Preparation and Characterization of Essential Oils Chitosan Nanoparticles: Physical, Structural, and Antioxidant Activity

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Abstract

One of the recent trends in food industry is the application of natural antioxidant agents. In this study, cinnamon, clove buds, laurel, basil and lemongrass essential oils have been extracted and encapsulated in chitosan nanoparticles using a two-step technique of emulsion-ionic gelation of chitosan with sodium tripolyphosphate (TPP). The obtained nanoparticles exhibited a regular distribution and spherical shape as observed by scanning electron microscopy (SEM). As determined the encapsulation efficiency (EE) and loading capacity (LC) of tested essential oils loaded chitosan nanoparticles were about (1.78, 35.63, 14.33, 0.97, 2.27)% and (0.94, 19.01, 5.06, 0.66, 1.51)% respectively, with a chitosan/essential oil ratio of 1:1 (w/w). In vitro release studies showed an initial burst effect followed by a slow essential oil release. The active ingredients of tested essential oils were prevented from being volatilized, significantly improving the chemical stability, so the scavenging activity of encapsulated EOs on DPPH radicals was in the range of 0.58%, 1.36%, 0.16%, 0.29% and 0.14% respectively. This technique could improve the efficiency of

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essential oils in food products and a delivery system for novel applications such as active packaging.
Key words: Antioxidant activity, Characterization, Chitosan, Essential oils, Nano-encapsulation.

تحضير وتوصيف جزئيات الكيتوزان النانوية للزيوت العطرية: فيزيائيا"، بنيويا" والنشاط المضاد للأكسدة

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الملخص

نظرا" لاعتبار تطبيق العوامل الطبيعية المضادة للاكسدة أحد أهم توجهات صناعة الأغذية مؤخرا"، تم في هذه الدراسة استخلاص الزيوت العطرية من القرفة، القرنفل، الغار، الريحان وعشبة الليمون بهدف كبسلتها في أغشية الكيتوزان النانومترية باستخدام التقنية ذات خطوتي الاستحلاب والتهلم الأيوني. حيث شوهد شكل الجزئيات النانوية المتشكلة باستخدام المجهر الماسح الالكتروني.

كما سجلت الجزئيات النانوية كفاءة كبسلة للزيوت العطرية المدروسة قدرها 0.97, 2.27)% (1.78, 35.63, 14.33, مع سعة تحميل قدرها (0.94, 19.01, 5.06, 0.66, 1.51)% على الترتيب بنسبة كيتوزان للزيت العطري بالمقدار 1:1 (وزن/وزن). أما دراسة تحرير الكبسولات النانوية للزيوت العطرية المحملة بها فقد أظهرت تحريرا" أوليا" عاليا" متبوعا" بتباطئ لمعدل التحرر مع الزمن. بتطبيق الكبسلة النانومترية منعت المكونات الفعالة للزيوت العطرية المدروسة من التطاير، مما أدى إلى تحسن كبير في استقرارها الكيميائي، وبالتالي نشاطها المضاد للأكسدة وفقا" للقيم (0.58, 1.36, 0.16, 0.29, 0.14)% على الترتيب.

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بحيث يمكن لهذه التقنية أن تحسن من كفاءة الزيوت العطرية في المنتجات الغذائية وبالتالي التوصل لتطبيقات جديدة لها كالتغليف الفعال.
الكلمات المفتاحية: نشاط مضاد للأكسدة، توصيف، كيتوزان، زيوت عطرية، كبسلة نانوية.

Introduction:

Owing to the potential hazards of artificial food additives to human health, natural, functional, and bioactive ingredients have drawn considerable attention because of their proven health benefits (Ranjan *et al*., 2017, 136). Essential oils (EOs) obtained from medicinal and herbal plants are recognized for their antioxidant properties. EOs from different herbs have been utilized as food flavors and medicines, and in the preparation of fragrances, which are volatile and fat soluble (Mathias *et al*., 2015, 85). The compositions of plant EOs are complex and contain high amounts of phenols, acetones, terpenes, alcohols, and aldehydes, which exhibit good antibacterial, antioxidant, and insecticidal properties (Sekhon, 2010, 8). Although the pharmacological effects of EOs have long been known, their application in the food industry is still limited (Gomes *et al*., 2017, 403). Encapsulation is an effective strategy to improve the sensitivity to degradation and promote the stability of the biologically active compounds in EO during the processing and storage stages (Alam *et al*., 2016, 50).

Nano-encapsulation is an emerging technology among the various techniques employed for encapsulation; it is widely employed in the food and nutrition industries. The nano-encapsulation of biologically active compounds has been demonstrated as a reliable and effective approach toward increasing the physical and chemical stabilities of active substances, thereby protecting them from volatilization and harmful environmental conditions, and extending the shelf life of food (Majeed *et al*., 2015, 58455). Additionally, it can improve water solubility, control delivery, facilitate absorption, and reduce toxicity and cost (Lammari *et al*., 2020, 11). Chitosan (CS) is a natural cationic polysaccharide with good biocompatibility, low toxicity, and excellent biodegradability, and is considered an ideal option for food packaging (Silvestre *et al*., 2011, 1775). It has been reported that CS,

as a nanoparticle (NP) carrier, can encapsulate EOs and improve their stability. Moreover, oxidation, as one of the important reactions that result in the corruption of food during processing and storage (Makwana *et al*., 2015, 172), shortens the shelf life of products by causing changes in flavor, texture, and color. Oxidation is not conducive to human health, and many reactive oxygen intermediates can induce a variety of diseases. However, most of the aforementioned studies focused on the antibacterial properties of NPs and their food preservation applications. Research on the structure, physical properties, and oxidation resistance of NPs is still rare. Finally, to the best of our knowledge, the literature on the antioxidant activities of EO encapsulated in CS nanoparticles is especially sparse.

This study aimed to characterize cinnamon, clove buds, laurel, basil and lemongrass CS- NPs prepared through oil in water emulsification and ion gelation techniques with a chitosan/essential oil ratio of 1:1(w/w), which are nontoxic, convenient, and controllable (Ribeiro-Santos *et al*., 2017). The characteristics of the nanocapsules were tested through scanning electron microscopy (SEM), encapsulation efficiency (EE), loading capacity (LC) and In vitro release studies. Furthermore, the oxidation resistance of the CS-NPs was evaluated. This study aims to provide considerable advances in the fields of food and agriculture.

Materials and methods:

Materials:

Low molecular weight chitosan >75% degree of deacetylation was supplied by Sigma-Aldrich (St. Louis, Mo, USA). Sodium tripolyphosphate (TPP), Tween 80, and 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) were purchased from Sigma Aldrich Co. (USA). Folin-Ciocalteu reagents and Gallic acid were supplied by Merck Chemicals Co. (Germany). All other chemicals utilized in this project were of analytical grade.

Preparation of EO-loaded chitosan particles
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EO-loaded chitosan nanoparticles were prepared according to a method modified from the ones described by (Amiri and Morakabati, 2017, 334) (Budama-Kilinc *et al*., 2018, 185). Briefly, aqueous and oil phase solutions were produced. Chitosan solution 1% (w/v) was prepared by agitating chitosan in an aqueous acetic acid solution 1% (v/v) at ambient temperature 25 ◦C overnight. Tween 80 was then added as a surfactant to the solution and stirred at 45 ◦C for 2 h to obtain a homogeneous mixture. Then oil phase is gradually dropped into the aqueous chitosan solution during homogenization to obtain an oil-in-water emulsion. TPP solution 0.4% (w/v) was then added drop wise into the agitated emulsion. Agitation was continuously performed for 40 min. The formed particles were collected by centrifugation at 9000 ×g for 30 min at 4 ◦C, and subsequently washed several times with deionized water (Jamil *et al*., 2016, 5). Finally, ultra-sonication was performed by a sonicator in an ice bath for 4 min resulting in a homogeneous suspension. The suspensions were immediately freeze-dried. Both chitosan nanoparticles and supernatant were stored at 4 ◦C until further analysis (Soto-Chilaca *et al*., 2019, 4062). Weight ratio of chitosan to essential oils (Chitosan: EO) of 1:1 was used for the present study (Mohammadi *et al*., 2015, 208).

Morphology of nanoparticles:

The morphology of the freeze-dried nanoparticles was studied by scanning electron microscopy (SEM). The frozen dried nanoparticles (1 mg) were dispersed in deionized water (20 mL) and sonicated for 4 min. One drop of the dispersion containing chitosan nanoparticles loaded with (cinnamon and clove buds EO) was placed on a glass plate and dried at room temperature and then examined. Representative SEM images were reported (Hadidi *et al*., 2020, 4).

Determination of Encapsulation Efficiency and Loading Capacity:
Encapsulated EOs were determined by UV–Vis spectrophotometry. Predetermined amounts of CS/ EO NPs were dispersed into 10 mL dehydrated ethanol and centrifuged at 12000 rpm for 20 min at 25 °C as reported by (Hosseinia *et al*., 2013, 53). The supernatant was analyzed for the EO content using UV–Vis spectrophotometry at a wavelength of 275 nm, respectively. The amount of EO was estimated by suitable calibration curve of pure EO in ethanol with R^2 of 0.99 for tested EOs. CS NPs was treated similarly and was used as a blank. Triplicate samples for each batch were recorded. Encapsulation Efficiency (EE)% and Loading Capacity (LC)% were estimated from Equations respectively. (EE)% = Total amount of loaded EO / Initial amount of EO * 100 **(1)** (LC)% = Total amount of loaded EO/Weight of NPs after freeze drying * 100 **(2)**

In vitro release studies:
Freeze-dried EO-loaded chitosan nanoparticles 20 mg were placed in a micro tube containing 5 mL of 60% phosphate buffer saline pH 7.4 + 40% ethanol and incubated at ambient temperature under gentle agitation. At specific time intervals, samples were centrifuged at 9000 rpm for 5 min at 25 ◦C; then a specific volume of supernatant was sucked out for analysis, and was replaced with an equivalent volume of fresh media (Keawchaoon and Yoksan, 2011, 168). To calculate the total cumulative amount of EO released loaded chitosan nanoparticles, EO concentration (ppm) in the release medium was measured at sampling time intervals by a UV–vis spectrophotometer at 275 nm and converted to the released amount (µg) considering the volume of the release medium (mL). Cumulative percentage of OEO released was obtained by dividing the cumulative amount of EO released at each sampling time point (Mt) to the initial weight of the EO loaded in the sample (M0)

$$\text{Cumulative release percentage} = \sum_{t=0}^{t} \frac{Mt}{M0} * 100 \qquad \textbf{(3)}$$

Stability of total phenolic contents of EOs in CS NPs

Folin–Ciocalteu method was used to estimate the total phenolic contents (TPC) of pure EO as well as in encapsulated one as reported by (Shetta *et al*., 2019, 736) with slight modifications. A pre-determined amount of pure EOs, CS NPs, encapsulated EOs were mixed with dehydrated ethanol to get a final concentration 30 $mg \cdot mL^{-1}$. 0.5 mL of each sample was shaken with 2.5 mL of (10% v/v) aqueous Folin–Ciocalteu reagent for 4 min. Then, 2 mL aqueous solution of (7.5% w/v) sodium carbonate was dropped to the mixture and left in the dark for 6 h at room temperature. Finally, samples were centrifuged at 10.000 rpm for 3min and the supernatant was removed for measuring the absorbance at 765 nm using a spectrophotometer. To estimate the (TPC), different concentrations of Gallic acid (0.01–0.1 $mg \cdot mL^{-1}$) in ethanol were treated like the samples estimating gallic acid standard calibration curve with R^2 of 0.99. The gallic acid equivalent (GAE) which expresses the amount of gallic acid in mg that is equivalent to 1 g of EO was estimated by the calibration curve.

Determination of the antioxidant activity:

The antioxidant activity of encapsulated EOs were examined using DPPH free radical scavenging assay as illustrated by (Ghahfarokhi *et al*., 2016, 1785) with slight modification. First, predetermined amounts of pure EOs, CS NPs and encapsulated EOs were dispersed in 2 mL of ethanol DPPH solution (0.05mM) and stored in the dark for 2 h at room temperature. The samples containing NPs were centrifuged for 3 min to take the supernatant for analysis. Spectrophotometer at 517 nm was used to measure the absorbance of samples considering dehydrated ethanol as a blank. Gallic acid was used as a reference standard and DPPH solution was used as the control. The DPPH radical-scavenging (%) was calculated using Equation (4).

DPPH scavenging activity% = A control−A sample / A control *100 **(4)**
Where A control is the DPPH absorbance and A sample is the sample absorbance.

Statistical analysis:

Results are reported as the mean ± SD for triplicate measurements. Statistical analysis of the data was carried out via one-way ANOVA followed by Tukey's test to compare the treatment means using MINITAB software (version 14). Statistical significance was expressed at $P < 0.05$.

Results and discussion:

Shape of EOs loaded chitosan nanoparticles:

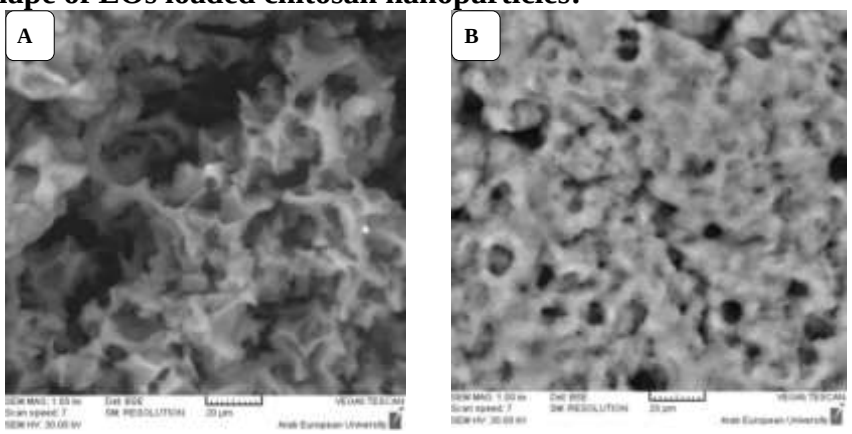

Figure 1. SEM images of (A) Cinnamon oil-loaded NPs (B) Clove oil-loaded NPs.

Toobtain information about the morphology of the cinnamon EO and clove buds EO loaded nanocapsules, scanning electron microscopy (SEM) analysis was performed. SEM analysis was carried out to visualize the shape of the nanocapsules. It can be inferred from (Fig. 1) A and B that the essential oil-loaded nanocapsules have spherical surface morphology (Su *et al*., 2020, 8).

Encapsulation efficiency and loading capacity

The percentage of EE and LC of different formulations were demonstrated in (Table. 1). The amount of loaded EO was determined using UV–vis spectrophotometry from the absorbance at 275 nm. The loading capacity (LC) and encapsulation efficiency (EE) were then calculated using Equations. (1) and (2), respectively, and tabulated in (Table. 1). From UV–vis spectrophotometry results, EE% of EOs ranged from 0.97% to 35.63%**.** However, maximum EE value was obtained for the sample prepared using clove buds EO (35.63%). The decrease of EE for the other samples might be explained due to the saturation of EO loading into chitosan nanoparticles. This finding was in agreement with previous reports (López-Menesesa *et al*., 2018, 600). The LC% of EOs was in the range of 0.66–19.01%, as determined from UV–vis spectrophotometry experiments. However, maximum LC% value was obtained for the sample prepared using clove buds EO (19.01%). This result was in agreement with the findings regarding the loading of carvacrol into chitosan–TPP nanoparticles, which have been reported by (Keawchaoon and Yoksan, 2011).

Table 1. EE% and LC% of tested EOs CS-NPs by UV–vis spectrophotometry.

Chitosan:EO mass ratio 1:1 (w/w)	UV–vis spectrophotometry	
	(EE)%	(LC)%
Cinnamon EO	1.78 ± 0.0057	0.94 ±0.017
Clove EO	35.63 ± 1.2935	19.01±0.6707
Laurel EO	14.33 ± 0.994	5.06 ±0.0341
Basil EO	0.97 ± 0.03	0.66 ±0.0208
Lemon grass EO	2.27 ± 0.0692	1.51 ±0.0169

Results were reported as mean ± SD, n = 3.

In vitro release studies of EOs from CS-NPs

The in vitro release profiles of EOs from the nanoparticles, prepared using a weight ratio of chitosan to EO 1:1 (w/w) were shown in (Fig. 2). The amount of EO released at different times was measured at 275 nm. EO release from nanoparticles takes place by several mechanisms

including surface erosion, disintegration, diffusion and desorption. The in vitro release profile of EOs from chitosan nanoparticles can be described as a two-step biphasic process, i.e., an initial burst release followed by subsequent slower release. The initial burst release was attributed to the EO molecules adsorbed on the surface of the particles and oil entrapped near the surface, as the dissolution rate of the polymer near the surface is high, the amount of EO released will be also high (Ghahfarokhi *et al*., 2017, 16). In the second stage, the release rate was relatively slow, or we could say that the release of EO reached plateau at this stage (Fig. 2). This might be due to the diffusion of the EO dispersed into the polymer matrix as the dominant mechanism. This stage has slower rate and thus resulting in nearly no additional release of EO at this stage. Further release of EO required the swelling and degradation of the compact chitosan–TPP nanoparticles. Hence, the results indicate that the chitosan–TPP nano-system is suitable for controlling the release of EOs.

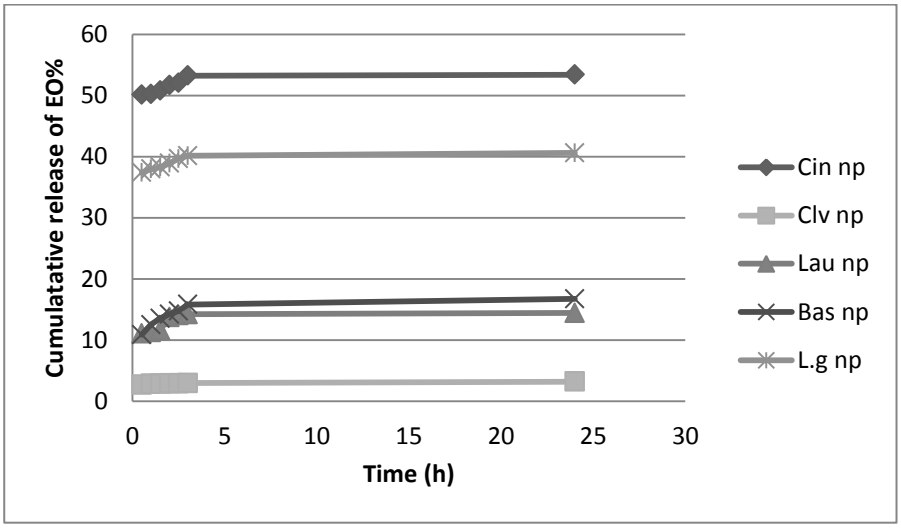

Figure 2. In-vitro release profiles of tested EOs from CS-NPs.

Stability of EOs phenolic contents in CS-NPs

The Folin-Ciocalteau assay is a method for the measurement of phenolic content in products. Folin-Ciocalteau reagent is composed mainly of sodium tungstate (VI) dihydrate and sodium molybdate (VI) dihydrate. The basic mechanism is oxidation with the phenolic group leading to the formation of the molybdenum blue and the tungsten blue that are quantitatively measured by spectrophotometer (Shetta *et al*., 2019). CS NPs and CS/EOs NPs were assayed for their TPC using Folin–Ciocalteu reagent in the range of (0.008, 0.017, 0.008, 0.007 and 0.007) mg GAE/g, respectively (Fig. 3).

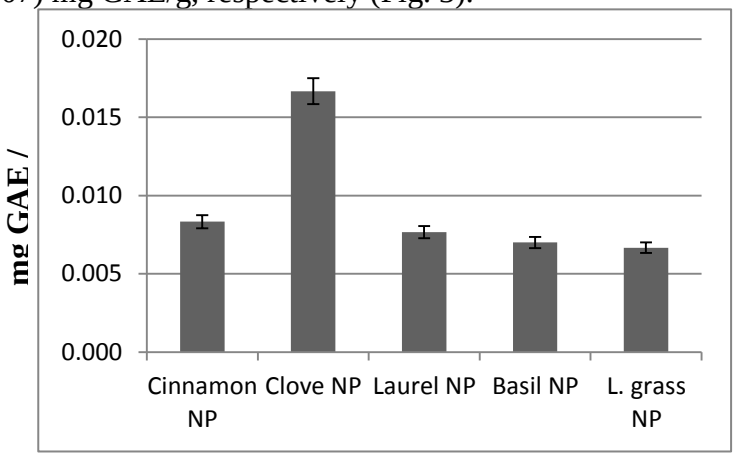

Figure 3. TPC of tested EOs CS-NPs expressed in (mg GAE/g sample).

Evaluation of antioxidant activity

Encapsulation process not only decreases the evaporation rate of volatile components in EOs, but also it enhances the antioxidant activity of these bioactive particles compared to their free forms as a result of protection against the adverse effects including oxygen and temperature (Hasani *et al*., 2020, 40).The DPPH radical scavenging assay is a method for the assessment of antioxidant activity. The mechanism of this method is based on the quenching of the single electron of DPPH by the antioxidant compounds and subsequently, the

solution is decolorized (Sotelo-Boy´as *et al*., 2015, 585). The free radical scavenging activity of pure EOs has been reported by others in the literature which is attributed mainly to the presence of monoterpenes, sesquiterpenes, and their oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols and oxides). The scavenging activity of encapsulated EOs on DPPH radicals was in the range of (0.58, 1.36, 0.16, 0.29 and 0.14)% respectively (Fig. 4).

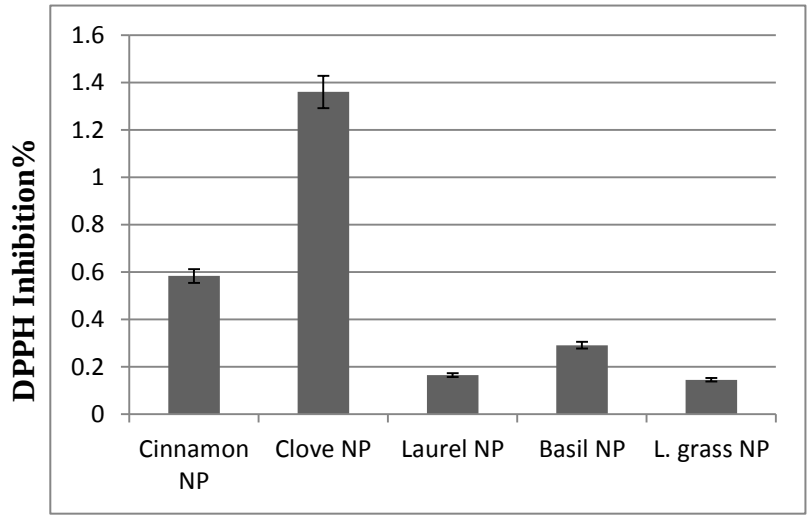

Figure 4. DPPH radical scavenging activity (%) of tested EOs CS-NPs.

Conclusion:

In this study, five EOs namely; Cinnamon, Clove buds, Laurel, Basil and Lemon grass were encapsulated in CS NPs with spherical surface morphology shown by SEM. The EE% of EOs NPs were about (1.78, 1.29, 0.99, 0.03, and 2.27)%, respectively, whereas, the LC% of EOs NPs were about (0.94, 19.01, 5.06, 0.66, and 1.51)%, respectively. Furthermore, in-vitro release studies of both EOs showed an initial burst effect and followed by a slow release at pH 7.4 conditions. Moreover, to study the stability of total phenolic contents (TPC), Folin–Ciocalteu was used and the results (0.008, 0.017, 0.008, 0.007

and 0.007) mg GAE/g respectively showed the ability of CS-TPP system to preserve the TPC of tested EOs NPs. The antioxidant activities of EOs-NPs were in the range of (0.58, 1.36, 0.16, 0.29 and 0.14)% respectively, where clove buds EO NPs assigned the best antioxidant activity because of having the highest TPC of tested EOs NPs. Finally, Based on these results (Ranjan *et al*., 2014, 12), the encapsulated EOs in CS NPs are promising candidates to be used in nutraceuticals, cosmetic and pharmaceutical applications.

References:

1. Alam A, Rizvi I, Sayeed U, Kalim, A. Khan M, Akhtar S, Farooqui A, and Siddiqui M (2016). **Application of nanotechnology in agriculture and food science**, World Journal of Pharmaceutical Sciences, 4: 45-54.
2. Amiri A, and Morakabati N (2017). **Encapsulation of Satureja khuzestanica Essential Oil in Chitosan Nanoparticles with Enhanced Antifungal Activity**, International Journal of Nutrition Food Engineering, 11: 331-336.
3. Budama-Kilinc Y, Cakir-Koc R, and Kaya, Z (2018). **Preparation and Cytotoxicity of Coriandrum sativum L. Oil-Loaded Chitosan Nanoparticles**, JOTCSA, 5: 179-190.
4. Ghahfarokhi M, Barzegar M, Sahari M, Ahmadi Gavlighi H, and Gardini F (2017). **Chitosan-cinnamon essential oil nano-formulation: Application as a novel additive for controlled release and shelf life extension of beef patties**, International Journal of Biological Macromolecules, 16: 1-32.
5. Ghahfarokhi M, Barzegar M, Sahari M, and Azizi, M (2016). **Enhancement of Thermal Stability and Antioxidant Activity of Thyme Essential Oil by Encapsulation in Chitosan Nanoparticles**, J. Agr. Sci. Tech, 18: 1781-1792.
6. Gomes L, Paschoalin V, and De-Aguila E (2017). **Chitosan Nanoparticles: Production, Physicochemical Characteristics and Nutraceutical Applications**, Revista Virtual de Quimica, 9: 387-409.
7. Hadidi M, Pouraminb S, Adinepourc F, Haghanib S, and Jafari S (2020). **Chitosan nanoparticles loaded with clove essential oil: Characterization, antioxidant and antibacterial activities**, Carbohydrate Polymers, 236: 1-8.
8. Hasani S, Ojagh S, Ghorbani M, and Hasani M (2020). **Nano-Encapsulation of Lemon Essential Oil Approach to Reducing the Oxidation Process in Fish Burger during Refrigerated Storage**, Journal of Food Biosciences and Technology, 10: 35-46.

9. Hosseinia S, Zandib M, Rezaeia M, and Farahmandghavic F (2013). **Two-step method for encapsulation of oregano essential oil in chitosan nanoparticles: Preparation, characterization and in vitro release study**, Carbohydrate Polymers, 95: 50– 56.
10. Jamil B, Abbasi R, Abbasi S, Imran M, Khan S, Ihsan A, Javed S, Bokhari H, and Imran M (2016). **Encapsulation of Cardamom Essential Oil in Chitosan Nano-composites: In-vitro Efficacy on Antibiotic-Resistant Bacterial Pathogens and Cytotoxicity Studies**, Frontiers in Microbiology, 7: 1-10.
11. Keawchaoon L, and Yoksan R (2011). **Preparation, characterization and in vitro release study of carvacrol-loaded chitosan nanoparticles**, Colloids and Surfaces B: Biointerfaces, 84: 163–171.
12. Lammari N, Louaer O, Meniai A, and Elaissari A (2020). **Encapsulation of Essential Oils via Nanoprecipitation Process: Overview, Progress, Challenges and Prospects**, Pharmaceutics, 12: 3-21.
13. López-Menesesa A, Plascencia-Jatomeaa M, Lizardi-Mendozad J, Fernández-Quirozb D, Rodríguez-Félixa F, Mouriño-Pérezc R, Cortez-Rochaa M, and Schinus molle L (2018). **Essential oil-loaded chitosan nanoparticles: Preparation, characterization, antifungal and anti-aflatoxigenic properties**, LWT - Food Science and Technology, 96: 597–603.
14. Majeed H, a Bian Y, Ali B, Jamil A, Majeed U, Khan Q, Iqbal K, Shoemakere C, and Fanga Z (2015). **Essential oil encapsulations: uses, procedures, and trends**, RSC Advances, 5: 58449–58463.
15. Makwana S, Choudhary R, and Kohli P (2015). **Advances in Antimicrobial Food Packaging with Nanotechnology and**

Natural Antimicrobials, International Journal of Food Science and Nutrition Engineering, 5: 169-175.
16. Mathias D, Amaral F, and Bhargava K (2015). **Essential Oil Nanoemulsions and Food Applications**, Advances in Food Technology and Nutritional Sciences, 1: 84-87.
17. Mohammadi A, Hashemib M, and Hosseinia S (2015). **Chitosan nanoparticles loaded with Cinnamomum zeylanicum essential oil enhance the shelf life of cucumber during cold storage**, Postharvest Biology and Technology, 110: 203–213.
18. Ranjan S, Dasgupta N, Chakraborty A, Samue S, Ramalingam C, Shanker R, and Kumar A (2014). **Nanoscience and nanotechnologies in food industries: opportunities and research trends**, J Nanopart Res, 16: 1-23.
19. Ranjan S, Dasgupta N, Chakraborty A, Samue S, Ramalingam C, Shanker R, Ribeiro-Santos R, Andrade M, Ramos de Melo N, and Sanches-Silva A (2017). **Use of essential oils in active food packaging: Recent advances and future trends**, Trends Food Sci. Technol, 61: 132-140.
20. Sekhon B (2010). **Food nanotechnology – an overview**, Nanotechnology, Science and Applications, 3: 1-15.
21. Shetta A, Kegere J, and Mamdouh W (2019). **Comparative study of encapsulated peppermint and green tea essential oils in chitosan nanoparticles: Encapsulation, thermal stability, in-vitro release, antioxidant and antibacterial activities**, International Journal of Biological Macromolecules, 126: 731–742.
22. Silvestre C, Duraccio D, and Cimmino S (2011). **Food packaging based on polymer nanomaterials**, Progress in Polymer Science, 36: 1766–1782.
23. Sotelo-Boy´as M, Valverde-Aguilar G, Plascencia-Jatomea M, Correa-Pacheco Z, Jim´enez-Aparicio A, Solorza-Feria J, Barrera-Necha L, and Bautista-Ba˜nos S (2015). **Characterization of Chitosan Nanoparticles Added with Essential Oils. In vitro**

Effect on Pectobacterium carotovorum, Revista Mexicana de Ingeniería Química, 14: 589-599.

24. Soto-Chilaca C, Mejía-Garibay B, Navarro-Amador R, Ramírez-Corona N, Palou E, and López-Malo A (2019). **Cinnamaldehyde-loaded chitosan nanoparticles: characterization and antimicrobial activity**, Biointerface Research in Applied Chemistry, 9: 4060–4065.
25. Su H, Huang C, Liu Y, Kong S, Wang J, Huang H, and Zhang B (2020). **Preparation and Characterization of Cinnamomum Essential Oil–Chitosan Nanocomposites: Physical, Structural, and Antioxidant Activities**, Processes, 8: 1-13.