

## Microencapsulation of Lime Peel Essential Oils (*Citrus aurantifolia*) with Complex Coacervation Methods using Gelatin/Sodium Alginate Coating

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### Abstract

Lime (*Citrus aurantifolia*) peel essential oils are complex metabolites mainly used as natural preservatives. The principal constituents include d-limonene,  $\beta$ -pinene, and terpineol. Moreover, essential oils (EOs) are potentially known volatile and unstable compounds, hence, they require proper packaging techniques, e.g., microencapsulation. The purpose of this study is to evaluate the binary methods of lime peel essential oils microencapsulation using complex coacervation. This involves the application of gelatin and sodium alginate coatings under two specific conditions, termed core/coating ratio 1:1.2, with modified stirring and core/coating ratio 1:2.0, with stirring speed of 600 rpm, respectively. The results showed the EOs preparation generated 0.70% yield, characterized by good quality, fresh aroma, and pale-yellow coloration. Also, microcapsules delivered 37.06% yield, 39.18% oil content, 31.53% encapsulation efficiency (EE), and less smooth-edged SEM surface under condition I. Meanwhile, condition II demonstrated 51.46% yield, 58.66% oil content, 92.04% encapsulation efficiency (EE), and flat-curved, SEM surface. Furthermore, it was carefully observed that condition II produced more superior microcapsules.

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

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### 1. INTRODUCTION

*Citrus aurantifolia* or lime are found in large quantities in tropical regions, including Asia (Razak *et al.*, 2013). Several industries in Indonesia process the fruit into a variety of foods and drinks, where the flesh and water are widely used as raw materials. Subsequent data analysis significantly shows the small-scale manufacturers generate maximum peel waste of 6 tons per month. However, the degradation process appears complicated and extended due to the presence of antimicrobial essential oils (Wahyudi *et al.*, 2017b).

EOs are aromatic compounds insoluble in water, but potentially dissolve in alcohol, ether, or natural oils (Preedy, 2016). The lime peel EOs are a complex mixture of approximately 400 compounds (Mahato *et al.*, 2017), including  $\beta$ -pinene, d-limonene, terpinen-4-ol, and  $\alpha$ -terpineol (Wahyudi *et al.*, 2017), and are separable through hydro-

distillation (Julaeha *et al.*, 2018; Costa *et al.*, 2014). However, traces of certain intrinsic biological properties such as spasmolytic, neuroprotective, and antioxidant are discovered, in addition to exhibiting antibacterial activities (Costa *et al.*, 2014; Enejoh *et al.*, 2015; Al-Aamri *et al.*, 2018; Jafari *et al.*, 2011), e.g., antiseptic, antispasmodic, aromatic, and carminative (Suryawanshi, 2011).

The oils comprise of volatile and non-volatile compounds ranging from 85-99% and 1-15%, respectively. These active materials are volatile and unstable to the application of oxygen, heat, or light (Mahato *et al.*, 2017), therefore instigating the need to adopt proper packaging technique for preservative purposes e.g. microencapsulation. The method uses a particular polymer to subsequently control the material flow gradually (Wahyudi *et al.*, 2017a; Julaeha *et al.*, 2018). The product known as microcapsule is characterized by particle size

between 0.2 to 5,000  $\mu\text{m}$  (Silva *et al.*, 2014).

The microencapsulation process performed using complex coacervation, describes the interaction between two or more opposing coatings (Timilsena *et al.*, 2018). This approach is widely applied in various industries due to large capacity to reserve contents and control possible material release (Tang *et al.*, 2020). Several contributing factors and parameters include pH, temperature, and coating comparison with capsule cores (Yan & Zhang, 2014). Furthermore, stirring the mixture appears to further impact the microcapsules, with the tendency to control particle size (Timilsena *et al.*, 2018).

The development of microcapsules involves the application of two coating types, termed natural materials and synthetic polymer. The addition of gelatin serves as a protein derivative from denatured collagen (Shaddel *et al.*, 2017). These proteins include amphoteric compounds with positive charges and pH between 8-9 located underneath the isoelectric point. Meanwhile, sodium alginate which includes polysaccharides is a sodium salt of alginic acid which is white to yellowish brown, and are obtained from brown algae extract with a negative charge at low pH (Devi *et al.*, 2012).

The application of alginate and gelatin have been widely reported although with varied core, such as sunflower oil (Devi & Kakati, 2013), buriti oil (Lemos *et al.*, 2017), olive oil (Devi *et al.*, 2012), and ginger EOs (Wang *et al.*, 2016). In addition, the introduction of lime peel EOs core is not yet achieved. Therefore, this paper significantly describes the microencapsulation results of lime peel EOs core using complex coacervation.

There has been a known performance of microencapsulation using a mixture of alginate and gelatin coatings, tween 80 emulsifiers, and glutaraldehyde crosslinkers by Devi *et al.* (2012). This study also captures a similar outcome, but the novelty was the use of a different core. Devi *et al.* (2012) used non-volatile olive oil core, a mixture of primary metabolites from fatty acids, while the volatile secondary metabolites and sesquiterpenes were the main EOs components. The resulting microcapsule characteristics are influenced by the difference, with the core/coating ratio 1:1.2 and 1:2.0 utilized under two stirring conditions.

## 2. MATERIALS AND METHODS

### Materials

Lime peel (*C. aurantifolia*) samples were obtained from Caringin Main Market, Bandung. The materials used are aquadest, glacial acetic acid (Merck), acetone (Merck), gelatin (PT. Subur Jaya Kimia, in Bandung), glutaraldehyde (Merck), *n*-hexane (Merck), sodium alginate (PT. Subur Jaya Kimia, in Bandung), anhydrous sodium sulfate (Merck), and tween-80 (PT. Subur Jaya Kimia, in Bandung). Glass equipment are commonly used in laboratories, scanning electron microscopy (SEM) JEOL brand type JSM-6510, GCMS brand Agilent type 7890A/5975C, and UV-Vis spectrometer brand Perkin-Elmer with Lambkin 35 type.

### Preparation and Determination of Physical Properties of Lime Essential Oils

The EOs preparation and physical property determination were generated based on density, refractive index, acid number, and alcohol solubility (Julaeha *et al.* 2018). Moreover, the chemical components of lime EOs were analyzed using GCMS and 35ms DB capillary column length of 30 m, diameter of 0.25 mm, and thickness of 0.25 micron liquid phase film. This was conducted by adjusting the temperature from 50-250°C, with an increase of 3.5°C/min. The injector was set to 250°C, while the amount of oil injected was 1  $\mu\text{L}$ .

### Essential Oils Microencapsulation

The Devi *et al.*, (2012) microencapsulation method modified the material type and core amount, as well as the stirring speed. This process also used separate alginate and gelatin coating of 2% in aquadest. Condition I commenced with the following steps: First, the gelatin solution (140 mL) was poured into a beaker and stirred at 1250 rpm speed to achieve a solution temperature of  $60 \pm 1^\circ\text{C}$ . Tween 80 (0.8 g) was then added along with EOs (3.5 g) in a dropwise pattern. Subsequently, sodium alginate solution (40 mL) was gradually introduced. The resulting mixture was then stirred for 15 minutes, and 2.5% acetic acid added to acquire a pH of 3.75. Furthermore, the liquid was cooled in an ice bath, while continuously stirred at 600 rpm speed to attain a solution temperature between 5–10°C, where glutaraldehyde (0.472 mL) was gently added. The solution was reheated to arrive between 35–40°C, while agitating for 3-4

hours, and stirred at 250 rpm under room temperature for 18 hours. The microcapsules formed were then filtered using a Buchner funnel. The final product was then washed with aquadest and *n*-hexane, before drying using a refrigerator.

Condition II showed a similar process, but the stirring speed was adjusted to 600 rpm, and without 18 hours stirring, although 2g core was added to produce a core/coating ratio of 1:2.0.

### Microcapsules Characterization

The microcapsules obtained are further characterized: morphology with Scanning Electron Microscope (SEM) and EOs content measurement in microcapsules by UV-Vis spectroscopy. The oil content in the microcapsules is calculated by the regression equation obtained from the standard curve.

Oil content value calculation, and the encapsulation efficiency (EE) is carried out using Equations (1) and (2).

$$\text{Oil content (\%)} = \frac{w_1}{w} \times 100\% \dots\dots\dots(1)$$

$$EE (\%) = \frac{w_1}{w_2} \times 100\% \dots\dots\dots(2)$$

Where, *w* = weight of microcapsules; *w*<sub>1</sub> = the actual amount of oil coated, *w*<sub>2</sub> = the oil added amount.

## 3. RESULTS AND DISCUSSION

### Preparation and Determination of Physical Properties of Lime Essential Oils

The EOs preparation results obtained a yield of 0.7% characterized by good quality, fresh aroma, and pale-yellow coloration using hydro-distillation for 3 hours. Table 1 shows the physical properties from the process.

**Table 1.** The physical properties of *C. aurantifolia* peel EOs resulting from preparation

Parameters	Values
Refractive index (25°C)	1.542
specific gravity (25°C) (g/L)	0.893
Acid number (%)	0.24
Solubility in alcohol	Soluble in ethanol 96% (Volume 5 mL)

Gamarra *et al.* (2006) reported the refractive index of *C. aurantifolia* between 1.479-1.485 and the specific gravity ranged from 0.847 to 0.882 g/L. On the contrary, this study recorded the refractive index and specific

gravity as 1.542 and 0.893 g/L, respectively. The difference proves the oil properties were influenced by various factors, including the geographical growth location, climate, species, maturity level, extraction methods, etc. (Darjazi, 2014). Currently, the Indonesian National Standard (SNI) related to the *C. aurantifolia* essential oil physical properties does not exist.

Table 2 represents 18 secondary metabolites of lime peel EOs, and the four main components highlighted as d-limonene with abundance (35.98%), β-pinene (9.02%), α-terpineol (8.12%) and citral (7.49%).

**Table 2.** *C. aurantifolia* peel essential oil composition

Compound	Time Retention	Composition (%)
α-Pinene	6.205	0.81
β-Pinene	7.922	9.02
β-Phellandrene	10.302	2.23
(+)-4-Carene	12.568	1.22
d-Limonene	10.016	35.98
o-Cymene	10.634	1.38
β-Ocimene	10.748	0.75
γ-Terpinene	11.441	1.14
2-Carene	12.568	1.61
β-Myrcene	19.337	0.20
Terpinen-4-ol	17.466	2.94
α-Terpineol	18.410	8.12
Citral	22.204	7.49
Nerolidol	16.282	0.20
Trans-α-bergamotene	25.208	0.89
Caryophyllene	25.328	2.03
γ-Elemene	25.746	0.55
β-Bisabolene	28.469	2.12

EOs constituent compounds are influenced by the physical properties, including increased refractive index due to more long-chain components or mechanisms containing oxygen. This causes the medium density to increase, so that the incident light becomes more difficult to refract. In addition, other physical properties such as specific gravity, are influenced by the constituent components weight fraction, while the acid number indicates the free acid level contained. Furthermore, most compounds forming EOs, including d-limonene and β-pinene are nonpolar, and are estimated to subsequently affect alcohol solubility.

Monoterpenes are the largest constituent of volatile compounds present in

EOs. Furthermore, citrus varieties, geographical location and the climate where plants grow are

**Essential Oils Microencapsulation**

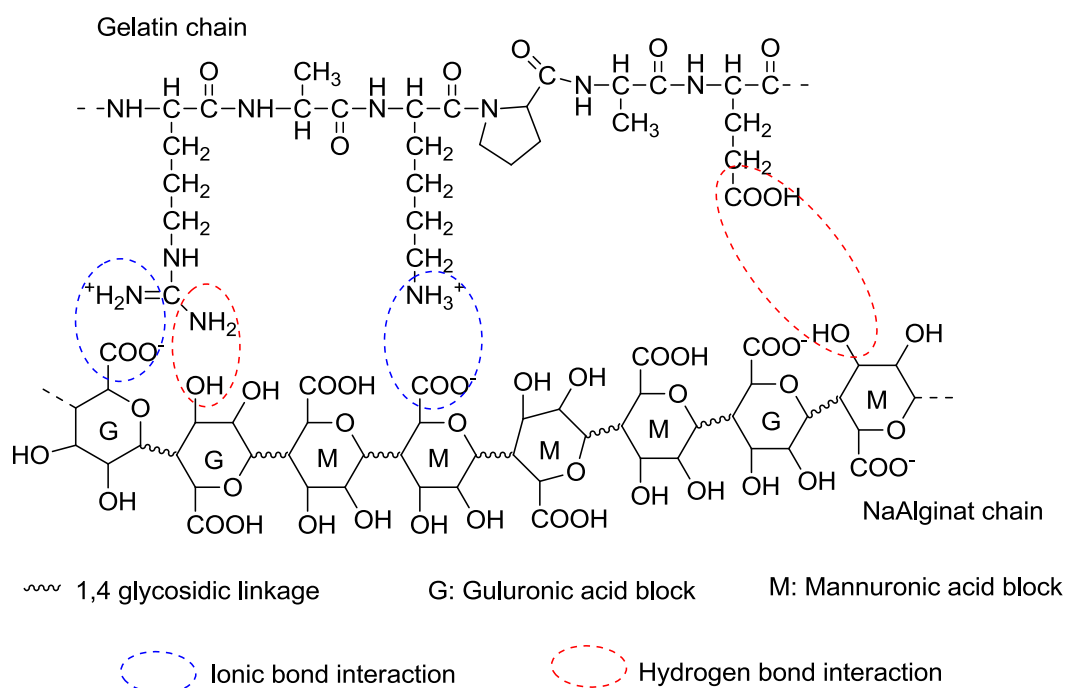
The application of alginate and gelatin combination on microencapsulation results to form excellent microcapsules are due to lack of aggregation, small particle size, and easy dispersion (Xiao *et al.*, 2014). Microcapsules are formed by the attraction between molecules with opposite charges, therefore resulting in insoluble colloidal phase formation. In addition, the surface tension tends to reduce and the core is coated by a polymer. The carboxyl groups in polysaccharides and amino groups in proteins interact to yield amide groups during the coacervation process. Figure 1 illustrates the reaction.

The use of crosslinking agents serves to stabilize the structure (Tang *et al.*, 2020), therefore, prompting the need to possess reactive ends toward specific functional groups. Also, glutaraldehyde is a typical example often used to form covalent bonds between carbonyl and amine groups present in glutaraldehyde and gelatin, respectively (Figure 2). The intensity is influenced by covalent bonds formation and interconnected sites availability.

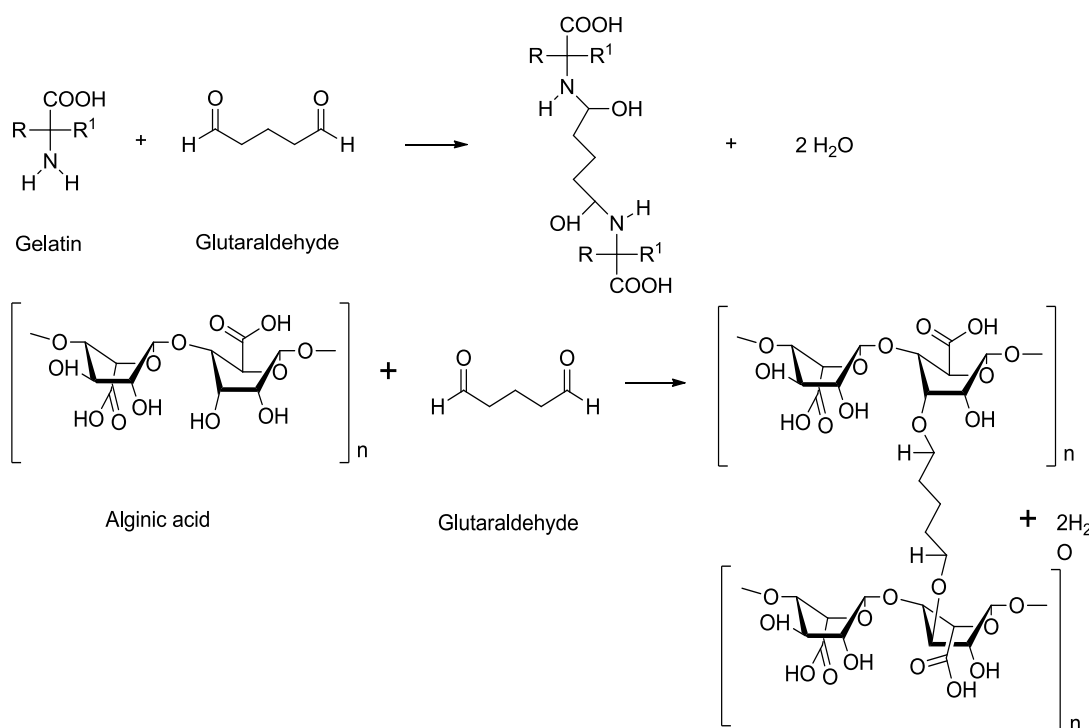
The stirring speed parameter helps to control the resulting particles size (Timilsena *et al.*, 2018), while the size distribution is affected

important factors in the composition and type of various compounds contained (Lin *et al.*, 2019). by the time duration (Rozenblat *et al.*, 1989). In addition, the core number is influenced by encapsulation efficiency with the tendency reported to decrease due to excess volatile oil core (Wang *et al.*, 2016). Excess uncaptured oil tends to stick to the microcapsule surface (Bastos *et al.*, 2020), causing a low oil content value due to residual oil.

Table 3 shows the microencapsulation conditions towards yield value, oil content, and EE. The oil content is absorbed in the microcapsules, while the EE states the oil penetration efficiency, although both parameters determine the success of the process. Table 3 also describes condition I and II produces 37.06% yield, 39.18% oil content, 31.53% EE, and 51.46% yield, 58.66% oil content, 92.04% EE, respectively. The results showed the oil content in condition II provided greater performance than condition I, therefore more oil is absorbed in condition II microcapsules. Furthermore, the higher EE value led to a more efficient process, and subsequently, condition II produced better outcome. These are caused by stirring speed effect, as rapid particle motion promotes microcapsules bursting, therefore, resulting in less homogenous size distribution (Figure 3).



**Figure 1.** Gelatin-sodium alginate bonds formation (Li *et al.*, 2011)

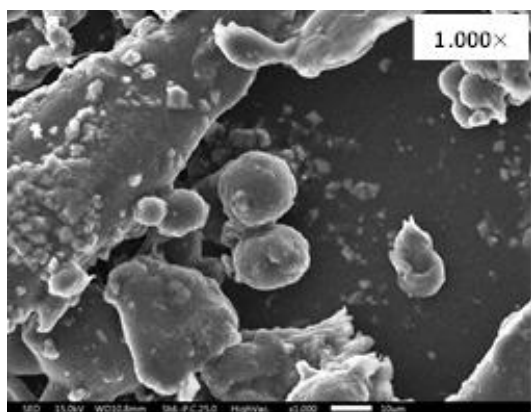


**Figure 2.** The mechanism of crosslinking glutaraldehyde with gelatin and alginate (Rojas, 2015)

Table 3. Microencapsulation conditions of *C.aurantifolia* peel essential oils

Stirring speed (rpm)	W (g)	W1 (g)	W2(g)	W3 (g)	Yield (%)	Oil content (%)	EE (%)
Condition I							
1250, 600, & 250	2.82	1.11	3.50	4.10	37.06	39.18	31.53
Condition II							
600	3.14	1.84	2.00	4.10	51.46	58.66	92.04

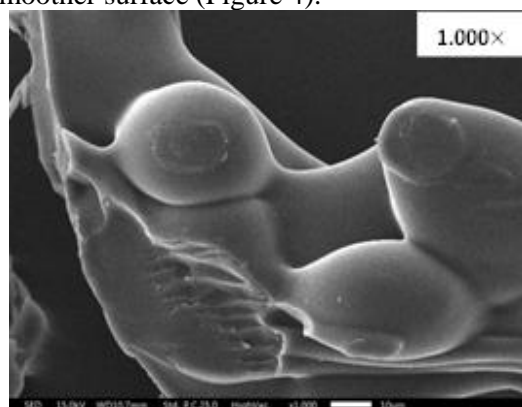
W: Microcapsule mass; W1: Encapsulated oil mass; W2; Essential oil mass added; W3: Total polymer



**Figure 3.** SEM micrographs of condition I microcapsules at magnification 1.000x

SEM measurement results support these conditions. However, condition I microcapsule morphology have less smooth surface and

inhomogeneous size distribution (Figure 3). Meanwhile, In condition II, the resulting microcapsules have a better round shape and smoother surface (Figure 4).



**Figure 4.** SEM micrographs of condition II microcapsules at magnification 1.000x



#### 4. CONCLUSION

This study concludes the investigation of lime (*C. aurantifolia*) peel EOs microencapsulation condition II (core ratio/coating 1:2.0 and stirring speed of 600 rpm) provides better microcapsule results compared to condition I (core/coating ratio 1:1.2 and stirring conditions modified). The outcome in condition II in yield, oil content, and EE were 51.46%, 58.66%; and 92.04%, respectively.

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