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Chemical Composition and Antioxidant Activities of Microencapsulated Rosemary and Clove Essential Oils

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Abstract : This study aimed to evaluate the influence of encapsulation by using some carrier materials on chemical compositions and antioxidant activities of rosemary and clove essential oils. Hydrodistillation essential oils (EOs) of rosemary and clove buds were separated and identified. GC and GC-MS identified 24 and 20 components in rosemary and clove EOs representing 94.93 % and 98.02 % of the total rosemary and clove EOs, respectively. The main components of rosemary EO were 1, 8-cineole (30.88%) followed by camphor (22.71%), α-terpineol (15.01%), α- pinene (8.78%) and camphene (4.31%). The major compound in the volatile oil of clove buds were Eugenol (81.77%) followed by β-Caryophyllene (5.97%) and eugenol acetate (5.19%). The effect of microencapsulation with some carrier materials (alginate, chitosan, carragenaan and carboxymethyl cellulose) on the chemical composition and antioxidant activities of rosemary and clove EOs were studied. Chitosan was characterized with its higher efficiency for microcapsulated EOs compared to other carrier material. The change in the chemical classes of EOs was observed after encapsulation. Clove EO exhibited a higher antioxidant activity and total phenolic content than rosemary EO. Also, encapsulated clove EO in chitosan and rosemary EOs in alginate exhibited higher antioxidant activity and total phenolic content compared to other investigated encapsulated EOs. After storage for 6 months, all encapsulated EOs exhibited an increase in antioxidant activity and total phenolic content except those encapsulated in alginate and CMC, respectively.
Keywords: Clove essential oil; rosemary essential oil; antioxidant activity; GC-MS; microencapsulation.

Introduction

Clove is belongs to the genus of Syzygium, family Syzygieae. Clove (*Syzygium aromaticum*) is one of the richest sources of phenolic compounds such as eugenol, eugenol acetate and gallic acid and posse's great potential for pharmaceutical, cosmetic, food and agricultural applications[1]. Rosemary (*Rosmarinus officinalis* L) belonging to plant family Labiatae is very common spice and its oil is used in fragrance flavor industry aromatherapy[2]. Rosemary essential oil provides antioxidant activity[3,4] antimicrobial and antitumour properties[5, 6]. Microencapsulation of essential oils or flavours has been reported for offering protection and to reduce volatilization as well as degradation. Yet another objective of encapsulating essential oils or flavours was to design solid particulate forms of liquid core material[7, 8] and to provide longer shelf life as a raw material which has better handling characteristics and is compatible with dry ingredients. Extended shelf life of microencapsulated flavours is attributed to limited exposure to temperature, moisture, oxidation and light during storage and processing[9, 10, 11.12, 13].

The main objective of this paper was to study the influence of encapsulation by using some carrier materials (alginate, carrageenan, chitosan and carboxy methyl cellulose) on chemical compositions and antioxidant activities of rosemary and clove essential oils.

2. Materials & Methods

2.1. Materials

The plant materials of clove buds (*Syzygium aromaticum*) and rosemary (*Rosmarinus officinalis*) were obtained from production medicinal and aromatic plant unit at National Research Centre, Cairo, Egypt. Sodium alginate, chitosan low MW, carrageenan, carboxymethyl 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.

2.2. Methods

2.2.1. Extraction of essential oil

One hundred grams of clove buds or rosemary were subjected to hydrodistillation using Clevenger apparatus for 4 hr to isolate their essential oils. Essential oil samples were stored at 0°C in air-tight containers after drying them over anhydrous sodium sulfate and filtered before analysis by GC and GC-MS.

2.2.2 Encapsulation of Essential Oils

Microencapsulation of oil was conducted using emulsion extrusion technique described by Chan[14]. Sodium alginate, *K*- Carrageenan, Carboxy Methyl Cellulose (CMC) 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 hrs to disengage bubble before use. Afterwards, polymer solution (100 ml) and EO (1 ml) were homogenized into a 200 ml beaker with stirring at a speed of 300 rpm for 10 hrs by a 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 loaded oil 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.

2.2.2.1. Microencapsulation efficiency

Encapsulation efficiency (EE) was determined according to the method described by Voncina et al. [15]

2.2.3. Determination of Phenolic Content

Phenolic content of initial, encapsulated and stored encapsulated essential oils of clove and rosemary 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 hr using a rotary evaporator (Heidolph-Laborota, Germany) to obtain the methanolic 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[16]. 200µl of methanolic extracts of clove or rosemary samples 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, spectrophotometer. 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).

2.2.4. Determination of Antioxidant Activity

Antioxidant activity of initial, encapsulated and stored encapsulated of clove and rosemary essential oils were determined. The DPPH radical-scavenging assay was carried out as previously reported by Grzegorczyk et al. [17]. Various concentrations of ethanol and ethanol extracts of clove and rosemary (50, 100, 150, and 200 µg/ml) were added to 4 ml of 0.1 mM 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:

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

2.2.5. 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 column DB5 (60 m 0.32 mm i.d. 0.25 l m film thickness). The oven temperature was maintained initially at 50 °C for 5 min, 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 componentswere calculated with reference to the retention time of aseries of alkanes (C6–C20) as external standard run at the same conditions.

2.2.6. 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[18].

2.2.7. Statistical analysis

The obtained results were evaluated statistically using the analysis of variance as reported by McClave & Benson [19].

3. Result and discussion

3.1 Effect of Microencapsulation on Composition of the Essential Oils

Volatile compounds of rosemary essential oil (EO) before and after microencapsulation with some carrier materials were identified and presented in Table 1. Twenty four components were identified in rosemary EO, representing 94.93% of total yield. The major compounds were 1, 8-cineole (30.88%) followed by camphor (22.71%), α- terpineol (15.01%), α- pinene (8.78%) and camphene (4.31%). Yang et al. [20] and Gharib & Teixeira da Silva [21] reported that 1, 8-cineol was the major compounds in rosemary EO. But in another study, α-pinene was the major compound followed by camphor and 1, 8-cineol[22, 23]. On the other hand, Boutekedjiret et al [24] and Benhabiles et al. [25] found that the major compounds of *Rosmarinus officinalis* EO that collected in Algeria were camphor, borneol, α-terpineol, bornyl acetate, β-caryophyllene, δ-candinene, muurolene and α-humulene. Differences in the volatile components could be related to climate and seasonal factors, origin and method of distillation. The antioxidant activity of rosemary EO may be due to the considerable concentration of 1, 8-cineol, camphor, α-pinene[21]. Wang et al.[26] reported that in addition to the major compounds, minor compounds may also contribute significantly to the activity of rosemary EO.

As shown in Table 1 oxygenated monoterpenes were the predominant chemical class followed by monoterpenes , sesquiterpenes, esters , phenyl propanoid and oxygenated sesquiterpene in initial and microencapsulated EOs. Total yield of monoterpenes showed remarkable decrease in all carrier materials of encapsulated rosemary EOs. While, the high content of oxygenated monoterpenes was noticed in all encapsulated oil samples, except which encapsulated with carrageenan. On the other hand, encapsulated EO with carrageenan showed the highest content of sesquiterpenes, oxygenated sesquiterpenes, phenyl propanoids and esters.

Table 1. Effect of microencapsulation rosemary essential oil with some carrier materials on its chemical constituents.

No	KI[a]	Compounds[b]	Relative area percentages(%)[c]				
			Initial EO	Encapsulated rosemary essential with			
				Alginate	Chitosan	Carrageenan	CMC
1	925	α-Thujene	0.14	--	--	--	--
2	936	α-Pinene	8.78	0.23	0.38	1.74	0.06
3	953	Camphene	4.31	1.00	1.75	0.94	--
4	979	β-Pinene	1.16	--	0.44	0.94	0.43
5	988	β-Myrcene	1.02	--	--	--	--
6	1005	α-Phellandrene	0.19	--	--	0.28	--
7	1018	α-Terpinene	0.29	--	0.06	0.31	0.11
8	1035	1,8-Cineole	30.88	12.29	8.61	10.24	26.49
9	1060	γ-Terpinene	0.31	0.37	0.11	--	--
10	1091	Terpinolene	0.35	0.27	0.21	0.34	0.35
11	1108	Linalool	1.71	3.10	2.92	0.83	0.22
12	1155	Camphor	22.71	24.24	20.97	7.84	30.01
13	1169	Borneol	0.19	--	0.24	0.76	2.05
14	1180	Terpinen-4-ol	1.31	26.77	29.99	2.95	19.27
15	1187	ρ-Cymen-8-ol	1.83	--	--	--	--
16	1203	α-Terpineol	15.01	19.33	19.98	30.02	11.90
17	1288	Bornyl acetate	1.94	1.49	2.34	10.76	--
18	1292	Thymol	0.46	1.91	2.55	2.92	0.27
19	1347	α-Cubebene	0.17	--	--	--	--
20	1360	Piperitenone	0.16	--	--	9.51	--
21	1375	α-Copaene	0.12	1.67	0.09	--	1.34
22	1427	β-Caryophyllene	1.39	0.33	0.93	6.32	--
23	1460	α-Humulene	0.11	--	0.84	--	--
24	1591	Caryophyllene oxide	0.39	0.21	0.81	6.52	0.72
		Monoterpenes	16.55	1.01	2.92	4.55	0.95
		Oxygenated monoterpenes	73.79	85.73	82.71	62.15	89.94
		Sesquiterpenes	1.79	2.0	1.86	6.32	1.34
		Oxygenated sesquiterpenes	0.39	0.21	0.81	6.52	0.72
		Phenyl propanoids	0.46	1.91	2.55	2.92	0.27
		Esters	1.94	1.49	2.34	10.76	--

[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.

--:not detected

Table 2. Effect of microencapsulation clove essential oil with some carrier materials on its chemical constituents.

No	KI[a]	Compounds[b]	Relative area percentages (%)[c]				
			Initial EO	Encapsulated clove essential with			
				Alginate	Chitosan	Carrageenan	CMC
1	1198	Dihydrocarvone	0.23	--	0.17	0.1	--
2	1238	Carvone	0.08	--	--	0.03	--
3	1258	P-Allylphenol	0.17	0.30	--	0.14	1.07
4	1267	(E) Geraniol	0.19	--	--	--	1.07
5	1289	Isobornyl acetate	0.06	--	--	0.03	--
6	1308	Carvacrol	0.10	--	--	--	--
7	1343	α-Cubebene	0.14	--	--	--	--
8	1367	Eugenol	81.77	88.36	95.6	89.47	58.43
9	1388	α-Elemene	1.63	--	--	--	--
10	1399	Methyleugenol	0.46	--	--	--	1.35
11	1411	α-Santalene	0.24	0.05	--	--	--
12	1426	β-Caryophyllene	5.97	0.07	1.16	0.19	--
13	1433	γ-Elemene	0.14	--	--	--	--
14	1442	Aromadendrene	0.11	0.10	--	--	--
15	1453	α-Humulene	1.01	--	--	0.06	--
16	1471	β –Santalene	0.05	0.05	--	--	--
17	1480	Curcumene	0.17	--	--	0.18	--
18	1499	Bicyclogermacrene	0.25	--	--	--	--
19	1521	Eugenol acetate	5.19	9.17	0.67	7.92	35.62
20	1578	Caryophyllene oxide	0.21	0.05	0.38	--	0.63
		Monoterpenes	0.31	--	0.17	0.04	--
		Oxygenated monoterpenes	0.19	--	--	--	1.07
		Sesquiterpenes	9.7	0.27	1.16	0.42	--
		Oxygenated sesquiterpenes	0.21	0.05	0.38	--	0.63
		Phenyl propanoids	82.5	88.66	95.6	89.62	60.85
		Esters	0.06	9.17	0.67	7.95	35.62

[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.

Volatile compounds of clove EO and their microencapsulated samples in alginate, chitosan, carrageenan and CMC were identified and presented in Table 2. Twenty components were identified in clove EO, representing 98.02% of the total clove EO. Eugenol (81.77%) was the major compound followed by β-Caryophyllene (5.97%) and eugenol acetate (5.19%).These results are in accord with previous literature [27, 28, 29]. Lee and Shibamoto [30] found that eugenol and eugenol acetate were the main antioxidant ingredients in clove EO. It is worth to be mentioned that, β-caryophyllene characterized with its anti-inflammatory activity as stated by Ghelardini et al.[31]. Many authors reported that the high level of eugenol contained in clove EO give it strong biological activity and is used traditionally as flavouring agent and antimicrobial material in food[32,33,34,35].

Phenyl propanoids were the dominant chemical classes in clove essential oil followed by sesquiterpenes , monoterpenes , oxygentated sequiterpene , oxygenated monoterpene and esters (Table 2). On other word, a decreasing in monoterpenes, oxygenated monoterpenes and sesquiterpenes in all encapsulated oil samples were observed. However, an increasing in the content of phenyl propanoids occurred in different selected carrier materials of encapsulated clove EO except in case of using CMC. Also, esters showed significant increase in all kinds of encapsulated clove EO. Furthermore, oxygenated sesquiterpenes were increased in case of using carrageenan encapsulated EO compared to other carrier materials. The change in the

concentrations of the EO composition among the encapsulated clove EO may be correlated to the interaction between the components of EO and carrier materials.

3.2. Efficiency of Micro-encapsulation (EE %)

The efficiency of microencapsulation rosemary and clove EOs by using some carrier materials was determined and graphically represented in Figs 1 and 2. From the obtained figures it could be noticed that, the highest encapsulation efficiency for both rosemary and clove EOs was found in case of encapsulation with chitosan, followed with alginate. While, the lowest value was happen in the encapsulated EOs with CMC. These differences in the encapsulation efficiency may be due to the change in physicochemical properties of the essential oil [36] .

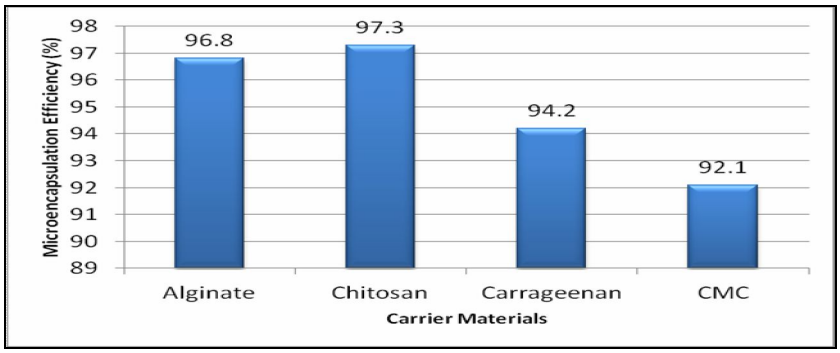

Figure 1: Efficiency of microencapsulation rosemary essential oil with some carrier materials.

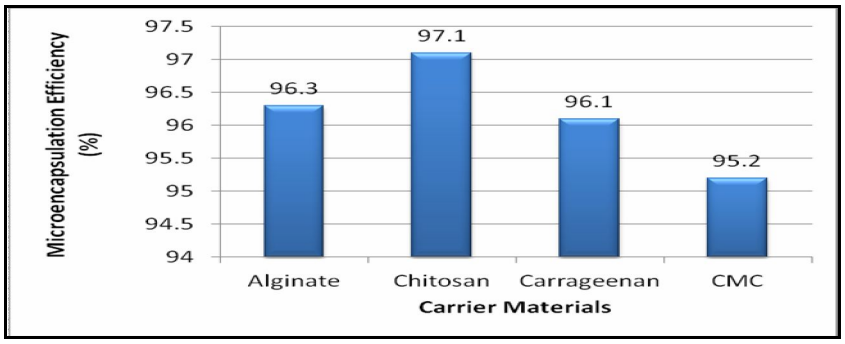

Figure 2: Efficiency of microencapsulation clove essential oil with some carrier materials.

3.3 Antioxidant Activity and total phenolics content of Clove and Rosemary Essential Oils

Antioxidant activity and total phenolic contents of clove and rosemary EOs were evaluated. Antioxidant activity of clove and rosemary EOs was assessed by DPPH assay and presented in Table 3. DPPH radical scavenging activity and total phenolic content was higher in EO of clove than rosemary, where they reached in clove EO to 89.24% and 425.5 µg/ g, while rosemary EO reached to 80.20 % and 390.1 µg/ g as Gallic acid respectively.

Table 3: Antioxidant activity (DPPH) and total phenolic contents (Gallic acid) of clove and rosemary essential oils.

Essential oils	Antioxidant activity (DPPH%)	Phenolic contents (µg/g) as GAE
Clove	$89.24^{a} \pm 0.103$	$452.5^{a} \pm 2.64$
Rosemary	$80.20^{b} \pm 0.248$	$390.10^{b} \pm 1.26$
LSD at 0.05	1.97	2.23

Where: GAE= Gallic acid

3.4 Effect of microencapsulation EO on antioxidant activity and total phenolic contents

Effects of microencapsulation clove and rosemary EOs with some carrier materials (alginate, chitosan, Carrageenan and CMC) on antioxidant activity and total phenolic contents were evaluated. Table 4 proved that the encapsulated clove EO in chitosan had the highest antioxidant activity (40%) and total phenolic content

(195µg/g as GAE) compared to encapsulated clove EOs in alginate, carrageenan and CMC. Also, encapsulated clove EO characterized with its higher antioxidant activity and total phenolic contents compared to the same carrier material in rosemary. While, the highest antioxidant activity (34.8 %) and total phenolic content (180 µg/ g as GAE) in encapsulated rosemary EO were found in alginate compared to other carrier materials of the same EO.

Table 4: Effect of microencapsulation of clove and rosemary essential oils with some carrier materials on antioxidant activity (DPPH) and total phenolic contents (Gallic acid).

Essential oils	Antioxidant activity (DPPH%)	Phenolic contents (µg/g as GAE)
Capsulated clove with		
CMC	35.5 ^{cd} ±0.82	178.5^{c} ±1.65
Chitosan	40.0^{a}±0.96	195.0 ^{a}±2.06
Carrageenan	36.5 ^{c} ±0.65	175.0 ^{d} ±1.53
Alginate	38.0 ^{b} ±0.86	185.5 ^{b} ±1.72
Capsulated rosemary with		
CMC	29.5 ^{f}±0.79	168.0 ^{e} ±1.13
Chitosan	32.8 ^{e}±0.56	174.0 ^{d} ±1.29
Carrageenan	31.6 ^{e} ±0.69	168.0 ^{e} ±1.34
Alginate	34.8 ^{d} ±0.76	180.0 ^{c} ±1.19
LSD at 0.05	1.47	1.79

3.4 Effect of storing microencapsulated EO for 6 months on antioxidant activity and total phenolic contents

The effect of storing microencapsulated clove and rosemary EOs for 6 months at room temperature on antioxidant activity (DPPH) and total phenolic contents (Gallic acid) were evaluated. As shown in Table 5 after storage for 6 months, all encapsulated EOs oils exhibited an increase in antioxidant activity and total phenolic content except that encapsulated rosemary and clove EOs in alginate and CMC matrixes compared to the same sample before storage (Table 4), respectively. The increase in antioxidant activity could be related to changes that occurred in concentration of EO components during storage.

Table 5: Effect of storage for 6 months on antioxidant activity (DPPH) and total phenolic contents (Gallic acid) of encapsulated essential oils.

Essential oils	Antioxidant activity (DPPH%)	Phenolic contents (µg/g) asGAE
Capsulated Clove with:		
CMC	36.5^{c}±0.77	185.0^{d}±2.18
Chitosan	40.0^{a}±0.83	202.5^{b}±1.85
Carrageenan	37.5b^{c}±0.89	200.0^{c}±1.72
Alginate	40.0^{a}±0.96	205.0 ^{a}±1.46
Capsulated Rosemary with:		
CMC	32.5^{d} ±0.52	172.5^{g}±1.65
Chitosan	38.5^{ab}±0.91	180.0^{e}±1.39
Carrageenan	34.0^{d}±0.82	170.0^{g}±1.70
Alginate	36.0^{c}±0.71	175.0 ^{f}±1.86
LSD at 0.05	1.69	1.74

Conclusion

The GC and GC-MS analysis revealed that 1, 8-cineole (30.88%) was the major compounds in rosemary essential oil followed by camphor (22.71%), α- terpineol (15.01%), α- pinene (8.78%) and camphene (4.31%). Whereas, the major compound in the volatile oil of clove were Eugenol (81.77%) followed by β-Caryophyllene (5.97%) and eugenol acetate (5.19%). The chemical composition concentration and antioxidant activity of essential oils were varied a as affected by microencapsulation process. Antioxidant activity and total phenolic content of encapsulated rosemary and clove EOs not declined but increased after storage for 6 months. Therefore, microencapsulation process prevent volatile compound of essential oils that responsible to antioxidant activity during storage for 6 months.

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