing calcium chloride solution (2 w/v%), KCl (2 w/v%), $FeCl_3$ (0.05 M) and TTP (5 w/v%), respectively by using an Inotech Encapsulator (Switzerland) with a 450- m nozzle. The resulting microcapsules were allowed to harden in cross-linking solutions for 3 hrs. The oil-

loaded polymer beads were collected from the cross-linking solutions using a sieve. Finally, the micro-beads were rinsed twice with distilled water; tissue paper was used to absorb the surface excessive water and oil onto the wet microcapsules.

Microencapsulation efficiency

Encapsulation efficiency (EE) was determined according to the method described by Voncina *et al*. (2009).

Determination of Phenolic Content

Phenolic content of initial, encapsulated and stored encapsulated essential oils of cumin and fennel were extracted as follows: One gram of initial or encapsulated essential oil samples on Sodium alginate, *K*-Carrageenan, Carboxy Methyl Cellulose (CMC) and chitosan were crushed, the 3 ml methanol was added and mixed for 10 min by ultrasonic. The obtained extracts filtered and centrifuged at 4000 r. p. m, for 10 min, the supernatant was concentrated under vacuum at 40^0C for 3 h using a rotary evaporator (Heidolph-Laborota, Germany) to obtain the essential oil methanolic crude extract. The crude extract was kept in dark glass bottles for three days at freezing point up till use.

Total phenolics contents were determined by the Folin–Ciocalteu method **(**Singleton *et al.*, 1999). 200µL of methanol extracts of samples with essential oil of fennel or cumin were added separately to 1 ml of 1:10 diluted Folin–Ciocalteu reagent and 800 µl of saturated sodium carbonate 75 g/L. The reaction mixture was incubated at 45°C for 40 min, and the absorbance was measured at 765 nm in Shimadzu, spectro-photometer. Gallic acid (0–50µg/ml) was used for the calibration curve. The results were expressed as Gallic acid equivalent, µg GAE/g dry weight and calculated as mean values (*n* = 3).

Determination of Antioxidant Activity

Antioxidant activity of initial, encapsulated and stored encapsulated of fennel and cumin essential oils were determined. The DPPH radical-scavenging assay was carried out, as previously reported by Grzegorczyk *et al*. (2007). Various concentrations of ethanol and ethanol extracts of fennel and cumin (50, 100, 150, and 200 µg/ml) were added to 4 ml of 0.1 m M DPPH solution in methanol and the reaction mixture was shaken vigorously. After incubation for 30 min at room temperature the absorbance was recorded at 517 nm. TBHQ (TBHQ, tertiary butyl hydroquinone) was used as a reference in the same concentration range as the test extract. A control solution, without a tested compound, was prepared in the same manner as the assay mixture. All the analyses were done in triplicate. The degree of decolorization indicates the radical-scavenging efficiency of the extract. The antioxidant activity of dates was calculated as an inhibitory effect (I %) of the DPPH radical formation as follows:

$$\text{Inhibition \%} = 100 \text{ X } A517_{(control)} - A\,517_{(sample)} / A\,517_{(control)}$$

Gas chromatographic (GC) analysis

GC analysis was performed by using the Hewlett–Packard model 5890 equipped with flame ionization detector (FID). Volatiles were separated using a fused silica capillary columnDB5 (60 m 0.32 mm i. d. 0.25 lm film thickness). The oven temperature was maintained initially at 50 °C for 5 min, and then programmed from 50 to 250 °C at a rate of 4°C/min. Helium was used as the carrier gas, at flow rate of 1.1 ml/min. The sample size was 2 µl, split ration 1:10, the injector and detector temperature were 220 and 250°C. The retention indices (Kovats index) of the separated volatile components were calculated with reference to the retention time of a series of alkanes (C6–C20) as external standard run at the same conditions.

Gas chromatographic-mass spectrometric (GC-MS) analysis

The analysis was carried out using a couple gas chromatography Hewlett–Packard 5890/mass spectrometry Hewlett–Packard-MS 5970. The ionization voltage was 70 eV, mass range m/z 39-400 amu. The GC condition carried out as mentioned above. The isolated peaks were identified by matching with data from

the library of mass spectra (National Institute of Standard and Technology) and comparison with those of authentic compounds and published data (Adams, 1995).

Statistical analysis

The obtained results were evaluated statistically using the analysis of variance as reported by McClave & Benson (1991).

RESULT AND DISCUSSION

Chemical composition of cumin and fennel EOs

The chemical composition of cumin seeds and fennel EOs were studied using GC and GC-MS. The main components of each EO, their relative percentage of the total chromatogram area and kovats index are presented in Tables 1, 2.

Table 1 showed that 22 compounds were identified; representing 86.77% of the total oil of cumin seeds and the yield was 3% (v/w). The most abundant compound was p-mentha1, 4-diene-7-al (34.30%) followed by cumin aldehyde (33.64%), p-mentha-1, 3-diene-7-al (8.99%), γ-terpinene (2.99%) and P-cymene (2.30%). This result agreed with Atta et al (1999) who stated that the major components of cumin seeds essential oil that obtained from different localities in Turky contained cuminal (19.6-27.0%), p-mentha-1,3-diene-7-al (4.3-12.3%), p-mentha-1,4-diene-7-al (24.5-44.9%), γ-terpinene (7.1-14.1%), p-cymene (4.6-12.0%) and β-pinene (2.9-8.9%). Also, Iacobellis et al (2005) reported that p-mentha-1, 4-diene-7-al, cuminal, γ-pinene and β-pinene were seen to be the major constitutes of cumin EO. Lis-Balchin et al (1998) and Pajohi Alamoti et al (2012) reported that Cumin aldehyde, menthone derivatives, γ-Terpinene and ρ-Cymene are responsible for biological effects in cumin EO. Also, Ruberto and Baratha (2000) confirmed that the monoterpenes, α-terpinene, γ-terpinene and p-cymene showed high antioxidant activities.

Table 1: Chemical composition of cumin seeds essential oil.

No	KI[a]	Compounds[b]	Relative area[c] (%)
1	927	α-Thujene	0.14
2	979	β-Pinene	1.87
3	1017	α-Terpinene	0.05
4	1025	P-Cymene	2.30
5	1059	γ-Terpinene	2.99
6	1089	Terpinolene	0.03
7	1102	2-Ethyl-1-hexanol	0.04
8	1141	Camphor	0.03
9	1168	Borneol	0.19
10	1183	Terpinen-4-ol	0.19
11	1195	Verbanol	0.80
12	1250	Cumin aldehyde	33.64
13	1291	p-Mentha-1,3-diene-7-al	8.99
14	1299	p-Mentha-1,4-diene-7-al	34.30
15	1342	1-Phenyl-1-butanol	0.08
16	1362	1-Phenyl-1,2-enthandiol	0.13
17	1426	β-Caryophyllene	0.06
18	1450	β-Farnesene	0.29
19	1480	β-Bisbolene	0.23
20	1500	Cyclogermacrene	0.27
21	1521	δ-Candinene	0.12
22	1606	Caryophyllene oxide	0.10

[a] Retention index: Kovats retention index relative to n-alkanes on column DB-5; [b]Compound identified by GC-MS(MS) and / or by Kovats index on DB5 (KI) and/ or by comparison of MS and KI of standard compounds run under similar GC-MS; [c] Value expressed as relative area percentages to total identified compounds.

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Sixteen compounds representing 97.10 % of the total fennel essential oil were separated and identified, and the obtained yield was 0.04% v/w (Table 2).The major components were estragole (79.92%), limonene (8.47%), fenchone (5.70%) and trans- anethole (1.64%). This result is in agreement with those found by Shahat et al (2011) who reported that fennel EO was rich in estragole (57.94%), limonene (20.64%), fenchone (7.22%) and trans-anethole (4.99%). On the other hand, Gulfraz et al (2008) and Aprotosoale et al (2010) reported that the major compounds of *F. vulgar* essential oil were trans-anethole followed by fenchone methyl chavical (estragole) and limonene. Fennel essential oil has been reported to possess antiflamatory, antioxidant and pro-oxidant activities (Miguel et al., 2010).

Table 2: Chemical composition of fennel seeds essential oil.

No	KI[a]	Compounds[b]	Relative area (%)[c]
1	935	α-Pinene	0.50
2	951	Camphene	0.04
3	973	Sabinene	0.94
4	1005	α-Phellandrene	0.09
5	1031	Limonene	8.47
6	1044	Eucatyptol	0.08
7	1058	γ-Terpinene	0.27
8	1093	Fenchone	5.70
9	1101	Linalool	0.11
10	1139	Camphor	0.58
11	1183	α-terpineol	0.29
12	1205	Estragole	76.92
13	1248	Cuminal	0.73
14	1259	p-Anisaldehyde	0.54
15	1288	Trans anethole	1.64
16	1426	β-Caryophyllene	0.20

[a] Retention index: Kovats retention index relative to n-alkanes on column DB-5;[b]Compound identified by GC-MS(MS) and / or by Kovats index on DB5 (KI) and/ or by comparison of MS and KI of standard compounds run under similar GC-MS; [c] Value expressed as relative area percentages to total identified compounds.

Microencapsulation of Essential Oils

Choosing the best wall materials and encapsulation technique are important steps in food encapsulation. Hydrogels such as alginate, carrageenan, chitosan and carboxy methyl cellulose are already permitted for use in pharmaceutical or food industries. The effect of previous encapsulation materials on efficiency and composition of entrapped essential oil were evaluated as follows:

Efficiency of Encapsulation

As shown in Figs 1 and 2, microencapsulation efficiency of cumin and fennel EOs in different carrier materials ranged between 92.3-97.0% and 93.4 to 98.1%, respectively being the lowest obtained value for *K*-Carrageenan (Car) and the highest obtained value for Alginate (Alg). The difference in the efficiency of encapsulation could due to the physicochemical properties of essential oils, which are determined by its composition and how the size of the molecules fit into the wall of carrier materials

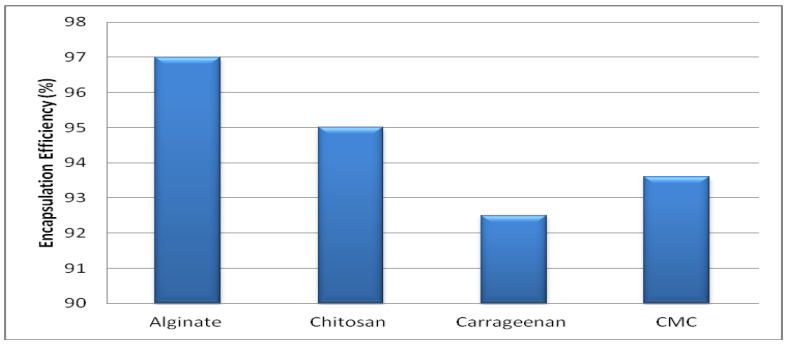

Figure 1: Encapsulation efficiency of cumin EO in different microencapsulated materials.

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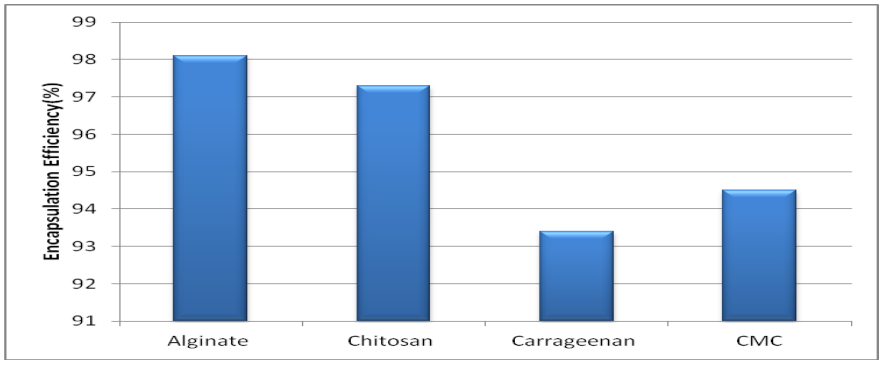

Figure 2: Encapsulation efficiency of fennel EO in some microencapsulated materials

Effect of Microencapsulation on Composition of the Essential Oils

The efficiency of encapsulation process for the main volatile compounds was evaluated by quantification calculation of the concentration of these compounds in the recovered essential from the microcapsules.

As shown in Figs 3 and 4 some changes in the percentages of the main compounds of cumin and fennel EOs were observed. Cumin EO encapsulated in CMC, Carrageenan and Alginate, comprised higher content of cumin aldehyde, P-cymene and γ-terpinene, respectively compared to their concentration in the initial EO (Fig 3). Also, the concentration of p-mentha-1, 3-diene-7-al in all the encapsulated oil samples was higher than that in the initial EO. On contrary, P-mentha1, 4-diene-7-al was encapsulated in lower relative concentration than in the initial EO.

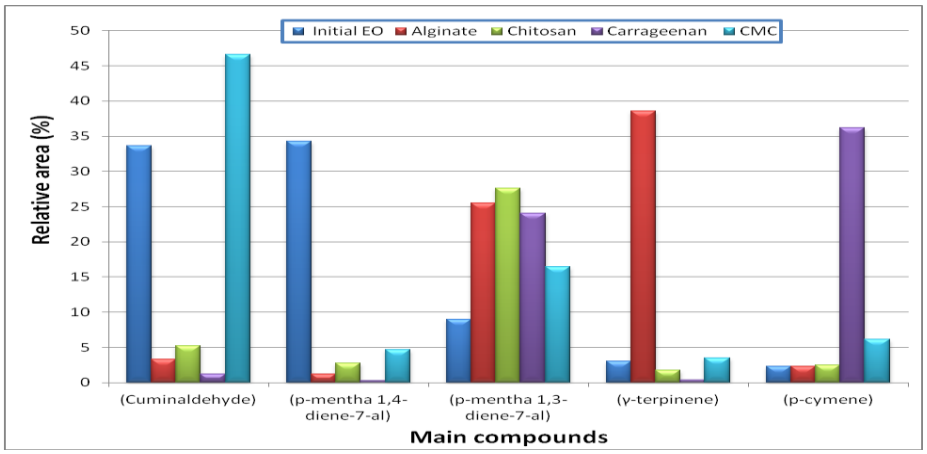

Figure 3: Main compounds percentage of Cumin essential oil before and after microencapsulation in alginate, chitosan, carrageenan and Carboxy methyl cellulose.

As shown in Fig 4 estragole, the main compound in fennel EO, showed decrease in encapsulated oil samples with chitosan, carrageenan and CMC compared to initial EO whereas, it showed no variation in encapsulated oil sample with alginate. Limonene was not detected or present in lower concentration in encapsulated oil samples compared to initial EO. While, the concentration of fenchone showed opposite trend. Also, Trans- anethole was encapsulated in lower relative concentration than in the initial EO.

Generally, the changes in the chemical composition of EOs after encapsulation may be due to different chemical structures, i.e. the properties of wall materials, the physicochemical properties of EOs components and binding capacity of the carrier materials with aroma components.

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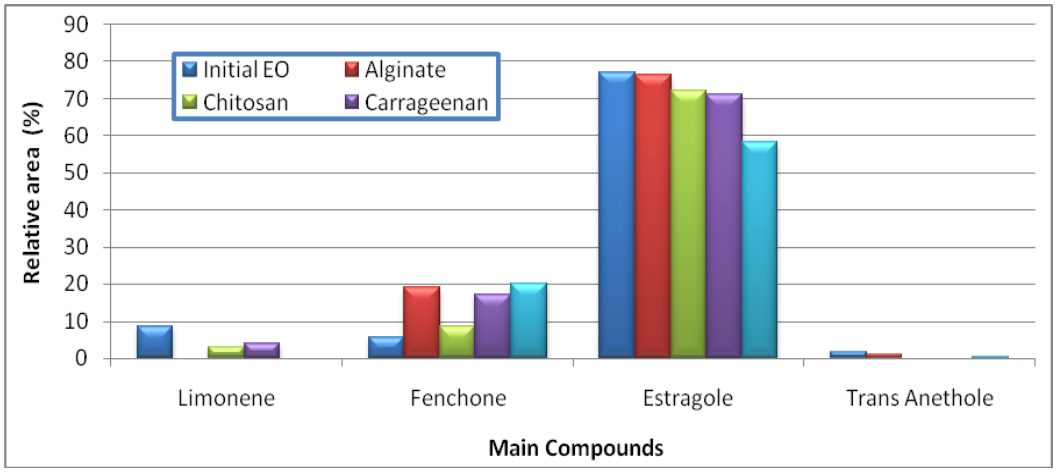

Figure 4: Main compounds percentage of Fennel essential oil before and after microencapsulation in alginate, chitosan, carrageenan and Carboxy methyl cellulose.

Antioxidant Activity and total phenolic contents of Cumin and Fennel essential oils:

Antioxidant activities of essential oil from aromatic plants are mainly attributed to their active compounds or to synergy among different oil constituents. Table 4 showed the antioxidant activity (DPPH) and total phenolic contents (Gallic acid) of cumin and fennel EOs. Results indicated that fennel EO characterized with its higher antioxidant activity (DPPH) reached to (78.87%) compared to cumin (74.50%) EO. Cumin EO had the highest total phenolic contents (276.15 µg GAE /g) compared to fennel EO (103.25 µg v GAE/g). The obtained results agreed with those reported by Foti et al. (1996), Muhammad et al (2013) and Meullemiestre et al (2014).

Table3: Antioxidant activity and total phenolic contents of cumin and fennel essential oils.

Essential oils	Antioxidant activity (DPPH %)	Phenolic contents (µg/g) as GAE
Cumin	$74.50^{b} \pm 0.707$	$276.15^{a} \pm 1.625$
Fennel	$78.87^{a} \pm 0.108$	$103.25^{b} \pm 0.918$
LSD at 0.05	1.048	6.406

Where: GAE= Gallic acid CE= Catechin

Effect of microencapsulation on antioxidant activity of cumin and fennel essential oils:

The effect of microencapsulation process on antioxidant activities and total phenolic contents of cumin and fennel EOs by using some selected polymers is clearly shown in Table 4.

As shown in Table 3 and 4 the antioxidant activities and the contents of total phenolic in the initial EOs were higher than those of the encapsulated oil samples. The results revealed that cumin EO that encapsulated in alginate had the highest scavenging effect on DPPH radical and total phenolic contents as compared to other encapsulated oil samples (Table 4). From the obtained results, fennel EO encapsulated in CMC exhibited higher antioxidant activity as compared with other encapsulated oil samples. Whereas, encapsulated essential oil in carrageenan had the highest contents of total phenolic.

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Table 4: Effect of microencapsulation on antioxidant activity and total phenolic contents of cumin and fennel essential oils.

Essential oils	Antioxidant activity (DPPH %)	Phenolic contents (µg/g) as GAE
	Capsulated cumin with	
CMC	35.0 ^{bc} ±0.14	130 ^{bc} ±0.52
Chitosan	32.5 ^{c} ±0.13	127 ^{c} ±0.51
Carrageenan	34.5 ^{b} ±0.14	133 ^{b} ±0.53
Alginate	36.6 ^{a} ±0.15	140 ^{a} ±0.57
	Capsulated fennel with	
CMC	18.7 ^{d} ±0.08	50 ^{e} ±0.20
Chitosan	17.5 ^{de}±0.07	53 ^{e} ±0.21
Carrageenan	17.0 ^{de}±0.04	58 ^{d} ±0.24
Alginate	16.2 ^{e} ± 0.07	51 ^{e} ±0.21
LSD at 0.05	1.731	3.462

Effect of storage for 6 months on antioxidant activities and total phenolic contents of encapsulated oil samples were studied. The results revealed that the encapsulated essential oils samples after 6 months exhibited slight decrease in the antioxidant activities, and total phenolic contents (Table 5).

Table 5: Effect of storage for 6 months on antioxidant activity and total phenolic contents of encapsulated oil samples.

Essential oils	Antioxidant activity (DPPH %)	Phenolic contents (µg/g) as GAE
	Capsulated cumin with:	
CMC	32.5^{a}±.0.13	120^{b}±0.48
Chitosan	30.0^{b}±0.12	115^{c}±0.46
Carrageenan	32.0^{ab}±0.13	122^{b}±0.49
Alginate	33.0^{a} ± 0.13	126 ^{a}± 0.49
	Capsulated Fennel with:	
CMC	17.5^{c}±0.07	49^{d} ± 0.20
Chitosan	16.0^{cd}±0.06	47^{d}±0.19
Carrageenan	15.5^{cd}±0.06	50^{d}±0.20
Alginate	15.0^{cd}±0.06	50^{d}±0.20
LSD at 0.05	2.948	3.462

CONCLUSION

Different carrier materials effect on the composition and antioxidant activities of cumin and fennel essential oils. The results revealed that cumin aldehyde, p-mentha 1, 4-diene-7-al, p-mentha 1, 3-diene-7-al, γ-terpinene and p- cymene were the major compounds in cumin EO. While estragole, limonene, fenchone and trans-anethole were the main components in fennel EO. The EOs that loaded with alginate beads has the highest microencapsulation efficiency. The changes in the concentration of compounds of volatile oils before and after encapsulation were observed. The antioxidant activity and the contents of total phenolic in the initial EOs were higher than those of the encapsulated oil samples. Storage of encapsulated oil samples for 6 months exhibited slight change in the antioxidant activity, and total phenolic contents.

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