



American Journal of
Food Technology

ISSN 1557-4571



Academic
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Research Article

Influence of Nano-encapsulation on Chemical Composition, Antioxidant Activity and Thermal Stability of Rosemary Essential Oil

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Abstract

Background: Herbs have been used for several purposes i.e., flavorings, beverages, repellents, fragrances, cosmetics and for their medicinal properties. Now a days, the interest in herbs has considerably increased, particularly as a natural source of flavor and antioxidants for the food and pharmaceutical industries. The quality and acceptability of food are related to flavor stability. It is well known that, manufacturing and storage processes, package materials and ingredient of foods often reduce aroma compound intensity. In order to minimize aroma degradation or loss during processing and storage, it is beneficial to encapsulate volatile ingredients prior to use in foods or beverages. **Objective:** This study aimed to investigate the effect of using different carrier materials on chemical composition, thermal stability and antioxidant activity of nano-encapsulated rosemary essential oil. **Methodology:** About 100 g of rosemary was subjected to hydro-distillation using Clevenger apparatus for 4 h to isolate its essential oil. Essential oil sample was analysis by GC-FID and GC-MS. The nano-encapsulation was procedure by homogenization technique in all samples. Encapsulation Efficiency (EE), Transmission Electron Microscopy (TEM), DPPH scavenging activity and total phenolic content of encapsulated essential oil samples were evaluated. Thermal stability assessment was done by Differential Scanning Calorimetric (DSC). **Results:** The results revealed that the highest release of total phenol (3349.4 $\mu\text{g GAE mL}^{-1}$) and DPPH scavenging activity of rosemary EO were found in case of using chitosan as carrier material for nano-encapsulation process compared to CMC or sodium alginate. Also, the nano-encapsulation process improved thermal stability of rosemary EO. The TEM of nano-encapsulated of rosemary EO in CMC showed the lowest nano-particle size (10-20 nm) compared with chitosan or sodium alginate. After the GC-MS analysis of the volatile compounds of rosemary EO revealed that 1,8-cineole (30.88%), camphor (22.71%), α -terpineol (15.01%), α -pinene (8.78%) and camphene (4.31%) were the major compounds in rosemary EO. The results showed an increasing content of oxygenated monoterpenes, sesquiterpenes, sesquiterpene oxide and ester in nano-encapsulated rosemary EO samples. An opposite behavior was observed in content of monoterpenes. **Conclusion:** In this study, the major components of rosemary EO were 1,8-cineole (30.88%), camphor (22.71%), α -terpineol (15.01%), α -pinene (8.78%) and camphene (4.31%). The type of wall materials has influence on the particles size and encapsulation efficiency and antioxidant activity of rosemary EO. The results indicated that nano-capsulation process increased the thermal stability of rosemary EO and could be useful as antioxidant for various thermal processing applications in industry.

Key words: Nano-encapsulation, rosemary essential oil, encapsulation efficiency, antioxidant activity, thermal stability

Received: October 11, 2016

Accepted: February 28, 2017

Published: April 15, 2017

Citation: Ahmed M.S. Hussein, Mohie M. Kamil, Shereen N. Lotfy, Khaled F. Mahmoud, Fathy M. Mehaya and Ayman A. Mohammad, 2017. Influence of nano-encapsulation on chemical composition, antioxidant activity and thermal stability of rosemary essential oil. *Am. J. Food Technol.*, 12: 170-177.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Since prehistoric times, herbs have been used for several purposes i.e., flavorings, beverages, repellents, fragrances, cosmetics and for their medicinal properties. Now a days, the interest in herbs has considerably increased, particularly as a natural source of flavor and antioxidants for the food and pharmaceutical industries.

The quality and acceptability of food are related to flavor stability. It is well known that, manufacturing and storage processes, package materials and ingredient of foods often reduce aroma compound intensity¹. In order to minimize aroma degradation or loss during processing and storage, it is beneficial to encapsulate volatile ingredients prior to use in foods or beverages².

Essential oils have the effect of an antioxidant but under inappropriate conditions may be loss the activity this is due to occurrence of demolition and degradation of essential oils. The encapsulation of essential oils nano-metric size capsules are used for the protection of the active components against environmental factors (e.g., oxygen, light, moisture and pH). But the effect of encapsulation of essential oil in nano-size on the chemical composition and antioxidant activity is limited. Encapsulation in nano-particles may increase the cellular absorption mechanisms and increasing bio-efficacy. Polymeric nano-carriers are classified as nano-capsules and nano-spheres. Nano-capsules consist of a polymeric wall and a core, which is commonly oily. Nano-spheres are matrix systems³. Different encapsulating materials i.e., biopolymers, like proteins and hydro-colloids, chitosan and sodium alginate were used in several applications. The release of EOs from carriers occurs through one of the following processes: Dissolution, desorption of the surface-bound/adsorbed functional ingredient, diffusion through the matrix, matrix erosion including enzyme degradation and a combination of these processes³.

Rosemary (*Rosmarinus officinalis* L.) is the most used and economically important aromatic and medicinal plant for its essential oil and phenolic compounds⁴⁻⁷. Antioxidant activity of rosemary extracts depends on their composition. Several investigators⁸⁻¹⁰ found that rosemary contains diterpenes, such as carnosic acid and carnosol that characterized with its antioxidant activity. Also, several flavonoids and phenolic compounds, such as hispidulin, cirsimaritin, apigenin, genkwanin, naringin, caffeic acid and rosmarinic acid were found in rosemary extracts^{5,6}.

Therefore, this study aimed to investigate the effect of using different carrier materials on chemical composition, thermal stability and antioxidant activity of nano-encapsulated rosemary essential oil.

MATERIALS AND METHODS

Materials: The plant material of rosemary (*Rosmarinus officinalis*) was obtained from production medicinal and aromatic plant unit at National Research Centre, Cairo, Egypt. Sodium alginate, chitosan low MW, carboxymethyl cellulose (CMC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Co. (St. Louis, Mo, USA). 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.

Methods: Extraction of essential oil: One hundred grams of rosemary was subjected to hydro-distillation using Clevenger apparatus for 4 h to isolate its essential oil. Essential oil sample was stored at 0°C in air-tight containers after drying over anhydrous sodium sulfate and filtered before analysis by GC-FID and GC-MS.

Encapsulation of essential oils: Nano-encapsulation of rosemary Essential Oil (EO) was conducted using homogenization technique as described by Donsi *et al.*¹¹ with some modifications. Where, polymer (sodium alginate or CMC or chitosan) was dissolved in distilled water or 1% solutions of acetic acid to produce polymer solutions with concentration of 2 w/v%, the solutions were left standing for 24 h to disengage bubble before use. Afterwards, polymer solution (10 g) and rosemary essential oil 0.4720 µL was primary emulsions by homogenized into a 250 mL beaker with stirring at a speed of 18000 rpm for 15 min. The EO was gradually added to the polymer solution during mixing until the desired EO loading was obtained. Crystallization of the essential oil droplets was attained by rapid cooling of the hot nano-emulsions in an ice-bath at the end of the homogenization processing.

Evaluation of DPPH scavenging activity: For evaluation of the antioxidant activity of initial EO and its extract from capsule, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method was used¹² with slight modifications. The assay was carried out by mixing 2 mL methanolic solution of the rosemary essential oil with 2.0 mL of a 0.02 mM methanolic DPPH solution at different concentrations (10, 15, 20, 25 and 30 µg mL⁻¹ essential oil). The mixture was then incubated in the dark for 30 min at 25°C and the absorbance at 517 nm was recorded as (A_{sample}), using spectrophotometer (Shimadzu UV-VIS 160 A). A blank experiment was also carried out applying the same procedure

to a solution without rosemary essential oil and the absorbance was recorded as (A_{blank}). The free radical scavenging activity of each solution was then calculated as percent inhibition according to the following Eq.1:

$$\text{Inhibition (\%)} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100 \quad (1)$$

Determination of the total phenolic content: The total phenolic content of initial EO and its extract from capsule were performed by spectrophotometry¹³. The reaction mixture was prepared by mixing 0.2 mL of the oil ethanol extract (1 mg mL⁻¹) with 1 mL of 10% Folin and Ciocalteu's reagent dissolved in water. After 6 min, 0.8 mL of 7.5% Na₂CO₃ was added. The blank contained distilled water instead of the extract. Absorbance was recorded at 740 nm after 2 h incubation at room temperature. The same procedure was repeated for the standard solution of gallic acid (GAE). The phenolic content in the samples was calculated from the standard curve and expressed as mg GAE g⁻¹ of extract, averaged from three measurements.

Encapsulation Efficiency (EE): Encapsulation Efficiency (EE) was determined according to the method described by Bae and Lee¹⁴. Fifteen milliliters of hexane were added to 1.5 g of rosemary encapsulated in a glass jar with a lid, which was shaken for 2 min at room temperature by hand to extract free EO. The obtained solvent mixture was filtered through a Whatman filter study No. 1 and the collected powder on the filter study was rinsed 3 times with 20 mL hexane. The obtained solvent was left to evaporate at room temperature and then evaporated at 60°C to reach constant weight. The free EO (non-encapsulated EO) was determined by mass difference between the initial flask and that containing the extract of EO residue¹⁵. Total oil was assumed to be equal to the initial oil, since preliminary tests revealed that all the initial oil was retained. Encapsulation Efficiency (EE) was calculated from Eq. 2:

$$\text{EE (\%)} = \frac{T_{\text{EO}} - F_{\text{EO}}}{T_{\text{EO}}} \times 100 \quad (2)$$

where, T_{EO} is the total EO content and F_{EO} is the free EO content.

Transmission electron microscopy (TEM): Morphology samples of rosemary EO encapsulated in chitosan, sodium alginate and CMC was examined by TEM. About 20 µL of diluted samples was placed on a film-coated 200 mesh copper

specimen grid for 10 min and the fluid excess was eliminated using filter study. The grid was then stained with one drop of 3% phosphotungstic acid and allowed to dry for 3 min. The coated grid was dried and examined under the TEM microscope. The samples were observed by operating¹⁶ at 160 kV.

Thermal stability assessment by Differential Scanning

Calorimetric (DSC): The DSC essential oil profile was determined using a differential scanning calorimeter, model 823E from Mettler Toledo. Ten milligrams samples were placed in aluminium crucibles. The samples were analyzed under a flow of nitrogen gas (40 mL min⁻¹). A dynamic scan was performed at a heating rate of 10°C min⁻¹ over a temperature range of 0-300°C. Evaporation enthalpies were calculated by peak area integration of DSC profiles and the results were compared with the estimated vaporization enthalpy of essential oils major components. The DSC data have an important role in determining reaction order, according to Hazra *et al.*^{17,18}.

Gas chromatographic (GC-FID) analysis: The 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 µm film thickness). The oven temperature was maintained initially at 50°C for 5 min, then programmed from 50-250°C at a rate of 4°C min⁻¹. Helium was used as the carrier gas, at flow rate of 1.1 mL min⁻¹. The sample size was 2 µL, split ratio 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. 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 data¹⁹.

Statistical analysis: All measurements were carried out in triplicate and expressed as the average of 3 measurements standard deviation. Calculations and construction of curves were performed using MS Office Excel, 2007.

Table 1: Effect of nano-encapsulation using CMC (CMC-EEO), chitosan (CH-EEO) and sodium alginate (A-EEO) on total phenol and antioxidant (DPPH) of rosemary essential oil (EO)

Parameters	Encapsulation in carriers (EEO) ($\mu\text{g mL}^{-1}$)									
	Initial EO ($\mu\text{g mL}^{-1}$)	CMC (CMC-EEO)			Chitosan (CH-EEO)			Sodium alginate (A-EEO)		
		Release ($\mu\text{g mL}^{-1}$)	(%)	EE (%)	Release ($\mu\text{g mL}^{-1}$)	(%)	EE (%)	Release ($\mu\text{g mL}^{-1}$)	(%)	EE (%)
Total phenol	6075.00	2020.6	33.26	80.59	3349.4	55.13	79.55	2555.6	42.06	70.71
DPPH	17224.08	5650.3	32.80	41.67	6094.1	35.38	36.49	6087.4	35.34	45.46

Total phenol content is expressed as $\mu\text{g GAE mL}^{-1}$ extract

RESULTS AND DISCUSSION

Total phenolic content and DPPH of nano-encapsulated rosemary essential oil: The effect of nano-encapsulation on total phenol and antioxidant (DPPH) of rosemary EO are clearly shown in Table 1. The obtained results indicated that initial rosemary EO characterized with its high total phenol ($6075 \mu\text{g mL}^{-1}$) and DPPH ($17224.08 \mu\text{g mL}^{-1}$). The release of rosemary EO from nano-encapsulated was showed in Table 1. Controlling and extension time for release of EO from nano-encapsulated is important to increase their useful life to knowing whether or not oil is present inside the nanoencapsules. The obtained results showed that carrier material of nano-encapsulation process was affected on release of EO, where total phenol after nano-encapsulation using CMC, chitosan and sodium alginate reached to 2020.6, 3349.4 and 2555.6 $\mu\text{g GAE mL}^{-1}$, respectively.

Essential oil of rosemary (EO) has been encapsulated into chitosan (CH-EEO), sodium alginate (A-EEO) and CMC (CMC-EEO) and the results of encapsulation efficiency were showed in Table 1. Encapsulation efficiency (EE%) reflects the real amount of rosemary EO that is encapsulated inside the wall materials. The results exhibited that encapsulated essential oil in CMC has the highest encapsulation efficiency (80.59%) for total phenol content and followed by encapsulated oil in chitosan (79.55%) and encapsulated oil in sodium alginate (70.71%). While, in DPPH radical scavenging activity were 45.46, 41.67 and 36.49% for A-EEO, CMC-EEO and CH-EEO, respectively. The increase in encapsulation efficiency can be explained by increase of surface area due to the smallest particle size and the type of wall material²⁰.

The DPPH values of the ethanol extracts of initial EO and its encapsulated essential oils (EEOs) in CMC, chitosan and alginate are presented in Table 1. As can be seen, the initial EO had the strongest activity against the DPPH radical ($17224.08 \mu\text{g mL}^{-1}$) followed by encapsulated EO in chitosan ($6094.1 \mu\text{g mL}^{-1}$), sodium alginate ($6087.4 \mu\text{g mL}^{-1}$) and CMC ($5650.3 \mu\text{g mL}^{-1}$). Therefore, it could be recommended using chitosan as a carrier material for nano-encapsulation

process of rosemary EO, where its release was higher in total phenol ($3349.4 \mu\text{g GAE mL}^{-1}$) and against the DPPH radical ($6094.1 \mu\text{g mL}^{-1}$).

Table 1 shows the EE% of encapsulated rosemary EO in chitosan for DPPH radical scavenging activity was 36.49%. This result revealed that CH-EEO has the lowest antioxidant capacity compared to CMC-EEO or A-EEO.

Transmission Electron Microscopy (TEM) of rosemary essential oil nano-encapsulated: The images obtained by SEM were used to observe the external morphology and the average size of nano-capsules. According to Fig. 1, Transmission Electron Microscopy (TEM) showed that the nano-particles of nano-encapsulated rosemary EO in chitosan are composed of a core phase entrapped in a shell material of a fairly constant thickness. Nano-capsules appear to be made up of spherical particles of about 37.51-58.60 nm in diameter. The external surface of each particle is almost regular and smooth, showing that chitosan polymer forms a continuous film surrounding the essential oil droplets. Rosemary oil nano-capsule TEM analysis showed homogeneous and spherical particles with similar diameters as determined by photon correlation spectroscopy.

Figure 2 shows that nano-capsule of rosemary EO in sodium alginate appeared to be made up of single spherical units was in the range of 34.39-57.27 nm diameter. The external surface of each unit was almost regular and smooth, showing that the polymer of sodium alginate forms a continuous film surrounding the rosemary EO droplets.

In Fig. 3, Transmission Electron Microscopy (TEM) showed that the nano-particles of rosemary EO capsulated in CMC are composed of a core phase entrapped in a shell material of a fairly constant thickness. Nano-capsules appear to be made up of spherical particles of about 10-20 nm in diameter.

The DSC of rosemary essential oil and its nano-encapsulated in different polymers: As the industrial usage of essential oils increase, the knowledge of thermal profile of such systems becomes fundamental, especially for product design.

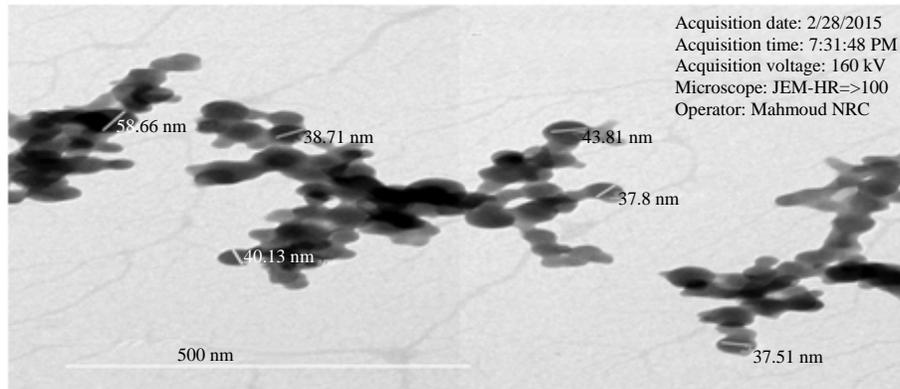


Fig. 1: Transmission Electron Microscopy (TEM) image of rosemary essential oil nano-encapsulated in chitosan

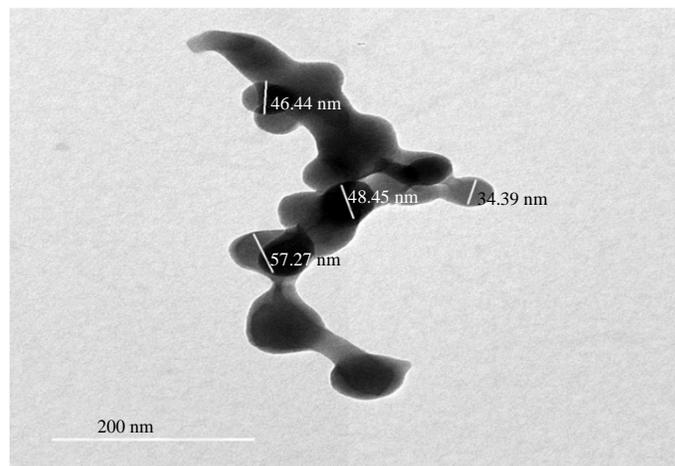


Fig. 2: Transmission Electron Microscopy (TEM) image of rosemary essential oil nano-encapsulated in sodium alginate

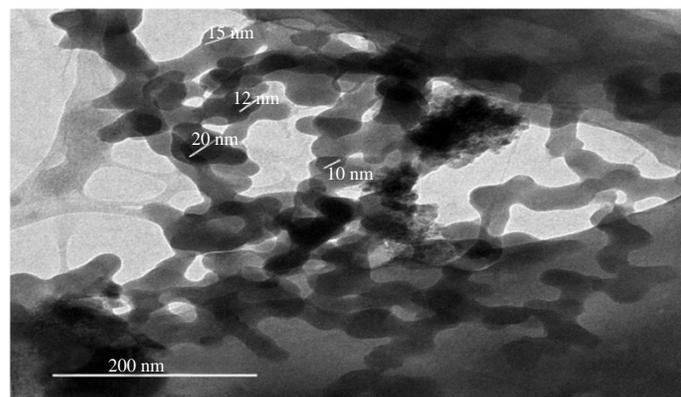


Fig. 3: Transmission Electron Microscopy (TEM) image of rosemary essential oil nano-encapsulated in CMC

The DSC curve profiles of initial rosemary essential oil and its oil nano-encapsulated in CMC, Chitosan and sodium alginate are presented in Fig. 4-7, respectively. Figure 4 shows the rosemary initial EO showed endothermic peaks

related to the process of evaporation at 47.03°C. This temperature is attributed to the decomposition temperature. Nano-encapsulated oil samples showed endothermic peaks related to the process of evaporation at 70, 84.05 and

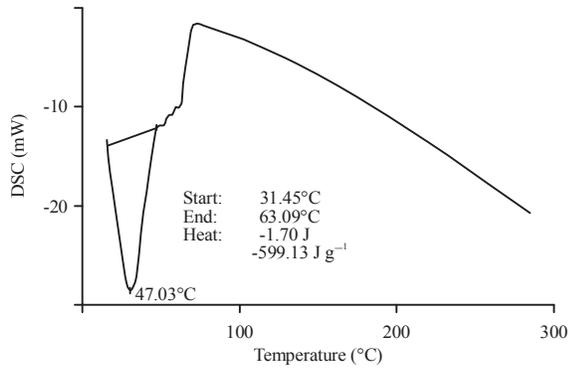


Fig. 4: Rosemary initial essential oil

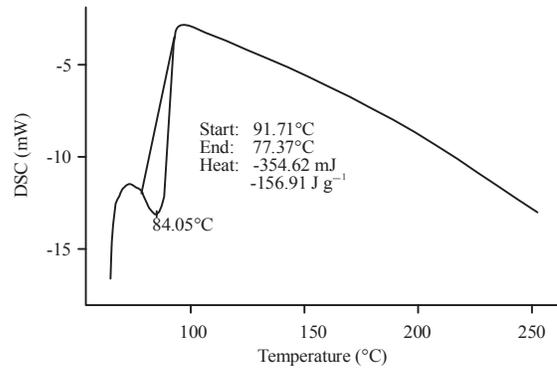


Fig. 6: Rosemary essential oil nano-encapsulated in chitosan

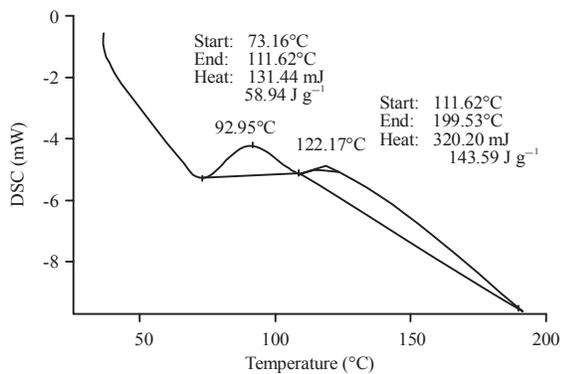


Fig. 5: Rosemary essential oil nano-encapsulated in CMC

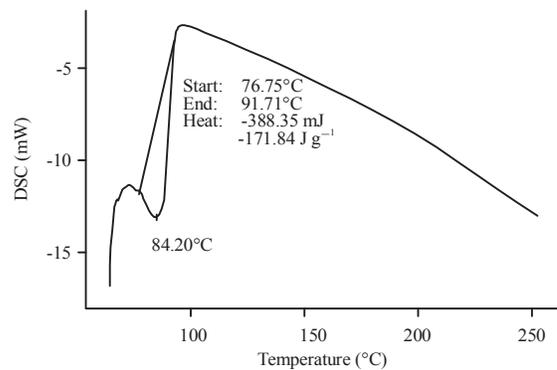


Fig. 7: Rosemary oil nanoencapsulated in sodium alginate

84.20°C in CMC, chitosan and sodium alginate, respectively. Comparing the calorimetric curve of initial oil with nano-encapsulated oil samples, it was observed that the nano-encapsulation process of the rosemary EO influences thermal stability performance.

Chemical composition of initial and nano-encapsulated rosemary essential oil:

The identified volatile compounds of rosemary EO before and after nano-encapsulation are listed in Table 2 according to their elution order on DB5 column. About 24 components were identified, representing 94.93%. Among of identified compounds were monoterpenes, oxygenated monoterpenes, sesquiterpenes, sesquiterpene oxide, phenyl propanoid and ester. Table 2 shows the major constituents being, 1,8-cineole (30.88%), camphor (22.71%), α -terpineol (15.01%), α -pinene (8.78%) and camphene (4.31%). These results are agreement with those of literature data but at different concentration of individual components²¹⁻²³. But in another study, the major components were camphor, borneol, α -terpineol, bornyl acetate, β -caryophyllene, δ -cadinene, muurolene and α -humulene²⁴. Gharib and da Silva²⁵ reported that the antioxidant activity of

rosemary EO may be due to the considerable concentration of 1,8-cineol, camphor and α -pinene.

It can be also observed that changes in the major compounds of rosemary EO occurred after nano-encapsulation. The results revealed that α -pinene and camphene were reduced in nano-encapsulated essential oil samples compared to initial EO. On contrary, camphor was encapsulated in higher concentration than in initial EO. Whereas, 1,8-cineol and α -terpineol encapsulated into chitosan and sodium alginate, respectively were in higher concentration than in initial EO. The changes may be related to conjugated essential oil with the polymers.

Figure 8 shows some changes in the percentages of main chemical classes of rosemary EO before and after nano-encapsulation were observed. For instance, the content of monoterpenes in all nano-encapsulated oil samples were the lowest compared with initial EO. On the contrary, the percentage of the oxygenated monoterpenes, sesquiterpenes, sesquiterpene oxide and ester were higher in all encapsulated oil samples than in the initial EO. The highest content of phenylterponied was obtained from nano-encapsulated oil in sodium alginate compared with initial EO and other

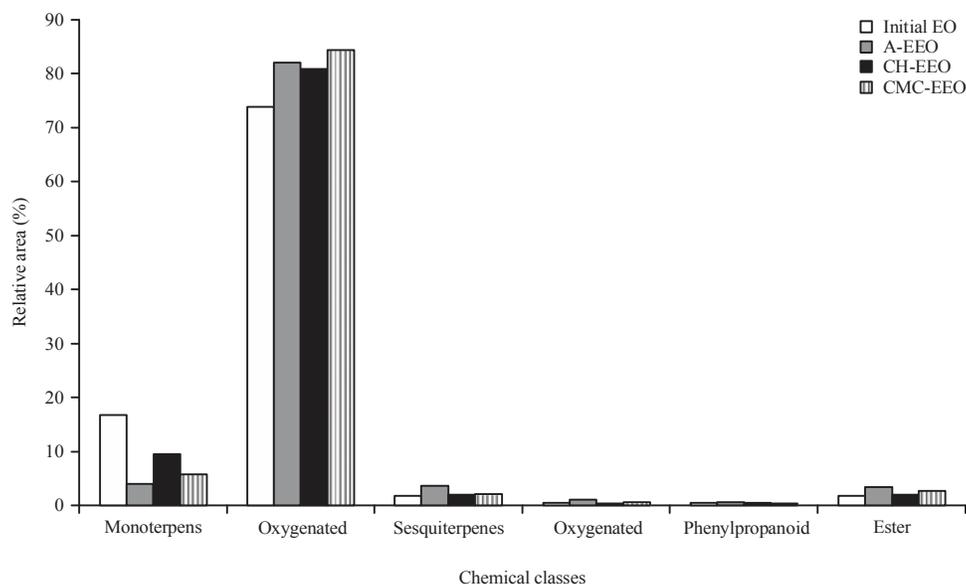


Fig. 8: Total area percentages of chemical class in rosemary essential oil before and after nano-encapsulation

Table 2: Chemical constituents of rosemary essential oil before and after nano-encapsulation

KI ^a	Compounds ^b	Relative area (%)			
		Initial EO	A-EEO	CH-EEO	CMC-EEO
925	α -thujene	0.14	-	0.04	-
936	α -pinene	8.78	0.96	5.45	2.27
953	Camphene	4.31	0.37	2.05	0.88
979	β -pinene	1.16	0.13	0.60	0.28
988	β -myrcene	1.02	1.53	0.72	2.05
1005	α -phellandrene	0.19	0.07	0.08	0.04
1018	α -terpinene	0.29	0.73	0.22	0.12
1035	1,8-cineole	30.88	11.90	40.88	30.10
1060	γ -terpinene	0.31	0.08	0.17	0.05
1091	Terpinolene	0.35	0.23	0.11	0.05
1108	Linalool	1.71	1.91	1.27	1.80
1155	Camphor	22.71	29.95	23.24	32.18
1169	Borneol	0.19	3.59	2.81	0.11
1180	Terpinen-4-ol	1.31	11.81	5.69	7.08
1187	ρ -cymen-8-ol	1.83	0.86	0.36	0.51
1203	α -terpineol	15.01	21.58	6.66	12.06
1288	Bornyl acetate	1.94	3.66	2.12	2.79
1292	Thymol	0.46	0.57	0.18	0.11
1347	α -cubebene	0.17	-	0.18	-
1360	Piperitenone	0.16	0.43	0.09	0.22
1375	α -copaene	0.12	0.46	-	-
1427	β -caryophyllene	1.39	3.18	1.95	2.01
1460	α -humulene	0.11	0.04	0.11	0.03
1591	Caryophyllene oxide	0.39	0.98	0.38	0.61

^aRetention index: Kovats retention index relative to n-alkanes on column DB-5, components were identified by retention indices (KI) with that of previously published data, A-EEO: Encapsulated rosemary essential oil with alginate, CH-EEO: Encapsulated rosemary essential oil with chitosan, CMC-EEO: Encapsulated rosemary essential oil with CMC

encapsulated oil samples. The main factors that affected encapsulation efficiency of encapsulated oil and flavors are the type of wall material, the properties of the core material (concentration and volatility), characteristics of the encapsulate (total solid, viscosity and droplet size)¹⁵⁻²⁶.

CONCLUSION

In this study, the major components of rosemary EO were 1,8-cineole (30.88%), camphor (22.71%), α -terpineol (15.01%), α -pinene (8.78%) and camphene (4.31%). The type of wall

materials has influence on the particles size and encapsulation efficiency and antioxidant activity of rosemary EO. The results indicated that nano-capsulation process increased the thermal stability of rosemary EO and could be useful as antioxidant for various thermal processing applications in industry.

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