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RESEARCH ARTICLE

Encapsulation of cinnamon leaf oil within chitosan: formulation and characterization

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Abstract: Chitosan microcapsules containing cinnamon leaf oil were formed by ionotropic gelation of chitosan by crosslinking with sodium tripolyphosphate. The microencapsulation method for oil loading was optimized by varying the amounts of polymer, oil and cross linker. The effect of varying process parameters on the encapsulation efficience (EE), oil loading capacity (LC) and release rate was monitored. The formulated microcapsules were characterized using Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR). SEM imaged clear spherical shaped oil loaded microcapsules with a crimpy surface while empty capsules had a smooth texture.FTIR spectra evidence for the successful encapsulation of oil within wall material. EE increased with increasing oil load while it decreased with increasing polymer and cross linker concentration. LC increased with increasing amounts of oil and crosslinker and showed a decrease with increasing polymer amounts. Release rate of oil increased with increasing oil loading whereas it decreased with increasing polymer and crosslinker amounts. Varying the process parameters has a direct impact on the EE, LC and release rate. The optimum formulation for cinnamon oil microcapsules is polymer, 1 g : oil, 3g: cross linker, 0.5 g with an EE of 91%, and a LC of 38%.

Keywords: Encapsulation, Cinnamon oil, Essential oil, Chitosan, Microcapsules.

INTRODUCTION

Essential oils (EOs) are aromatic and volatile active substances extracted from different plant parts. They have gained a huge interest in various industries due to their important biological activities.Antimicrobial and antioxidant properties of many essential oils, such as garlic, cinnamon, thyme, oregano, clove, basil, coriander, citrus peel, eucalyptus, ginger, rosemary, and peppermint, among others, have been demonstrated under in-vitro conditions (Soliman *et al.*, 2013).

However, their industrial applications are limited due to the volatile nature and ease of decomposition of active substances to heat, oxygen, light and moisture when applied directly. Thus, a suitable formulation which avoids the above drawbacks and at the same time allows sustainable release of the oil is needed. Encapsulation protects the active substances from physical and chemical interactions and allows sustainable release of active substance. On the other hand, it increases the bioavailability of active substances by means of sustained release. Many core materials like live cells, adhesives, flavors, agrochemicals, enzymes, pharmaceuticals etc., can be encapsulated using microencapsulation. Nano encapsulation is of high interests as the delivery of bioactive compounds into various sites of the body is highly dependent on the particle size. Encapsulated EOs have many applications in food, agriculture, pharmaceutical and textile industries.

Cinnamon (*Cinnamomum zeylanicum*) belongs to Lauraceae family which is native to Indonesia, but also it is cultivated in India and Sri Lanka. The active components of cinnamon leaf oil are eugenol, trans-β-caryophyllene, benzyl benzoate, linalool, and cinnamic alcohol acetate. Cinnamon oil is known to possess many biological properties including antioxidant, antidiabetic, antimicrobial and lipid lowering properties (Patel *et al.*, 2010).

In encapsulation, selection of the wall material depends on the active ingredient, the system in which it is going to be applied and the release mechanism. The use of various wall materials such as pectin, sodium alginate, modified cellulose, gelatin, sodium caseinate and soybean protein isolate etc. was reported (Davidov-Pardo *et al*., 2008). Further, chitosan (CS) has gained much attention as the wall material in many applications, because of its biodegradability, bio-compatibility and low toxicity (Bakry *et al.,* 2016). CS is obtained by deacetylation of chitin which is found in shells of arthropods such as crabs, shrimps, lobsters, and insects. Chitin is also produced extracellularly by the cell walls of fungi and brown algae. Formulation of CS microcapsules can be done by different methods. Among those methods, ionotropic gelation is an effective method which is organic solvent free, nontoxic and scalable. Ionotropic gelation involves interaction of ionic polymer with oppositely charged ion to initiate crosslinking. This study focuses on development of a microencapsulated formulation of cinnamon leaf oil by oil-in–water emulsification followed by ionotropic gelation in order to increase the bioavailability of the oil. The effects of variation of amount of polymer, oil and the cross linker

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on the encapsulation efficiency (EE), loading capacity (LC) and release rate and the optimum formulation for best encapsulation are reported here.

MATERIALS AND METHODS

Materials

Chitosan, medium molecular weight with viscosity of 36.5 cps was purchased from Biotech Surinodo, Indonesia. Acetic acid was purchased from Sigma Aldrich (Molecular weight of 60.05 g/mol with 99.5% assay). Tween 80 (Molecular weight of 604.822 g/mol, density of 1.06 - 1.10g/mL) was used without further purification. The core material, cinnamon leaf oil (CNO) was purchased from the local market.

Formulation of oil encapsulated chitosan microcapsules using ionotropic gelation method

CS solution in 1% acetic acid (w/v) (100 mL) was taken into a beaker. To this, oil was added dropwise with slow agitation (200 rpm) using an overhead stirrer (IKAW 20 digital) at room temperature. Then mixture was agitated using high speed (1000 rpm) for 10 minutes to obtain a good emulsion. Then the speed was decreased to 200 rpm and sodium tripolyphosphate(STPP) was added dropwise. The solution was stirred at the same speed for further 30 minutes. The microcapsules were centrifuged at high speed (6000 rpm) for 15 minutes. The supernatant was discarded, and the resulting pellet was washed with ethanol. MCs were freeze dried using the freeze dryer (LABONCO) and stored in the refrigerator until further use.

Optimization of process parameters

Various parameters were altered to find the optimum formulation for the MCs; polymer (1-2%), oil loading (1-3g) and STPP (0.5-1%).

CHARACTERIZATION OF MCs

Particle size measurement of CNO-CS-MCs

The particle sizes of the prepared MCs were measured using the particle size analyzer (DLS) (CILA – Nano DS). The sizes were determined using water as the dispersing medium.

Scanning electron microscopy (SEM) study of MCs

MCs were observed under a light microscope as they were formed. The samples were sputtered with gold and surface characteristics of MCs were studied using a scanning electron microscope. (ZEISS EVO LS15)

Fourier transform infrared (FT-IR) analysis

FT-IR measurements of CS films, CNO, CS-MC, CNO-CS-MC were performed on FT-IR spectrometer (Perkin Elmer, Spectrum Two). The spectra were obtained in the wavenumber range of 4000 – 750 cm^{-1} at a resolution of 4 cm^{-1}. Oil loaded, and empty capsules were made into KBr pellets before analyzing in the FT-IR instrument. IR spectrum of CNO was obtained using the ATR (Attenuated Total Reflection) accessory of the instrument. CS film was prepared by air drying a CS solution (1%) on a glass plate.

Oil release studies

The study was carried out according to the method described by Devi and Maji (2009). A series of oil solutions of CNO (1-10 mg/L) in Tween 80 (0.1%, w/v) were made. The solution with the highest concentration was scanned in the range 200– 400 nm (UV-Visible spectrophotometer – Agilent Technologies, Cary 60) to obtain the wavelength with maximum absorption. A prominent peak was observed at 260 nm for CNO. A calibration curve was made using the above concentration series by plotting the absorbance value at λ_{max} against the concentration of the oil. MCs (1.00 g) were placed in Tween 80 solution (0.1%, w/v) (100 mL). The solution was shaken from time to time. The solution was maintained at room temperature during the whole experiment. An aliquot (5.00 mL) was taken from the solution at 30 min intervals for 8 hours and it was assayed spectrophotometrically at λ_{max} of the oil. In order to maintain a constant volume, fresh Tween 80 (0.1 %, w/v) (5.00 mL) was added after every measurement. Each determination was carried out in triplicate.

Determination of encapsulation efficiency(EE) and oil loading capacity (LC)

Known weight of wet microcapsules (1.00 g) were ground in a mortar. It was transferred to a volumetric flask containing Tween 80 solution (0.1%, w/v) (100 mL). This was kept overnight. An aliquot of known volume (5.00 mL) was taken from the solution and assayed spectrophotometrically at 260 nm. The EE and LC were calculated using the calibration curve and the below formula (Soliman *et al.*, 2013).

$$\text{EE } (\%) = \frac{W_0}{W_I} \times 100$$

$$\text{LC } (\%) = \frac{W_0}{W_{MS}} \times 100$$

W_0 = Quantity of loaded EO in known amount of MCs,

W_{MS} = Quantity of MCs,

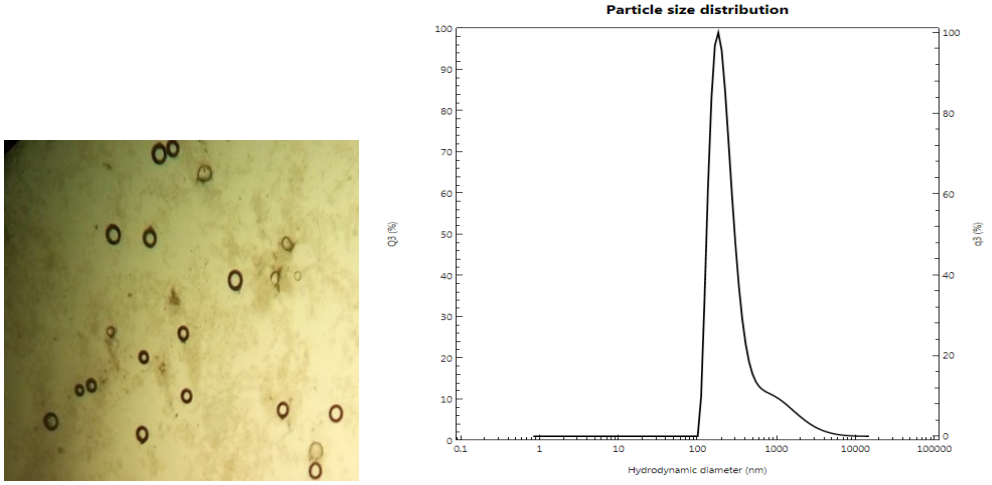
W_I = Initial quantity of EO

RESULTS AND DISCUSSION

Microstructure of MCs

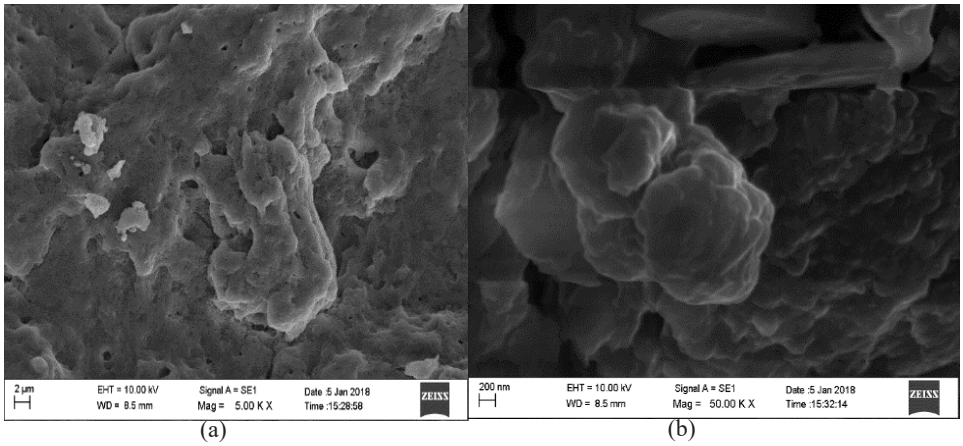
CNO-CS-MCs observed under optical microscope show a spherical shape. Encapsulated oil can be observed in the interior of the microcapsule (Figure 1a). Oil vesicles which are not encapsulated can be seen clearly without walls.The formation of thicker walls can be observed at higher CS and STPP concentrations. According to the DLS measurements (Figure 1b) MCs are polydispersed and the majority of MCs in the range 0.1 to 1µm.

The SEM images of MCs are given in Figure 2. MCs are spherical in shape. A significant difference can be observed between the surface and the appearance of oil loaded (CNO-CS-MC) and plain MCs (CS-MC). The surface of CS-MCs exhibits a smooth texture and MCs



(a) (b)

Figure 1: (a) Optical microscopic image (10 ×100) (b) Particle size distribution of CNO-CS-MCs (CNO, 1.0 : CS, 1.0 : STPP, 0.5 g).



(a) (b)

Figure 2: Scanning electron microscopic images of (a)CS-MC (b) CNO-CS-MC(3.0 g CNO, 1.0g CS, 0.5g STPP).

arein a layered structure [Figure2(a)]. CNO-CS-MC has a crimpy surface with bursting appearance confirming the successful encapsulation of oil by the wall material.

FT-IR analysis

In order to support the encapsulation of oil in CS, MCs were analyzed by FT-IR (Figure 3). The IRspectrum of CS film shows major peaks for N-H and O-H stretch (3400 cm^{-1}), C-H stretch (2919 cm^{-1}), amide II carbonyl stretch (1649 cm^{-1}) and N-H bending vibration of amine I (1582 cm^{-1})(Hou *et al.*, 2015).

The new peak at 1150 cm^{-1} in CS-MC evidences for the crosslinking of CS with phosphate group of STPP(Gierszewska-Drużyńska *et al.*, 2011). Furthermore, N-H stretching and N-H bending vibration of amine I of CS film have shifted to 3412 cm^{-1} and 1530 cm^{-1} in CS-MCs respectively indicating the formation of linkage between the phosphoric group of STPP and ammonium groups of chitosan in CS-MCs (Hou *et al.*, 2015). In the IR spectra of CNO, C-H stretching peaks at 2983 cm^{-1}, C=O stretching peak at 1670 cm^{-1} and C-O peak at 1119 cm^{-1}, specify few bands in oil. No significant difference is observed in the position of the major bands of the oil and in the oil loaded MCs indicating the absence of any significant interaction between oil and wall material. Thus, we can conclude successful encapsulation of oil in chitosan. Similar results have been observed in previous studies (Devi *et al.*, 2009).

Optimisation of process parameters

The effect of varying amount of polymer, crosslinker and oil load on the EE and LC of MCs were investigated. The results are summarized in Table 1.

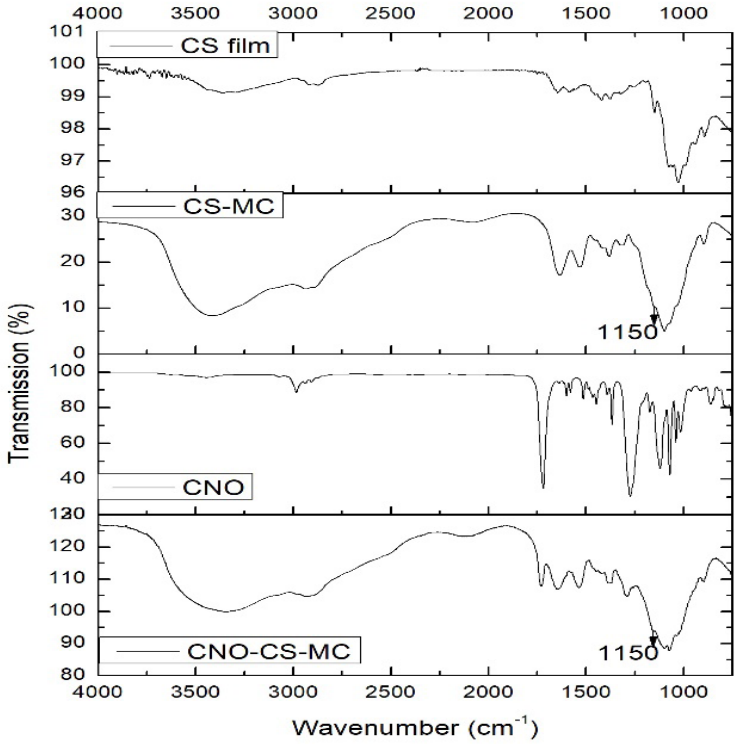


Figure 3: FTIR spectra of CS film, CS-MCs, CNO and CNO-CS-MCs.

Table 1: Effects of variation of oil loading, amount of CS and STPP on the EE and LC of microcapsules.

Amount (g)			EE (%)*	LC (%)*
CS in 100 mL	CNO	STPP in 100 mL		
1.0	1.0	0.50	70.50 ± 2.23	11.30± 0.25
1.0	2.0	0.50	81.65 ± 1.34	32.02± 0.05
1.0	3.0	0.50	90.85 ± 3.18	38.28± 0.46
1.0	1.0	0.75	69.56 ± 3.34	36.20± 0.16
1.0	1.0	1.00	56.67 ± 5.23	40.25± 0.05

*Each value represents the mean of three replicates ± SE (n=3).

Variation of CS concentration

Three concentrations of CS (1.0, 1.5 and 2.0 %) were tried while keeping oil loading (1.0 g) and STPP amount (0.5 g) constants. As expected, loading capacity decreases with increasing CS concentration. The crosslinked polymer forms a dense network structure with proper pore sizes to hold the oil vesicles. When the amount CS increased, the space occupied by the CS increased causing a decrease in the free volume within the polymer matrix. This causes a compact structure with smaller pores resulting in a decrease of oil vesicles that could be entrapped in the pores. As a result, loading capacity decreases with increasing polymer concentration. These results are in agreement with those reported by Devi *et al.,* (2009). At 1 and 1.5% CS concentrations, EE is found to be the same whereas with further increase of polymer amount (2%) a decrease on EE was observed. Devi *et al.,* (2009) and Soliman *et al.,* (2013) reported an initial increase of EE followed by a decrease in EE, with increasing the polymer concentration. However, they have used lower initial concentrations of polymer than the initial polymer concentration used in this study. When reaching 1 g of CS, the optimum amount of polymer for MC formation may have been reached which explains the difference in observations in this study to previously reported studies (Devi *et al.*, 2008 and Soliman *et al.*, 2013). As the CS concentration increases, excess CS is available to encapsulate the oil vesicles. This increases the EE. Increase of polymer amount increases the viscosity of the solution. When the viscosity of the solution is too high, the efficiency of emulsion formation decreases thus decreasing EE. After covering the vesicles, remaining CS increases the thickness of the MC by forming multiple layers (Devi *et al.*, 2009). The release rate decreases with increasing CS concentration due to the increase in wall thickness of the MCs[Figure 4(a)](Devi *et al.*, 2009).

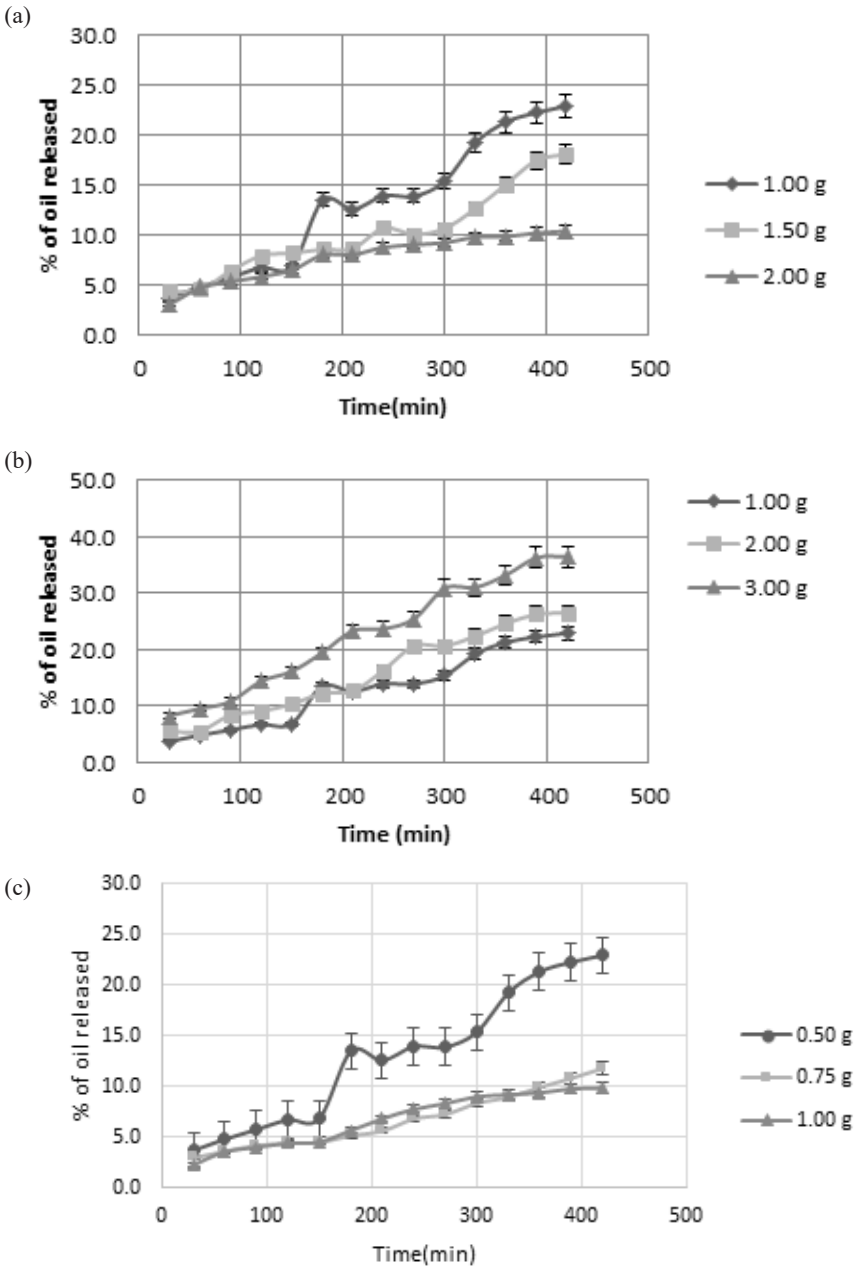


Figure 4: Variation of process parameter on release profile (a) amount of polymer (b) oil loading (c) amount of cross linker.

Variation of oil loading

Three oil loads (1.0, 2.0 and 3.0 g) were tried keeping CS (1.0 g) and STPP (0.5 g) amounts constant. The results show that with increasing oil load, EE, LC and release rate increase [Table 1 and Figure 4(b)]. At low oil load, dispersion force of the stirrer is more efficient resulting in generation of smaller oil vesicles. There is sufficient amount of polymer in the mixture to encapsulate these oil vesicles. With increasing oil load, the dispersion force becomes lower thus forming larger oil vesicles, consequently higher EE. Since a fixed amount of polymer is used, polymer would encapsulate all the larger oil vesicles at the expense of wall thickness. This is evident from the increase of release rate with increasing oil load. As the wall gets thinner, the diffusional path for the oil to travel becomes shorter. This would cause higher release rates at higher oil loading. At very low oil load, many MCs contain fewer oil vesicles below their normal capacity. With the increase of oil load, number of oil vesicles in MCs increase to reach their full capacity which results in an increase of loading capacity. Results are in agreement with those reported by Devi *et al.,* (2009).

Variation of STPP concentration

Table 1 shows the effect of variation of STPP concentration (0.5, 0.75 and 1.0 g) on EE and loading capacity at CS, 1.0 g : oil, 1.0 g and 30 minute crosslinking time. The loading capacity increases with the increasing STPP concentration. This may be due to the formation of a more compact solid matrix structure due to increased STPP concentration which leads to the increased number of formed MCs. Formation of more cross-linking improves the oil retention capacity of the MCs. However, the EE decreases with

increasing STPP concentration. This may be attributed to formation of a more densed cohesive network by increased amount of crosslinks between CS chains which results in smaller pore sizes. Hence the amount of oil that could be entrapped in these pores will be decreased. Soliman *et al.,* (2013) has observed an initial increase in EE followed by a decrease with increasing cross linker concentration. Again, their initial cross linker concentration is lower than that of used in this study (0.5%) and they too have observed the highest EE at 0.5% cross linker concentration followed by a decrease of EE with further increase of concentration. When the STPP concentration is high the wall of MC becomes compact. This resulted in a decrease of diffusion rate through the microcapsule wall which decrease the release rate (Figure 4(c)) (Devi *et al.*, 2009).

CONCLUSIONS

Ionotropic gelation can be used for the formulation of oil loaded MCs. Varying the process parameters have an direct impact on the EE, LC and release rate. The optimum formulation for microcapsules is polymer, 1 g : oil, 3g: cross linker, 0.5 g with an EE of 91%, and a LC of 38%.

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