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Formulation and characterization of Lemon Peel Essential Oil Loaded Corn Starch Nanoemulsion

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ABSTRACT: Lemon (*Citrus limon*) belonging to the family *Rutaceae*, is the third most cultivated citrus fruit after orange and mandarin. Lemon processing industry produces a huge amount of waste as lemon peel and lemon seed which are generally discarded. The lemon peel is a rich source of bio active compounds that possess significant antibacterial and antioxidant property. Lemon peel essential oil which is extracted from lemon peel can be used as a natural preservative in diverse food systems to extend the shelf life of the product. Direct addition of lemon peel essential oil in food system has several limitations such as high volatility and strong organoleptic property of the essential oil. This can be overcome by encapsulating the lemon peel essential oil in a suitable delivery system. Nanoemulsion coating solution can be formulated using lemon peel essential oil and a biological polymer (corn starch). Lemon peel essential oil loaded corn starch nanoemulsion (CS-LPEO) was formulated by using different concentration of lemon peel essential oil (0.3, 0.5 and 0.7%) and corn starch (2 and 3%). The particle size of the nanoemulsions were in the range of 340.10 to 358.40 nm having polydispersity index ranging from 0.3 to 0.5. The zeta potential of the nanoemulsion varied from -34.00 to -45.00 mV. The encapsulation efficiency was as high as 83.85% among the different treatments. The whiteness index of the CS-LPEO was around 50.92 and the viscosity of the nanoemulsion was between 10.80 to 18.30 cP. The FTIR absorption peak of the LPEO and corn starch was compared with that of CS-LPEO nanoemulsion and the morphology of the CS-LPEO nanoemulsion was observed through scanning electron microscope. By this, the lemon peel essential oil was entrapped using a suitable edible delivery system that can be directly used in the food system for preservation process.

Keywords: Lemon peel essential oil, corn starch, nanoemulsion.

INTRODUCTION

Agriculture and food processing industries generate a huge amount of by-products as wastes such as peel, rind, stem, stalk, seeds and leaves as wastes. The waste from the food industries should be valorized as this poses a major environmental treat. Citrus fruits which belongs to the family *Rutaceae* comprises of 17 species which includes *Citrus indica, Citrus latipes, Citrus aurantium, Citrus macroptera, Citrus megaloxycarpa, Citrus jambhiri, Citrus ichangenesis* and *Citrus reticulate*. *Citrus limon* is the third most important citrus fruit following *Citrus sinensis* and *Citrus reticulate.* Generally, citrus fruits are consumed as such or processed into juice. Citrus processing industries produce a large amount of waste as citrus peel and citrus seeds. Citrus peel can be used to extract essential oil by various methods such as cold pressing, hydro-distillation, steam distillation and solvent extraction. Citrus essential oil contains a mixture of terpinoids, terpenes and other aromatic and aliphatic compounds (Yazgan *et al*., 2019).

Lemon peel essential oil contains natural antimicrobial and antioxidant properties. It is effective against several food borne pathogens such as *E. coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Camphylobacter* (Calo *et al*., 2015). Lemon peel essential oil contains limonene, which is responsible for the antimicrobial property and also responsible for the distinct aroma of the lemon fruit. The bioactive compounds in citrus peels namely limonene, citral, linalool, α pinene, β pinene, β myrcene and γ terpinene can be used to treat various health problems such as spasms, fever, respiratory problems, cardiovascular diseases, gastrointestinal problems or anxiety (Martínez-Abad *et al*., 2020).

Lemon peel essential oil has good scope for application as natural preservative in food system. Lemon peel essential oil can be used as edible coating over whole or cut fruits and vegetables to extend the shelf life of the product both in quality and safety aspects. Direct incorporation of lemon peel essential oils in food products becomes a formidable challenge because of its hydrophobic nature, high volatility, low stability, high susceptibility to environmental conditions and strong organoleptic property. This can be overcome by encapsulating the essential oils in a suitable delivery system compatible with the food system (Prakash *et al*., 2018). Biological polymer such as polysaccharides, proteins, lipid or gum can be used to carry the essential oil. Polysaccharide such as corn starch can be used as a biological polymer to carry the essential oil as the corn starch yield colorless, odorless, tasteless and non-toxic costing solution.

Hence, the present study aims the formulation and characterization of corn starch edible nanoemulsion loaded with lemon peel essential oil.

MATERIALS AND METHODS

Procurement and processing of lemon peel

Procurement of lemon peel. Lemon (*Citrus limon*) peel was procured from the local juice shops of Madurai who were in prior advised on good processing practices to assure the hygienic collection of the citrus peel.

Processing of lemon peel. The lemon peel was separated from the endocarp (inner pulp) of the fruits and cut into small pieces (approximately 1×1cm) and dried by shade drying method until the sample reaches the moisture content of 10 per cent after which the samples were powdered and sieved using a sieve of British Standard (BS) 60 mesh sieve to get uniform size particle.

Extraction of lemon peel essential oil by hydro distillation method. The extraction of essential oil from lemon peel was carried out by hydro distillation method which was carried out in Clevenger apparatus for about 3 hours at 95°C using the solid solvent ratio of 1:20. The sample material was directly immersed in water and the solid-liquid mixture was heated until boiling under atmospheric pressure. The extraction was carried out from the first drop of distillate until the amount of essential oils stabilized.

Preparation and characterization of edible nanoemulsion coating solution loaded with Lemon Peel Essential Oil (LPEO)

Preparation of corn starch loaded with lemon peel essential oil (CS-LPEO) edible nanoemulsion. Edible nanoemulsions (T_1 to T_6) were formulated with corn starch solution (2 and 3 percent) as wall material, lemon peel essential oil (0.3, 0.5 and 0.7 per cent) as core material and Tween 20 (concentration as same as the concentration of the lemon peel essential oil) as an emulsifier.

A modified method of Abbasi *et al*. (2021) was followed for the preparation of CS-LPEO nanoemulsion coating solution. Corn starch solution was prepared using Sigma Aldrich corn starch. Corn starch (2 and 3%) and Glycerol (1 and 1.5%) were dissolved in distilled water and heated at 90°C with constant stirring for 10 minutes until gelatinization and allowed to cool at 40°C. Coarse emulsion was prepared by mixing the corn starch solution with lemon peel essential oil at 0.3, 0.5 and 0.7 per cent which was previously mixed well with Tween 20 (v/v with respect to the volume of essential oil) as surfactant in ultrasonic bath sonicator for 10 minutes with a known amount of distilled water. The mixture was homogenized well using a High Shear Homogenizer (220-240V power: 50Hz frequency) at 8000 rpm for 15 minutes. Nanoemulsion coating solution was obtained by subjecting the above emulsion to high pressure homogenizer (model: FPG 11300, Stansted Fluid Power Ltd., Essex, UK) at 200 MPa for 5 passes.

Standardization of edible nanoemulsion coating solution loaded with LPEO. Based on the percentage of corn starch and essential oil, the following treatments (Table 1) are standardized for the development of edible nanoemulsion of citrus peel essential oil.

Table 1: Standardization of edible nanoemulsion coating solution loaded with LPEO.

Treatments	Corn starch	Glycerol	Tween 20	Essential oil
T_1	2	1	0.3	0.3
T_2	2	1	0.5	0.5
T_3	2	1	0.7	0.7
T_4	3	1.5	0.3	0.3
T_5	3	1.5	0.5	0.5
T_6	3	1.5	0.7	0.7

Physico chemical characterization of corn starch loaded with lemon peel essential oil (CS-LPEO) edible nanoemulsion. The physicochemical properties such as particle size, polydispersity index, zeta potential, encapsulation efficiency, *in vitro* release percentage, color value, whiteness index and viscosity of CS-LPEO nanoemulsion (T_1 to T_6) were analysed by the procedure given below.

Particle Size Analysis and Poly Dispersity Index (PDI) of edible nanoemulsion loaded with LPEO. The particle size of the lemon peel essential oil nanoemulsions was measured using Nano particle analyzer sz-100 at 633 nm, 25°C. Water (refractive index-1.333) was used as a dispersive medium for all the samples. The 0.5µl of sample was suspended in 10ml of water and subjected to sonication process for 5 minutes in a water bath type sonicator and the readings were taken immediately for better dispersion of the samples. The average droplet size (z- average) and PDI was recorded. The PDI value is the measure of heterogeneity in the droplet size distribution. The PDI values close to 0 indicate homogenous size distributions, whereas PDI values close to 1 indicate heterogenous size distributions (Noori *et al*., 2018).

Zeta potential of edible nanoemulsion loaded with LPEO. Electrophoretic mobility of high pressure homogenized edible nanoemulsions was estimated using the Nano particle analyzer sz-100. The zeta potential measurement was carried out on the diluted solutions (0.5µl sample in 10 ml of water) at neutral pH (pH 7). Using a syringe, 1 mL of the appropriate solution was injected in the measurement vessel (special tank U-shaped). The vessel was then positioned in the optical drive of the apparatus. Temperature was set at 25°C and voltage applied was 3.9V. Duration of analysis was approximately 10 minutes (Noori *et al.,* 2018).

Encapsulation efficiency of edible nanoemulsion loaded with LPEO. Encapsulation efficiency of the edible nanoemulsion can be determined by using the following equation.

Encapsulation efficiency (%) = (EO loaded/ EO total) ×100

where [EO loaded] = [EO]tot -[EO] free (total and free contents of essential oil in the nanoemulsion suspension). The total amount of essential oil in the nanoemulsion was determined by Double beam UV-VIS Spectrophotometer 2201 over wavelengths ranging from 250 to 450 nm (λ_{max} 274). 1 ml of edible nanoemulsion was treated with 9 ml of acetonitrile and the mixture was centrifuged at 3500 rpm for 15 minutes. An aliquot (4 ml) of the supernatant was diluted with 20 ml of acetonitrile and the amount of essential oil was derived by the absorbance at 274 nm. The free essential oil content in the nanoemulsion was determined by ultra filtration technique. A total of 5 ml of the nanoemulsion was ultra filtered using a membrane filter (0.25µm) and 2 ml of the filtrate was diluted with 20 ml of acetonitrile. The absorbance was recorded at 274 nm using a spectrophotometer (Granata *et al*., 2021).

The study of the *in vitro* release of LPEO from the edible nanoemulsion. The *in vitro* release profile of LPEO from the nanoemulsion was studied using phosphate buffer solution (pH 7.4). The LPEO loaded nanoparticles (750 µl) from the aqueous suspension was separated by centrifugation at 10,000 rpm for 5 minutes at 25°C. The separated nanoparticles were resuspended in the buffer solution (1200 µl) and incubated at 25°C under general shaking. At definite time interval, samples were withdrawn and centrifuged at 10,000 rpm for 5 minutes at 25°C. 100 µl of the supernatant was mixed with 3 ml of ethanol and the *in vitro* release of LPEO was recorded spectrophometrically at 275 nm (Esmaeili and Asgari 2015). Cumulative percentage of LPEO release was calculated using the equation given below.

$$\text{Cumulative release percentage} = \sum_{t=0}^{t} \frac{M_t}{M_0} \times 100$$

Where

M_t - cumulative amount of LPEO released to each sampling time point,

t- time of release of LPEO-loaded in the nanoemulsion

M_0- initial weight of the LPEO-loaded in the nanoemulsion.

Color value and whiteness index of the edible nanoemulsion loaded with LPEO. The colour values of the developed nanoemulsions were recorded using the Hunter lab meter. The $L*$ value was used to measure the lightness ranging from 0 to 100. The + $a*$ value represents red,- $a*$ represents green, $+b*$ represents yellow and $-b*$ represents blue. Three readings were taken for each sample. The results presented were the means of three values. Distilled water was used as reference (Farahmandfar *et al*., 2020).

Whiteness indices of samples were calculated by formula given below (Das *et al*., 2020).

Whiteness Index= 100- $[(100-L^*)^2 + a^{*2} + b^{*2}]^{1/2}$

Viscosity of the edible nanoemulsion coated loaded with LPEO. Viscosity of edible nanoemulsions was analyzed by Brooke field viscometer using spindle No. 62 at 100rpm. 100ml of the nanoemulsion was placed in beaker for viscosity analysis. Values (cP) were recorded after 30 s for 3 times. The sample holder was cleaned after each reading and values were recorded (Rao and McClements 2012).

Study of the Fourier Transform Infrared Spectroscopy (FTIR) profiles of edible nanoemulsion loaded with LPEO. The FTIR profiles of corn starch, tween 20, lemon peel essential oil and corn starch essential oil nanoemulsion were recorded and corrected by air background using a JASCO ATR-FTIR equipped with spectrum software (Spectra measurement JASCO/B006661794, Tensor27, Bruker, Bremen, Germany). The spectra were generated in absorption mode (1655-1615 cm^{-1}) with the resolution of 4 cm^{-1} (Liu and Liu, 2020).

Study of the morphology of edible nanoemulsion loaded with LPEO using Scanning Electron Microscope (SEM). A piece of the lemon peel essential oil loaded edible nanoemulsion film was attached to a cylinder stub. The film specimen was dried at 40°C overnight and coated with platinum using an ion sputter. The coated film was further investigated for its microstructure using a scanning electron microscope with an acceleration voltage of 5 kV (Elshamy *et al*., 2021).

RESULTS AND DISCUSSION

Yield of lemon peel essential oil. The yield of lemon peel essential oil (LPEO) from shade dried lemon peel (*C. limon*) by hydro-distillation method was about 1.5 per cent.

Physicochemical characteristics of corn starch loaded with lemon peel essential oil (CS-LPEO) edible nanoemulsion. The physicochemical properties such as particle size, polydispersity index, zeta potential, encapsulation efficiency, *in vitro* release percentage, color value, whiteness index and viscosity of CS-LPEO nanoemulsion (T_1 to T_6) formulated with different concentration of corn starch (2 and 3 percent) and lemon peel essential oil (0.3, 0.5 and 0.7 per cent) were analysed and the results were presented in Table 2 and 3.

Table 2: Physicochemical characteristics of citrus peel essential oil edible nanoemulsion.

Treatments	Particle size (nm)**	PDI	Zeta potential (mV)	Encapsulation efficiency (%)**	*In vitro* release percentage		
					Initial burst release at 0-3hrs (%)	Steady state release 3-12 hrs (%)	Stationary phase release 12-48hrs (%)
T_1	345.40±3.54^{bcd}	0.40±0.00	-34.00±0.71	60.00±0.08^{e}	57.68±0.76^{f}	78.27±0.50^{f}	81.45±2.54^{f}
T_2	346.10±4.82^{bc}	0.50±0.01	-42.40±0.63	81.00±0.16^{b}	59.73±0.32^{def}	81.44±0.49^{e}	85.74±1.10^{e}
T_3	358.40±4.93^{d}	0.40±0.00	-45.00±0.55	82.00±2.73^{b}	60.45±0.53^{bc}	85.41±0.78^{cd}	88.69±0.96^{de}
T_4	340.10±3.08^{b}	0.30±0.00	-36.90±0.45	76.66±0.26^{c}	61.36±0.20^{abcd}	88.76±0.77^{bc}	92.25±2.13^{c}
T_5	349.00±3.41^{bcd}	0.40±0.00	-39.00±0.84	82.85±1.07^{b}	61.54±0.37^{abcd}	91.93±0.43^{a}	95.68±2.34^{ab}
T_6	355.10±0.44^{cd}	0.30±0.00	-45.00±0.24	83.85±2.42^{b}	62.74±0.91^{abc}	94.64±0.26^{a}	98.34±2.34^{a}

Values are means of three replicates, Means in the same column followed by different superscripts are significantly different at P<0.05 among the treatments.

T_1	0.3% LEO + 1% Corn starch	T_4	0.3% LEO + 2% Corn starch
T_2	0.5% LEO + 1% Corn starch	T_5	0.5% LEO + 2% Corn starch
T_3	0.7% LEO + 1% Corn starch	T_6	0.7% LEO + 2% Corn starch

Table 3: Whiteness index, color value and viscosity of citrus peel essential oil edible nanoemulsion

Treatments	Whiteness index	Color value			Viscosity (cP)
		L*	a*	b*	
T_1	49.25±0.20	47.61±1.87	-0.80±0.19	-1.00±0.21	10.80±0.02
T_2	49.34±1.47	47.81±2.07	-0.82±0.00	-0.93±0.00	10.95±0.32
T_3	49.75±2.03	46.19±0.17	-1.34±0.13	-1.33±0.02	11.25±0.12
T_4	49.79±2.42	48.62±2.41	-0.70±0.10	-0.83±0.12	16.50±0.11
T_5	50.02±0.48	48.63±0.56	-0.84±0.25	-0.83±0.18	17.84±0.36
T_6	50.92±0.51	48.90±0.76	-0.94±0.16	-1.07±0.04	18.30±0.21

Values are means of three replicates

T_1	0.3% LEO + 1% Corn starch	T_4	0.3% LEO + 2% Corn starch
T_2	0.5% LEO + 1% Corn starch	T_5	0.5% LEO + 2% Corn starch
T_3	0.7% LEO + 1% Corn starch	T_6	0.7% LEO + 2% Corn starch

Particle size, polydispersity index (PDI) and zeta potential of CS-LPEO edible nanoemulsions. The term nanoemulsion refers to oil-in-water (o/w) emulsions with mean droplet diameters between 50 and 1000 nm. Typically, droplet sizes range from 100 to 500 nm. Particle size and polydispersity index are crucial markers for describing the quality, stability, homogeneity, and dispersibility of nanoemulsions. The particle size of the nanoemulsions was in the range of 340.10 nm to 358.40 nm. The size of the treatment T_1 was 345.40 nm, T_2 was 346.10 nm, T_3 was 358.40 nm, T_4 was 340.10 nm, T_5 was 349.00 nm and T_6 was 355.10 nm.

The PDI can range from 0 to 1, where 0 (zero) stands for monodisperse system and 1 for a polydisperse particle dispersion. The PDI of the LPEO nanoemulsions of the present study were in the range of 0.3 to 0.5 which denotes the monodispersed (homogenous) distribution of particles in nanoemulsion.

The zeta potential is a method of measuring surface charge of particles when it is placed in liquid medium. It provides an accurate description of an emulsion droplet's electrical properties. Zeta potential is used for predicting dispersion stability. The zeta potential of corn starch based nanoemulsion loaded with LPEO was in the range of -34.00 to -45.00 as the surface charge of the corn starch molecule was negative. Zeta potential of ±30 mV is believed to be sufficient for ensuring physical stability of nanoemulsion. High zeta potential nanoemulsion particles are electrically stabilized; while, low zeta potential nanoemulsion particles are more prone to flocculation and coagulation.

The particle size of nanoemulsion formulated with banana starch and lemongrass essential oil was in the range of 180 to 596 nm and banana starch and rosemary essential oil in the range of 164 to 676 nm (Restrepo *et al.*, 2018). Manzoor *et al.*, (2021) reported the particle size of the nanoemulsion formulated with sodium alginate and ascorbic acid to be in the range of 220 to 335 nm. The PDI of sodium alginate based nanoemulsion loaded with *Citrus sinensis* essential oil was determined as 0.3 by Das *et al*., (2020). Salvia-Trujillo *et al*., (2013) had reported the zeta potential of lemongrass oil-alginate nanoemulsion as -46 mV.

Encapsulation efficiency of CS-LPEO edible nanoemulsions. Encapsulation efficiency (EE) is a measure of the ability of a nanocarrier to retain the drug/active ingredient and deliver an adequate amount of the component to the targeted site (Che Marzuki *et al*., 2019). The EE of nanoemulsion formulated with 2 per cent corn starch and 0.3, 0.5 and 0.7 per cent LPEO was 60.00, 81.00 and 82.00 per cent, respectively whereas the EE of nanoemulsion formulated with 3 per cent corn starch with 0.3 per cent LPEO was 76.66 per cent, with 0.5 per cent LPEO was 82.85 per cent and with 0.7 per cent LPEO it was 83.85 per cent. The higher EE of corn starch nanoemulsion was found in treatment T_6 (83.85%).

***In vitro* release of CS-LPEO edible nanoemulsions.** Drug release from particles generally occurs by various mechanisms such as disintegration, surface erosion, desorption and diffusion. The dominant mechanism of drug release is mainly by diffusion of drug from the particles into the medium (Esmaeili and Asgari, 2015). The release behavior of LPEO from CS-LPEO nanoemulsion in phosphate buffer (pH 7.4) was characterized by three different stages (initial burst release, steady state release and stationary phase release). The initial burst of LPEO from the nanoemulsion occurred within 3 hours of the test period. The LPEO release from nanoemulsion in phosphate buffer was in the range of 57.68- 62.74 per cent. This might be due to instant diffusion and weak interaction functional group of LPEO attached to the surface layer of nanoemulsion. The second steady state release of drug happened between 3-12 hours. The release percentage was higher at this phase and reached upto 94.64 per cent of release of LPEO. The final stationary phase release of drug occurred between 12-48 hours in which there was only a small increase in release percentage after which the release of LPEO reached a plateau. The release of LPEO was significantly higher ($P<0.05$) in T_6 when compared with other treatments.

Color value and whiteness index of CS-LPEO edible nanoemulsions. The color parameters (L*, a*, b*) and the whiteness index of the LPEO loaded nanoemulsions were determined. It was observed that the whiteness index of treatments T_1 to T_3 was in the range of 49.25 to 49.75 and T_4 to T_6 was in the range of 49.79 to 50.92. Salvia-Trujillo *et al*. (2013) reported that the whiteness index of nanoemulsion formulated with sodium alginate loaded with lemongrass essential oil was 42.79. It was also reported that the whiteness index of nanoemulsion prepared from pectin with oregano essential oil was 55.79, thyme essential oil was 43.29, lemongrass essential oil was 39.89 and mandarin essential oil was 38.61 (Guerra-Rosas *et al*., 2016).

Viscosity of CS-LPEO edible nanoemulsions. The viscosity of an emulsion is highly dependent on the compositions of its surfactant, water, and oil components, as well as their concentrations. From the results, it was found that the viscosity of the corn starch LPEO edible nanoemulison was in the range of 10.80 to 18.30 cP. Salvia-Trujillo *et al*., (2015) reported that the viscosity of coarse emulsions formulated with sodium alginate and essential oils (lemongrass, clove, tea tree, thyme, geranium, marjoram, palmarosa, rosewood, sage or mint) ranged from 22.95 to 36.05 mPa.s, whereas the viscosity of the nanoemulsions were in the range of 9.77 and 14.95 mPa.s. which may be due to the droplet size of the nanoemulsion.

Fourier Transformation Infrared Spectroscopy (FTIR) analysis of CS-LPEO edible nanoemulsions. The FTIR spectroscopy is based on an infrared beam that travels through a material, where it is primarily absorbed and part of it is transmitted. The resulting spectrum gives the sample a molecular fingerprint by displaying the molecule absorption and transmission. Each sample fingerprint has distinct absorption peaks that correspond to the frequencies of vibrations between the atoms of the material. Because each material is a unique combination of atoms, no two compounds produce the same infrared spectrum. As a result, infrared spectroscopy can positively identify various materials. Furthermore, the size of the peaks in the spectrum indicates the amount of material present in the sample (Silva *et al*., 2012).

The FTIR spectrum of LPEO, Tween 20, corn starch and CS-LPEO is given in Fig. 1. From the spectrum of LPEO, the stretching vibration of C=O was observed at 1643 cm^{-1}. Peaks of aromatic C-H out-of-plane bend was observed at 886 cm^{-1} and 797 cm^{-1} and a peak of aromatic C-H in plane bend was present at 949 cm^{-1} which evinces the presence of limonene, α-pinene; β-pinene and γ-terpinenes in the LPEO. The above mentioned peaks are not observed in other spectra which confirmed the encapsulation of LPEO into corn starch in the nanoemulsion. This result was in agreement with Li *et al*. (2018) who had reported the FTIR spectra of citrus essential oil had a stretching vibration of 1646 cm^{-1} and the peak corresponds to the presence of limonene at 886 cm^{-1}. Similar studies of FTIR spectra of lemon essential oil was determined by Nunes *et al*., (2021) who had observed the aromatic rings at 797 cm^{-1}, 886 cm^{-1} and 948 cm^{-1} that indicated the presence of limonene, α-pinene, β-pinene and γ-terpinenes respectively.

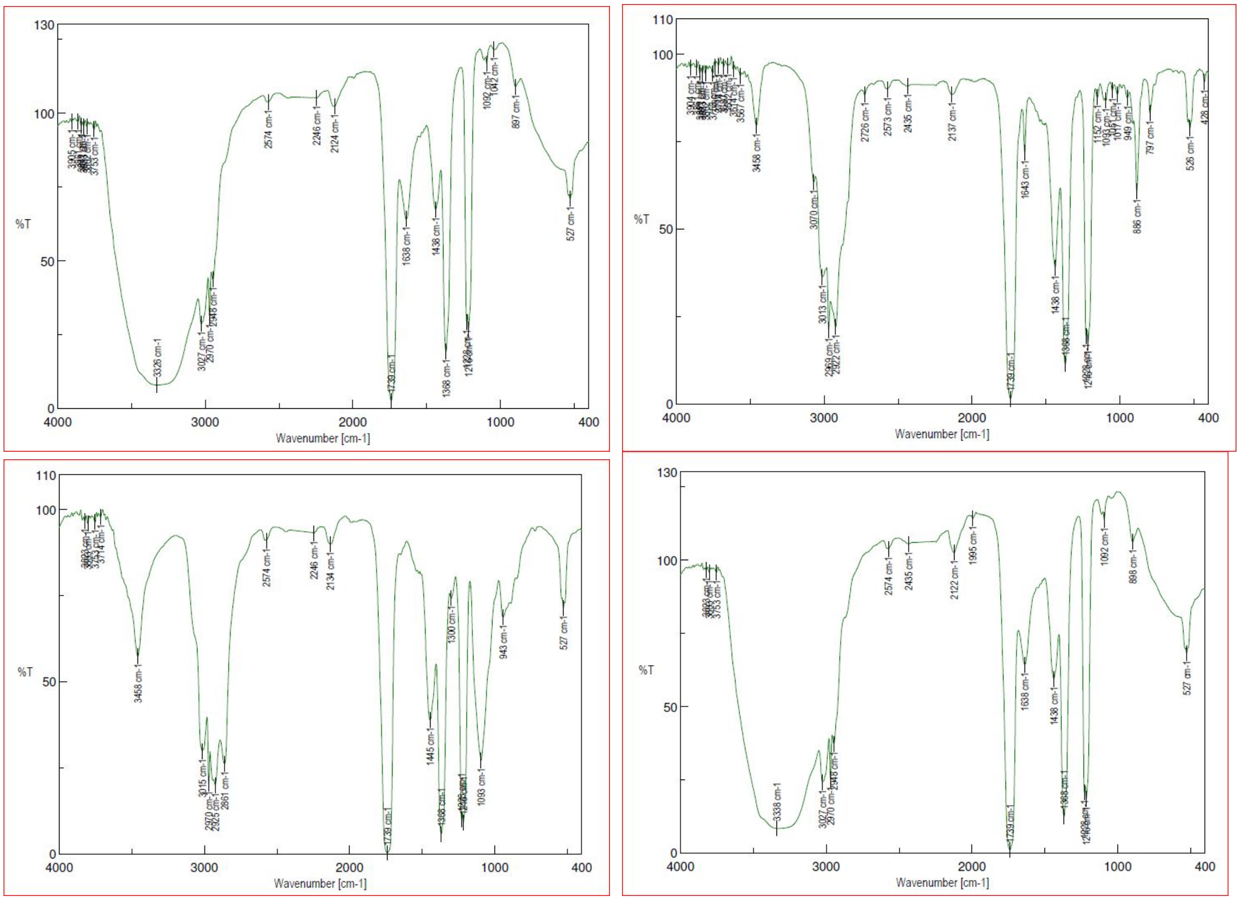

Fig. 1. FTIR spectra of Corn starch, LPEO, Tween 20, and CS-LPEO nanoemulsion.

The FTIR spectrum of Tween 20 indicated the presence of –OH stretch at 3458 cm^{-1}, asymmetric and symmetric methylene stretch at 2925 cm^{-1} and 2861 cm^{-1}. Presence of carbonyl stretching (C=O) was found at 1739 cm^{-1}, and stretch vibration of C-O was present at 1093 cm^{-1}. Ortiz-Tafoya and Tecante (2018) evaluated the FTIR spectra of Tween 20 and reported the presence of O-H stretching at 3476 cm^{-1}, asymmetric and symmetric methylene stretching vibrations at 2920/2860 cm^{-1}, carbonyl stretching at 1734 cm^{-1} and stretch vibration of $–CH_2$-O-CH_2 at 1095 cm^{-1}.

The FTIR spectrum of corn starch revealed the presence of hydrogen bonded –OH stretching vibration at 3326 cm^{-1}. Presence of methyl asymmetric/symmetric stretching and methylene asymmetric/symmetric stretching at 2970 $cm^{-1}$and 2948 cm^{-1} are the characteristic peaks of corn starch. A peak at 897 cm^{-1} represented the C-H bending out of plane ring of polysaccharides. Similar result was reported by Ji *et al*., (2018) for corn starch. The peak at 3285 cm^{-1} represented the hydrogen-bonded –OH groups of starch whereas the peak at 2,900 cm^{-1} attributed to the CH_2-asymmetric stretching of – CH_2OH groups in starch.

In CS-LPEO nanoemulsion spectrum, the peak was shifted from 3326 cm^{-1} to 3338 cm^{-1} (O-H stretching) and 897 cm^{-1} to 898 cm^{-1} (C-H bending out of plane ring of monosaccharides). A new peak was observed at 1995 cm^{-1} of C=C=C stretching. The distinctive peak of LPEO was not present in the CS-LPEO nanoemulsion, indicating that corn starch had been used to encapsulate the essential oil in the nanoemulsion formulation.

Morphology of CS-LPEO edible nanoemulison using Scanning Electron Microscope (SEM). The SEM can generate high-resolution images of a sample's surface. The SEM images have a distinct three-dimensional appearance and are useful for determining surface structure (Silva *et al*., 2012). The size of the nanoemulsion measured in the micrograph (200 nm - 300 nm) was in accordance with the mean particle size of the nanoemulsion measured (Fig. 2). The shape of the nanoemulsion was somewhat spherical in CS-LPEO nanoemulsions. This result was in accordance with the result reported by Wu *et al*. (2016) who reported the shape of citrus essential oil loaded nanoemulsion as spherical. The size and shape of the final droplets were greatly influenced by the ratio of wall material (corn starch) and core material (LPEO) used. Distribution of nanoemulsion was fairly dense in the nanoemulsions. The surface of the nanoemulsion was rough that might be due to the presence of LPEO.

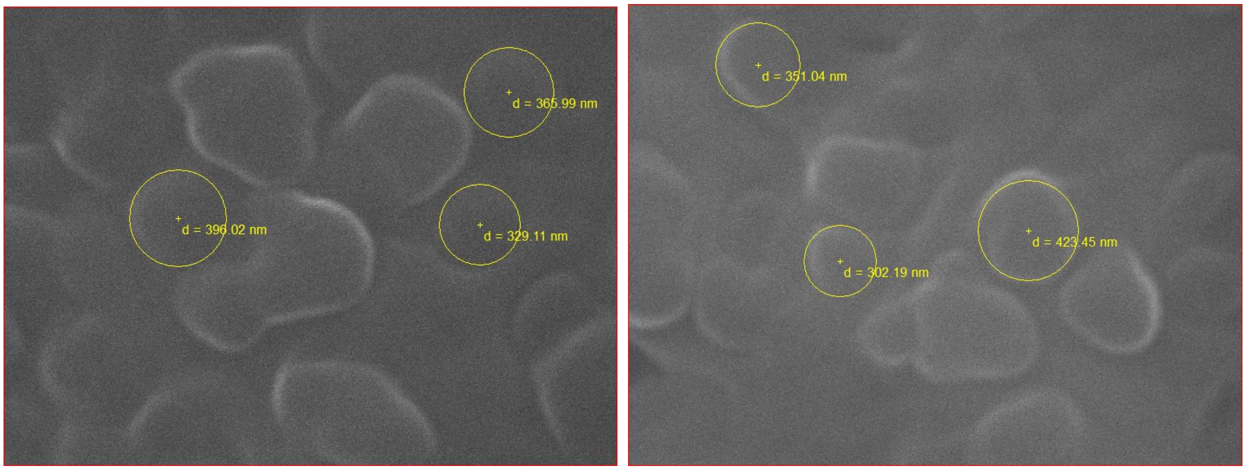

Fig. 2. Surface morphology of lemon peel essential oil loaded corn starch nanoemulsion.

CONCLUSION

Edible nanoemulsion solutions can be formulated using corn starch (2 and 3 per cent) and lemon peel essential oil (0.3, 0.5 and 0.7 per cent). Characterization of the edible nanoemulsion indicated that the nanoemulsion formulated with 0.7 per cent of lemon peel essential oil and 3 per cent of corn starch had higher encapsulation efficiency and *in vitro* release percentage. Therefore, the formulated edible nanoemulsion solution can be used as an edible coating over whole or cut fruits and vegetables.

FUTURE SCOPE

The formulated edible nanoemulsion can be applied as coating material in minimally processed or fresh cut fruit in order to extend the shelf life of the product. Edible nanoemulsion film can also be developed using the formulated edible nanoemulsion solution.

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