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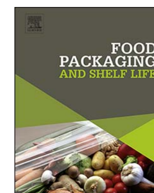

A coating based on clove essential oils encapsulated by chitosan-myristic acid nanogel efficiently enhanced the shelf-life of beef cutlets

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ABSTRACT

In the present study, a coating was investigated through the encapsulation of clove essential oils (CEOs) by chitosan (CS)–Myristic acid (MA) nanogel. The results of radical scavenging activity showed that the antioxidant activity of the CEOs was increased by encapsulation. Subsequently, the antibacterial activity of the coatings, i.e., free CEOs, CS–MA nanogel, and CS–MA nanogel encapsulated CEOs against *Salmonella enteric* Ser. Enteritidis was evaluated on inoculated beef samples. The results obtained showed that the CS–MA nanogel encapsulated CEOs was more effective compared with its free counterpart in controlling Salmonella population on beef under refrigerated storage. More specifically, the CS–MA nanogel encapsulated CEOs at only 2 mg/g beef was found promising in reducing *S. enteric* Ser. Enteritidis while it led to minimal unfavorable impacts on meat color values during prolonged storage. Overall, the findings achieved indicated that the encapsulation could considerably improve the performance of CEOs.

1. Introduction

Today as a result of critical diseases caused by contaminated foodstuffs around the world, there are growing demands for healthier and safer products (Severino et al., 2015). In particular, the survival of microorganisms in foods is of crucial importance, as it can result in decomposition and deterioration of the quality of food products while also posing health threats (Mazzarrino et al., 2015). Among various food-born pathogens, Salmonella is known to be one of the most-widely found types of pathogenic bacteria in beef, eggs, and poultry meat or the products containing these items (Pasquali, Klein, Reich, Manfreda, & Valero, 2016; Vardaka, Yehia, & Savvaidis, 2016).

Physical treatments such as thermal processing are efficiently capable of inactivating pathogenic bacteria in foodstuffs, however, they could also result in deteriorated nutritional and organoleptic properties of the foodstuff treated (de Souza et al., 2015). Therefore, chemical additives have been used for decades to address such contaminations but concerns have also arisen over the safety of these additives and consumers have increasingly demanded for the use of natural products as alternative preservatives in foods (Vardaka et al., 2016). Hence, in recent years there has been a growing interest in the application of non-thermal and non-chemical methods to reduce the pathogens load while the quality of the produce is also preserved. Among these methods, lots of efforts have been put into the industrial applications of essential oils (EOs) as antimicrobial agents (Caner & Yuceer, 2015;Ndoti-Nembe, Vu, Han, Doucet, & Lacroix, 2015). EOs are secondary metabolites of aromatic plants and are normally formed in leaves and stems of these plants. EOs have long been used as flavoring agents in food and drinks while owing to their antimicrobial compounds, they could also be potentially used as natural factors to preserve foods (Bakkali, Averbeck, Averbeck, & Idaomar, 2008;Nerio, Olivero-Verbel, & Stashenko, 2010). Among various EOS, clove (*Syzygium aromaticum*) EO has been shown to possess promising both antibacterial and antioxidant activities (Fu et al., 2007; Matan et al., 2006;Nassan, Mohamed, Abdelhafez, & Ismail, 2015) (Fu et al., 2007; Gülçin, Elmastaş, & Aboul-Enein, 2012; Nassan et al., 2015).

Although EOs are considered as safe (GRAS), their use is often limited by organoleptical criteria (Lambert, Skandamis, Coote, & Nychas, 2001). For this reason, it is necessary to apply the minimum inhibitory concentration (MIC) of a given EOs in order to

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avoid any unfavorable impacts on the quality of foods. In addition to that, EOs are highly volatile and are susceptible to chemical degradation and modification in the presence of ambient oxygen and light (Marques, 2010;Parris, Cooke, & Hicks, 2005). In recent years, to overcome the above-mentioned obstacles associated with the application of EOs, their encapsulation in nanoscale delivery systems has attracted a great deal of attention and has been found to improve the EOs bioactivity through the activation of passive cell absorption mechanisms (Beyki et al., 2014).

Among nanoscale delivery systems, nanogels are considered very promising due to their high loading capacity, high stability, and release properties (Khalili et al., 2015). From the structure point of view, nanogels are three dimensional, cross-linked networks of polymer chain formed *via* covalent linkages or self-assembly processes (Beyki et al., 2014). Among the various biopolymers used for the production of nanogels, chitosan (CS) is one of the most suitable options obtained by alkaline deacetylation of chitin and is composed of glucosamine and N-acetyl glucosamine monomers (El-hamahmy, ElSayed, & Odero, 2017;Khalili et al., 2015). CS nanogels can be prepared by physical or covalent crosslinking. Physical crosslinking is based on ionic gelation method. However, the produced particles by ionic gelation method are not water dispersible at physiological pH. In better words, the stability of these particles is negatively impacted by changes in the pH of the environment as they are formed through electrostatic interactions. Consequently, covalent crosslinking is more favorable than physical crosslinking. The most commonly-used crosslinkers of CS are aldehydes, epoxides, etc. Some of these crosslinkers, such as glutaraldehyde, are toxic (Pujana, Pérez-Álvarez, Iturbe, & Katime, 2012). Thus, using non-toxic alternative crosslinker such as myristic acid (MA) ($C_{14}H_{28}O_2$) is desirable to prepare CS nanoparticles. Several studies have shown the efficacy of modified CS, as matrix for food coatings to preserve food quality against microorganisms (Beyki et al., 2014;Kanatt, Rao, Chawla, & Sharma, 2013; Khalili et al., 2015; Severino et al., 2015).

In spite of all the aforementioned efforts, and to the best our knowledge, there is no report on the simultaneous application of CEOs and nanogel as coating with an aim to increase the shelf life of meat as a high spoilable food. Accordingly, the present study was first set to generate a nanogel through the formation of amid linkages between the existing amino groups of CS, and the carboxyl group of MA. Subsequently, the synthesized CS–MA nanogel was used to encapsulate clove essential oils (CEOs). Subsequently, the antioxidant and antimicrobial activities of both CEOs and CS–MA nanogel were evaluated *in vitro*. Finally, in order to evaluate the potential synergistic effects of both CEOs and CS–MA nanogel in the elimination of *Salmonella enterica* Ser. Enteritidis, an *in vivo* study was conducted in which encapsulated CEOs in CS–MA nanogel was used as a coating in a real food system, i.e., beef.

2. Materials and methods

2.1. Materials

CS was purchased from Sigma (USA). 1-*E*thyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was obtained from Fluka (USA). Acetic acid, Tween 80, glycerol, myristic acid (MA), and ethanol were purchased from Merck (Germany). The EOs of *Syzygium aromaticum* (clove) was purchased from Barij Essence Co. (Iran) (Table 1). Mueller–Hinton agar (MHA), eosin methylene blue lactose sucrose (EMB) agar and nutrient broth (NB) were purchased from Merck (Germany).

2.2. Nanogel formulation and analysis

CS–MA nanogel was prepared through the formation of amide bonds between MA and CS through an EDC-mediated reaction according to the method described by of Khalili et al. (2015).

Table 1
Composition of the clove essential oils (CEOs).

Main Component	Composition (%)
Eugenol	63.4
Caryophyllene	16
Eugenyl acetate	13.1

To verify the formation of CS–MA nanogels, Fourier transformation infrared (FTIR) spectrum at 20 °C and at the range of 500–4000 cm^{-1} was achieved using an FTIR-430 (Jascow, Japan). The size and morphology of the obtained CS–MA nanogel was analyzed using scanning electron microscopy (SEM) on a Philips: XL30 (Netherlands, http://www.philips.com). Samples were prepared as described by Beyki et al. (2014).

2.3. Encapsulating of CEOs in CS–MA nanogel

The encapsulation of CEOs in CS–MA nanogel was carried out according to the method elaborated in our previous study (Zhaveh et al., 2015). Briefly, the CEOs were dissolved in ethanol (1:1, v/v) and mixtures of the nanogels (10000 mg/l) and the CEOs (5000 mg/l), were prepared by sonication (70 kHz) for 5 min. The encapsulation efficiency was measured using a Shimadzu spectrophotometer (Japan, http://www.shimadzu.com), based on the optical density spectra of the essential oils (i.e., 400–650 nm). The spectroscopic readings were performed after precipitating the nanogel by centrifugation (10000 × *g*; 15 min).

2.4. Radical-scavenging activity

2,2-diphenyl-1-picrylhydrazyl(DPPH) radical scavenging activity (RSA) assay was performed by using the method described by Hatamnia et al. (Hatamnia, Abbaspour, & Darvishzadeh, 2014) with some modifications. Briefly, free CEOs, CS–MA nanogel, and CS–MA encapsulated oils were separately added to 3 ml of the methanolic solution containing 0.1 mM of DPPH and were kept under darkness at room temperature. Then, the absorbance value was determined at 515 nm at various time intervals by a spectrophotometer (model Unico UV 2100 made in USA). RSA% was calculated using the following equation (Eq. (1)):

$$RSA\% = \frac{A_0 - A_s}{A_0} \times 100 \quad (1)$$

Where A_0 is the absorbance value of the control reaction and A_s is the absorbance value of the sample. Moreover, EC50 (μg/ml DPPH), representing the effective concentration at which 50% of the DPPH radicals are scavenged, was calculated for free CEOs and CS–MA encapsulated CEOs by interpolation from linear regression analysis.

2.5. Preparation of bacterial inoculum

Salmonella enterica Ser. Enteritidis (ATCC:9270) was provided by the Razi Vaccine and Serum Research Institute (Tehran, Iran) on nutrient agar slants and kept at 4 °C. Working cultures were prepared by transferring a single colony of *S. enterica* Ser. Enteritidis from the nutrient agar to 10 ml of nutrient broth (NB) and the cultures were incubated at 37 °C. The 18 h old culture broth (approximately 10^9 CFU/ml) was washed three times with sterile saline solution (0.85%, w/v). The precipitate of the *S. enterica* Ser. Enteritidis population was resuspended in NB to achieve a suitable population (Akbar & Anal, 2014). Inocula were periodically confirmed by means of plate counts on EMB agar, incubated at 37 °C for 24 h.

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