

# Microencapsulation of *Litsea cubeba* Essential Oil in $\beta$ -Cyclodextrin Using Paste and Co-Precipitation Methods

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## ABSTRACT

Microencapsulation of *Litsea cubeba* essential oil (LCEO) with  $\beta$ -cyclodextrin (BCD) was carried out using the paste and co-precipitation methods at various oil concentrations and ratios of LCEO to BCD adapted from a central composite design to determine the effect of these factors on the microencapsulation efficiency, microencapsulation yield, surface oil and recovery of the finished product. In addition, the effect of different levels of water activity and temperature on the oxidative stability of the powder was investigated. Treatment 4 using the paste method with 90% LCEO and a ratio of LCEO to BCD of 15:85 was found to be more efficient compared to samples produced by the co-precipitation method. This condition provided an oil loading of 102 mg.g<sup>-1</sup> of powder, 74.7% microencapsulation yield, 71.9% microencapsulation efficiency, 94.8% recovery of LCEO powder and surface oil with 4.08 mg.g<sup>-1</sup> of powder. Statistical analysis indicated that the temperature and water activity had significant effects on the peroxide value. Moreover, there was an interaction between these two factors. The LCEO powder had higher oxidative stability with the lowest peroxide value of 4.9 meq.kg<sup>-1</sup> when it was stored at 10 °C and a water activity of 0.53 whilst the accelerated conditions of 50 °C and a water activity of 0.64 resulted in the highest peroxide value (13.6 meq.kg<sup>-1</sup>) and lower oxidative stability. It was notable that except for the samples stored at a water activity of 0.64 and temperatures of 30 and 50 °C, the other samples did not exceed the limit for the peroxide value of 10 meq.kg<sup>-1</sup> which is the acceptable limit for edible vegetable oils.

**Keywords:** microencapsulation, *Litsea cubeba*,  $\beta$ -cyclodextrin, oxidative stability, peroxide value

## INTRODUCTION

*Litsea cubeba* (Lour.) is an evergreen tree or shrub in the Lauraceae family and produces *Litsea cubeba* essential oil (LCEO) which is thin in consistency and greenish-yellowish in color and is extracted from the flower, leaf and fruit of the plant having a fresh, sharp, lemon-like scent and is in some ways similar to citronella (Anonymous, 2014). The amounts of *Litsea cubeba* oil extracted by hydrodistillation from fruits and leaves are in

the range of 13.9 and 4.0%, respectively, with a total of 53 compounds being reported in the leaf oil, of which the main component is 1, 8-cineol (57.6%) whereas the main compound of the extract from fruit is citral (68.9%), which in turn consists of neral (31.3%) and geranial (37.6%) (Ho *et al.*, 2010). *Litsea cubeba* (LC) is a plant widely distributed in Southeast Asia, Japan, Taiwan and Southern China and has been used as a flavor enhancer in foods and the beverages industry, cosmetics and cigarettes (Luo *et al.*, 2005). Since

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ancient time, LCEO has been applied in the field of herbal medicine due to its healing properties. LCEO essential oil is also used commonly in skin care as it is effective for acneic skin types and dermatitis and is non-irritating and antiseptic, while it can also be used for its antifungal (Luo *et al.*, 2004; Yang *et al.*, 2010), insecticidal and insect repellent properties in agro-industry (Noosidum *et al.*, 2008; Ko *et al.*, 2009). as well as an anti-inflammatory and cytotoxic agent in medicine (Lin *et al.*, 2007).

LCEO also has been applied as a raw material in the production of citral, ionone, methyl ionone, herbal medicine, aromatherapy, perfumes and vitamins such as A, E, and K by the chemical industry (Luo *et al.*, 2005; Hu *et al.*, 2014). Some researchers have studied the functional properties of LCEO such as antimicrobial activity (Wang and Liu, 2010; Liu and Yang, 2012) and antioxidant activity (Hwang *et al.*, 2005; Lin *et al.*, 2007). LCEO provides a unique mild flavor similar to lemon grass. However, the incorporation of essential oils into products by the food industry has been restricted because of either its low oxidative stability which is related to its high volume of unsaturated fatty acids or its poor solubility and miscibility within aqueous-based matrices (Avramenko, 2013). Factors including the production process, storage time, packaging materials and the combination of food ingredients may interfere with the flavor, causing lipid rancidity and an off-flavor or a reduction in the flavor and aroma intensity (Lubbers *et al.*, 1998). Therefore, a microencapsulation technique is suggested to suppress aroma degradation or loss during food processing and storage.

Microencapsulation is defined as a technique by which one material or a mixture of materials (core material) is entrapped within another material (coating material or supporting material or encapsulant) and was first described by Green and Scheicher (1955) for the production of carbonless copying paper. Nowadays its use has expanded and has been accepted by the chemical,

cosmetic, pharmaceutical and food sectors as well as others including the printing industry (Augustin *et al.*, 2001; Heinzen, 2002).

Inclusion complexation using  $\beta$ -cyclodextrin (BCD) is of interest for the encapsulation of flavoring compounds, especially essential oil. Cyclodextrins have been used widely to form inclusion complexes to enhance the bioavailability of insoluble active compounds by increasing their solubility, dissolution, permeability and stability (Szente *et al.*, 1993; Chaudhary *et al.*, 2012). Cyclodextrins are mostly used to improve the water solubility of hydrophobic compounds including essential oils, food colorings, liquid essences and vitamins by converting them into stable powder (Martin Del Valle, 2003). This technique improves the physicochemical properties and the shelf life of the guest molecules by protecting them against oxidation, heat and light degradation, in combination with undesired elements, hydrolysis and the risk of evaporation, while also providing the controlled release of the active ingredients and the suppression of undesired off flavor and odors (Szejtli, 1982; Patington, 1986; Nagai and Ueda, 1996; Hedges and McBride, 1999; Bhandari *et al.*, 2001; Villaverde *et al.*, 2004; Cravotto *et al.*, 2006). Among various encapsulation techniques, molecular inclusion or inclusion complexation is preferred due to its simplicity, low cost and the production of finely finished nanopowders (Hedges *et al.*, 1995). In the aqueous phase, the BC cavity (slightly polar) is engaged by H<sub>2</sub>O molecules which can simply be substituted with nonpolar, active compounds by noncovalent interactions and stabilized (Shahidi and Han, 1993). There are three different methods to attain complex inclusion, including co-precipitation, paste and spray drying methods (Patington, 1985). In the co-precipitation method, the cyclodextrin solution is heated beforehand. Then, the guest molecules are added and stirred gently for hours until precipitation is achieved. In the spray drying method, the complex inclusion is obtained by diffusing the mixture of guest and

host molecules in a high temperature chamber. In the paste method, core material is mixed with the BCD using shear force or kneading.

Encapsulation of LCEO using BCD has not been reported elsewhere. Therefore, this research compared the encapsulation efficiency of LCEO entrapped in BCD using the paste and co-precipitation methods and the stability of the encapsulated LCEO powder was also investigated.

## MATERIALS AND METHODS

### Encapsulation of *Litsea cubeba* essential oil in $\beta$ -cyclodextrin using paste method

A paste method was derived from that of Bhandari *et al.* (1999) with some modifications. A precise amount of BCD was moistened with distilled water to form a paste in a dough mixture and then a predetermined quantity of LCEO (sourced from the Royal Project Foundation, Khunwang Center, Chiang Mai, Thailand) dissolved in ethanol was added and mixed well. The ratios of LCEO to BCD used were 5:95, 10:90 and 15:85. Based on these factors, nine trials were generated (Table 1) similar to those of central composite design (CCD) 2 factors. The mixture was blended for 15 min using a dough mixer. The paste was then dried in an oven at 40 °C for 24 hr. The powder was ground, sieved using a flour

sifter and allowed to air dry at 25 °C for 24 hr at room temperature to reach its equilibrium moisture content. The microencapsulated LCEO powder was then kept in an airtight plastic bag at 25 °C.

### Encapsulation of *Litsea cubeba* essential oil in $\beta$ -cyclodextrin using co-precipitation method

A co-precipitation method was derived from Bhandari *et al.* (1998). BCD (4, 8 and 12 g) was dissolved in a mixture solution of ethanol to water (1:2). The volumes were then adjusted to 100 mL. The solutions were heated to 50–55 °C. The LCEO dissolved in the ethanol (4, 8 and 12%) was then gently added into a warm BCD solution using the different ratios of LCEO to BC (5:95, 10:90 and 15:85). Based on these factors, 15 trials were generated (Table 2) similar to those of CCD 3 factors. During addition, the mixture was continuously stirred using a magnetic stirrer. After addition, heating ceased and eventually the mixture was covered with aluminum foil and agitated for 4 hr and then stored at 4 °C for 24 hr. The precipitated LCEO-BCD complex was obtained by vacuum filtration and then dried in an oven at 40 °C for 24 hr. The powder was ground, sieved using a flour sifter and allowed to air-dry at 25 °C for 24 hr at room temperature to reach its equilibrium moisture content. The microencapsulated LCEO powder was then kept in an airtight plastic bag at 25 °C.

**Table 1** Derived trials generated similar to central composite design 2 factors (paste method).

TRT	LCEO (%)	Ratio of LCEO to BCD
1	50	5:95
2	90	5:95
3	50	15:85
4	90	15:85
5	70	10:90
6	41.72	10:90
7	98.28	10:90
8	70	2.93:97.07
9	70	17.07:82.93

LCEO = *Litsea cubeba* essential oil.

BCD =  $\beta$ -Cyclodextrin.

**Table 2** Derived trials generated similar to central composite design 2 factors (co-precipitation method).

Treatment	LCEO (%)	BCD (%)	Ratio of LCEO to BCD
1	4	4	5:95
2	12	4	5:95
3	4	12	5:95
4	12	12	5:95
5	4	4	15:85
6	12	4	15:85
7	4	12	15:85
8	12	12	15:85
9	1.28	8	10:90
10	14.72	8	10:90
11	8	1.28	10:90
12	8	14.72	10:90
13	8	8	1.6:98.40
14	8	8	18.4:81.60
15	8	8	10:90.00

LCEO = *Litsea cubeba* essential oil.

BCD =  $\beta$ -Cyclodextrin.

### Some chemical and physical properties of microencapsulated *Litsea cubeba* essential oil

#### Moisture content determination

The moisture contents of the BCD and the microencapsulated powder were determined following the method of Association of Official Analytical Chemists (1990). The analysis was performed in duplicate in each analysis and calculated using Equation 1:

$$\text{Moisture content (\%)} = \frac{W1 - W2}{W1} \times 100 \quad (1)$$

where W1 is the mass of powder prior to drying and W2 is the weight of powder after drying (both measured in grams).

#### Recovery of powder analysis

The total recovery was calculated according to Equation 2:

$$\text{Total recovery (\%)} = \frac{W2}{W1} \times 100 \quad (2)$$

where W<sub>1</sub> is the mass of the initial materials (dry basis) and W<sub>2</sub> is the weight of encapsulated powder (dry basis) both measured in grams.

#### Surface oil extraction

The surface oil of the powder was measured by washing 2 g of powder with 20 mL of hexane using an orbital shaker (Ks501 Digital; IKA-Werke GmbH and Co.; Staufen, Germany) for 15 min at room temperature. The supernatant was then filtered through Whatman no. 1 filter paper and the powder collected on the filter paper was rinsed twice with 15 mL of hexane for 10 min, consecutively (Bae and Lee, 2008). The solution containing the extracted oil was transferred to a clean beaker and left to evaporate for a while under a laminar hood and then eventually was dried at 60 °C until constant weight was achieved. The amount of surface oil was then calculated (Jafari *et al.*, 2008). The analysis was done in duplicate for each analysis.

#### Total oil extraction

The total oil load in the powder was determined using the hydro-distillation method (Padukka *et al.*, 1999). The microencapsulated powder (5 g) was hydro-distilled with distilled water (250 mL) for 3 hr. The extracted oil was collected gradually into a cylinder and then the

volume of oil extracted was read directly from the graduated arm cylinder and multiplied by the density for gravimetric determination (density of the LCEO was  $0.886 \text{ g.cm}^{-3}$  at  $20 \text{ }^{\circ}\text{C}$ ). The analysis was performed in duplicate for each analysis.

### Microencapsulation efficiency and microencapsulation yield

To evaluate the microencapsulation process, the microencapsulation efficiency (ME) and the microencapsulation yield (MY) were determined using Equations 3 and 4, respectively:

$$\text{ME (\%)} = \frac{\text{Mass of total extracted oil Oil on the microcapsule surface}}{\text{Amount of initial essential oil used}} \times 100 \quad (3)$$

$$\text{MY (\%)} = \frac{\text{Weight of total extracted flavor oil}}{\text{Weight of initial essential oil used}} \times 100 \quad (4)$$

### Storage stability of microencapsulated *Litsea cubeba* essential oil

A sample (20 g) of microencapsulated LCEO powder was spread on Petri dishes placed in desiccators containing a saturated solution of 11 M NaCl,  $\text{NaNO}_2$  and NaCl, for water activity (aw) determination of 0.53, 0.64 and 0.75, respectively (Labuza, 1975; Labuza et al., 1985). The humidity of the desiccators was balanced to the desired level prior to inserting the samples. The desiccators were covered to avoid light oxidation during storage. The desiccators were then stored for 5 wk at 10, 30 and  $50 \text{ }^{\circ}\text{C}$ , respectively. The samples were collected and analyzed weekly for peroxide values (PV) using the method Cd 8b-90 (American Oil Chemists' Society, 2004) with some modification which reduced the amount of sample (2g) applied as well as dissolving the LCEO powder into acetic acid/chloroform in a water bath ( $50 \text{ }^{\circ}\text{C}$ ) for 5 min to release the oil from the BCD complexes. The PV was analyzed based on the total extracted oil (oil loading included inside inclusion plus surface oil). The analysis was performed in duplicate.

### Statistical analysis

A complete randomized design with two

replications in each analysis was performed. The mean differences were determined using Duncan's multiple range tests at the 95% confidence level. A  $3 \times 3$  factorial design with two replications in each analysis was carried out for PV determination.

## RESULTS AND DISCUSSION

### Microencapsulation of *Litsea cubeba* essential oil using paste method

Microencapsulation of LCEO in BCD using the paste method involved a kneading process. The mixture of water and BCD was kneaded to produce a high consistency dough, related to the interaction between the water molecules and the BCD cavity. The addition of LCEO during kneading led to a reduction in the water interaction within the complex resulting in the formation of a lower consistency dough (Bhandari *et al.*, 1998). The BCD cavity can be easily occupied by LCEO with a non-covalent bond followed by replacement of the excess water. All powders produced by the paste method were in the form of agglomerates after the drying process so they needed to be milled and sieved to obtain uniform and flowable powders. It was observed that an increase in the concentration of LCEO and the ratio of LCEO to BCD led to greater smoothness of the final powder, which was consistent with the study conducted by Bhandari *et al.* (1998). All the samples had a pale yellow color compared to the white color of the original BCD powder due to the interaction of the LCEO with the BCD either as an oil load inside the inclusion or following adsorption on the surface of the BCD molecules (subjective observation). Some properties of the microencapsulated LCEO in the BCD using the paste method are presented in Table 3.

### Powder recovery

The powder recovery is the percentage of obtained powder compared to the initial solid input. As was expected, the mass of finished powder was slightly less than the mass of the

**Table 3** Properties of microencapsulated *Litsea cubeba* essential oil (LCEO) in  $\beta$ -cyclodextrin (BCD) using paste method.

Treatment	LCEO (%)	Ratio of LCEO to BCD	Oil loading (mg.g <sup>-1</sup> of powder)	MY (%)	ME (%)	Recovery powder (%)	Surface oil (mg. g <sup>-1</sup> of powder)
1	50	5:95	23.9 <sup>g*</sup> ±0.28	94.4 <sup>b</sup> ±0.52	91.2 <sup>a</sup> ±0.57	97.8 <sup>g</sup> ±0.14	0.84 <sup>a</sup> ±0.11
2	90	5:95	41.6 <sup>f</sup> ±0.51	91.5 <sup>c</sup> ±0.42	89.8 <sup>b</sup> ±0.64	96.8 <sup>bc</sup> ±0.42	1.38 <sup>bc</sup> ±0.25
3	50	15:85	72.0 <sup>d</sup> ±0.66	88.9 <sup>d</sup> ±0.45	86.2 <sup>c</sup> ±0.66	96.2 <sup>cde</sup> ±0.28	2.34 <sup>c</sup> ±0.31
4	90	15:85	102.0 <sup>a</sup> ±0.82	74.7 <sup>g</sup> ±0.64	71.9 <sup>f</sup> ±0.62	94.8 <sup>f</sup> ±0.57	4.08 <sup>e</sup> ±0.20
5	70	10:90	64.0 <sup>e</sup> ±0.90	89.4 <sup>d</sup> ±0.35	86.4 <sup>c</sup> ±0.42	96.4 <sup>bcd</sup> ±0.14	2.22 <sup>c</sup> ±0.17
6	41.72	10:90	40.6 <sup>f</sup> ±0.44	92.6 <sup>c</sup> ±0.31	89.8 <sup>b</sup> ±0.35	97.0 <sup>b</sup> ±0.28	1.26 <sup>ab</sup> ±0.30
7	98.28	10:90	83.0 <sup>c</sup> ±0.55	84.1 <sup>e</sup> ±0.28	81.3 <sup>d</sup> ±0.42	95.9 <sup>de</sup> ±0.42	2.76 <sup>d</sup> ±0.20
8	70	2.93:97.07	19.6 <sup>b</sup> ±0.21	95.4 <sup>a</sup> ±0.48	91.8 <sup>a</sup> ±0.45	98.1 <sup>a</sup> ±0.14	0.78 <sup>a</sup> ±0.08
9	70	17.07:82.93	97.0 <sup>b</sup> ±0.49	77.1 <sup>f</sup> ±0.64	74.3 <sup>e</sup> ±0.28	95.5 <sup>ef</sup> ±0.28	3.60 <sup>de</sup> ±0.16

ME = Microencapsulation efficiency.

MY = Microencapsulation yield.

\* = Values with the same lowercase superscript letters in each column are not significantly different (P ≥ 0.05).

initial ingredients due to the loss of LCEO-BCD complexes during kneading using the dough mixer, due to evaporation, and due to degradation of volatile LCEO during the drying step. The statistical comparison of data illustrated significant differences between treatments. The lowest recovery was found in treatment 4, followed by treatment 9, which was 94.8 and 95.5%, respectively while treatment 8 had the highest recovery (98.1%), followed by treatment 1 (97.8%). These results may have been due to the lower amount of LCEO entrapped in the inclusion complexes. Moreover, it was recognized that the decrease in the powder recovery was directly proportional to the increase in the amounts of LCEO added to the mixture. Thus, as the concentration of the LCEO or the initial ratio of LCEO to BCD increased, the possibility of degradation or evaporation of the LCEO entrapped within the agglomerates or adsorbed on the surface of the BCD molecules increased during microencapsulation processing and the drying step. The results are shown in Table 3.

#### Oil loading of $\beta$ -cyclodextrin

The oil loading is expressed as the amount of oil included in the  $\beta$ -cyclodextrin molecule (total oil minus the surface oil). There was a significant difference between treatments. The maximum amount of oil entrapped in the BCD molecules occurred in treatments 4 and 9 (102 and 97.0 mg of oil per gram of BCD, respectively), whereas treatments 8 and 1 had the lowest oil loading (19.6 and 23.9 mg of oil per gram of BCD, respectively). The results from treatment 4 were 90% LCEO and 15:85 for the ratio of LCEO to BCD, which were in the range of the maximum oil loading for BCD with essential oil of 8-12% (Pagington, 1986). Bhandari *et al.* (1998) also reported that the total lemon oil load of the BCD complexes was 97.1 mg of oil per gram of BCD using the paste method. In addition, Westing *et al.* (1988) stated that the total oil content of orange oil-BCD complexes was 82 mg of oil per gram of BCD. Furthermore, Szente and Sziptli (1988)

reported the total lemon oil load in the complex was 98 mg of oil per gram of BCD. The small differences in the inclusion capacity of the BCD might have been due to the addition of different quantities of essential oils, different stirring and/or kneading times, polarity compatibility and the molecular weight of the essential oils components. Statistical analysis of data illustrated that the oil loading increased significantly as the concentration of the LCEO and the ratio of LCEO to BCD increased. The results are presented in Table 3.

#### **Surface oil of the microcapsule**

The amount of LCEO oil on the surface of the BCD microcapsules measured by washing the powder with hexane (Bae and Lee, 2008; Jafari *et al.*, 2008) were in the range 0.78–4.08 mg per gram of powder. The highest surface oil content (4.08 mg.g<sup>-1</sup>) was found in treatment 4, whereas the lowest value (0.78 mg.g<sup>-1</sup>) was found in treatment 8. Duncan's multiple range tests indicated that the mean values for treatments 1 and 8 were not significantly different. The amount of surface oil volatiles significantly increased as the initial oil concentration and the ratio of oil to BCD increased. A portion of the oil which was not encapsulated within BCD molecules but entrapped within the solid matrix of agglomerates could not be extracted by the washing the powder with hexane; therefore, the actual amounts of surface oils were higher than the values reported. It is important to note that the Soxhlet method may extract greater amounts of oil from microcapsules compared to simply washing with a solvent (Bhandari *et al.*, 1998). This was consistent with the studies of Szente and Szijtli (1988) and Bhandari *et al.*, (1998) who reported that the further addition of LCEO resulted in an increase in the surface oil due to exceeding the theoretical maximum oil loading of the BCD cavity. The results are shown in Table 3.

#### **Microencapsulation efficiency and microencapsulation yield**

The microencapsulation efficiency is expressed as the percentage of oil entrapped within the structure of the BCD and the microencapsulation

yield is the amount of essential oil recovered by hydro-distillation from the microcapsules compared to the initial amount added. There was a significant difference between treatments for ME and MY (Table 3). The highest ME was found in treatment 8 (91.8%) and the lowest value was found in treatment 4 (71.9%). Duncan's multiple range tests illustrated that the mean values for treatments 1 (91.2 ± 0.57) and 8 (91.8 ± 0.45) were not significantly different. The highest MY value was found in treatment 8 (95.4%) while the lowest was found in treatment 4 (74.7%). This may have been caused by the excessive oil residue adsorbed on the surface of the BCD microcapsules, leading to the loss of oil by degradation, oxidation and evaporation within microencapsulation processing and the drying step. Although treatment 4 had the lowest MY (74.7%) and ME (71.9%) values, the most important factor is the oil loading (102 mg.g<sup>-1</sup>); thus, treatment 4 was chosen for the further experiment.

#### **Microencapsulation of *Litsea cubeba* essential oil using co-precipitation method**

The recovered powders had a light yellow color in comparison to the white color of the original BCD. This difference in color might have been due to either the inclusion of LCEO pigments into the BCD cavity or the adsorption of oil on the surface of the microcapsules. The intensity of the pale yellow color of the powders produced by the paste method was higher than those from the co-precipitation method due to the higher amount of LCEO remaining either on the surface of the BCD molecules or within agglomerates. Moreover, it was observed that the smoothness and fineness of the final powder increased as the concentration of the LCEO or the ratio of LCEO to BCD was elevated (subjective observation) which may have been caused by the reduction of water interaction with the hydroxyl groups of the secondary and primary faces of the BCD molecules throughout the complex. Some properties of the microencapsulated LCEO in

the BCD using the co-precipitation method are presented in Table 4.

#### **Complexation and powder recovery**

The recovery of the LCEO powder at the equilibrium state is shown in Table 4. Statistical analysis showed that there was a significant difference between treatments. Increasing the LCEO and the ratio resulted in an increase in the recovery percentage. The highest value was found in treatment 14 (88%), followed by treatment 10 (87.9%), while the lowest was in treatment 11 (66%), followed by treatment 6 (68%). Moreover, there was no significant difference between treatments 6 and 11 and between treatments 10 and 14. It was also observed that a higher ratio of LCEO to BCD in the solution contributed to better inclusion and stronger linkage, resulting in the reduced solubility and greater stability of the LCEO-BCD complexes (Bhandari *et al.*, 1998). In addition, there were some important factors that may have contributed to the reduction of powder recovered, including the amount of oil left in the solution after forming complexes due to reaching the equilibrium level of the oil between the aqueous solution and the cavity of the BCD and also the loss of noncomplexed BCD in the solution. A portion of the LCEO, which might be adsorbed on the surface of microcapsules or entrapped within agglomerates, might have been lost during the drying step.

#### **Oil loading of $\beta$ -cyclodextrin**

The statistical comparison indicated that the maximum oil loading occurred in treatment 11 (97.4 mg of oil per gram of BCD), while treatment 13 had the lowest value (16.5 mg of oil per gram of BCD), which was in the range of the maximum loading (8-12%) for BC reported by Pagington (1986). Bhandari *et al.* (1998) also stated that the oil loading of lemon oil-BCD complexes was 96.8 mg of oil per gram BCD using the co-precipitation method. The results are presented in Table 4.

The higher oil loading might have been caused by either the addition of greater amounts of LCEO or longer stirring times, which contributed

to the higher co-crystallization of the BCD. Moreover, the physicochemical properties of oil, including the molecular weight of the essential oil components, polarity and the compatibility of the oil components with the non-polar cavity of the BCD have profound effects on the inclusion and subsequently, on the oil loading (Bhandari *et al.*, 1998; Martin del Valle, 2003).

#### **Surface oil of the microcapsule**

The amount of surface oil (hexane washing method) was in the range 0.35–1.66 mg per gram of powder. The highest surface oil was found in treatment 11 (1.66 mg of oil per gram of powder), whereas the two lowest were found in treatments 9 and 3 (0.35 and 0.36 mg per gram of powder, respectively). The amount of surface oil increased significantly as the concentration of the LCEO and the starting ratios of LCEO to BCD increased. Westing *et al.* (1988) reported that the surface oil content for orange oil-BCD, determined using Soxhlet extraction with pentane, was 431 mg per 100 gram of final powder, which was higher than the results found in the current experiment which might have been caused by the different oil extraction process used. The results are represented in Table 4.

#### **Microencapsulation efficiency and microencapsulation yield**

The maximum ME occurred in treatment 3, (78.7%), while the lowest efficiency was found in treatment number 11 (15.7%). Similarly, the maximum MY value was found in treatment 3 (80.2%) and the minimum value was found in treatment 11 (16%). It was observed that the amount of essential oil added into the system was higher than the equilibrium level reported by Pagington (1986), leading to a noticeable reduction in the ME. Statistical analysis showed that treatment 14 had the highest inclusion efficiency as well as the highest value of recovery powder, indicating the optimum concentration of LCEO and BCD, including the ratio of LCEO to BCD. The results are shown in Table 4.

**Table 4** Properties of microencapsulated *Litsea cubeba* essential oil (LCEO) in  $\beta$ -cyclodextrin (BCD) using co-precipitation method.

Treatment	LCEO (%)	BCD (%)	Ratio of LCEO to BCD	Oil loading (mg/g of powder)	MY (%)	ME (%)	Recovery powder (%)	Surface oil (mg/g of powder)
1	4	4	5:95	43.81 <sup>*</sup> ±0.14	72.3 <sup>c</sup> ±0.33	71.5 <sup>c</sup> ±0.41	81.7 <sup>cd</sup> ±0.28	0.45 <sup>i</sup> ±0.11
2	12	4	5:95	79.8 <sup>e</sup> ±0.28	49.2 <sup>f</sup> ±0.15	48.7 <sup>h</sup> ±0.17	83.2 <sup>bc</sup> ±0.44	0.89 <sup>ef</sup> ±0.23
3	4	12	5:95	18.4 <sup>i</sup> ±0.42	80.2 <sup>a</sup> ±0.53	78.7 <sup>a</sup> ±0.47	73.0 <sup>e</sup> ±0.18	0.36 <sup>i</sup> ±0.44
4	12	12	5:95	34.9 <sup>k</sup> ±0.28	55.7 <sup>d</sup> ±0.49	55.1 <sup>f</sup> ±0.38	78.8 <sup>d</sup> ±0.23	0.40 <sup>ij</sup> ±0.35
5	4	4	15:85	83.4 <sup>d</sup> ±0.28	48.4 <sup>f</sup> ±0.38	47.9 <sup>h</sup> ±0.34	86.1 <sup>ab</sup> ±0.35	0.98 <sup>d</sup> ±0.50
6	12	4	15:85	92.5 <sup>b</sup> ±0.52	18.6 <sup>h</sup> ±0.25	18.3 <sup>i</sup> ±0.18	68.7 <sup>f</sup> ±0.40	1.35 <sup>b</sup> ±0.22
7	4	12	15:85	39.4 <sup>i</sup> ±0.71	56.9 <sup>d</sup> ±0.48	56.3 <sup>e</sup> ±0.42	79.5 <sup>d</sup> ±0.33	0.44 <sup>i</sup> ±0.20
8	12	12	15:85	75.8 <sup>f</sup> ±0.49	42.5 <sup>g</sup> ±0.35	42.1 <sup>i</sup> ±0.45	83.2 <sup>bc</sup> ±0.54	0.87 <sup>f</sup> ±0.38
9	1.28	8	10:90	18.1 <sup>lm</sup> ±0.14	77.8 <sup>b</sup> ±0.78	76.3 <sup>b</sup> ±0.64	73.9 <sup>e</sup> ±0.25	0.35 <sup>j</sup> ±0.22
10	14.72	8	10:90	82.0 <sup>d</sup> ±1.20	43.0 <sup>g</sup> ±0.34	42.5 <sup>i</sup> ±0.17	87.9 <sup>a</sup> ±0.20	0.95 <sup>de</sup> ±0.55
11	8	1.28	10:90	97.4 <sup>a</sup> ±0.85	16.0 <sup>i</sup> ±0.17	15.7 <sup>k</sup> ±0.20	66.1 <sup>f</sup> ±0.46	1.66 <sup>a</sup> ±0.60
12	8	14.72	10:90	48.7 <sup>h</sup> ±0.28	70.7 <sup>c</sup> ±0.47	69.9 <sup>d</sup> ±0.45	81.5 <sup>cd</sup> ±0.13	0.57 <sup>h</sup> ±0.33
13	8	8	1.6:98.4	16.5 <sup>m</sup> ±0.71	77.3 <sup>b</sup> ±0.41	75.5 <sup>b</sup> ±0.38	73.9 <sup>e</sup> ±0.27	0.39 <sup>ij</sup> ±0.30
14	8	8	18.4:81.6	86.0 <sup>c</sup> ±0.90	42.2 <sup>g</sup> ±0.24	41.7 <sup>i</sup> ±0.37	88.0 <sup>a</sup> ±0.30	1.14 <sup>c</sup> ±0.35
15	8	8	10:90	65.1 <sup>g</sup> ±0.14	52.8 <sup>e</sup> ±0.34	52.2 <sup>g</sup> ±0.49	80.1 <sup>cd</sup> ±0.14	0.74 <sup>g</sup> ±0.45

ME = Microencapsulation efficiency.

MY = Microencapsulation yield.

\* = Values with the same lowercase superscript letters in each column are not significantly different ( $P \geq 0.05$ ).

### Comparison between paste and co-precipitation methods

Although the paste method provided a greater amount of surface oil than the co-precipitation method, which might lead to poor storage stability, other factors such as oil loading, microencapsulation efficiency, microencapsulation yield and the percentage of powder recovery were significantly higher than those from the co-precipitation method (Table 5). The oil loading was the main criterion used to select the method and optimum treatment. Moreover, with respect to simplicity, processing time, cost and the size of production, the paste method was considered better than the co-precipitation method. Thus, the paste method was chosen for further experiment.

### Storage stability of microencapsulated *Litsea cubeba* essential oil in $\beta$ -cyclodextrin

The rate of lipid oxidation (autoxidation)

is associated with two main factors, water activity and glass transition in an amorphous food system (Nelson and Labuza 1992). Water plays a noticeable role in glass transition because it can plasticize the amorphous structure of carbohydrates as well as food matrices. The glassy state is attributed to less free volume in a polymer matrix in comparison with the rubbery state that has been reported to retard the oxidation of lipids. There have been only a few studies reported involving the influence of water activity on the oxidation of volatile compounds of microencapsulated lipids or essential oils (Anker and Reineccius 1988; Beristain *et al.*, 2002, Soottitantawat *et al.*, 2004). Therefore, the role of the water activity and storage temperature on the stability of microencapsulated LCEO was investigated and the results are shown in Figure 1 and Table 6.

There was a significant difference among all treatments. The highest peroxide value (13.6

**Table 5** Comparison of LCEO microencapsulation by using paste and co-precipitation methods.

Method	Oil loading (mg.g <sup>-1</sup> of powder)	MY (%)	ME (%)	Recovery powder (%)	Surface oil (mg.g <sup>-1</sup> of powder)
Paste	102.0 <sup>a</sup> ±0.82	74.7 <sup>a*</sup> ±0.64	71.9 <sup>a</sup> ±0.62	94.8 <sup>a</sup> ±0.57	4.1 <sup>a</sup> ±0.20
Co-precipitation	86.0 <sup>b</sup> ±0.90	42.2 <sup>b</sup> ±0.24	41.7 <sup>b</sup> ±0.37	88.0 <sup>b</sup> ±0.30	1.1 <sup>b</sup> ±0.35

ME = Microencapsulation efficiency.

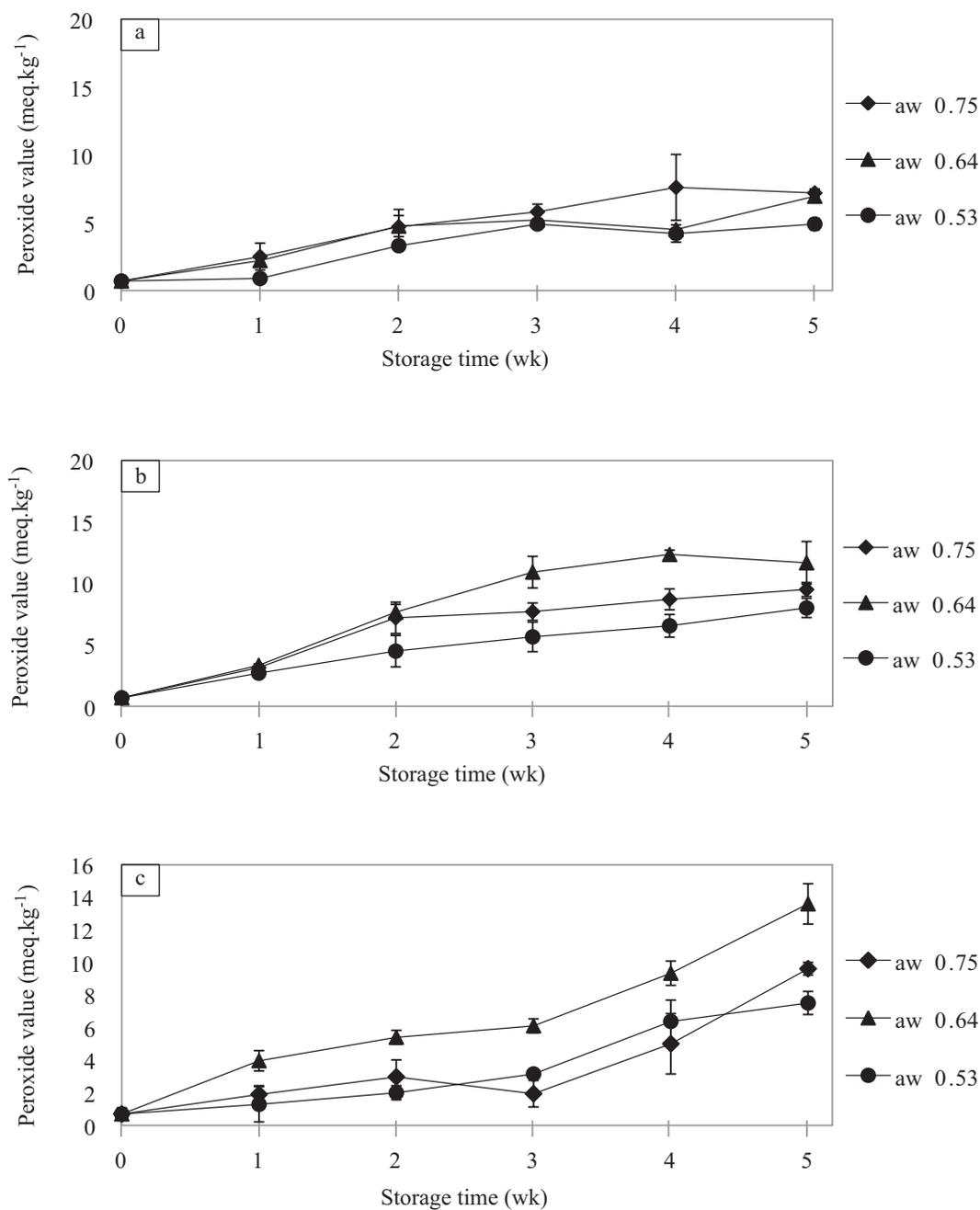
MY = Microencapsulation yield.

\* = Values with the same lowercase superscript letters in each column are not significantly different ( $P \geq 0.05$ ).

**Table 6** Peroxide value (PV) changes during 5 wk storage under different conditions.

Temperature	Water activity	PV at week 0 (meq.kg <sup>-1</sup> )	PV at week 5 (meq.kg <sup>-1</sup> )	$\Delta$ PV (meq.kg <sup>-1</sup> )
10 °C	0.53	0.7	4.9	4.2 <sup>e*</sup> ±0.50
	0.64	0.7	6.9	6.2 <sup>d</sup> ±0.63
	0.75	0.7	7.2	6.5 <sup>d</sup> ±0.51
30 °C	0.53	0.7	8.0	7.3 <sup>cd</sup> ±0.62
	0.64	0.7	11.7	11.0 <sup>b</sup> ±1.09
	0.75	0.7	9.5	8.8 <sup>c</sup> ±0.71
50 °C	0.53	0.7	7.5	6.8 <sup>d</sup> ±0.72
	0.64	0.7	13.6	13.0 <sup>a</sup> ±1.30
	0.75	0.7	9.6	9.0 <sup>c</sup> ±0.95

\* = Values with the same lowercase superscript letters in each column are not significantly different ( $P \geq 0.05$ ).



**Figure 1** Oxidative stabilities of microencapsulated *Litsea cubeba* essential oil in  $\beta$ -cyclodextrin as a function of storage time measured under different water activities (aw) and temperatures: (a) 10 °C; (b) 30 °C; and (c) 50 °C.

meq.kg<sup>-1</sup>) was found at water activity 0.64 and 50 °C, whereas the lowest (4.9 meq.kg<sup>-1</sup>) was obtained when the powder was kept at a water activity of 0.53 at 10 °C. The rate of oxidation was substantially accelerated as the temperature increased. Samples kept at 10 °C had the lowest oxidation rate at all water activities tested (Table 6). As the temperature increased, the peroxide value increased sharply, reaching 6.95 and 7.19 meq.kg<sup>-1</sup> for water activity levels of 0.64 and 0.75, respectively. Temperature and water activity had a significant effect on the oxidative stability of the powder by increasing the rate of lipid peroxidation and also there was an interaction between these two factors. The samples exposed to the water activity levels of 0.63 and 0.75 had faster oxidation rates than those of the water activity level of 0.53 for all storage temperatures. All the samples except for samples stored at a water activity of 0.64 and temperatures of either 30 and 50 °C, showed acceptable limits for the peroxide value which was lower than 10 meq.kg<sup>-1</sup> applicable for edible vegetable oils (American Oil Chemists' Society, 2004).

Interestingly, a slight decrease was observed for a water activity level of 0.53 at 30 and 50 °C, where the peroxide value dropped from 8.0 to 7.5 meq.kg<sup>-1</sup>, while at a water activity of 0.75, the peroxide value remained steady at 9.5 meq.kg<sup>-1</sup>. As shown in Figure 1, an increase in the temperature caused a sizeable increase in the rate of lipid oxidation. At week 5 of storage at 30 and 50 °C, there were sizable decreases in the peroxide values either as the water activity increased from 0.64 to 0.75 or decreased from 0.64 to 0.53. These results paralleled the studies of Toure *et al.* (2007) and Reed *et al.* (2002). Consistent with the study of Soottitawattawat *et al.* (2004), it was observed that oxidation increased significantly with increasing water activity up to 0.64, but a further increase in the water activity resulted in lower oxidation rates. This phenomenon may have occurred due to either a function of the water molecule as a barrier against oxidation or the rehydration of the

powder following adhesion of microcapsules in a rubbery state, which limited the oxygen intake and subsequently retarded the oxidation reaction. This result was in agreement with results reported by Whorton and Reineccius (1995). They stated that flavor loss increased with increasing water activity until structural collapse took place.

## CONCLUSION

The microencapsulation of *Litsea cubeba* essential oil in BCD using the paste method under conditions of 90 % LCEO and a ratio of 15:85 for LCEO to BCD provided a higher oil loading (102 mg of oil per gram of BCD), recovery of powder (90.9%) and microencapsulation efficiency (71.9%) than those under the co-precipitation method. The encapsulated powder was most stable when it was kept at a water activity of 0.53 and at 10 °C which produced the lowest peroxide value. Further investigation must be done to better understand the synergy between temperature and water activity to achieve better oxidative stability of the product and to minimize the oxidation of oil during processing and storage.

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