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Original Article

The effect of carboxymethyl cellulose coating incorporated with clove oil nanoemulsion on quality of shrimp (*Litopenaeus vannamei*) during refrigerated storage

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Abstract

Background: The quality of shrimp, as high perishable seafood, can readily be effected by microbial, chemical, and physical alterations during storage. **Aims:** In this study, carboxymethyl cellulose (CMC) coating either alone or incorporated with variable levels of clove oil nanoemulsion (CNE) (10, 20, and 30 mg/ml) was developed to sustain the quality of shrimp during refrigerated storage. **Methods:** Changes in microbial, chemical, textural, color (L*, a*, b*), and sensory parameters of the studied groups evaluated through distinct experimental approaches. **Results:** Based on our results, the pattern of increase in the aerobic plate count (APC) and psychrotrophic bacterial count (PBC) in different groups were reported as the following order: CMC + CNE < CMC < control. Also, CMC and CNE integration decreased the upward trend of total volatile basic nitrogen (TVB-N), and thiobarbituric acid reactive substances (TBARS) compared to that of the control group during storage at 4°C for 10 days ($P<0.05$). During the storage period, the decrease of the L* value in the control group was greater than the others; whereas, the lowest a* and b* values were observed in this group ($P<0.05$). Besides, the textural and sensory properties of coated shrimp were significantly more acceptable ($P<0.05$). **Conclusion:** Integration of CNE in CMC coating promisingly improves the quality of shrimp during refrigerated storage.

Key words: Carboxymethyl cellulose, Clove, Coating, *Litopenaeus vannamei*, Nanoemulsion

Introduction

Shrimp is one of the most popular seafood. Due to the high content of free amino acids and other soluble non-nitrogen compounds, shrimp is susceptible to both microbial and chemical spoilage during storage (Mastromatteo *et al*., 2010). Therefore, applying preservative methods is a crucial step for extending the shelf life and maintain its quality and safety.

Essential oils (EOs) are plants secondary metabolites with confirmed antioxidant and antimicrobial effects (Burt, 2004). Clove (*Syzygium aromaticum*), an ancient medicinal plant, has natural antimicrobial and antioxidant agents; it encompasses a variety of active compounds (e.g., eugenol, eugenyl acetate) that is attractive for food packaging as generally recognized a safe (GRAS) (Li *et al*., 2006). Eugenol (phenylpropanoid), the active component of clove essential oil (CEO), efficiently inhibits amylase, and protease productions by Gram-positive bacteria as well as enzyme activity in Gram-negative bacteria through interaction with the hydroxyl group (Burt, 2004). The potential of clove oil nanoemulsion (CNE) incorporation into biodegradable films for expanding the shelf life of shrimp and fish has been examined in several studies (Ejaz *et al*., 2017; Dehghani *et al*., 2018).

Despite their potentials for usage in the food industry, EOs have some limitations such as low water solubility, and high volatility. To overcome these limitations, EOs have been incorporated into a variety of biopolymer-based films and coatings (Moradi *et al*., 2011). Edible films and coatings with biodegradability and environment-friendly properties have recently received much attention. There is a wide range of edible coatings among which linear, non-toxic, water-soluble, and anionic polysaccharide derivative of cellulose, carboxymethyl cellulose (CMC), has broad applications in food and pharmaceutical industries (Tongdeesoontorn *et al*., 2011). Considering the aforementioned features, CMC is a promising candidate for fabric usage in the food industry.

Essential oils emulsification improves their water-solubility, stability, and biological activities. Besides, the emulsified EOs particle size has a great impact on their functional properties (Acevedo-Fani *et al*., 2015).

Nanoemulsions are a sub-group of emulsions ranging from 20 to 200 nm (Shah *et al*., 2010) that commonly are used as delivery systems for bioactive lipids, drugs, antioxidants, and antimicrobial agents (Joe *et al*., 2012). Owning a more specific surface area, nanoemulsions

have more biological properties and greater antimicrobial activities (McClements and Rao, 2011).

There is limited information on the antimicrobial and antioxidant activities of nanoemulsions on the quality of shrimp. Therefore, the purpose of this study was to investigate the effect of CMC based coatings incorporated with CNE on the quality of white leg shrimp (*Litopeneous vannamei*) during refrigerated storage.

Materials and Methods

CEO analysis

Pure CEO provided from a local company (Nourhan, Shiraz, Iran) and analyzed by Beifen 3420A gas chromatograph (Beijing Beifen-Ruili Analytical Instrument Co., Ltd., Beijing, China) according to the method described by Dehghani *et al*. (2018).

CNE Preparation

Briefly, 6 ml of CEO was mixed with 4.5 ml of tween80 (Sigma_Aldrich, Germany) as a surfactant and 4.5 ml of ethanol 96% (Sigma_Aldrich, Germany) as a co-surfactant. The mixture, then, was kept in a closed bottle for 1 h at 86°C. Finally, the volume was adjusted to 100 ml with distilled water. This emulsion was then sonicated by ultrasound (TOMY UD-201, Japan) at a power of 200 W and a 20 kHz frequency for 15 min continuously (Hamouda *et al*., 1999; Joe *et al*., 2012). The droplet size was measured every 2 days up to 10 days using dynamic light scattering (DLS) instrument (W3325, Microtrac, USA) at 25°C and scattering angle of 90°.

To assess the stability of CNE emulsion, it was centrifuged at 3400 g and 25°C for 30 min every 2 days up to 10 days and checked visually for the emulsion breakdown (Gahruie *et al*., 2017; Ozogul *et al*., 2017).

CMC and CMC-CNE preparation

To make the CMC gel, CMC powder was (Wealthy, China) gradually added to deionized water (1% W/V) while agitating the solution by magnetic stirring (Wisemix, Germany) at 80°C for about 45 min to achieve a clear solution.

For a generation of CMC gel incorporated with CNE, the different concentrations of the CNE were mixed with CMC gel (W/V) and then homogenized at 20,000 rpm for 5 min (Homogenizer, DI18B, Germany).

Treatment groups

Shrimps with a sample size of 50-60 shrimps/kg were immediately collected after the catch from a shrimp farm (Bandar-Abbas, Iran) and transported to the laboratory in less than 6 h on the crushed ice. They were randomly divided into five groups, including control (uncoated group), CMC group (coated with CMC), and three groups CNE10 (coated with CMC + 10 mg/ml CNE), CNE20 (coated with CMC + 20 mg/ml CNE), and CNE30 (coated with CMC + 30 mg/ml CNE). Each group consisted of three subgroups, and each subgroup contained 50 shrimps. They were dipped in the gels for 15 min (shrimp/gel ratio of 1:1 (w/v) at 4°C) except for the control group, dipped in the deionized water. They were then drained for 1 min and packed in polyethylene bags and stored at 4°C ± 1 for 10 days. Sampling was carried out every 2 days of storage for further analysis.

Microbiological analysis

Three shrimps were assessed from each subgroup. They, at first, were completely minced in sterile conditions and homogenized in normal saline in decimal dilutions. Second, to enumerate the bacteria, aerobic plate count (APC) and psychrotrophic bacterial count (PBC) were done using plate count agar (Merck, Germany), and the plates were incubated for 2 days at 37°C and 10 days at 7°C, respectively.

Chemical analysis

Chemical analyses were performed on the minced shrimps. pH was measured using a digital pH meter (CG824, Germany) according to López-Caballero *et al*. (2007).

Total volatile basic nitrogen (TVB-N) content was determined in the minced samples by the steam distillation as described by Abbasvali *et al*. (2016).

The thiobarbituric acid reactive substances (TBARS) value was determined according to Benjakul and Bauer method (2001). TBARS value expressed as mg malondialdehyde per kg of samples based on a standard curve of MDA (0-2 mg/kg).

Texture profiles analysis

A texture analyzer (Brookfield, USA) was used for evaluation of shrimp's hardness (g), by applying force on the 2nd segment of shrimp (after peeling) with a cylindrical plunger of 0.4 cm diameter and bar probe of 0.1 P and a speed of 60 mm/min to a depth of 70% deformation.

Color properties

A box (50 × 50 × 60 cm) with interior white color equipped with a light source (20-W fluorescent light lamp, Natural Daylight, Cixing, Zhenjiang, China) was used to evaluate the color parameters of shrimps according to Hunter L*, a*, b* system (L*: brightness, a*: redness-greenness, and b*: yellowness-blueness) (Yam and Papadakis, 2004). Briefly, simple digital images were taken with a Sony color digital camera (DCR-SR65E/SR85E, Tokyo, Japan) that located at a 30 cm constant distance from the surface of the samples in the box, and finally, all surfaces of digital images were selected and analyzed in the Lab mode by using the Photoshop version 8.0.

Sensory and melanosis evaluations

The sensory characteristics were evaluated through visual inspection by twelve trained panelists in the same conditions every 2 days. Melanosis, odor, color, and the overall acceptability were scored using a 4-point descriptive scale from 1 (spots over the entire shrimp), 2

(spots on the carapace), 3 (few small spots on the carapace), 4 (substantially without black spots) for the degree of melanosis, and 1 (dislike very much) to 4 (like very much) for other sensory characteristics.

Statistical analysis

All experiments were performed in three independent samples and analyzed by one-way ANOVA using SPSS software version 25. Significant differences were defined as $P<0.05$. Kruskal-Wallis test used for non-parametric data. Also, for means comparison and comparison between treatments, Duncan's multiple range tests and Pearson correlation test were applied, respectively.

Results

CEO composition

According to GC-MS analysis, allyl-6-3 methoxyphol (48.75%), eugenol acetate (21.6%), and eugenol (11.61%) were the main compounds of CEO (Table 1).

Table 1: Chemical composition of clove essential oil (CEO)

Compound	%
Allyl-6-3 methoxyphol	48.75
Eugenol acetate	21.6
Eugenol	11.61
Trans-Caryophyllene-4,7,10	11.44
Cycloundecatriene,1,1,4,8-tetramethyl	1.41
Caryophyllene oxide	1
Trans-anethole	0.36
Alpha-Copaene	0.3
Alpha-Cubebene	0.13

Droplet size and stability of CNE

The mean droplet size of clove emulsion was 1.67 ± 0.43 µm and after sonication reached 59.8 ± 2.26 nm. During 10 days of storage, the mean particle size of CNE increased and reached 183.7 ± 9.47 nm and also no phase separation (creaming) was observed after centrifugation.

Microbiological analysis

Figure 1 presents the APC and PBC changes in the different groups during storage. Overall, all groups showed a rising trend in APC during the storage period among them the control group had a quicker pattern (8.00 ± 0.01) compared to other groups ($P<0.05$). By increasing the concentration of the CNE, the APC significantly decreased ($P<0.05$); CNE30 had the lowest APC (2.95 ± 0.12) ($P<0.05$). The rising pattern of APC for the other groups was obtained as follows: CNE20 < CNE10 < CMC < control (Fig. 1A). The lowest PBC (3.01 ± 0.06) was resulted for CNE30, as well (Fig. 1B, $P<0.05$).

Chemical analysis

On day 0, pH values were not significantly changed among the experimental groups ($P>0.05$) (Fig. 2A). On the 10th day of storage, the lowest pH was obtained in the CNE30 group (7.72 ± 0.02) ($P<0.05$), and the highest pH observed in the CNE10 (7.82 ± 0.01) and control (7.85 ± 0.02) groups ($P<0.05$).

The TVB-N content were increased over the storage period in all groups (Fig. 2B) with the greatest increase in the control (70 ± 0.46) ($P<0.05$). Intriguingly, TVB-N productions were significantly reduced in CNE30, CNE20, and CNE10 groups ($P<0.05$).

The effects of CMC and different CNE concentrations on TBARS value of shrimp are presents in Fig. 2C. TBARS contents of coated groups were significantly lower than the control (1.17 ± 0.01) ($P<0.05$). There was no significant difference between CNE30 (0.01 ± 0.00) and CNE20 (0.03 ± 0.00) in TBARS formation ($P>0.05$).

Texture profile analysis

The texture hardness of the control and CMC groups was lower than other groups from day 2 up to the end of storage ($P<0.05$). Conversely, the hardness of shrimps treated by CNE10 (613.33 ± 41.12), CNE20 (681 ± 21.07), and CNE30 (759.5 ± 33.03) was markedly improved at the 10th day of storage, respectively (Fig. 2D).

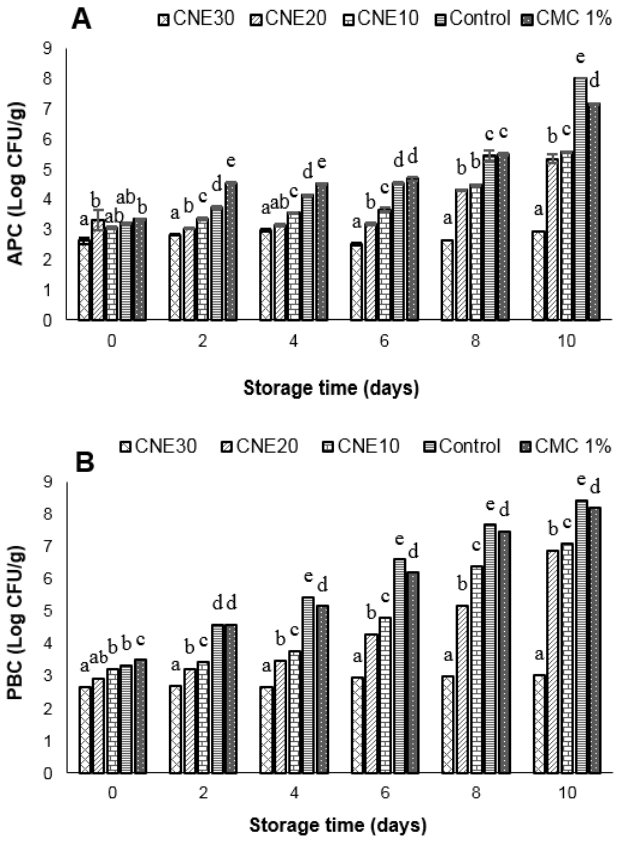

Fig. 1: Mean ± SD of aerobic plate count (APC) (**A**) and psychrotrophic bacterial count (PBC) (**B**) of shrimps treated with carboxymethyl cellulose (CMC) coating incorporated with clove oil nanoemulsion (CNE) at three levels during 10 days of refrigerated storage. Control: Non-coated, CMC 1%: Carboxymethyl cellulose 1%, CNE30: CMC 1% + 30 mg/ml CNE, CNE20: CMC 1% + 20 mg/ml CNE, and CNE10: CMC 1% + 10 mg/ml CNE. In each sampling day, different small letters indicate significant ($P<0.05$) differences

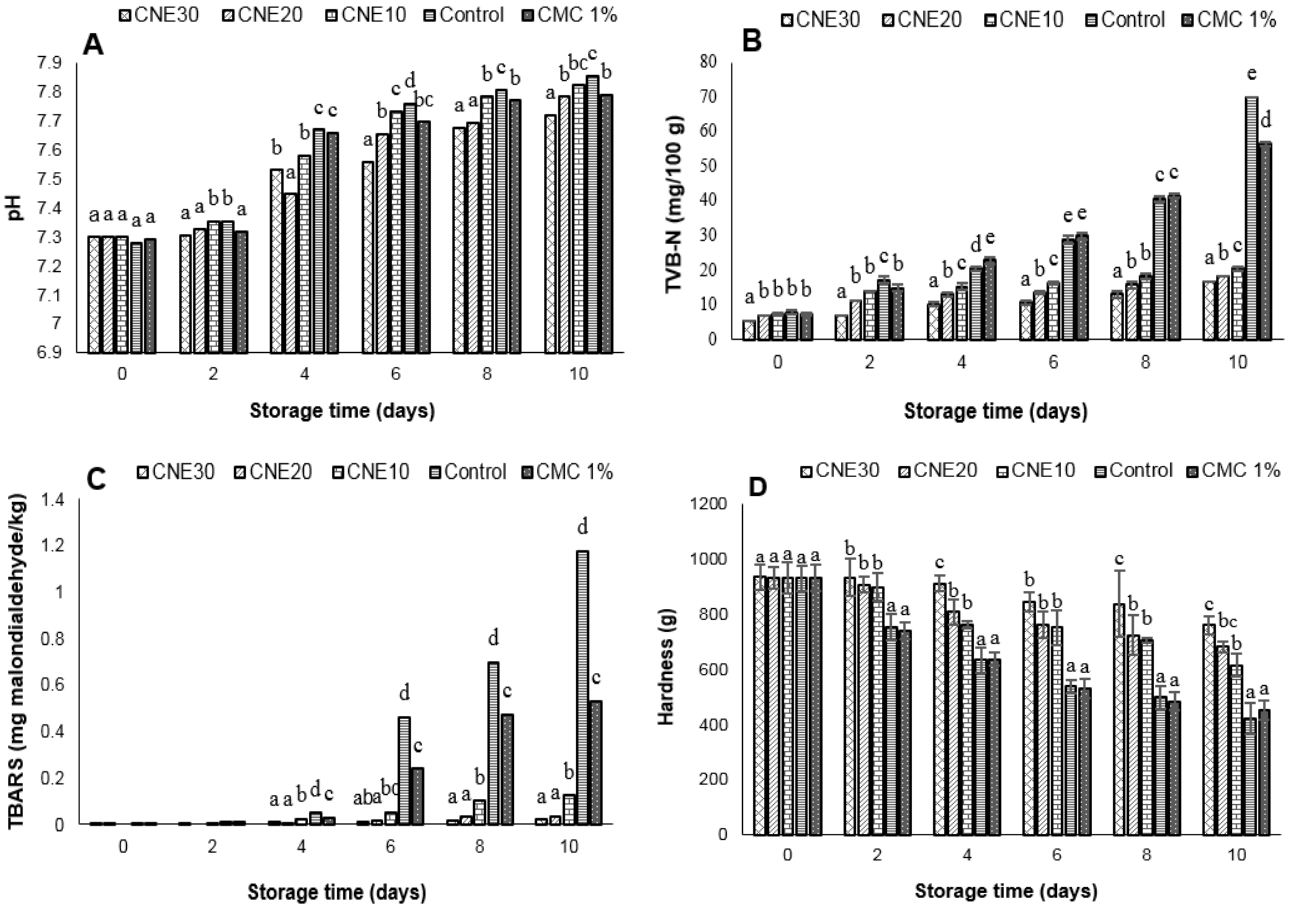

Fig. 2: Mean ± SD of pH (**A**), total volatile base nitrogen (TVB-N) (**B**), thiobarbituric acid reactive substances (TBARS) (**C**), and hardness (**D**) of shrimps treated with CMC coating incorporated with clove oil nanoemulsion (CNE) at three levels during 10 days of refrigerated storage. Control: Non-coated, CMC 1%: Carboxymethyl cellulose 1%, CNE30: CMC 1% + 30 mg/ml CNE, CNE20: CMC 1% + 20 mg/ml CNE, and CNE10: CMC 1% + 10 mg/ml CNE. In each sampling day, different small letters indicate significant (P<0.05) differences

Table 2: The effect of CMC coating incorporated with CNE at three levels on color properties (L*a*b*) of shrimps during 10 days of refrigerated storage

Color properties	Days of storage	Treatments				
		Control	CMC	CNE10	CNE20	CNE30
L*	0	56.33 ± 1.15^{a}	56.00 ± 1.73^{a}	53.67 ± 1.53^{a}	53.33 ± 2.52^{a}	56.33 ± 0.58^{a}
	2	44.33 ± 1.53^{a}	53.33 ± 0.58^{c}	50.00 ± 1.00^{b}	52.00 ± 1.00^{c}	59.33 ± 0.58^{d}
	4	42.00 ± 2.65^{a}	51.67 ± 1.53^{b}	49.67 ± 1.53^{b}	50.67 ± 1.53^{b}	56.67 ± 1.15^{c}
	6	42.00 ± 1.00^{a}	50.67 ± 0.58^{b}	49.33 ± 3.79^{b}	50.33 ± 2.52^{b}	52.00 ± 4.36^{b}
	8	40.67 ± 1.15^{a}	50.00 ± 3.61^{b}	48.67 ± 3.06^{b}	50.33 ± 1.15^{b}	50.67 ± 2.52^{b}
	10	33.67 ± 3.51^{a}	50.00 ± 1.00^{b}	46.67 ± 2.08^{b}	49.33 ± 1.53^{b}	50.00 ± 0.00^{b}
a*	0	3.00 ± 0.00^{a}	3.33 ± 0.58^{a}	3.33 ± 0.58^{a}	3.33 ± 1.15^{a}	3.00 ± 1.00^{a}
	2	2.33 ± 0.58^{a}	3.00 ± 0.00^{ab}	4.00 ± 0.00^{b}	3.33 ± 1.53^{ab}	3.67 ± 0.58^{ab}
	4	2.33 ± 0.58^{a}	3.00 ± 0.00^{ab}	4.33 ± 0.58^{b}	3.33 ± 1.53^{ab}	3.67 ± 0.58^{ab}
	6	2.33 ± 0.58^{a}	3.33 ± 0.58^{a}	3.67 ± 1.15^{a}	4.33 ± 2.08^{a}	4.33 ± 0.58^{a}
	8	2.00 ± 1.00^{a}	3.33 ± 0.58^{bc}	4.67 ± 0.58^{d}	4.67 ± 0.58^{d}	4.33 ± 0.58^{cd}
	10	2.67 ± 0.58^{a}	3.67 ± 0.58^{b}	4.67 ± 0.58^{b}	4.33 ± 0.58^{b}	4.00 ± 0.00^{b}
b*	0	14.00 ± 0.00^{a}	14.33 ± 1.53^{a}	14.00 ± 1.00^{a}	14.00 ± 1.00^{a}	14.33 ± 2.08^{a}
	2	14.00 ± 0.58^{a}	14.33 ± 1.73^{b}	14.67 ± 0.58^{b}	14.67 ± 0.58^{b}	14.33 ± 0.58^{b}
	4	10.67 ± 2.08^{a}	12.33 ± 1.15^{ab}	14.33 ± 1.53^{b}	14.67 ± 1.53^{b}	13.33 ± 0.58^{b}
	6	10.33 ± 1.15^{a}	14.33 ± 1.15^{b}	14.67 ± 0.58^{b}	14.67 ± 1.53^{b}	14.67 ± 2.08^{b}
	8	9.33 ± 1.15^{a}	14.33 ± 0.58^{b}	15.00 ± 0.58^{b}	14.33 ± 1.53^{b}	14.33 ± 2.08^{b}
	10	7.67 ± 3.06^{a}	14.33 ± 2.08^{b}	15.00 ± 1.00^{b}	14.67 ± 0.58^{b}	14.33 ± 0.58^{b}

Data are given as mean values ± standard deviation (n=3). L*: Brightness, a*: Redness-greenness, b*: Yellowness-blueness, CMC: Carboxymethyl cellulose 1%, CNE10: CMC 1% with 10 mg/ml CNE, CNE20: CMC 1% with 20 mg/ml CNE, and CNE30: CMC 1% with 30 mg/ml CNE. Different letters ($^{a, b, c, d}$) within a row indicate significant differences (P<0.05)

Table 3: The effect of CMC coating incorporated with CNE at three levels on the sensory attributes of shrimps during 10 days of refrigerated storage

Sensory attributes	Days of storage	Treatment groups				
		Control	CMC	CNE10	CNE20	CNE30
Odor	0	4.0 ± 0.00[a]	4.0 ± 0.00[a]	4.0 ± 0.00[a]	4.0 ± 0.00[a]	4.0 ± 0.00[a]
	2	3.2 ± 0.25[b]	3.4 ± 0.51[b]	4.0 ± 0.00[a]	3.8 ± 0.52[a]	4.0 ± 0.00[a]
	4	2.3 ± 0.52[b]	2.5 ± 0.58[b]	3.5 ± 0.71[a]	3.5 ± 0.60[a]	3.8 ± 0.25[a]
	6	2.1 ± 0.67[b]	2.1 ± 0.45[b]	3.2 ± 0.60[a]	3.2 ± 0.58[a]	3.4 ± 0.52[a]
	8	1.0 ± 0.00[b]	1.2 ± 0.94[b]	3.1 ± 0.71[a]	3.0 ± 0.60[a]	3.3 ± 0.49[a]
	10	1.0 ± 0.00[b]	1.0 ± 0.00[b]	3.0 ± 0.52[a]	3.2 ± 0.84[a]	3.2 ± 0.58[a]
Melanosis	0	4.0 ± 0.00[a]	4.0 ± 0.00[a]	4.0 ± 0.00[a]	4.0 ± 0.00[a]	4.0 ± 0.00[a]
	2	3.3 ± 0.75[b]	4.0 ± 0.00[a]	4.0 ± 0.00[a]	4.0 ± 0.00[a]	4.0 ± 0.00[a]
	4	2.5 ± 0.87[b]	4.0 ± 0.00[a]	4.0 ± 0.00[a]	3.8 ± 0.58[a]	4.0 ± 0.00[a]
	6	2.1 ± 0.60[b]	4.0 ± 0.00[a]	4.0 ± 0.00[a]	4.0 ± 0.00[a]	4.0 ± 0.00[a]
	8	1.0 ± 0.00[b]	3.7 ± 0.68[a]	3.3 ± 0.75[a]	3.3 ± 0.87[a]	3.5 ± 0.71[a]
	10	1.0 ± 0.00[b]	3.3 ± 0.58[a]	3.3 ± 0.87[a]	3.2 ± 0. 58[a]	3.3 ± 0.49[a]
Overall acceptability	0	4.0 ± 0.00[a]	4.0 ± 0.00[a]	4.0 ± 0.00[a]	4.0 ± 0.00[a]	4.0 ± 0.00[a]
	2	3.2 ± 0.58[b]	3.2 ± 0.68[b]	4.0 ± 0.00[a]	4.0 ± 0.00[a]	4.0 ± 0.00[a]
	4	2.4 ± 0.94[b]	2.3 ± 0.78[b]	3.5 ± 0.71[a]	3.5 ± 0.25[a]	3.7 ± 0.78[a]
	6	2.2 ± 0.60[b]	2.1 ± 0.60[b]	3.3 ± 0.49[a]	3.4 ± 0.52[a]	3.5 ± 0.61[a]
	8	1.0 ± 0.00[b]	1.0 ± 0.00[b]	3.0 ± 0.45[a]	3.2 ± 0.58[a]	3.3 ± 0.58[a]
	10	1.0 ± 0.00[b]	1.0 ± 0.00[b]	3.0 ± 0.60[a]	3.1 ± 0.67[a]	3.0 ± 0.45[a]

Data are given as mean values ± standard deviation (n=3). CMC: Carboxymethyl cellulose 1%; CNE10: CMC 1% with 10 mg/ml CNE, CNE20: CMC 1% with 20 mg/ml CNE, CNE30: CMC 1% with 30 mg/ml CNE. Different letters ([a, b]) within a row indicate significant differences (P<0.05)

Color properties

Table 2 represents the color changes (L*, a*, b*) values in different experimental groups. The L* value (brightness) declined in all groups at the end of storage; the control group showed the lowest L* value (33.67 ± 3.51) (P<0.05). There was no significant difference in L* values between CMC (50.00 ± 1.00), CNE10 (46.67 ± 2.08), CNE20 (49.33 ± 1.53), and CNE30 (50.00 ± 0.00) (P>0.05). For all groups, the initial a* and b* values were around 3.33 and 14.33, respectively. There was no significant difference in a* and b* values between CMC, CNE10, CNE20, and CNE30, on the 10th day of storage (P>0.05). Surprisingly, the lowest a* (2.67 ± 0.58) and b* (7.67 ± 3.06) values observed in the control group (P<0.05).

Sensory and melanosis analysis

Sensory and melanosis analysis of different experimental groups is summarized in Table 3. At the end of the storage, the control group had the lowest score in color and melanosis (P<0.05), however, there was no significant difference in color and melanosis scores between other groups (P>0.05). In terms of odor and overall acceptability, lower scores and consequently lower acceptability were observed in control and CMC groups from the 2nd day (P<0.05).

Discussion

In the present study, we have evaluated the effect of CMC coating incorporated with CNE on the quality of shrimp (*L. vannamei*) during refrigerated storage.

The mean particle size of CNE after sonication was 59.8 ± 2.26 nm. Various parameters including sonication conditions and oil concentration, affect the particle size of nanoemulsions. Different publications reported varied mean droplet diameters (range of 8.69-671 nm) for CNE (Li and Chiang, 2012; Shahavi *et al*., 2016; Zhang *et al*., 2017). Also, clove oil nanoemulsions were adequately stable during the storage time. CNE particle size remained below 200 nm until the 10th day of storage, and also no phase separation (creaming) was observed after centrifugation.

The minimum APC and PBC were reported in shrimps treated with CMC incorporated with CNE. Eugenol has been suggested to act as an antimicrobial agent by disturbing the production of an essential enzyme and damaging the cell wall of bacteria, resulting in the leakage of lipid and protein components (Helander *et al*., 1995). Similar to previous studies (Dehghani *et al*., 2018), our GC-MS data showed that eugenol and eugenol acetate as the main compounds of CEO. Various reports have indicated the antimicrobial effect of plant extracts and EOs. Dehghani *et al*. (2018) reported that the application of Farsi gum-based coating containing clove and Shirazi thyme EOs emulsion in fish fillets stored at refrigerator temperature could reduce the APC, PBC, and lactic acid bacterial population in comparison with the control group. Also, Zhang *et al*. (2017) suggested cloves/cinnamon EOs nanoemulsion for their appropriate antibacterial activity against *E. coli*, *B. subtilis*, *S. typhimurium*, and *S. aureus* strains. According to our results, CNEs, with active compounds and more specific surface areas, have great antimicrobial activities to inhibit the growth of APC and PBC during the storage period.

On the 10th day of storage, the highest pH values

were indicated in CNE10 and the control groups (P<0.05). Based on previous studies, an increase in pH has been associated with the microbial growth and accumulation of bases (López-Caballero *et al*., 2007). However, despite the lower microbial count of CNE10 compared to the control group, this group had the highest pH among all samples. Similar results were reported by Huang *et al*. (2012) and Basiri *et al*. (2015) suggesting pH as an unreliable indicator for shrimp's quality.

TVB-N content increased over the storage period in all groups with the greatest increase in the control (P<0.05). TVB-N is related to the ammonia production (primary, secondary, and tertiary amines) following the activity of spoilage bacteria and endogenous enzymes (Huang *et al*., 2012). Significant positive correlations between TVB-N content, APC (r=0.88, P=0.00), and PBC (r=0.84, P=0.00) could confirm preceding results. Therefore, the sluggish rising of the TVB-N production in the groups treated with CNEs validates the CNE capability of lowering the bacterial count.

Oxygen plays a main role in TBARS, a parameter determining the final concentration of lipid oxidation in foods. Our result showed that samples coated with CMC either alone or incorporated with CNE had lower TBARS values in comparison with the control (P<0.05). Acting as an oxygen barrier, CMC can protect against lipid oxidation (Varela and Fiszman, 2011). Also, exponentially lower TBARS value in groups treated with CNE could be attributed to the antioxidants eugenol and other phenolic compounds of CNE. Generally, EOs manifest their antioxidants activity by curbing the radical chain formation, reacting with free radicals, and breaking down the peroxides (Jouki *et al*., 2014).

Hardness value is one of the main sensory parameters, considerably affecting consumer acceptability. In this study, the hardness of control and CMC groups was lower than other groups (P<0.05). The hardness is decreased by the proteolytic activity of endogenous or microbial proteases and collagenase (Diaz-Tenorio *et al*., 2007). So, CMC and control groups with greater microbial counts had the lowest hardness. A negative correlation between the APC and hardness of different groups (r=-0.77, P=0.00) confirms the previous results.

In terms of color property assessment, polyphenol oxidase reacts with phenols and finally forms insoluble black pigments, melanosis, that reduced the L* values. This reduction was faster in the control group compared to the coated groups. Generally, coating with CMC either alone or in combination with CNE prevented the polyphenol oxidase activity or improved the L* value presumably by acting as an oxygen barrier (Varela and Fiszman, 2011). Also, the lowest a* and b* values observed in the control group on the 10th day of storage might be explained by the appearance of noticeable black spots on the surface of shrimps, consequently decreasing the lightness. The application of CMC coating could slow down the incremental increase in the values of a* and b*, in the shrimps. Likewise, Dehghani *et al*. (2018) reported that changes in the total color difference in rainbow trout fillets coated by Farsi gum incorporated with clove and thyme emulsions were lower than non-coated ones. To recap, coating with CMC either alone or incorporated with CNEs could contribute to the color values throughout the storage period.

The results of the sensory evaluation were in good agreement with those of microbial analyses. Generally, higher sensorial scores is explained by CNE and CMC properties such as antioxidant and antimicrobial characteristics, and an oxygen barrier in comparison with the control group (Simpson *et al*., 1997; Varela and Fiszman, 2011; Dashipour *et al*., 2015).

To sum up, according to the lower APC, PBC, TVBN, and higher hardness (factors which influenced by microbial growth) in shrimps treated with CNE, the nano-emulsification of CEO efficiently enhance its antimicrobial activity. The incorporation of CNE and CMC could retard the chemical reactions and improve sensory changes in coated shrimps. The higher quality of shrimps treated with 30 mg/ml CNE is due to the high contents of eugenol and other phenolic compounds. Therefore, this study advocates that CMC coating activated by CNEs can be used as a natural bio-preservative for shrimps to progress their quality and shelf life during cold storage.

References

Abbasvali, M; Ranaei, A; Shekarforoush, SS and Moshtaghi, H (2016). The effects of aqueous and alcoholic saffron (*Crocus sativus*) tepal extracts on quality and shelf-life of pacific white shrimp (*Litopeneous vannamei*) during iced storage. J. Food Qual., 39: 732-742.

Acevedo-Fani, A; Salvia-Trujillo, L; Rojas-Graü, MA and Martín-Belloso, O (2015). Edible films from essential-oil-loaded nanoemulsions: Physicochemical characterization and antimicrobial properties. Food Hydrocoll., 47: 168-177.

Basiri, S; Shekarforoush, SS; Aminlari, M and Akbari, S (2015). The effect of pomegranate peel extract (PPE) on the polyphenol oxidase (PPO) and quality of Pacific white shrimp (*Litopenaeus vannamei*) during refrigerated storage. LWT-Food. Sci. Technol., 60: 1025-1033.

Benjakul, S and Bauer, F (2001). Biochemical and physicochemical changes in catfish (*Silurus glanis Linne*) muscle as influenced by different freeze-thaw cycles. Food Chem., 72: 207-217.

Burt, S (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. Int. J. Food. Microbiol., 94: 223-253.

Dashipour, A; Razavilar, V; Hosseini, H; Shojaee-Aliabadi, S; German, JB; Ghanati, K; Khakpour, M and Khaksar, R (2015). Antioxidant and antimicrobial carboxymethyl cellulose films containing *Zataria multiflora* essential oil. Int. J. Biol. Macromol., 72: 606-613.

Dehghani, P; Hosseini, SMH; Golmakani, MT; Majdinasab, M and Esteghlal, S (2018). Shelf-life extension of refrigerated rainbow trout fillets using total Farsi gum-based coatings containing clove and thyme essential oils emulsions. Food. Hydrocoll., 77: 677-688.

Diaz-Tenorio, LM; Garcia-Carreno, FL and Pacheco-Aguilar, R (2007). Comparison of freezing and thawing treatments on muscle properties of whiteleg shrimp

(*Litopenaeus vannamei*). J. Food Biochem., 31: 563-576.

Ejaz, M; Arfat, YA; Mulla, M and Ahmed, J (2018). Zinc oxide nanorods/clove essential oil incorporated Type B gelatin composite films and its applicability for shrimp packaging. Food Packag. Shelf Life. 15: 113-121.

Gahruie, HH; Ziaee, E; Eskandari, MH and Hosseini, SMH (2017). Characterization of basil seed gum-based edible films incorporated with *Zataria multiflora* essential oil nanoemulsion. Carbohydr. Polym., 166: 93-103.

Hamouda, T; Hayes, MM; Cao, ZH; Johnson, K; Wright, DC and Baker Jr, JR (1999). A novel surfactant nanoemulsion with broad-spectrum sporicidal activity against *Bacillus* species. J. Infect. Dis., 180: 1939-1949.

Helander, IM; Alakomi, HL; Latva-Kala, K; Mattila-Sandholm, T; Pol, I; Smid, EJ and von Wright, A (1998). Characterization of the action of selected essential oil components on Gram-negative bacteria. J. Agr. Food Chem., 46: 3590-3595.

Huang, J; Chen, Q; Qiu, M and Li, S (2012). Chitosan-based edible coatings for quality preservation of postharvest whiteleg shrimp (*Litopenaeus vannamei*). J. Food Sci., 77: C491-C496.

Joe, MM; Chauhan, PS; Bradeeba, K; Shagol, C; Sivakumaar, PK and Sa, T (2012). Influence of sunflower oil based nanoemulsion (AUSN-4) on the shelf life and quality of Indo-Pacific king mackerel (*Scomberomorus guttatus*) steaks stored at 20 °C. Food Control. 23: 564-570.

Jouki, M; Yazdi, FT; Mortazavi, SA; Koocheki, A and Khazaei, N (2014). Effect of quince seed mucilage edible films incorporated with oregano or thyme essential oil on shelf life extension of refrigerated rainbow trout fillets. Int. J. Food Microbiol., 174: 88-97.

Li, PH and Chiang, BH (2012). Process optimization and stability of D-limonene-in-water nanoemulsions prepared by ultrasonic emulsification using response surface methodology. Ultrason. Sonochem., 19: 192-197.

Li, JJ; Ji, BP; Zhou, F and Li, B (2006). Study on the extraction, main component and antimicrobial activity of clove and cinnamon essential oil. J. Food Sci., 27: 64-68.

López-Caballero, ME; Martínez-Alvarez, O; Gómez-Guillén, MDC and Montero, P (2007). Quality of thawed deepwater pink shrimp (*Parapenaeus longirostris*) treated with melanosis-inhibiting formulations during chilled storage. Int. J. Food Sci. Tech., 42: 1029-1038.

Mastromatteo, M; Danza, A; Conte, A; Muratore, G and Del Nobile, MA (2010). Shelf life of ready to use peeled shrimps as affected by thymol essential oil and modified atmosphere packaging. Int. J. Food Microbiol., 144: 250-256.

McClements, DJ and Rao, J (2011). Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. Crit. Rev. Food Sci. Nutr., 51: 285-330.

Moradi, M; Tajik, HR; Rohani, SM and Oromiehie, AR (2011). Effectiveness of *Zataria multiflora* Boiss essential oil and grape seed extract impregnated chitosan film on ready-to-eat mortadella-type sausages during refrigerated storage. J. Sci. Food Agric., 91: 2850-2857.

Ozogul, Y; Yuvka, İ; Ucar, Y; Durmus, M; Kösker, AR; Öz, M and Ozogul, F (2017). Evaluation of effects of nanoemulsion based on herb essential oils (rosemary, laurel, thyme and sage) on sensory, chemical and microbiological quality of rainbow trout (*Oncorhynchus mykiss*) fillets during ice storage. LWT-Food Sci. Technol., 75: 677-684.

Shah, P; Bhalodia, D and Shelat, P (2010). Nanoemulsion: a pharmaceutical review. Sys. Rev. Pharm., 1: 24-32.

Shahavi, MH; Hosseini, M; Jahanshahi, M; Meyer, RL and Darzi, GN (2016). Clove oil nanoemulsion as an effective antibacterial agent: taguchi optimization method. Desalin. Water Treat., 57: 18379-18390.

Simpson, BK; Gagne, N; Ashie, INA and Noroozi, E (1997). Utilization of chitosan for preservation of raw shrimp (*Pandalus borealis*). Food Biotechnol., 11: 25-44.

Tongdeesoontorn, W; Mauer, LJ; Wongruong, S; Sriburi, P and Rachtanapun, P (2011). Effect of carboxymethyl cellulose concentration on physical properties of biodegradable cassava starch-based films. Chem. Cent. J., 5: 6.

Varela, P and Fiszman, SM (2011). Hydrocolloids in fried foods. A review. Food Hydrocoll., 25: 1801-1812.

Yam, KL and Papadakis, SE (2004). A simple digital imaging method for measuring and analyzing color of food surfaces. J. Food Eng., 61: 137-142.

Zhang, S; Zhang, M; Fang, Z and Liu, Y (2017). Preparation and characterization of blended cloves/cinnamon essential oil nanoemulsions. LWT-Food. Sci. Technol., 75: 316-322.