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Preparation and Characterization of *Citrus Aurantifolia* Lime Oils Microcapsules by Complex Coacervation Technique

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Abstract: *The Lime oil of Citrus aurantifolia was microencapsulated by coacervation technique employing gelatine and alginates as the shells, and calcium chloride as cross linker. Optimization of core/shell ratio, speed and temperature of stirring was carried out. Structure and shape of the microcapsules were characterized by particle size analyzer (PSA) and scanning electron microscope (SEM) respectively. The properties of microcapsules such as, yield, particle size distribution, oil content, oil load, and encapsulation efficiency were also determined. Upon condition of ratio core/shell 0.49, speed and temperature of stirring at 600 rpm and 35 °C respectively, the microcapsules of LOs with improved performance were achieved with efficiency of 46% and oil content of 78%.*

Keywords*: microencapsulation, complex coacervation, lime oil, Citrus aurantifolia*

1. Introduction

Encapsulation is a technique of enclosing droplets or small particles of a sensitive substance (called core) within a continuous thin film of polymer shell. Thus, the core is protected from the effects of environmental changes and retarded from being released [1, 2]. Microencapsulation can be carried out by several methods, such as phase separation, phase change polymerization of materials in situ, mini emulsion polymerization, coacervation, or solvent evaporation [3] while considering some essential factors including: coating material and capsule core, core/shell ratio, viscosity, type of solvent, addition of additives [4]. In addition, it also requires such careful control of temperature, time, speed of stirring [5, 6], *p*H, and others [7].

Citrus aurantifolia essential oil (LOs) has been increasingly utilized for various needs in everyday life, including for: cosmetics, health, cleaning, dentistry, antiseptics, perfumes, insect repellents, adhesives, and flavor since it has substantial amounts of beneficial compounds of terpene secondary metabolites (monoterpenes and sesquiterpenes both oxygenated and non-oxygenated), alcohols, esters, ethers, lactones, ketones, phenols, and hydrocarbons [8, 9]. Thus, it exhibits various activities, such as antimicrobial, repellent, astringent, anthelmintic, diuretic, antiviral, antiseptic [10], antioxidants [11], and insecticides [12]. However, the oil is chemically unstable and susceptible to oxidative deterioration, especially when exposed to light, changes in moisture and temperature, and oxygen in the air [13-16]. Therefore, microencapsulation technique is employed to suppress or retard oxidation process and increase a range of applications in the health, commercial, textile, agriculture, and other fields [14].

In term of fairly simple process of microencapsulation at ambient temperatures, and high loading capacity of oil core [17], complex coacervation method has been utilized frequently. The method employs one or more charged coating polymers which combine proteins and polymers such as Arabic protein soybean [13, 18], gelatine-gum Arabic [1], and alginate-gelatine [19]. Microencapsulation of

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various essential oils with such method with charged coating polymers of alginate-gelatine have been reported for clove oil [20], olive oil [19], and ginger oil [7]. However, to the best of author's knowledge none of data reported the encapsulation of LOs by those biopolymers, apart of applying double coating of konjac glucomannan and gum Arabic [21], gum acacia and gelatine [22], and chitosan and gum arabic [23].

The main objective of this paper was to prepare and characterize microcapsules of *C. aurantifolia* essential oil (LOs) coated by two biopolymers of gelatine and sodium alginate for improved LOs microcapsules performance, and thus, be extensively applied in daily needs of live.

2. Materials and methods

2.1 Materials

Analytical grade of anhydrous sodium sulfate and glacial acetic acid was supplied by Merck, while technical grade of Tween 80, gelatine, alginate, calcium chloride, and *n*-hexane were purchased at local chemical store and used without further purification. Lime oils (LOs) were obtained from *C. aurantifolia* peels by hydrodistillation.

2.2 Preparation of Lime Oil (LOs)

Preparation of LOs was based on a literature procedure [24]. The peels of *C. aurantifolia* separated from the fruit were washed thoroughly with running tap water and cut into small pieces. The peels were then distilled using hydrodistillation technique at 100°C for 3 h. The LOs was separated from water, dried over anhydrous sodium sulfate, and stored at 5°C in a sealed vial. The procedure was repeated to obtain LOs sufficiently.

2.3 Determination of Physical Properties of LOs

The physical properties of LOs obtained were determined according to standard parameter of the previous published data [24], including density, refractive index, acid number, and solubility in alcohol.

2.4 Microencapsulation with Various Weights of LOs

Microencapsulation procedure was modification of the two established methods [16, 19]. The amount of core material (LOs) addition was varied from 3.0; 3.5; 4.0; 4.5; to 5.0 grams, while 2% (w/v) of shell mixture of gelatine and alginate (3.5:1) were dissolved in distilled water. 0.8 g of tween 80 and 0.18 g of calcium chloride were employed as emulsifier and cross linker respectively. Firstly, the aqueous solution of gelatine was stirred at 60 ± 1°C and speed of 600 rpm. Certain weight of LOs was added dropwise to the solution and followed by sodium alginate solution. The mixture was further stirred for 15 min, and the *p*H was then adjusted to 3.75 with addition of 2.5 % of glacial acetic acid. Furthermore, the solution was cooled to 5 - 10°C on an ice bath, and then calcium chloride was added slowly. The solution was further stirred for 3 - 4 h at 35 - 40°C. Upon cooling process at 25°C, the microcapsules were formed and then filtered. The microcapsules were washed with 3 × 20 mL of distilled water and n-hexane, successively and finally dried in the refrigerator.

2.5 Variation of Stirring Temperature

The microencapsulation procedure was carried out in the same way as described with the optimal amount of LOs obtained previously. However, the temperature was varied at 35°C, 40°C, 45°C, 50°C and 55°C upon addition of cross linker.

2.6 Variation of Stirring Speed

The stirring speed was varied at 400, 500, 600, 700, and 800 rpm upon microencapsulation process of the optimal amount of LOs and stirring temperature obtained previously.

2.7 Characterization of Microcapsules

The microcapsules obtained were characterized based on their particle size and distribution using Beckman Coulter LS 13 320 Particle Size Analyzer (PSA) equipped with optical Fraunhofer, morphology using Leica DME microscope and Scanning Electron Microscope (SEM) JEOL JSM-6510, and LOs content using UV-Vis Perkin-Elmer Lambda type 35. The oil content of microcapsule was calculated by regression equation obtained from the standard curve. The oil load and oil content were calculated using Eq. (1) and (2) respectively, while encapsulation efficiency was with Eq. (3).

$$\text{Oil Load (\%)} = w2/w3 \times 100 \quad (1)$$
$$\text{Oil Content (\%)} = w1/w \times 100 \quad (2)$$
$$\text{EE (\%)} = w1/w2 \times 100 \quad (3)$$

where, w1 = the actual weight of LOs in microcapsules, w2 = the added weight of LOs, w3 = the total weight of polymer including the cross linker agent, w = the total weight of the microcapsule.

2.8 Data Collection and Analysis

The physical properties of *C. aurantifolia* LOs, the properties of LOs microcapsules affected by various weights of LOs, stirring temperatures, and stirring speeds were expressed as mean ± standard deviation (SD). Analysis of statistical difference of particle diameter (PSA data) among treatments were performed on RStudio [25]. Shapiro-Wilk test was employed to evaluate the normality of data. The non-parametric method of Kruskal-Wallis rank sum test was used to assess significant differences among treatments [26]. If the Kruskal-Wallis rank sum test rejects the null hypothesis then Dunn's test was conducted as the post Hoc test. All statistical analyses were carried out at a confidence level of 95%.

3. Results and discussions

3.1 Preparation of Lime Oils (LOs)

Preparation of LOs was carried out for 3 h using the hydrodistillation method to yield 0.5% of clear yellowish oil with a fresh aroma of lime fragrance. Moreover, the physical properties of LOs were also determined (Table 1). Inclusively, the results were in agreement with the literature data [27].

Table 1. Physical properties of LOs of *C. aurantifolia*

Parameter	Lime oil (LOs)*
Density (g/mL)	0.8604 ± 0.0299
Refraction index	1.5236 ± 0.0420
Acid number (%)	0.5216 ± 0.0161
Solubility in 90% alcohol	(1 : 4.5 mL) ± 0.0

*Data were expressed as mean ± SD (n = 3)

The chemical composition of the LOs, reported by Wahyudi et al. [28], contained predominantly of d-limonene (39.23%), β-pinene (22.82%), citral (5.63%), and α-terpineol (3.74%). The limonene content was found below average (32 - 98%) among other lime oils, which might be due to many factors such as, plantation geographic, maturity level, genotype, soil type, climate, and type of extraction process [8, 9, 29, 30].

3.2 Microencapsulation

The LOs microcapsules with various amounts of LOs were produced by employing two biopolymers of gelatine and alginate 2% (w/v) as shell materials with fixed ratio of 3.5:1, tween 80 as emulsifier, and calcium chloride as cross linker showed distinctive particle size of microcapsules which ranged from 52 to 178 μm in diameter (Figure 1). In particular, the increasing weight of LOs above 3 g did not enlarge the particle diameter significantly, yet statistical test of differentiation revealed the substantial influences

of various LOs content toward particle size distribution. In general, the particles diameter, relied on the core size and wall thickness [31], were in agreement with the standard of microcapsules (0.2 – 5.000 µm) [32]. Other essential parameters of the resulted microcapsules such as, microcapsule yield, oil content, oil load, and encapsulation efficiency were also determined (Table 2).

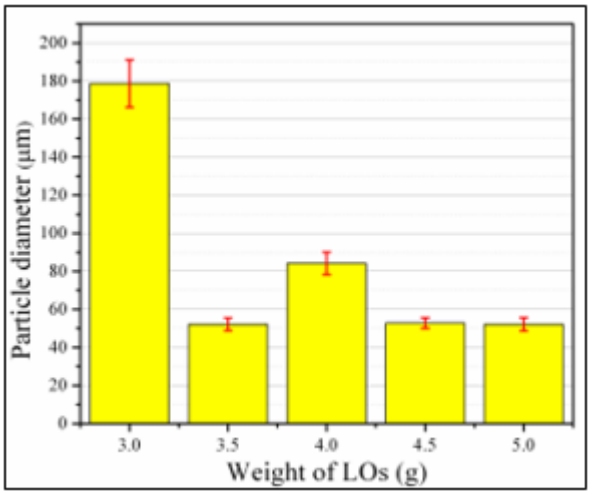

Figure 1. Average size distribution of microcapsules ± SD at various weights of LOs. Statistical test of significant differences with 95% confidence level. Value of $p < 2.2 \times 10^{-16}$ (Kruskal-Wallis test), p ($1.1 \times 10^{-3} – 2.9 \times 10^{-66}$) (Dunn test) were significantly different among all treatments

Table 2. Effect of various weights of LOs on the properties of microcapsules

Weight of microcapsule (g) W*	Weight of encapsulated oil (g) W1	Weight of added LOs (g) W2	Total polymer (g) W3	Yield (%)*	Oil content (%)*	Oil load (%)	EE (%)*
1.75 ± 0.17	0.94	3.0	3.6	26.52 ± 2.63	53.41 ± 5.91	83	31.20 ± 5.08
2.23 ± 0.06	1.23	3.5	3.6	31.46 ± 0.78	55.14 ± 3.62	97	35.17 ± 2.33
2.40 ± 0.06	1.37	4.0	3.6	31.54 ± 0.79	57.06 ± 5.19	111	34.24 ± 3.92
2.56 ± 0.03	1.49	4.5	3.6	31.65 ± 0.38	58.02 ± 5.82	125	33.02 ± 2.91
2.73 ± 0.05	1.65	5.0	3.6	31.71 ± 0.52	60.42 ± 10.81	138	32.91 ± 5.55

Stirring speed 600 rpm, stirring temperature 40 °C, *data were expressed as mean ± SD (n = 3).

The data (Table 2) showed that the microcapsules yield resulted from various weight of LOs ranged from 26 - 31%. Increasing of LOs amount more than 3.5 g (oil load 97%) did not indicate linier correlation to the yield remarkably. In fact, efficiency of the microcapsules decreased by 1% per - 0.5 g of added LOs which might be due to a higher percentage of loss during isolation. Low oil loads allowed the dispersion force by stirrer more effective than at high oil loads, and led to the formation of smaller oil vesicles [33]. In addition, the SEM image (Figure 2) confirmed that morphology of the microcapsules of 3.5 g of LOs weight more distinctly formed instead of 4.5 g one, even so the shape of the 4.5 g one was fairly more rounded and homogeneous with some agglomeration. As anticipated, the agglomeration was due to excessive oil in the system which could cause partial emulsification and alter electrostatic interaction between two biopolymers of gelatine and alginate [13]. Furthermore, un-emulsified oil also caused the microcapsules become sticky and broken [19].

3.3 Effect of Stirring Temperature on Microencapsulation

Since formation of microcapsule structure is affected by the cross-linker agent and its stirring temperature [34], the effect of various stirring temperatures upon addition of calcium chloride cross-linker to the properties of microcapsules such as, yield, oil content, oil load, and encapsulation efficiency, was explored (Table 3). Upon microencapsulation process of optimized LOs weight previously obtained (3.5

g), elevating gradual temperature of stirring by 5ºC up to 55ºC tend to decrease yield and efficiency of the encapsulation. In contrast, decreasing the temperature by 5 to 35ºC increased the yield and efficiency up to 10%. The result was supported by published data [35] which reported the stabilization of the microcapsules system at low temperatures.

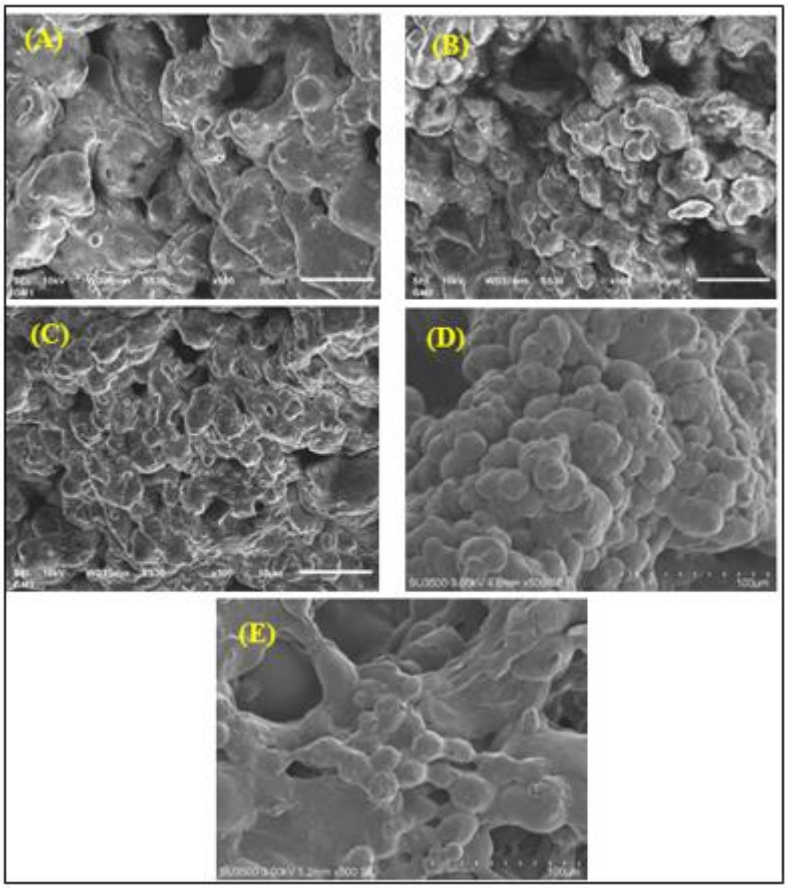

Figure 2. SEM images of microcapsules at various weights of LOs (a) 3.0 g, (b) 3.5 g, (c) 4.0 g, (d) 4.5 g, and (e) 5.0 g

Table 3 Effect of various stirring temperatures on the properties of microcapsules

T of stirring (°C)	Weight of microcapsules (g) W*	Weight of encapsulated oil (g) W1	Weight of added LOs (g) W2	Total polymer (g) W3	Yield (%)*	Oil content (%)*	Oil load (%)	EE (%)*
35	2.44 ± 0.11	1.57	3.5	3.6	34.41 ± 1.52	64.74 ± 19.34	97.22	45.00 ± 12.95
40	2.15 ± 0.11	1.24	3.5	3.6	30.23 ± 1.58	62.42 ± 14.77	97.22	35.30 ± 10.83
45	1.62 ± 0.22	1.04	3.5	3.6	21.74 ± 3.89	63.30 ± 8.19	97.22	29.68 ± 7.62
50	1.67 ± 0.07	1.05	3.5	3.6	23.47 ± 1.02	62.82 ± 6.60	97.22	29.90 ± 3.17
55	1.92 ± 0.10	1.18	3.5	3.6	27.09 ± 1.47	61.21 ± 3.95	97.22	33.62 ± 2.52

Stirring speed of 600 rpm, *data were expressed as mean ± SD (n = 3)

Further analysis of the result obtained upon treatment of various stirring temperature was performed to identify the particle size and its morphology. In spite of the fact that particle diameter of the microcapsules formed at 35ºC was slightly smaller than at 40ºC (Figure 3), the morphology of the microcapsules was predominantly more homogeneous and rounder (Figure 4). However, data analysis of

significant differences showed that temperature of stirring barely affected distribution of particles diameter.

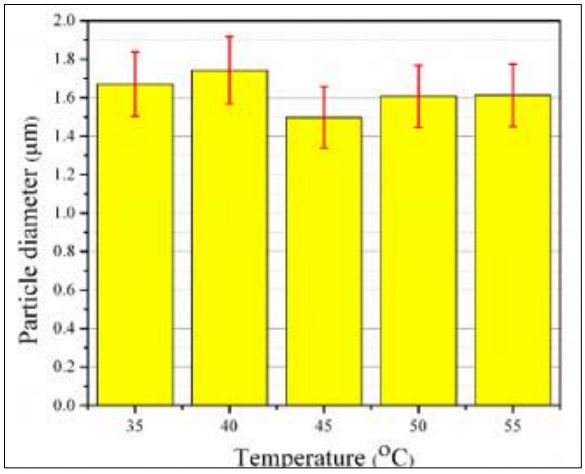

Figure 3. Average of size distribution ± SD at various stirring temperatures: 35; 40; 45; 50; and 55 °C. Statistical test of significant differences with 95% confidence level. Value of p = 0.7721 (Kruskal-Wallis test) was not significantly different among all treatments

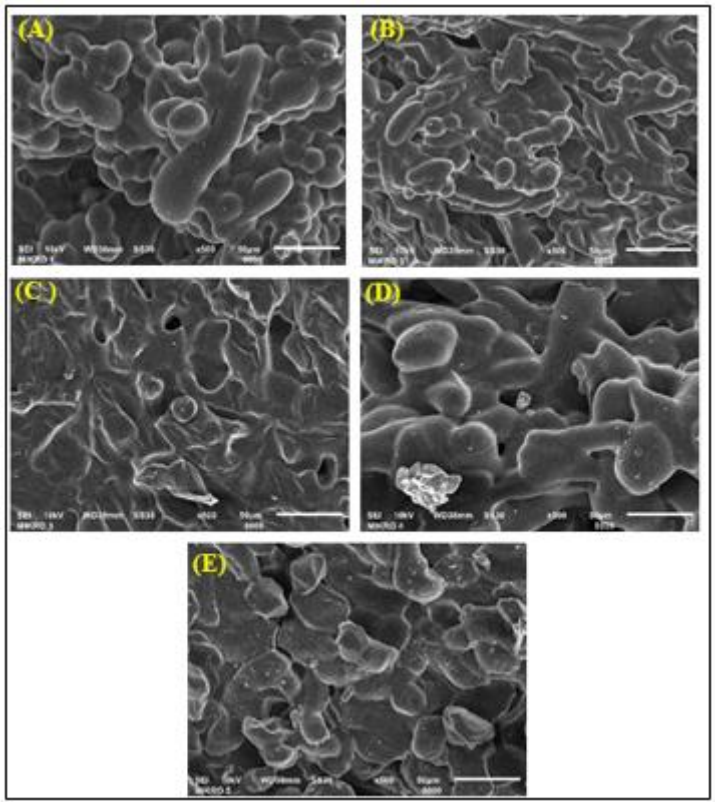

Figure 4. SEM images of microcapsules at various stirring temperatures: (a) 35°C, (b) 40°C, (c) 45°C, (d) 50°C, and (e) 55°C

3.4 Effect of Stirring Speed on Microencapsulation

To improve oil content and efficiency of the microcapsules obtained from the previous optimal condition, more treatment parameter was adjusted. The PSA result (Figure 5) revealed that various speed of stirring produced microcapsules in the range size of 1.40 - 1.52 µm with the yield of 23 - 30% (Table 4). In principle, the microcapsule diameters were not substantially influenced by the treatments statistically. Optimal oil content with highest encapsulation efficiency was achieved at 600 rpm stirring speed. Accelerating the speed above 600 rpm just destroyed the encapsulated oil, thus decreased the oil content and efficiency. Based on PSA data (Figure 5) and SEM images (Figure 6), the best microcapsules gave ratio mean/median close to 1. To end with, the results were supported by previous finding [5, 36], where the speed and length of stirring were not only control the size of microcapsules but also their size distribution.

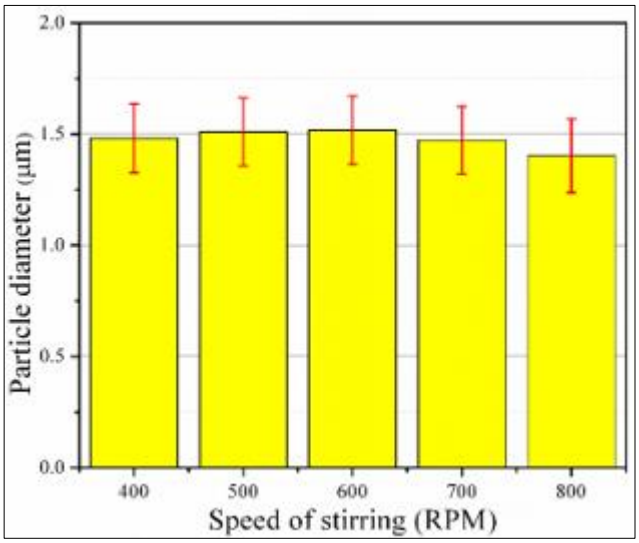

Figure 5. Average of size distribution ± SD at various stirring speeds 400; 500; 600; 700; and 800 rpm. Statistical test of significant differences with 95% confidence level. Value of p = 1 (Kruskal-Wallis test) was not significantly different among all treatments

Table 4. Effects of various stirring speeds on the properties of microcapsules

Speed of stirring (rpm)	Weight of microcapsules (g) W *	Weight of encapsulated oil (g) W1	Weight of added LOs (g) W2	Total polymer (g) W3	Yield (%)*	Oil content (%)*	Oil load (%)	EE (%)*
400	1.74 ± 0.39	1.10	3.5	3.6	24.51 ± 5.49	63.49 ± 4.80	97.22	31.43 ± 6.80
500	2.14 ± 0.79	1.35	3.5	3.6	30.14 ± 11.13	63.39 ± 5.56	97.22	38.63 ± 14.85
600	2.06 ± 0.47	1.62	3.5	3.6	29.01 ± 6.62	77.84 ± 6.67	97.22	46.38 ± 14.04
700	1.85 ± 0.21	1.36	3.5	3.6	26.06 ± 2.89	72.75 ± 8.14	97.22	38.76 ± 8.34
800	1.66 ± 0.19	0.79	3.5	3.6	23.33 ± 2.64	46.40 ± 7.06	97.22	22.48 ± 1.01

Stirring temperature was 35°C, *data were expressed as mean ± SD (n = 3)

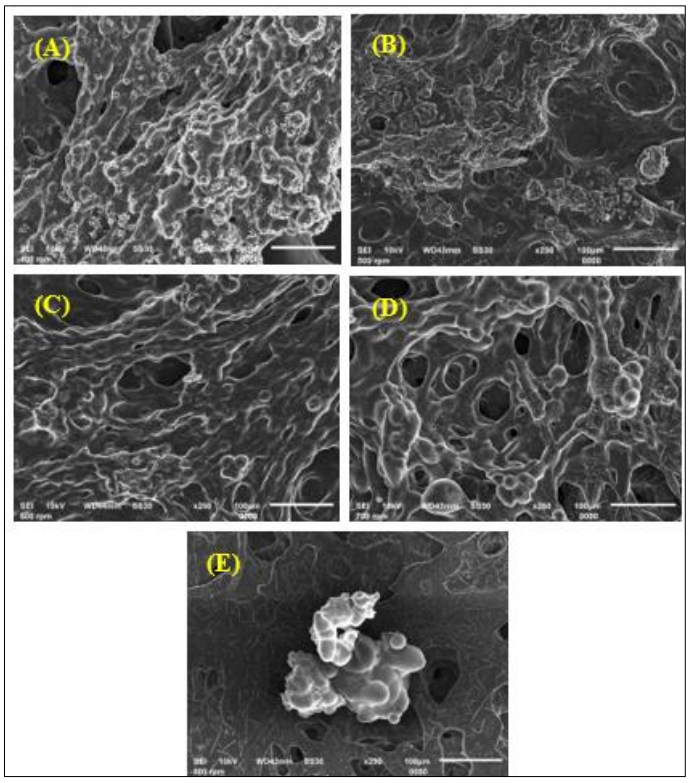

Figure 6. SEM images of microcapsules at various stirring speeds: (a) 400 rpm; (b) 500 rpm; (c) 600 rpm; (d) 700 rpm; and (e) 800 rpm

4. Conclusions

The Lime Oils microcapsules were successfully prepared and characterized at optimum condition of ratio core/shell of 0.49, speed and temperature of stirring of 600 rpm and 35°C respectively, with efficiency of 46% and oil content of 78%. Physical properties of the microcapsules such as, yield, oil content, encapsulation efficiency, particle size distribution, and morphology were substantially improved. Consequently, it could be more extensively applied for daily need of live, including by incorporation onto functional cotton fabrics or cosmetics, thus enhance their added values economically.

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