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Encapsulation efficiency and thermal stability of lemongrass (*Cymbopogon citratus*) essential oil microencapsulated by the spray-drying process

[1,2]Nguyen, P.T.N., [1,2]Vo, T.T., [1,2]Tran, T.Y.N., [3]Le, T.H.N., [4]Mai, H.C., [5]Tran, T.T. and [1,2*]Long, G.B.

[1]*Center of Excellence for Biochemistry and Natural Products, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam*
[2]*NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam*
[3]*Department of Chemical Engineering, HCMC University of Technology, VNU-HCM, Ho Chi Minh City, Vietnam*
[4]*Department of Chemical Engineering & Processing, Nong Lam University of Ho Chi Minh City, Viet Nam*
[5]*Food Technology Department, College of Agriculture, Can Tho University, Can Tho City, Vietnam*

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Abstract

Encapsulation is a potential technique that is widely studied and applied in the preservation of biological compounds and living cells. Lemongrass (*Cymbopogon citratus*) essential oil is a biological compound that emits a strong aroma and contains robust antibacterial compounds, hence its applications in various areas such as food, pharmaceuticals and cosmetics. However, lemongrass essential oil is in a liquid form, so it is easy to evaporate and oxidize when being exposed to temperature variations, oxygen, and light. Hence, the present study was aimed to determine the effects of heating temperature and time on the stability of microencapsulated lemongrass essential oil powder. In this research, microencapsulated powder was produced by the spray-drying method using maltodextrin (30%, w/w) as the wall material and lemongrass (1.5%, w/w) as the core material. Thereafter, the effects of heat during processing, including temperature (60°C to 180°C) and treatment time (from 20 to 80 mins) were evaluated. The results showed that when the temperature and the time increased, the color of powder became darker and OR values were rapidly reduced. The selected optimal temperature and time was 100°C and 20 mins. The encapsulated essential oil maintained its main components including nerol, citral a, citral b, and geraniol, even after being heated.

1. Introduction

Nowadays, studies on plants natural compounds have become more extensive because they contain many biological compounds (Alitonou *et al*., 2006). The *Cymbopogon* genus is an aromatic tall grass with long, thin leaves. It originated in India and is grown in countries such as Vietnam, China, Thailand and West India. Moreover, it is found in tropical and subtropical regions of Asia, Africa and America (Lonkarbr *et al*., 2013). This genus includes approximately 140 species and is applied in many different areas such as food, cosmetics and medicine (Chanthai *et al*., 2012). The lemongrass leaves can be used to produce tea or powders. Furthermore, one of the most popular products derived from lemongrass is essential oil (Eos) extracted using many different methods: steam distillation, hydrodistillation and microwave-assisted distillation (Hien *et al*., 2018). Beside main components such as citral and geraniol, lemongrass EO also contains various compounds such as β-caryophyllene, limonene, geranyl acetate, methyl eptanone and linalool, depending on environmental, genetic, and geographic conditions (Khanuja *et al*., 2005; Xu *et al*., 2013).

Many studies have shown that lemongrass EO exhibited antimicrobial, anti-inflammatory, anticancer and allelopathic properties (Alitonou *et al*., 2006; Kumar *et al*., 2008). However, lemongrass EO is a volatile compound so it is susceptible to change and lost when exposed to external environments such as light, temperature or oxygen. One of the popular technologies used to increase the stability of lemongrass EO is microencapsulation because it could prevent chemical reactions and limit the loss of these components in lemongrass EO (Zanetti *et al*., 2018).

*Corresponding author.
Email: *blgiang@ntt.edu.vn*

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Microencapsulation technologies include physical processes (such as spray chilling/cooling, spray drying and extrusion) and chemical processes (such as coacervation, molecular inclusion, co-crystallization, and interfacial or *in situ* polymerization) (Schapoval *et al*., 2010).

In recent years, spray-drying microencapsulation has become a favorable method due to low cost and the ability to protect the contained oils against ambient conditions, including temperature, light, oxygen and humidity without causing changes or loss of the oil's quality (Veiga *et al*., 2019; Assadpour and Jafari, 2019). For example, a study conducted by Tomazeli Junior *et al*. (2018) showed that spray-drying of essential thyme oils did not result in any changes in the chemical compounds and antimicrobial activity of this EO. Another study by Granados-Vallejo *et al*. (2019) also proposed that the shelf-life and stability of green coffee oils have been improved upon spray-drying microencapsulation. Of all the factors that determine the efficiency of spray-drying, temperature and time length of processing and preservation are known to play a significantly important role; as studies have shown that high temperature within a prolonged period of time can readily damage the wall material of the encapsulates (Dunstan *et al*., 2002; Tonon *et al*., 2010; Sun *et al*., 2020). However, up to the present, there has not been any research to examine the effects of these factors on the stability of microencapsulated powders, especially microencapsulated lemongrass EO powders.

Therefore, in this study, the effects of the temperature and time of spray-drying microencapsulation on lemongrass EO were investigated and evaluated in terms of the retention of lemongrass EO (OR), the powder color, the chemical composition and the powder particle morphology.

2. Materials and methods

2.1 Chemicals and reagents

Lemongrass EO obtained from Tien Giang province, Vietnam was distilled using the steam distillation method and purified using the fractional distillation method. Maltodextrin (12DE, Roquette, France) were used as the materials. Tween 80 (Xilong, China) were used as an emulsifier.

2.2 Microencapsulation by spray-drying

The process of EO microencapsulation was based on the methodology of Saénz (2009) with modifications. Maltodextrin as the wall material was dissolved in distilled water at a concentration of 30% solids. This solution was kept for 12 hrs at room temperature to allow the polymer molecules to completely saturate. Then, the emulsions were prepared by adding lemongrass EO and Tween 80 with an amount equal to 5% weight of essential oil. The dispersions were homogenized using a basic homogenizer (Ultra-Turrax IKA T18, Wilmington, North Carolina, USA) with stirring at 6000 rpm for 20 mins. These emulsions were dried with spray-dryer equipment (YC-015; Shanghai Pilotech Instrument and Equipment Co., Ltd, Shanghai, China) equipped with a two-fluid nozzle atomizer. The process was performed under the inlet temperature of 140°C and feed rate of 120 mL/h. The obtained powder was collected and stored in sealed glass bottles at 25°C for further analysis.

2.3 Thermal treatment

The obtained powder was measured for moisture content before applying thermal treatment. A total of forty-eight aluminum plates (10 cm in diameter) was prepared, put in 25 g of microencapsulated lemongrass EO powder and uncovered. All plates were subjected to heating under various temperature levels (60, 100, 140, 180°C) in a drying oven (Memmemert UN55, Schwabach, Germany). At each the temperature levels, after a period of 20, 40, 60 and 80 mins, the samples were removed, cooled and weighed. The microencapsulated products were determined for oil retention (OR) by distilling the microencapsulated powder in a Clevenger-type apparatus for 4 hrs. The EO was extracted from the water phase using ethyl ether. All experiments were conducted three times.

2.4 Characterization of the microcapsules

2.4.1 Moisture content

The moisture content of microencapsulated lemongrass essential powder was estimated by drying at 105°C in the oven (Vord-460-D, Thermoline Scientific Equipment Pty Ltd., NSW, Australia) to constant weight using the method of AOAC International (1995).

2.4.2 EO retention (OR)

The OR of microencapsulated powder before and after the heating process was calculated by diluting the microencapsulated powder before and after heating for 4 hrs in a Clevenger-type apparatus and applying the following equation:

$$\text{Oil retention (\%)} = \frac{X}{Y}$$

Where:

$$X\ (\text{mL/g}) = \frac{\text{volume of lemongrass essential oils in powders after heating (mL)}}{\text{weight of dry matter after heating (gram)}}$$

$$Y\ (\text{mL/g}) = \frac{\text{volume of lemongrass essential oils in powders before heating (mL)}}{\text{weight of dry matter before heating (gram)}}$$

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2.4.3 Identification of EO components

The chemical composition of the EO samples was analyzed by a SCION SQ gas chromatograph (Bruker Daltonics Inc., Billerica, MA, USA) equipped with a Rxi -5MS capillary column (30 m x 0.25 mm, film thickness 0.25µm) (Restek, Bellefonte, PA, USA). The temperature of the injector was adjusted at 250°C. Helium was the carrier gas with a constant flow rate of 1 mL/min.

2.4.4 Particle morphology determination

The particle morphology was determined using scanning electron microscopy (SEM). Samples were directly deposited on carbon conductive tape on aluminum SEM stubs and were coated with a thin gold layer using gold sputtering.

2.4.5 Color measurement

Color of powders after the heating process was measured using colorimeter (DP-9000, Hunter Associates Laboratory, Reston, VA, USA) fitted with an aperture (25 mm in diameter). Lightness (L), redness (a) and yellowness (b) as well as darkness (Lo), greenness (ao) and blueness (bo) were determined to calculate the total color difference using the equation as described previously (Saenz *et al*., 2009; Sarpong *et al*., 2019; Al-Ghamdi *et al*., 2020).

2.5 Statistical analysis

All experiments were conducted in triplicates. The data were expressed in mean±standard deviation (SD) and obtained from two-way ANOVA (Statgraphics Technologies Inc., The Plains, VA, USA) with the level of significance at 5%.

3. Results and discussion

3.1 Properties of lemongrass essential oils

Lemongrass EO is a viscous liquid with dark yellow or dark amber color. GC-MS analysis has shown the presence of 9 compounds accounting for 89.949% of the total EO. The major components were citral (49.076%), myrcene (30.492%), farnesol (1.898%), piperitone (0.627%), limonene (0.299%) and 6-methyl-5-hepten-2-one (1.460%) while the minor components were geraniol (3.762%) and citronella (2.335%). The quality of lemongrass EO is generally determined by its citral content (Mohamed Hanaa *et al*., 2012). Citral (3,7-dimethyl-2,6- octadienal) is used as a starting material for manufacturing perfumes in cosmetics and flavoring in the food industry. Nerol is used for bouquetting citrus flavor (Joy *et al*., 2006). Myrcene, geraniol, linalool and citronellol separated from lemongrass oil can be used in fragrance and flavoring production (Fahlbusch *et al*., 2003; Lasekan and Lasekan, 2012). Comparing with the chemical constituents of this lemongrass EO with the previous study by Tajidin (2012), its composition was relatively similar to that of Indian oils, with the same content of citral and geraniol. The result also indicated a high percentage of myrcene content in the oil (30.492%) which is similar to the study in West Indian lemongrass EO (Skaria *et al*., 2006). Boukhatem *et al*. (2013) has also shown similar results when extracting EO from lemongrass leaves, in which the main constituents included geranial (28.93%), neral (24.30%), and myrcene (23.92%).

3.2 Properties of lemongrass essential oils

The color of microencapsulated lemongrass EO powders at different temperatures and time was shown in Table 1.

Between 60 and 100°C, the powder color retained its characteristic white color after drying. However, when the temperature increased from 140 – 180°C, the color significantly changed into darker yellow. Similarly, the powder color also became dark yellow as the treatment time prolonged.

The color of microencapsulated EO powders influenced by heating temperature and time were shown in Table 2. It is clear that b value of color parameter achieved the highest range value (app. 13.4±0.086) and the L values reduce increasingly (app. 95.310±0.923) at 180°C. These results revealed that the microencapsulated powders would have a deep yellow color than at 60°C (b value app. 7.827±0.174, L value 99.176±0.570). Figure 1 displayed the change of TCD values at different time and temperatures. The TCD values increased rapidly from 0.768 to 7.007, comparing with the control sample when changing temperatures from 140 to 180°C. In contrast, from 60 to 100°C, TCD values were not significantly

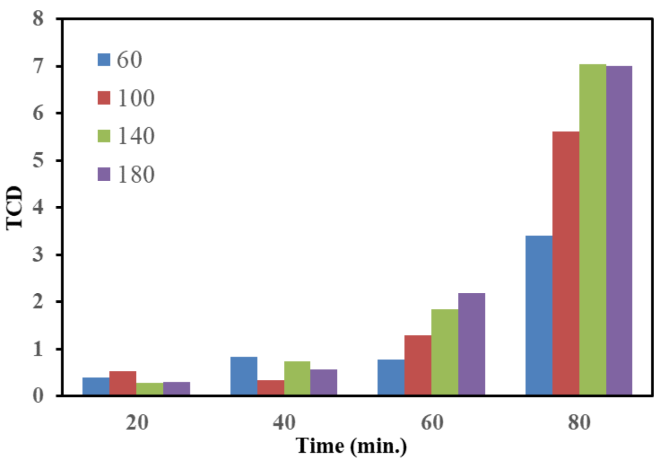

Figure 1. Total color difference values of lemongrass EO at different time points and temperatures as compared to the control sample.

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Table 1. Image of color of microencapsulated lemongrass EO powders at different temperature and time

Time (mins)	Temperature (°C)			
	60	100	140	180
20				
40				
60				
80				

Table 2. Effects of temperature and treatment time on lemongrass EO powder color.

Temperature (°C)	Time (min)	L value	TCD	a value	b value
Control sample	0	99.46±0.553	0	-5.72±0.066	7.78±0.025
60°C	20	99.213±0.898	0.391	-5.630±0.000	8.070±0.138
	40	99.106±0.775	0.529	-5.617±0.029	8.160±0.252
	60	99.203±0.143	0.289	-5.617±0.029	7.863±0.274
	80	99.176±0.570	0.3	-5.633±0.076	7.827±0.174
100°C	20	98.706±1.352	0.825	-5.610±0.114	8.097±0.451
	40	99.133±0.783	0.345	-5.610±0.075	7.803±0.146
	60	98.853±1.371	0.733	-5.643±0.052	8.183±0.534
	80	99.220±0.495	0.575	-5.640±0.089	8.297±0.359
140°C	20	98.950±0.935	0.768	-5.680±0.114	8.353±0.425
	40	99.110±0.886	1.292	-5.687±0.052	9.023±0.502
	60	98.580±0.366	1.84	-5.630±0.099	9.393±0.436
	80	98.426±0.404	2.178	-5.660±0.049	9.697±0.245
180°C	20	97.873±0.207	3.407	-5.540±0.000	10.790±0.504
	40	96.466±0.669	5.603	-5.323±0.038	12.500±0.489
	60	95.373±0.567	7.045	-5.143±0.076	13.490±0.345
	80	95.310±0.923	7.007	-5.177±0.038	13.400±0.086

changed. The situation can be explained by browning reactions or caramelization of sugars, which has also been found in the study of Quek *et al*. (2007), Sachin *et al*. (2010) and Chong and Wong (2015) when conducting spray-dried Sapodilla (*Manilkara zapota*), watermelon, ginger powders, respectively.

3.3 Properties of lemongrass essential oils

The effects of heating temperature and time on OR of encapsulated lemongrass EO powder were demonstrated in Figure 2.

As shown in Figure 2, treatment temperature and time had significant effects on the OR values of the microencapsulation powder ($p<0.05$). When the temperature increased from 60 to 180°C and the treatment time increased from 0 to 80 mins, the OR values decreased. Although the decrease in OR values seemed insignificant during the first 20 mins of treatment time (60°C: 92.854%, 100°C: 92.015%, 140°C: 80.722% and 180°C: 67.523%), it tended to continue drastically towards the end of the heating process (60°C: 79.051%, 100°C: 65.953%, 140°C: 53.800% and 180°C: 27.153%).

The inclusion of essential oils in cyclic

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oligosaccharides or oligosaccharides (e.g. *b*-cyclodextrin, maltodextrin) was proposed to improve the stability and decrease its volatility and degradation (Schapoval *et al*., 2010). When the temperature was increased up to 100°C, moisture moves by diffusion to the surface of the particles and drags the EO along with it, leading to reduced OR values (Rulkens and Thijssen, 2007). The increasing temperature may also associate with accelerated chemical reactions and formation of free radicals which leads to autoxidation and hydroperoxides decomposition (Choe and Min *et al*., 2006). The van's Hoff law expresses that chemical reaction rates increase approximately doubles when the temperature rises by 10°C, Abdul Aziz *et al*. (2015) discussed a temperature effect on microencapsulation of other Eos. Recently, the researchers were pointed out that the effects of spray-drying temperature and treatment time on other Eos (Obón *et al*., 2009). The above results have shown the efficiency of the microencapsulation lemongrass EO by spray-drying technique using maltodextrin as wall material to help to enhance the product thermal stability. Nevertheless, as mentioned above, higher temperature (140 - 180°C) could cause browning reactions or caramelization of sugars, leading to the destruction of wall material and loss of EO inside (Miao and Roos, 2004; Nunes and Mercadante, 2007). Therefore, it was necessary to select an appropriate processing and storage temperature for the product. The combination of data obtained from the color measurement and OR values of microencapsulated powders suggested that the suitable temperature for maintaining the stability of microencapsulated lemongrass essential oil powder must be below 100°C in 20 mins; OR value observed at 92.015% after 20 mins.

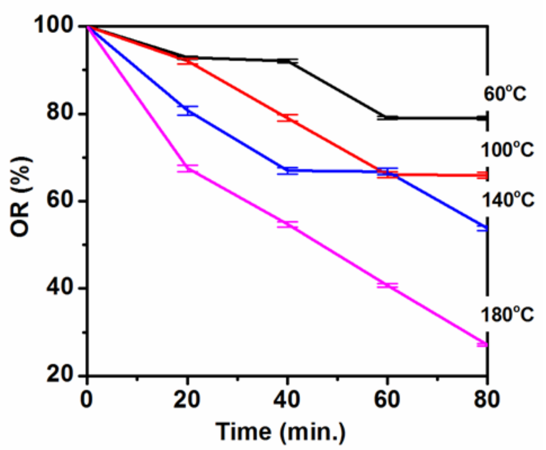

Figure 2. Effect of heating temperature and time on OR of lemongrass EO.

3.4 Properties of lemongrass essential oils after thermal treatment

The properties of lemongrass essential oils after heating at 100°C in 20 mins were analyzed by gas chromatography-mass spectrometry (GC-MS). Table 3 compares the composition of lemongrass essential oil in powders before and after heated at 100°C in 20 mins.

Table 3. Compositions of Lemongrass EO before and after thermal treatment

Compounds	Content before heating (%)	Content after heating (%)
Myrcene	30.492	17
Citral	49.076	37.634
Geraniol	3.762	1.589
Farnesol	1.898	0.309
Piperitone	0.627	2.684
Citronella	2.335	4.681
Limonene	0.299	0.255
6-methyl-5-hepten-2-one	1.46	1.061
Total	89.949	65.213

Analysis of lemongrass EO composition after heating process showed that the main compounds included: citral (37.634%), myrcene (17%), geraniol (1.589%), citronella (4.681%) and limonene (0.255%). Despite the general decline of all components upon heat application at 100°C in 20 mins, except for piperitone and citronella, the content of citral and myrcene remained the highest. When heating up to 100°C, moisture moved by diffusion to the surface of the particles and drags along the EO. Loss of citral on this study was related to its volatility since no additional peaks were found on chromatograms. Shahpour *et al*. (2008) found that as the drying temperature increases, the content of monoterpenes in EO gradually decreased while sesquiterpenes increased to a noticeable amount. This may be due to the low molecular weight of monoterpenes in comparison with sesquiterpenes, which would leave the particles more rapidly at high temperature. In addition, certain heat-sensitive terpenoid classes, particularly terpenes and aldehydes, are also prone to structural transformations occurring at elevated temperatures (Turek and Stintzing, 2013). Tomaino *et al*. (2005) reported that heating has caused terpenic conversion reactions to the EO. Microencapsulation is an advanced technology which helps to protect bio-compounds against the heat effect. According to the obtained results, the uses of wall material made of maltodextrin combined with spray-drying technology increased the durability of compounds in lemongrass EO under heating conditions.

3.5 Powder morphology

Morphology of the powder particles after heating at 100°C in 20 mins and control sample (not heated) were shown in Figure 3. The two samples have similar concentrations of wall material (30% w/w) and core

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material (1.5% w/w). The particles from two obtained powders had various sizes. However, in terms of morphology, most of the particles have a rounded external surface and no apparent fissures or cracks, which plays an important role in the protection and core retention (Carneiro *et al*., 2013). In addition, particle surfaces were also shriveled and concave, as reported previously (Rajabi *et al*., 2015). Increasing temperature also led to faster water evaporation and formation of the particles with a smooth surface, although not clearly observed in Figure 3b (Alamilla-Beltrán *et al*., 2005). In this study, the heating process has been conducted within a short period of time (20 mins) and OR values have not decreased significantly (about 8%). Thus, no significant changes in particle size and surface were visualized after heating.

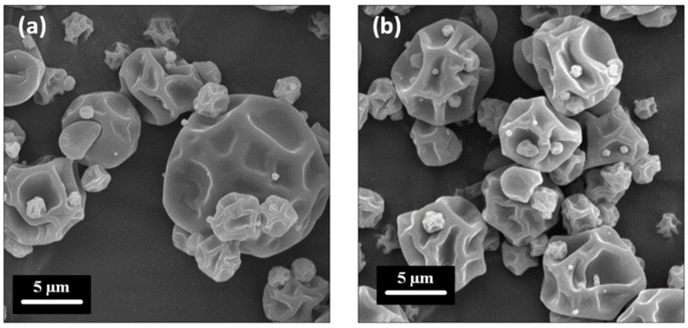

Figure 3. SEM observation of the particles containing lemongrass EO using maltodextrin as wall materials after heating at 100°C in 20 mins. (a) Control sample and (b) Heated sample.

4. Conclusion

Lemongrass EO has been widely used in folk medicine for the treatment of anxiety, memory deficit, and cancer thanks to its high antioxidant activity and antibacterial properties. Therefore, this study determined the effects of temperature and heating time on microencapsulated lemongrass essential oil based on the retention of lemongrass EO (OR), powder color, the chemical composition and particle morphology after the heating process. The lemongrass EO was successfully microencapsulated with maltodextrin as a carrier material by using spray drying. After heating was completed, the EO was obtained using hydro-distillation and analyzed by GC-MS. The decrease of color was observed in all powder samples after thermal treatment. Besides, high temperature also results in a reduction of OR values. The lowest OR (27.153%) was observed at powder subjected to thermal treatment of 180°C in 80 mins. Optimal temperature and time were selected as 100°C and 20 mins, respectively. However, the phytochemical screening of lemongrass EO before and after heating revealed the major presence of citral, myrcene, geraniol and citronella. The SEM image showed that all the samples have similar morphology and most of the particles have a rounded external surface and no apparent fissures or cracks. The microencapsulation process is proven to be highly effective in preserving the active substances present in lemongrass EO after heating.

Conflict of interest

The authors declare no conflict of interest.

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