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Influence of process variables on essential oil microcapsule properties by carbohydrate polymer–protein blends

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ABSTRACT

Carbohydrate polymer–protein blends *Zanthoxylum limonella* oil (ZLO) loaded microcapsules were prepared by multiple emulsion solvent evaporation technology and the influence of various processing variables on the properties of ZLO loaded microcapsules were examined systematically. It was found that the internal aqueous alginate phase volume, external aqueous gelatin phase volume and concentration of surfactant in external aqueous gelatin phase have a significant influence on microcapsules properties. The essential oil-loaded microcapsules were smooth and spherical in shape as revealed by scanning electron micrograph. Results of Fourier transform infrared (FTIR) spectroscopy indicated stable character and showed the absence of chemical interaction between the microencapsulated oil and carbohydrate polymer–protein blends. Differential scanning calorimetry (DSC) study revealed the antioxidant nature of ZLO in the microcapsules. The release rate of ZLO loaded microcapsules was analyzed by UV–vis spectrophotometer. 83.80% of oil encapsulation efficiency was obtained depending upon the processing variables. Thus, proper control of the processing variables involved in this technology could allow effective incorporation of essential oil into the core of the carbohydrate polymer–protein blends matrix.

1. Introduction

Zanthoxylum limonella oil (ZLO) exudes in the process of extracting *Z. limonella* seed, which is used for mosquito repellent application. However, the controlled release formulation of ZLO has been extensively reported with the aid of polymeric matrix system by coacervation process for encapsulation purpose (Hussain & Maji, 2008; Maji & Hussain, 2009). The release characteristics of ZLO microcapsules in glutaraldehyde crosslinked gelatin (Maji, Baruah, Dube, & Hussain, 2007) as well as the permeation characteristic of coacervated crosslinked gelatin–acacia membranes to various active agents (Jalsenjak & Kondo, 1981; Nixon & Wang, 1989) have also been mentioned in the literature. But to the best of our knowledge, no reports are available that describe the multiple water-in-oil-in-water (w/o/w) emulsion solvent evaporation technique for the effective incorporation of ZLO into polymeric microcapsules.

The technique of emulsion solvent evaporation offers a versatile, easy, and practical method for the manufacture of microspheres because it requires only mild conditions such as ambient temperature and constant stirring. Moreover, this technique seems to involve a relatively simple process, final product characteristics depends mainly on the formulation and process variables. However, it has been little investigated by w/o/w emulsion solvent evaporation technology. Thus, the objective of our present investigation was to encapsulate ZLO into microcapsules by carbohydrate polymer–protein blends by multiple w/o/w emulsion solvent evaporation technique and to study systematically the effect of various processing variables which are likely to influence the properties of ZLO-loaded microcapsules such as percentage yield, particle size, surface morphology, oil entrapment efficiency, and in vitro oil release behaviors. The compatibility of entrapped oil into carbohydrate polymer–protein blends matrix was evaluated through DSC, and FTIR spectroscopy analysis.

2. Methods

2.1. Materials

Essential oil was obtained from *Z. limonella* seed (exterior portion of Tezpur city) under the Sonitpur district of Assam. Gelatin (Extra Pure), ethyl cellulose (Ethocel, Ethoxy Content: 48–49.5%, 18–22 cps viscosity), sodium alginate (0.1%, w/v, aqueous solution exhibits 1.15 cps viscosity), Amaranth, Certified (Acid Red 27,

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Dye Content-90%) were procured from HiMedia Laboratories Private Limited, Mumbai, India. Methylene chloride was purchased from Qualigens Fine Chemicals, Mumbai, India. Tween 80 was purchased Merck Specialities Private Limited, Mumbai, India. All other reagents obtained commercially were of analytical grade and used as received. Double distilled water was used throughout the course of investigation.

2.2. Essential oil extraction

Shade dried seeds of *Z. limonella* for 3–4 days was extracted by steam distillation process in order to obtain essential oil (ZLO). The obtained essential oil (ZLO) was separated from aqueous phase and dried by treatment with anhydrous sodium sulphate. Then the dried essential oil (ZLO) was transferred into an amber colored glass container and kept at 4 °C for further use.

2.3. Development of ZLO-loaded microcapsules by multiple emulsion technique

ZLO-loaded carbohydrate polymer–protein blends microcapsules were prepared by w/o/w emulsion solvent evaporation technology from an aqueous system containing Tween 80 as emulsion stabilizer. Initially an internal aqueous phase (w_1) of 1.5% (w/v) sodium alginate (pH 10.0) solution was uniformly dispersed in essential oil phase (o) containing 25 ml of methylene chloride solution of 1.5% (w/v) ethyl cellulose with the aid of surfactant (Tween 80) and emulsified under high agitation for 10 min at 800 rpm to form an water-in-oil (w_1/o) primary emulsion. The resulting primary water-in-oil (w_1/o) emulsion was again emulsified in 0.10% (w/v) external aqueous solution (w_2) of gelatin (pH 6.2) containing Tween 80 under high agitation at 500 rpm to form multiple w_1/o/w_2 type emulsion (pH 6.0). The stirring was continued for a period of 3 h to allow evaporation of the organic solvent and leads to the formation of microcapsules. The discrete microcapsules were filtered, washed with cold double distilled water (3× 100 ml). Finally those were air-dried at room temperature for overnight and stored in secador (Sicco, Germany) desiccators until further characterization and investigation. The following variations in process parameters were investigated:

1. Internal aqueous alginate phase volume (w_1): 3 and 6 ml.
2. External aqueous gelatin phase volume (w_2): 50 and 100 ml; while the internal aqueous alginate phase volume was 6 ml.
3. Concentration of Tween 80 in external aqueous phase: 0.2%, and 0.4% (w/v). While the internal aqueous alginate phase volume was 6 ml.

2.4. Analysis of ZLO and construction of calibration curve

A stock solution of ZLO standard solution was prepared in double distilled water containing 0.1% Tween 80 and scanned in the range of 200–400 nm by using double beam UV–vis spectroscopy (Thermo Electron Corporation, UV-1, Great Britain). A sharp peak at 228 nm was observed. Working standard solutions were prepared by diluting the stock solution with the same solvent to obtain different concentrations. The absorbance values at 228 nm wavelength were obtained and plotted on *Y*-axis against concentrations on *X*-axis and slope of the calibration curves was obtained.

2.5. Identification of multiple emulsion structure

Amaranth, a water soluble dye, was used to identify the structure of multiple emulsions. The secondary w_1/o/w_2 type emulsion was colored with amaranth and a drop of multiple emulsions was put on a microscopic slide and observed under co-axial trinocular microscope (Coslab, HL-23T, India). An optical combination of 10× eyepiece and 10× objective was used for the detection of emulsion structure. The multiple emulsion structure was captured with a 12-megapixel digital camera (Nikon Coolpix, S-3000, Japan).

2.6. Percent yield of microcapsules

The percent yield of the microcapsules was calculated as a percentage of the total amounts of polymers and essential oil employed during preparation. The percentage yield of the microcapsules was calculated by using the following formula (Banerjee et al., 2012):

$$\%\,\text{yield} = \left[\frac{\text{Actual amount of microcapsules obtained}}{\text{Total (amount of oil + amount of polymer)}}\right] \times 100$$

2.7. Determination of microcapsule size

Particle size analyses of the dried ZLO-loaded carbohydrate polymer–protein blends microcapsules were done by optical microscopy method (Coslab, HL-23T, India). A standard stage micrometer was used to calibrate the eye-piece micrometer. Dried microcapsules were placed in a glass slide and the number of divisions of the calibrated eye piece was counted. The particle size of the microcapsules was calculated by using the following formula (Banerjee et al., 2010):

$$\text{Calibration factor} = \left(\frac{\text{Eye piece micrometer}}{\text{Stage micrometer}}\right) \times 10$$

2.8. Determination of oil encapsulation efficiency

Accurately weighed, ZLO-loaded microcapsules were dissolved in minimum quantity of methylene chloride; then a required volume of double distilled water containing 0.1% (w/v) Tween 80 was added and stirred for overnight with magnetic stirrer to remove organic phase. After that, the solution was filtered, and analyzed spectrophotometrically at 228 nm. Reliability of the results was judged by conducting triplicate study. The % oil encapsulation efficiency was calculated by using the following formulae (Maji et al., 2007):

$$\text{Encapsulation efficiency}\,(\%) = \left(\frac{\text{Actual amount of ZLO entrapped in the microcapsules}}{\text{Theoretical amount of ZLO entrapped in the microcapsules}}\right) \times 100$$

2.9. Determination of in vitro oil release study

A known amount of dried ZLO loaded microcapsules, were suspended in a known volume of double distilled water containing 0.1% (w/v) Tween 80. The oil release studies were conducted using the same size fractions of microcapsules to get comparable results. The dispersion was rotated at 50 rpm with a magnetic stirrer at a constant rate and the temperature was set at 25 ± 2 °C. At predetermined times interval 5 ml of aliquot sample was withdrawn and replenished with fresh medium solution. The aliquots were analyzed using a double beam spectrophotometer (UV1, Thermo Spectronic, Great Britain) at 228 nm. Cumulative percentage of ZLO released was plotted against function of time in hour (Fig. 2). Each determination was carried out in triplicate.

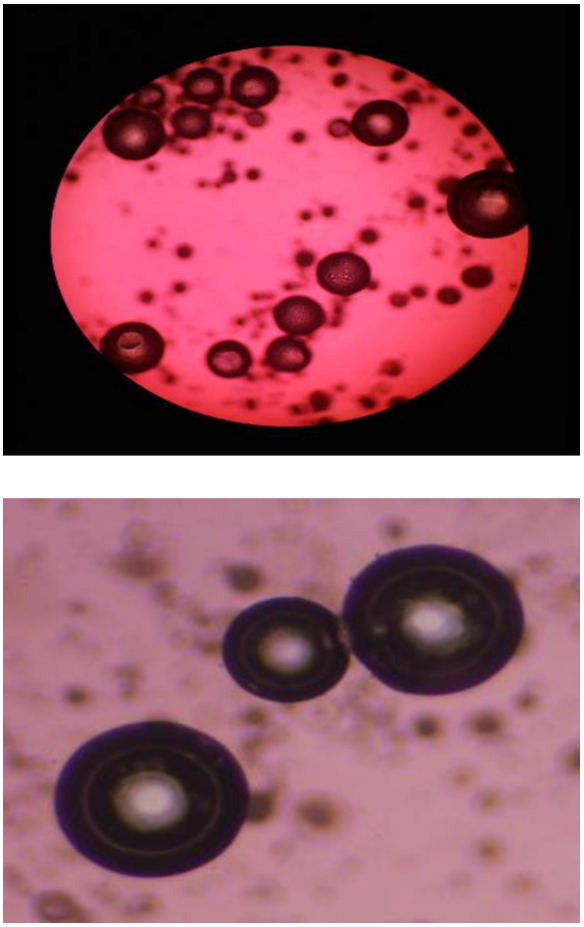

Fig. 1. Microscopic examination of multiple (water-in-oil)-in-water emulsion structure; oil phase (black); aqueous phase (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.10. Scanning electron microscopy (SEM)

The shape and surface characteristics of ZLO loaded microencapsulated formulations were examined using a scanning electron microscope (Jeol-Datum, JSM-6390, Tokyo, Japan) at required magnifications with the secondary electron image (SEI) as a detector and an accelerated voltage of 5 kV.

2.11. Fourier transform infrared (FTIR) measurements

FTIR measurements was done to detect any chemical interactions between essential oil with carbohydrate polymer–protein blends. FTIR (Model FTIR-8400s, Shimadzu, Japan) spectra of essential oil (ZLO) and ZLO loaded microcapsules were crushed with potassium bromide and pellets were formed under a hydraulic pressure of 600 kg/cm. The FTIR measurements were recorded in the wave number region between 4000 and 600 cm^{-1} at a resolution of 4 cm^{-1} using KBr pellets.

2.12. Differential scanning calorimetry (DSC)

DSC (DSC Q10 TA Instruments) thermograms of essential oil and ZLO loaded microcapsules were recorded. Each sample were heated in the range between 20 °C and 250 °C at a heating rate of 10 °C/min in an inert nitrogen atmosphere with a flow rate of 50 ml/min.

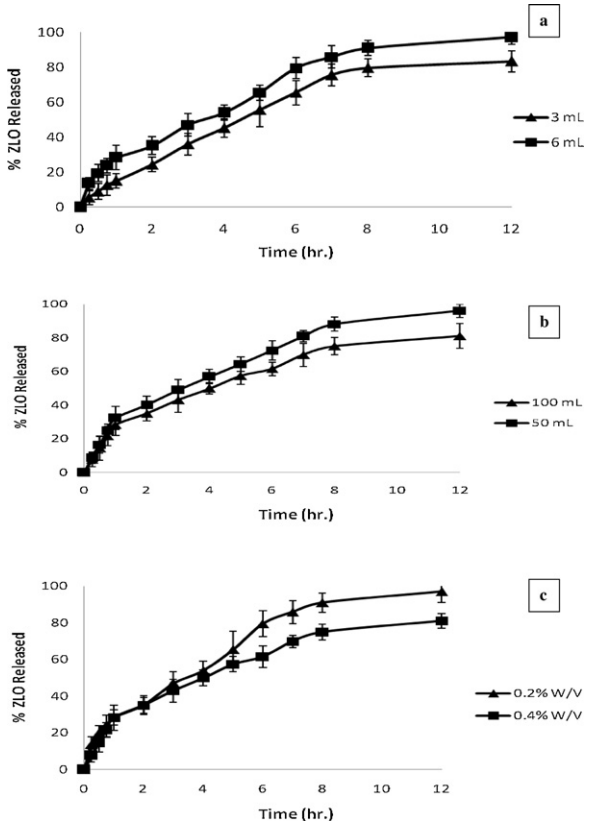

Fig. 2. Release profiles of ZLO-loaded microcapsules: (a) internal aqueous alginate phase volume (w_1); (b) external aqueous gelatin phase volume (w_2); (c) concentration of Tween 80 in external aqueous phase.

3. Results and discussion

Carbohydrate polymer–protein blends microcapsules of ZLO were developed by w/o/w multiple emulsion solvent evaporation technology. The formation of multiple emulsion structure by this technology has been represented in Fig. 1. A representative group and single SEM micrograph of ZLO-loaded microcapsules appeared spherical in shape with smooth external surfaces has been displayed in Fig. 3a and b, respectively. As several variations in process parameters were involved for the development of ZLO-loaded microcapsules from multiple emulsion templates. So, it can be assumed that the characteristics of ZLO microcapsules could be altered. Thus, the focal point of this research work was specially intended for the systematic examination of these variations in process parameters which could influence the properties of ZLO containing microcapsules.

3.1. Influence of internal aqueous alginate phase volume

As the internal aqueous phase volume of the primary w/o type emulsion increased the percentage yield of the microcapsules was decreased from 88.64% to 82.01%. This may be due to the increase in internal aqueous alginate phase volume caused increase in mean particle diameter of the formulations from 209.41 to 223.17 μm (Table 1). This might increase the number of dispersed phase droplets size of the primary w/o type emulsion in a fixed volume of organic phase, and the probability of coalescence between the dispersed droplets increases. This resulted increase in size of the ZLO microcapsules. Similar observations have been reported (Jeffery, Davis, & O'Hagan, 1993; Schlicher, Postma, Zuidema, Talsma, & Hennik, 1997). The oil entrapment efficiency of the ZLO loaded microcapsules showed an increasing tendency from 33.73% to

Table 1
Impact on variation in process parameter on in vitro physico–chemical characteristics of ZLO loaded carbohydrate polymer–protein blends microcapsules.

Variation in process parameter	% yield value	Mean size (μm)	% oil entrapment efficiency (±SD, $n = 3$)
Internal aqueous alginate phase volume (ml)			
3	88.64	209.41	33.73 ± 1.63
6	82.01	223.17	67.71 ± 2.68
External aqueous gelatin phase volume (ml) [while internal aqueous alginate phase volume =6 ml]			
50	86.07	241.06	76.43 ± 2.38
100	89.17	367.38	69.34 ± 3.72
Concentration of Tween 80 in external aqueous gelatin phase (%, w/v) [while internal aqueous alginate phase volume =6 ml]			
0.2	84.35	235.05	73.18 ± 5.57
0.4	87.97	217.14	83.80 ± 3.31

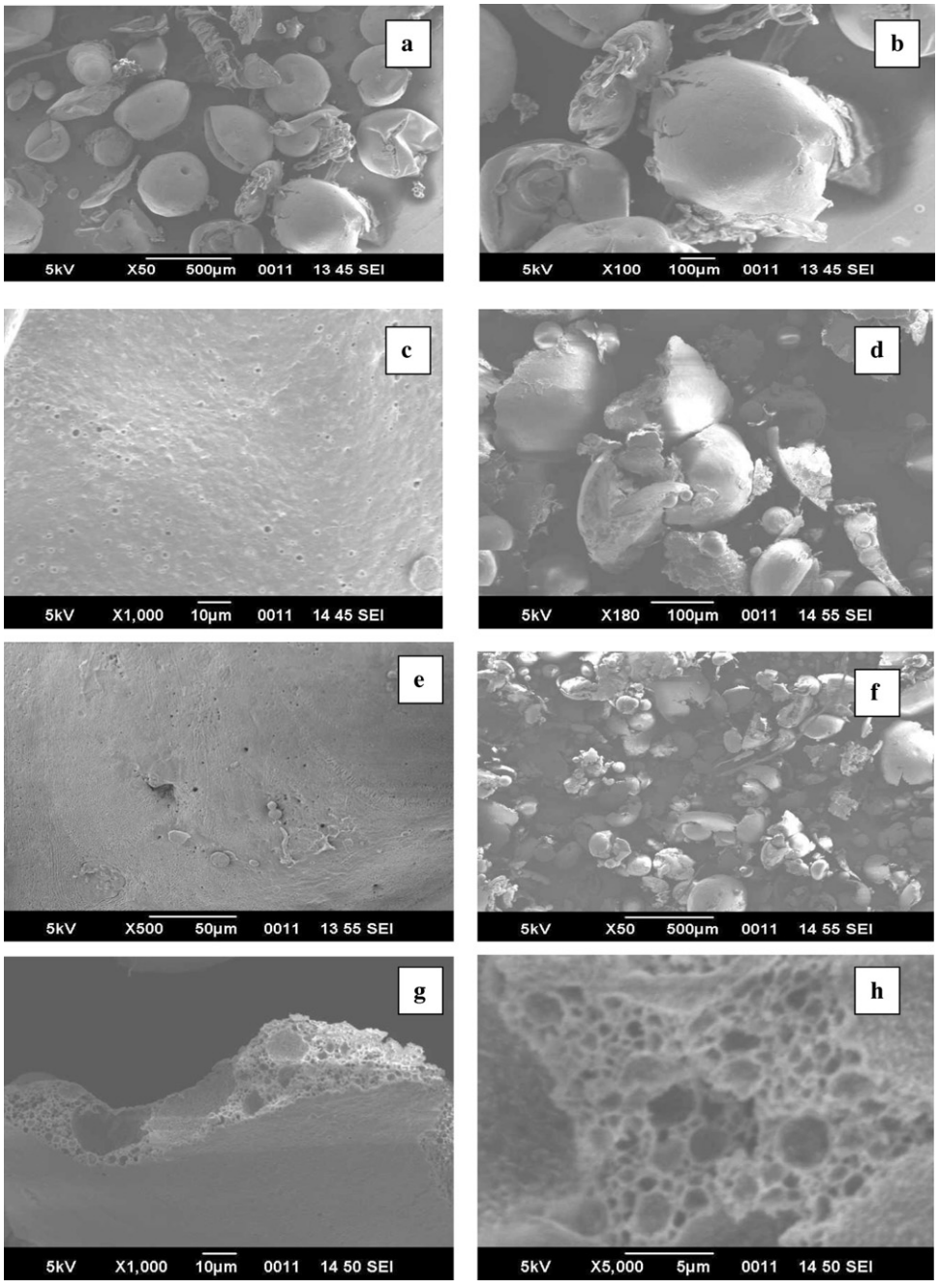

Fig. 3. Scanning electron micrographs of ZLO-loaded microcapsules: (a) group micrograph of prepared microcapsules (50× magnification); (b) single micrograph of prepared microcapsules (100× magnification); (c) 6 ml internal aqueous phase volume; (d) 100 ml external aqueous phase volume; (e) 0.4% (w/v) Tween 80; (f) fate of microcapsules after dissolution; (g) porous surface of microcapsules after dissolution; (h) matrix surface after dissolution.

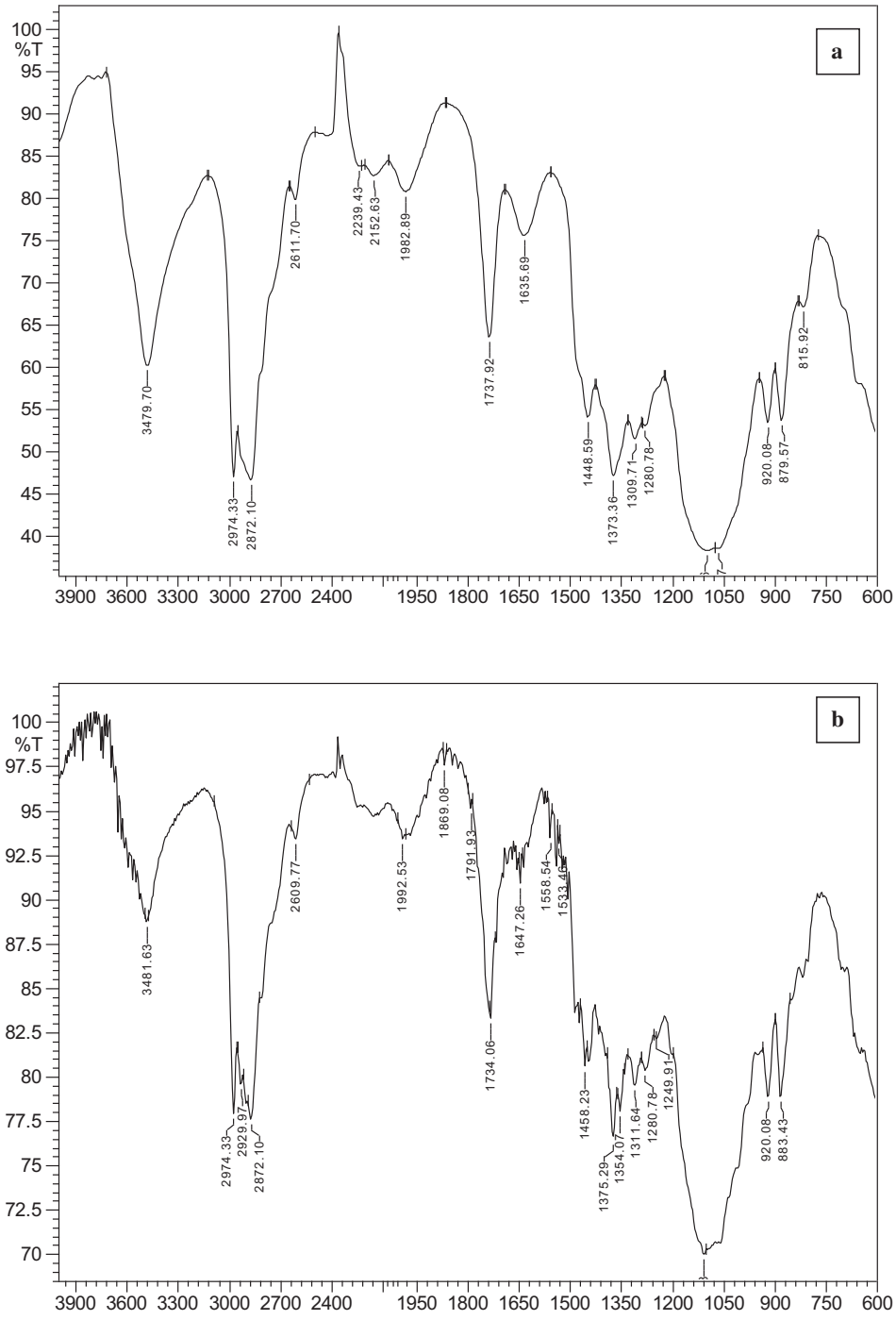

Fig. 4. FTIR spectra of (a) pristine ZLO, and (b) ZLO-loaded microcapsules.

67.71% (Table 1) due to the increase in volume mean diameter of the particle size, this helps to penetrate larger amount of essential oil into the core of the carbohydrate polymer–protein blends matrix. ZLO loaded microcapsules prepared with higher volume of internal aqueous phase released its content at a much faster rate than those prepared with lower volume of internal aqueous phase in the dissolution media (Fig. 2a). This sort of release behavior may be due to an increase in the volume of internal aqueous phase in the primary emulsion, the porosity of the microcapsules increased as evident by SEM micrograph (Fig. 3c) and directed to the faster oil release rates associated with the use of higher internal aqueous phase volume (Crotts & Gwan Park, 1998).

3.2. Influence of external aqueous gelatin phase volume

As the external aqueous phase volume increased from 50 to 100 ml resulted in an increase in percent yield from 86.07% to 89.17% and particle size from 241.06 to 367.38 μm of the microcapsules, but lower the oil entrapment efficiency from 76.43% to 69.34% (Table 1). This kind of phenomena may be due to the higher rate of oil leaching from the internal aqueous phase volume of primary w/o emulsion to the external aqueous phase volume of the secondary w/o/w type emulsion, because of increase in external aqueous phase volume there was a decrease in mixing efficiency due to the viscosity of the oil phase which may be likely due

to decrease in agitation and ultimately, the particle size of the microcapsules increased. A reduction in mixing efficiency probably produced an increase in the size of emulsion droplets which could from large microcapsules. An increase in volume of the external aqueous gelatin phase decrease the oil encapsulation efficiency of the carbohydrate polymer–protein blends microcapsules. The emulsion droplets probably more freely moved in the medium and the oil was extracted at a higher rate by the external aqueous gelatin phase volume. The release behaviors of ZLO-loaded microcapsules in the dissolution media with increasing external phase volume has been presented in Fig. 2b. In 50 ml volume of external aqueous phase higher oil release profile was observed than prepared with 100 ml because of the smaller particle size in lower external aqueous phase volume. No significant effect of external phase volume was observed on the gross morphology of ZLO-loaded microcapsules (Fig. 3d).

3.3. Influence of Tween 80 concentration in external aqueous gelatin phase volume

Tween 80 is regarded as a nonionic; hydrophilic surfactant having a hydrophilic lipophilic balance (HLB) value of 14.8 was used here to stabilize the multiple w/o/w emulsions by reducing the interfacial tension between the two phases and allowed higher oil entrapment (83.80%) at a concentration of 0.4%. Two different concentration of this surfactant (0.2% and 0.4%, w/v) in external aqueous gelatin phase solution were prepared. The production yield was gradually increased from 84.35% to 87.97% with increasing concentration of Tween 80 (Table 1). Particle mean diameter also considerably altered as a function of surfactant concentration in the external aqueous gelatin phase. When the concentration was increased, a significant reduction in particle size was noticed (Table 1). At higher surfactant concentration, the rate at which the emulsion stabilizer molecules diffused at the primary emulsion droplets external aqueous gelatin phase interface was probably higher and localized at the surface of emulsion droplets. This could provide a better protection of droplets from coalescence and ultimately resulted in formation of smaller emulsion droplets at higher surfactant concentrations. As the solvents evaporated form the system, these droplets hardened to form the microcapsules. Therefore, the size of the finally obtained microcapsules became dependent upon the size of emulsion droplets formed during the agitation process (Blanco-Prieto et al., 1994). At low surfactant concentration, small emulsion droplets were not stable and the resulting microcapsules were larger in size than those prepared with higher surfactant concentration. Fig. 2c illustrated that as the surfactant concentration in the continuous phase was increased, the oil release rate from the microcapsules tended to be slower in dissolution medium due to rigidization of carbohydrate polymer–protein blends matrix as evident by SEM micrograph (Fig. 3e). Surface analysis of the ZLO-loaded microscapsuless after dissolution study revealed a large number of pores which supported micropore diffusion controlled release mechanism (Fig. 3f–h).

3.4. FTIR analysis

The compatibility of the entrapped oil with the polymers was evaluated qualitatively through FTIR analysis. FTIR spectrum of pristine ZLO (Fig. 4a) exhibited distinctive peaks at 1635.69–1737.92 cm^{-1} due to the carbonyl stretching band of ZLO. Several strong vibration peaks appeared at 1448.59 cm^{-1}, 1373.36 cm^{-1}, 1280.78 cm^{-1}, 1167.56 cm^{-1} and 1060.87 cm^{-1} due to CH_2 asymmetric deformation, CH_2 symmetric deformation, C—N, C—C and C—O stretching vibration respectively (Maji et al., 2007). Similar vibration peaks of ZLO were detected in the spectrum of ZLO-loaded microcapsules with minor differences in frequencies

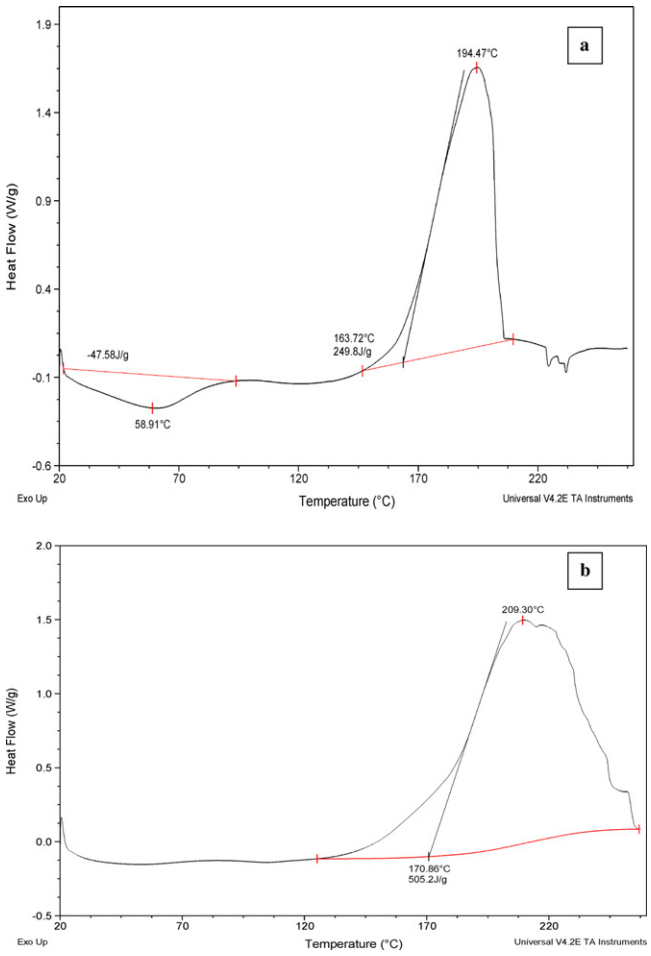

Fig. 5. DSC thermograms of (a) pristine ZLO, and (b) ZLO-loaded microcapsules.

(Fig. 4b). This FTIR results suggested that the presence of essential oil into the polymeric matrix was apparently stable in and did not interact with carbohydrate polymer–protein blends matrix components.

3.5. DSC analysis

DSC analysis is valuable for the study of the thermostability of entrapped oil in the carbohydrate polymer–protein blends matrix. Fig. 5a and b represents the DSC thermogram of pure essential oil (ZLO) and ZLO-loaded microcapsules. ZLO showed a sharp exothermic peak at a temperature of 194.47 °C (Fig. 5a). However, the DSC thermogram of ZLO-loaded microcapsules did not showed a sharp peak at the same temperature, moreover it showed peak at 209.30 °C (Fig. 5b) which may be related to the auto-oxidation process of the samples (Liolios, Gortzi, Lalas, Tsaknis, & Chinou, 2009). The tested formulations showed improved antioxidant action rater than its pure form. The modified antioxidant activity of ZLO loaded formulations by this w/o/w multiple emulsion solvent evaporation technology implies that the proper control of the variation in process parameters could allow possible link formation between the carbohydrate polymer–protein blends from an emulsion template.

4. Conclusion

This investigation suggested that the use of internal aqueous alginate phase volume in this double emulsion solvent evaporation technology may be an alternative approach for the effective incorporation of essential oil in carbohydrate polymer–protein blends

microcapsules. The influence on variation in process parameters of ZLO-loaded microcapsules has provided an understanding of the effects on different evaluation parameters. Proper control of such variation in process parameters enabled the formulation spherical in nature with a desired micron size range. This carbohydrate polymer–protein blends ZLO loaded microcapsules were able to provide prolong oil release dependent on internal and external aqueous phase volume as well as surfactant concentration in external aqueous phase volume. FTIR and DSC analysis indicated the absence of chemical interaction between the entrapped oil with polymer and revealed the antioxidant nature of the entrapped oil after microencapsulation, respectively. Therefore, it may be concluded that proper control of the variation in process parameters involved in this technology showed a slow and prolonged release of essential oil from the polymeric core, indicating the potential for controlled release mosquito repellent application.

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