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Original research

Preparation and properties of ginger essential oil nanocapsules

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ABSTRACT

Ginger herb has remarkable antimicrobial and antioxidant activities. In the present study, the aim is to investigate the effect of chitosan as an encapsulation on the physicochemical characteristics of ginger essential oil. Encapsulation process with the ratios of chitosan to ginger essential oil (1:0, 1:0.4, 1:0.8 and 1:1.2 (w/w)) and sodium tripolyphosphate concentrations (0.5 and 1% w/v) was performed by emulsion-gel method. Encapsulation efficiency, loading capacity, particle size distribution and zeta potential tests were carried out on samples. Also, Fourier-transform infrared spectroscopy, total phenolic compounds, free radical scavenging and minimum inhibitory concentration (MIC) tests were done on selected and control samples. According to the results of physical tests, the optimal sample was selected with the ratio of chitosan to essential oil (1:0.8 w/w) and salt concentration (0.5% w/v). This nanocapsule exhibited high encapsulation efficiency (23.1 %), suitable particle size (734 nm) and zeta potential (29.2 mV). Application of chitosan nanocapsule containing ginger essential oil indicated more MIC on *Escherichia coli* (0.97 µg/ml), *Staphylococcus aureus* (1.9 µg/ml), *Salmonella typhimurium* (3.90 µg/ml) and *Pseudomonas aeruginosa* (0.97 µg/ml) compared to other control samples. Also, the antioxidant activities (97%) and the amount of total phenolic compound (980 mg/g) of optimal chitosan nanocapsule were significantly improved. The application of chitosan nanocapsules has led to the improvement of the functional properties of the encapsulated ginger essential oil and is suggested as a natural alternative to chemical additives.

Keywords: Nanocapsules; Ginger; Phenolic compounds; Antimicrobial properties

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1. Introduction

In recent years, more attention has been paid to traditional medicines derived from different herbaceous species and their therapeutic characteristics throughout the world, because the use of natural products as antioxidant and antimicrobial compounds is desirable. Spices are known for their health-promoting ingredients such as vitamins, minerals, antioxidants, antibacterial and probiotics (Policegoudra et al., 2007; Dehghani et al., 2019). Ginger has been one of the most well-known spices in the world for over 2000 years. The main region of this plant is Asia, it has strong and branched roots and is cultivated all over the world due to its medicinal and decorative properties (Rondanelli et al., 2020). In the evaluation of fresh ginger, about 63 components have been identified and the most important components include volatile and non-volatile antioxidant compounds in its rhizome (Srinivasan, 2017). Ginger has medical uses as a remedy for cancer, arthritis, skin and heart diseases (Choia et al., 2017). Antimicrobial properties investigation of ginger essential oil indicated that the essential oil with acetate had an effect on spoilage microorganisms and the essential oil at a concentration of 200 mg/L for 24 h almost completely destroyed all microorganisms in environment (Malu et al., 2009). Essential oils (EOs) have been widely applied for many years (Gonçalves da Rosa et al., 2020). In different circumstances, the application of natural aromatic of plants as a flavoring factor is permitted (Hematian et al., 2020).

Ginger is considered for its antimicrobial and antioxidant characteristics, but the instability of its activities against environmental factors such as oxygen, light, moisture and pH during the production and storage process has limited its use (Muhialdin et al., 2020). Therefore, the use of new methods seems necessary to protect natural compounds. For this purpose, nanoencapsulation technique is used to increase the stability of

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bioactive compounds against adverse environmental factors and interference of compounds. *Hyssopus officinalis* and *Eryngium caeruleum* M. Bieb. and their compounds were produced based on thin film hydration method and the results indicated that the antioxidant and antimicrobial activities of the essential oils increased after encapsulation and were recommended for industrial use (Nouri, 2020). Production of Brazilian propolis essential oil nanoemulsions indicated that the antioxidant and antimicrobial activities of the essential oil were increased and this nanoemulsion is used as a tasteless preservative (Seibert et al., 2019). Zein nanocapsules containing *Origanum vulgare Linneus* and *Thymus vulgaris* essential oils had greater antimicrobial activity and thermal resistance in baking processes, compared to these free essential oils (Gonçalves da Rosa et al., 2020).

Some techniques specifically used for encapsulation of the ginger essential oil include gelatin containers with different acids, cyclodextrin using steam distillation, and spray or roller dryers (Gaonkar et al., 2014). In the present study, chitosan polysaccharide has been used for the basis of encapsulation.

Chitosan is a cationic amino polysaccharide. It is partly deacetylated more than 50% and called chitin, if this percentage is low, the compound is chitosan (Karimi et al., 2020). Nowadays, chitosan has various applications in medicine, biomedical, pharmaceutical, agriculture, biological, environment and in food technology such as, binding, food formulations, bioencapsulation, thickening, stabilizing, gelling, clarifying and antimicrobial factor, so more attention has been paid to chitosan (Nouri et al., 2016; Mahdizadeh Barzoki et al., 2019).

Ginger is one of the valuable plants in Iran. Field studies illustrated that ginger essential oil has not been encapsulated with chitosan nanocapsules, so it is necessary to carry out this research. The aim of this study is to investigate and compare the increased stability of antimicrobial and antioxidant activities of nanocapsules with control compounds.

2. Material and Methods

2.1. Materials

In the present study, the used chitosan had a degree of deacetylation (89.34%) and a molecular weight (806.931 Da). Fresh ginger was purchased from local markets in Tehran, Iran. Tween 60 and analytical grade methanol, aceton, chloroform, glacial acetic acid, sodium carbonate, Folin–Ciocalteu reagent, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), sodium tripolyphosphate (STP) and Müller-Hinton broth were purchased from Sigma-Aldrich.

Staphylococcus aureus PTCC 1431, *Escherichia coli* PTCC 1399, *Salmonella typhimurium* ATCC 14028 and *Pseudomonas aeruginosa* PTCC 1430 were taken from the Microorganism Collection Center of Iran Scientific and Industrial Research Organization.

2.2. Extraction of ginger essential oil

Ginger is prepared in such a way that the rhizome (ginger root) was freshly purchased, after several times it was washed, peeled, crushed and dried in an oven at 50 °C for 72 h. Then it was powdered in a mixer and kept at a cold temperature. In the present study, cold solvent method was used to essential oil the ginger (Su et al., 2007). In this method, rhizome powder obtained from the previous stage was mixed with acetone solvent and before filtration and separation by centrifugation (4000 rpm, 15 min) was stirred well at ambient temperature with a mixer. A rotary apparatus was used at 50 to 60 °C to remove the solvent of the ginger essential oil. It was kept in a dark glass container at cold temperature for the required time.

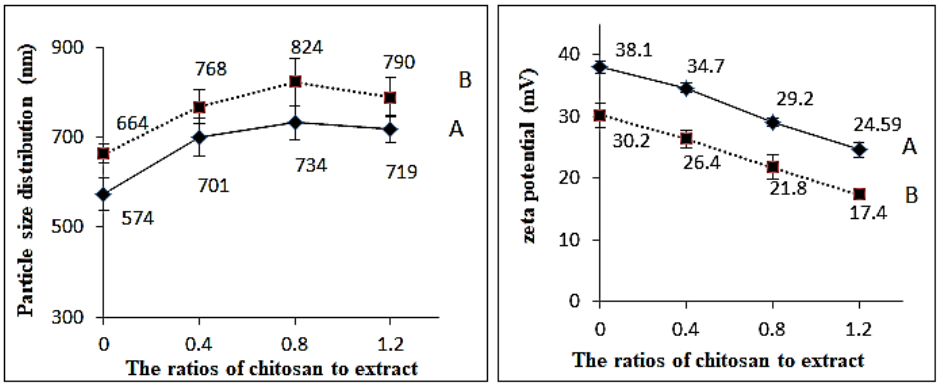
2.3. Production of chitosan nanocapsules

The formation of nanocapsules was carried out in two stages of emulsion production (oil in water) and ionic gelling (Ko et al., 2002; Gopalakrishnan et al., 2014). In the first step, in order to produce an emulsion, Tween 60 emulsifier was added to a homogeneous solution of chitosan and acetic acid (1% v/v), the solution was well stirred, then the ginger essential oil was gradually added at different concentrations with stirring (1 h at ambient temperature) to obtain different ratios of chitosan to ginger as 1:0, 1:0.4, 1:0.8 and 1:1.2 (w/w). In the second step, STP solution at concentrations of 0.5 and 1% (w/v) was added to the emulsion solution and stirring was continued for 1 h at ambient temperature. At the end of the process, a centrifuge was used to separate the produced particles (10,000 rpm, 10 min). The precipitated part was washed several times with deionized water and then dried well in a vacuum dryer and stored in dark glass containers at a cool temperature for the required time (Table 1).

Table 1. Loading capacity (%) and encapsulation efficiency (%) of different treatments.

Codes	Treatment		Loading capacity (%)	Encapsulation efficiency (%)
	The ratio of chitosan to extract	STP concentration		
1	1:0	0.5	0	0
2	1:0.4	0.5	12.7±0.7^{c}	19.6±0.9^{d}
3	1:0.8	0.5	14.2±0.3^{d}	23.1±1.1^{e}
4	1:1.2	0.5	15.1±1.0^{d}	16.4±0.8^{c}
5	1:0	1	0	0
6	1:0.4	1	2.3±0.2^{a}	9.7±0.6^{b}
7	1:0.8	1	4.7±0.4^{b}	10.5±0.4^{b}
8	1:1.2	1	5.6±0.7^{b}	6.1±0.5^{a}

Means with different letters within a column are significantly different ($p < 0.05$).



(1-1) (1-2)

Fig. 1. Diagrams of (1-1) particle size changes and (1-2) zeta potential changes in different ratios of chitosan and essential oil. A: No. 1 to 4 (salt concentration of 0.5% w/v), B: No. 5 to 8 (salt concentration of 1% w/v).

2.4. *Determination of encapsulation efficiency and loading capacity of nanocapsules*

Dispersion of the solutions was stirred with hydrochloric acid solution at 95 °C for 30 min and heating was carried out to break the structure and release the nanocapsules. The cooled solution was mixed with methanol solution before centrifugation (9000 rpm, 2 min) (Calvo et al., 1997; Luo et al., 2011). Standard calibration curve was obtained by plotting the desired concentrations of ginger essential oil in methanol solution (0.1, 0.4, 0.7, 1 and 1.3 mg /ml), (vertical axis) versus absorption values (horizontal axis). The adsorption intensities of the samples were measured from lower to higher concentrations at 281 nm, respectively, and then Eqs. 1 and 2 were used.

$$\text{Encapsulation efficiency percentage} = \frac{\text{amount of loaded ginger essential oil}}{\text{initial amount of ginger essential oil}} \times 100 \quad (1)$$

$$\text{Loading capacity percentage} = \frac{\text{amount of loaded ginger essential oil}}{\text{amount of sample}} \times 100 \quad (2)$$

2.5. *Determination the particle size distribution and zeta potential*

Particle size distribution and zeta potential tests were performed after diluting the samples at 25 ± 1 °C using an electron laser to determine the measurement of nanocapsules and zeta potential (Wu et al., 2005). In the present study, the average particle size was measured based on the mean number, surface and volume distributions.

2.6. *Fourier-transform infrared spectroscopy (FTIR) test*

A dry powder sample with powdered potassium bromide was compressed into a glass tube and placed in an infrared spectrometer to prepare a solid sample. The desired spectrum was reported in the region (400-4000 cm^{-1}) (Mathew & Abraham, 2008).

2.7. *Measurement of total phenolic*

Folin–Ciocalteu method was used to measure phenolic compounds. 100 µl of the filtered solution samples were mixed with 1 ml of 10 % normal Folin–Ciocalteu and shaken for 5 min, then 600 µl of sodium carbonate (20 %) was added to the solution and kept at ambient temperature and in the dark for 2 h. Then the absorbance of the solution was observed by spectrophotometer at a wavelength of 760 nm against the control sample (distilled water), (Hematian et al., 2020).

2.8. *Determination of free radical scavenging by DPPH Assay*

1.25 ml of the methanolic solution of samples containing chitosan, control, optimized nanocapsule (0.05 g/ml) was mixed with 1.5 ml of DPPH methanolic solution (0.004%), and then it kept at ambient temperature and in the dark for 90 min. Then the absorbance was read at 517 nm against methanol-water 1:1 as a control. The control sample was prepared according to the above method. The difference was that instead of ginger solution, 1.25 ml of methanol was mixed with 1.5 ml of DPPH solution. The antioxidant activity of ginger solution was expressed as a percentage of inhibition using Eq. 3 (Fadda et al., 2014).

$$\text{Free radical trapping percentage} = \frac{(A_c - A_s)}{A_c} \times 100 \quad (3)$$

In this equation, A_c and A_s are control adsorption and sample adsorption, respectively.

2.9. *Microbial analysis*

In the present study, bacterial strains were chosen such as gram-positive (*staphylococcus aureus* PTCC 1431) and gram-

negative (*Escherichia coli* PTCC 1399, *Salmonella typhimurium* ATCC 14028 and *Pseudomonas aeruginosa* PTCC 1430). In order to maintain the vital activity of the achieved strains, adequate cultures were obtained from them every month and stored in the refrigerator. The mentioned bacterial strains were superficially cultured the day before microbial analysis in Müller-Hinton broth to be in their logarithmic phase after one night of incubation (37 °C). Microbial tests of samples including chitosan, control, optimized nanocapsule and ginger essential oil were carried out based on measurement of minimum inhibitory concentration (MIC) against microorganisms of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* by dilution method in a tube. In this method, 0.5 ml of the solution of all samples mentioned above was added to the test tubes containing 0.5 ml of autoclaved Müller-Hinton broth. After mixing, the combination was transferred to the second tube, this process was continued and each tube contained the sample solution and half the concentration of the previous tube. 50 µl of the microstructure suspension was added to each tube and a control sample was obtained next to the samples. The samples were incubated for one day in each tube with vertex at 37 °C. The turbidity was calculated in samples with a spectrophotometer at 625 nm (Costa et al., 2012).

2.10. Statistical analysis

The statistical population was designed from 8 different treatments and each treatment with 3 replications (24 samples). The dependent variables (studied tests) on the changes of independent variables such as increasing concentrations of chitosan nanocapsules and STP were investigated as independent variables using factorial test (two-factors). The mentioned statistical processes were performed by Minitab (version 16) statistical software (Minitab Inc., State College, Pennsylvania, USA).

3. Results and Discussion

3.1. Encapsulation efficiency and loading capacity

Encapsulation efficiency and loading capacity of nanocapsules are a measure of the particle capacity in holding and strength release of the compounds within the structure, which process conditions affect them such as purity of raw material, temperature, duration and required energy for mixing, type and salt content, zeta potential, gelling point, etc. (Shu & Zhu, 2000; Ngo et al., 2011; Gaonkar et al., 2014).

In the present study, chitosan powder was considered as a substrate to preserve ginger essential oil and in order to determine the amount of released ginger essential oil from chitosan encapsulation, a calibration diagram of five ginger essential oil concentrations (0.1, 0.4, 0.7, 1 and 1.3 mg/ml) was plotted in terms of adsorption intensity and Eq. 4 was obtained.

$$Y = 11.206X - 0.8151 \ (R^2 = 0.91) \quad (4)$$

By obtaining the amount of loaded ginger and Eqs. 1 and 2, the encapsulation efficiency and loading capacity are shown in terms of the obtained percentage in Table 1, the encapsulation efficiency and loading capacity of the obtained samples have a range of 6.1 to 23.1% and 2.3 to 15.1%, respectively. The results of Table 1 indicated that the percentage of loading capacity expanded with enhancing the amount of consumed essential oil, but higher amounts of salt (1%) did not exhibit an increase in the loading capacity percentage.

It is probable that the loading capacity at higher values of the binder (STP) occupies a larger volume of the prototype, so the overall particle values are greater and the loading capacity fraction due to the larger denominator indicates a smaller number (Nouri & Khodaiyan, 2020[b], 2003; Woranuch & Yoksana, 2013). There is another possibility that a larger volume of STP will lead to more water absorption from the essential oil and shrinkage, so the numerator (loaded essential oil content) is a smaller number and the sample exhibits lower values of this factor (Ajun et al., 2009; Wisuitiprot et al., 2011).

The results indicated that the encapsulation efficiency percentage has higher values than 1 % when the amount of STP consumption is 0.5%, also with increasing the ginger essential oil concentration, this percentage enhanced to a certain point (Table 1). The highest encapsulation efficiency percentage belonged to No. 3 (23.1%) but with increasing the essential oil concentration (ratio 1:1.2) in the same conditions, ie No. 4 (16.4%), this percentage significantly decreased ($p < 0.05$). It is possible that in higher amounts of primary ginger essential oil due to load limitation on chitosan nanocapsules, no larger amounts of the essential oil can be encapsulated (Yoksan et al., 2010). The increase in STP levels may also be related to the same contraction and shrinkage in the loading formula (Jang & Lee, 2008; Ajun et al., 2009; Wisuitiprot et al., 2011).

The highest encapsulation efficiency percentage is observed when the most suitable amount of bonds between ammonium and hydroxyl groups are obtained in chitosan and ginger essential oil, respectively. The encapsulation efficiency of the essential oil in chitosan nancapsules did not necessarily increase with enhancing the desired composition percentage of the essential oil, these results are the same as the reports of some previous researchers (Shah et al., 2009; Yoksan et al., 2010; Luo et al., 2011). Table 2 indicates the results of applying various methods and compounds to encapsulate in chitosan biopolymer over the range of loading and encapsulation efficiency percentage by previous researchers.

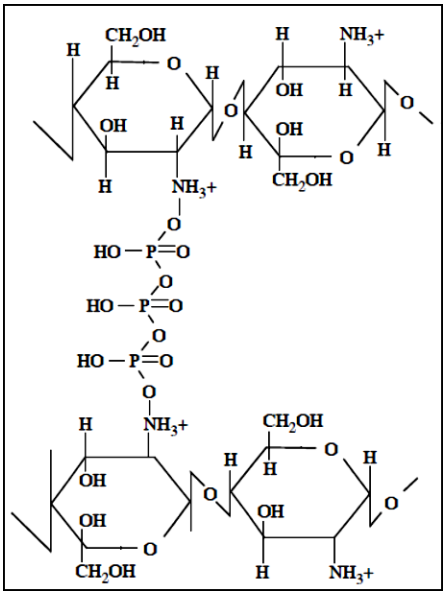


Fig. 2. Binding chitosan to STP (Vandana & Sahoo, 2009).

Table 2. The results of applying various methods and compounds to encapsulate in chitosan biopolymer over the range of loading and encapsulation efficiency by previous researchers.

Researches	Loaded chitosan compound	Loading capacity (%)	Encapsulation efficiency (%)
Xu and Du (2003)	Bovin Serum Albumin	17- 45	19.4- 30.2
Yoksan et al. (2010)	Ascorbyl Palmitate	8.46- 19.78	38.9- 76.6
Alishahi et al. (2011)	Vitamin C		30.2- 70.1
Woranuch and Yoksan (2013)	Eugenol	0.40- 12.80	1.1- 20.2
Gopalakrishnan et al. (2014)	Ellagic Acid		37.2- 50.1
Sanna et al. (2015)	Poly lactic- Glycolic Acid	0.09- 0.50	9.22- 52.5
Hasheminejad et al. (2019)	Clove essential oil	0-6.18	31-45.77

Table 3. The results of applying different encapsulation conditions on particle size and zeta potential characteristics by previous researchers.

Researchers	Loaded chitosan compounds	Particle Size (nm)	Zeta potential (mv)
Wu et al. (2005)	Glycerinated Ammonium	160- 250	43.2- 35.4
Yoksan et al. (2010)	Ascorbyl Palmitate	500- 2500	
Alishahi et al. (2011)	Vitamin C	185- 585	49.3- 62.3
Woranuch and Yoksan (2012)	Eugenol	586- 974	37.7- 16.2
Sanna et al. (2015)	Poly lactic- Glycolic Acid	840- 19790	
Hasheminejad et al. (2019)	Clove essential oil	129-1287	22-39

3.2. Particle size distribution and zeta potential

Particle size and their zeta potential are among the characteristics that determine the type and application of nanocapsules (Shu & Zhu, 2000; Yoskan et al., 2010; Gaonkar et al., 2014; Sanna et al., 2015). According to Fig. 1(1-1), the particle size of No. 1 to 4 with less STP concentration (0.5%) and No. 5 to 8 with a higher salt concentration (1%) have ranges of 574 to 734 nm (Fig. 1 (1-1A)) and 664 to 824 nm (Fig. 1 (1-1B)), respectively. The amount of used compounds in the process (increasing the ratio of chitosan to essential oil and STP percentage) had a significant effect on particle size distribution. This index is significantly higher than other samples in prepared treatments with higher STP concentration (Fig. 1 (1-1B)) ($p < 0.05$).

Significant increase in particle size at the level of 0.05 from 574 to 664 nm in produced No. 5 compared to No. 1 (also other samples in Fig. 1 (1-1A) compared to their corresponding point in Fig. 1 (1-1B)) can be attributed to the increase in particle size after the process according to the definition of this index. In such an operation, the chitosan particles may have aggregate and form hydrogen bonds with the desired salt, but since the salt concentration was high, the bonds occupied a larger volume (Lang Tsai et al., 2008; Ajun et al., 2009).

Therefore, the result is the production of larger nanocapsules compared to the corresponding points in Fig. 1 (1-1A). Also, in both graphs, an increasing trend of particle size was observed, which is due to the increase in the amount of ginger essential oil (encapsulated compound). The results indicated at higher ratios of essential oil (0 to 1.2), the particle size was reduced, probably (No. 4) due to lower encapsulation efficiency than the previous treatment (No. 3) the ability to carry the essential oil in this amount for chitosan nanocapsules was not possible and less essential oil is encapsulated, which leads to smaller particle size (Ajun et al., 2009; Keawchaoon & Yoksan, 2011).

The amount of zeta potential in each food indicates the stability of particles and hydrocolloid systems, which decreases the system stability (Wu et al., 2005). The effect of essential oil addition on system stability was monitored through changes in the zeta potential of the desired nanocapsules. Fig. 1 (1-2) indicated the zeta potential in diagrams A and B in the range of 38.10 to 24.59 mV and 30.2 to 17.40 mV, respectively. Fig. 1 (1-2B) indicated that produced nanocapsules without essential oil, containing 0.5 % of STP concentration (No. 1) has the highest stability and zeta potential (Fig. 1 (1-2A)), but with increasing the salt percentage, this factors decreased (Fig. 1 (1-2B)).

Due to the production stage of nanocapsules (No. 1), NH_3^+ groups of chitosan have established hydrogen bonds with STP phosphoryl groups (Fig. 2). These relatively stable bonds have led to the production of a stable system (Nouri & Khodiyan, 2020b). However, with increasing the amount of consumed STP as a result of increasing the groups with negative charge (No. 5), the stability of the system decreases. Also, the results of both diagrams indicated that with presence of essential oil and beginning of encapsulation process of the essential oil in chitosan polymer, the zeta potential and the stability have reduced.

This is probably due to the presence of double bonds in the linear structure of phenolic and terpene compounds in the essential oil and their covalent bonds to the amine or ammonium groups remaining in the medium. Therefore, increasing the amount of essential oil has led to interference in the electrostatic balance and instability of the system compared to its original state (Wu et al., 2005; Woranuch & Yoksana, 2013).

The results of applying different encapsulation conditions on particle size and zeta potential characteristics by previous researchers are shown in Table 3. Research results have shown that the range of -20 to 40 mV zeta potential is stable for chitosan biopolymer (Nouri & Khodaiyan, 2020a) and the results of present study are also in this range.

3.3. Determination of the optimal point to produce nanocapsule

The optimal sample with a ratio of chitosan/essential oil (1:0.8 w/w), and concentration of 0.5% STP (w/w) were selected according to the results. This sample had the highest encapsulation efficiency (23.1%) and loading capacity (14.2%). It also had suitable zeta potential (29.2 mV) and particle size (734 nm), which indicates the stability of this compound. As a result, it was selected as the final treatment and the FTIR was used to identify the types of

chemical bonds and functional groups of the encapsulated compound.

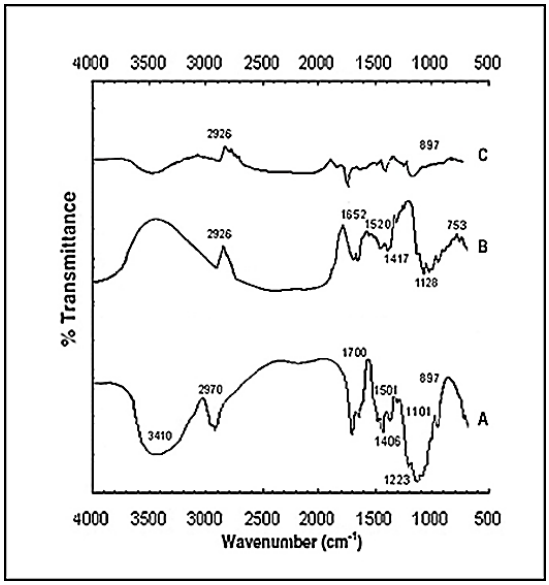


Fig. 3. FTIR diagrams of treatment samples No. 1 (A), ginger essential oil (B) and No. 3 (C).

3.4. FTIR of nanocapsules and essential oil

Fig. 3 indicated the structure of No. 1 and 3. Fig. 3A, belongs to chitosan nanocapsules with 0.5 % STP (No. 1). This diagram had main absorption peaks (in the same ranges with the chitosan structure) in the ranges of 3410, 2970, 1700, 1501 and 1406 cm^{-1}, which represent the bonds of the hydrazine group NH-(-NH_2), methylene methylidine CH-(CH_2), amide 1 or C=O-NH_2, amide 2 or alkyl hydrazine (N-H bend of R-NH_2) and methylene methylidine CH-(CH_2), respectively. A peak was also observed in the range of 897 cm^{-1} for pyranose rings. Diagram 3A has new peaks compared to the original chitosan sample (control). These peaks include phosphoryl bonds (P=O) and (P-O) in the range of 1223 to 1159 cm^{-1}, which indicate the presence of STP (phosphorous groups) and chitosan (carbon groups) in the ionic gel stage.

Previous researchers have observed peaks in similar ranges (Qi et al., 2004; Yoksan et al., 2010; Al-Remawi, 2012). Fig. 3B belonged to ginger essential oil and has the main absorption peaks in the range of 2926 and 1127 cm^{-1}, which indicate the bonds of methylene (CH_2) and methyl (CH_3) groups, respectively. In Fig. 3B, the peaks in the range 1652 cm^{-1} represent the water groups H_2O, amide 1C=O-NH_2 and cyclic aromatic carbonyl compounds (C=O). Peaks in the range of 1530 and 1417 cm^{-1} indicate the aromatic and cyclic structures in the desired essential oil, respectively. Peaks in the range of 753 cm^{-1} are associated to structural compounds such as pyrrole.

Previous researchers have observed similar peaks for ginger essential oil (Purnomo et al., 2010; Aris & Morad, 2014; Zhao et al., 2015), But diagram 3C was shown for the optimal sample (ratio of chitosan to ginger essential oil 8:0.1). This graph was structurally similar to diagram 3A, but in comparison, new peaks were observed, which indicate the presence of ginger essential oil. Peaks are in the range of 2926 and 897 cm^{-1}, which represent methylene (CH_2) groups in the structure of ginger essential oil and pyranose rings in chitosan nanocapsules, respectively. Both of these peaks in nanocapsules clearly indicate the encapsulation process of ginger essential oil in chitosan nanocapsules.

3.5. Evaluation of antioxidant activities

The antioxidant activity of herb essential oils including polyphenol compounds is due to their capacity to be donors in hydrogen atoms or electrons and to capture the free radicals. One of the tests utilized to confirm the ability of the ginger essential oil compounds to act as donors of hydrogen atoms is DPPH analysis (Stoilova et al., 2007). This analysis was performed for all samples at constant concentration and the results of Table 4 indicated that ginger has more phenolic compounds and antioxidant activities than chitosan with a significant difference. Since there is a positive correlation between the amount of phenolic compounds and DPPH percentage, the highest amount of this percentage appeared in the optimal sample (97%), because the presence of chitosan with low antioxidant effects and nanocapsule have been effective in increasing the antioxidant activities of ginger.

Table 4. Total phenol content and DPPH radical scavenging activity of different samples.

Samples	Total phenolic (mg/g)	DPPH (%)
Chitosan	58±11.23[a]	16 ±9.02[a]
Ginger	840±20.13[b]	80 ±12.83[b]
Control (No. 1)	67±23.70[a]	32 ±7.12[a]
Optimized nanocapsule (No. 3)	980±20.21[b]	97 ±6.45[b]

Means with different letters within a column are significantly different ($p < 0.05$).

3.6. Minimum Inhibitory Concentration (MIC) Testing

One of the main objectives of present study was to determine MIC against target microstructures for the control (No. 1) and optimized nanocapsule (No. 3), the results were shown in Table 5. Ginger and chitosan have inhibitory properties on *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*. Control (No. 1) and optimized nanocapsule (No. 3) have the least to greatest effect on the destruction of target microorganisms, respectively. Chitosan is also an effective compound in inhibiting gram-positive and gram-negative bacteria. There is a significant difference in inhibiting the growth of target bacteria between the control and optimal samples because there was no ginger extract in the control sample, but in the optimal sample of nanocapsules, the loading capacity (%) and encapsulation efficiency (%) were the highest. The presence of extract in nanocapsules had an effect on increasing the inhibitory property. On the other hand, the optimal sample with higher difference contains more phenolic compounds than the control sample. According to previous studies, the amount of phenolic compounds affects the increase of antimicrobial properties (Wen et al., 2003). Among the available microorganisms, *Escherichia coli* and *Salmonella typhimurium* were the most susceptible and resistant bacteria against the samples, respectively (Table 5).

Table 5. Determination of the MIC of different samples against target microstructures.

Samples	MIC (µg/ml)				Microorganisms
	0.97	1.9	3.90	7.81	
chitosan			×		*Escherichia coli*
				×	*Salmonella typhimurium*
				×	*Staphylococcus aureus*
			×		*seudomonas aeruginosa*
Control (No. 1)		×			*Escherichia coli*
				×	*Salmonella typhimurium*
			×		*Staphylococcus aureus*
		×			*seudomonas aeruginosa*
Optimized nanocapsule (No. 3)	×				*Escherichia coli*
			×		*Salmonella typhimurium*
		×			*Staphylococcus aureus*
	×				*Pseudomonas aeruginosa*
Ginger	×				*Escherichia coli*
			×		*Salmonella typhimurium*
		×			*Staphylococcus aureus*
	×				*Pseudomonas aeruginos*

Factors affecting the occurrence of antimicrobial activities are:

Chitosan structure: From the past to present, various inhibitory mechanisms have been proposed for chitosan biopolymer against the growth of microorganisms, which are not accurate and proven description, but the most probable theory is that chitosan is converted to cationic polysaccharides in acidic media, which bonded with the negative groups in the wall of gram positive and negative bacteria. It also interfered with the entry and exit of various compounds into bacterial cells, and destructed microorganisms (Qi et al., 2004; Vasconez et al., 2009; Kong et al., 2010; Zhong et al., 2011; Bonilla et al., 2013; Nouri et al., 2015).

Nanostructure: Nanocapsules always have a greater inhibitory effect on microbial growth than larger particles because nanocapsules have a higher surface to volume ratio, so they have more surfaces for attach to microstructures, impair membrane permeability, and make changes to microbial cells. Antimicrobial chitosan nanocapsules have the same conditions like other antimicrobial nanocapsules (Qi et al., 2004; Kong et al., 2010; Ngo et al., 2011).

Essential oil structure: Different processes have been proposed on the effect of inhibitory properties of essential oils on bacteria. For example, terpene and phenolic compounds damage the cell membrane of microorganisms and destruct it. They also have a detrimental effect on electron transfer disruption, protein and nucleic acid synthesis in the membrane and ultimately lead to inhibition of bacterial activity (Tassou et al., 2000; Gokoglu et al., 2007; Yeh et al., 2014).

Synergistic role: The use of compounds such as essential oils along with compounds that naturally have antimicrobial or antioxidant activities leads to an increase in their desired properties, for example, this role in the essential oils such as monoterpenes, eugenol, carvacrol, thymol and cinnamon have been observed alongside chitosan biopolymers (Zivanovic et al., 2005; Bakkali et al., 2008; Yoksan et al, 2010; Hu et al., 2015). Acetic acid has been used in the production of chitosan solution, and these acidic conditions (pH < 6) can be effective along with the destroying mechanisms.

4. Conclusion

Nowadays, the consumption of synthetic preservatives is associated with a negative outlook, so most manufacturers have turned to the use of natural preservatives. In the present study, encapsulated ginger essential oil in chitosan nanocapsules was used to produce a natural preservative. FTIR peaks have demonstrated the encapsulation of ginger essential oil in chitosan nanocapsules. The results of microbial test indicated that *Pseudomonas aeruginosa* and *Escherichia coli* have the highest susceptible around the encapsulated sample, while *Salmonella typhimurium* has the lowest susceptible. Regarding antioxidant activities, a significant increase of 80 to 97% compared to the control sample was observed. The optimal use of this essential oil is in the form of nanocapsule in industry. The general results of the present study illustrated that encapsulation was an efficient and significant process in improving the antioxidant and antimicrobial activities of ginger essential oil and can be used as a natural preservative in industry. It is suggested to draw more attention to the essential oils encapsulation process of other native plants in the country to minimize the use of chemical preservatives in the food industry.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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