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Research Article

Effect of saffron nanoemulsion on the shelf life extension of shrimp, *Penaeus semisulcatus* using an ultrasonic homogenizer

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Abstract

As the prolongation of shelf life in fish processing is one of the industrial importance, it seems that food and aquatic scientists have been moving toward a point where more novel and efficient methods for refraining from food wastage like integrative systems using nanotechnology-based approaches. *Penaeus semisulcatus*(n=24) were covered with saffron nanoemulsionat 0% (control, group 1), 3% (group 1), and 5%(group 2).They were kept at 4 and 8°C until further testing. The samples were assessed for microorganisms, sensory quality, peroxide value (PV), and pH.The lowest total count of bacteria, coliform count, and psychrophilic count were observed in the shrimp samples covered with 3 and 5% saffron nanoemulsions stored at 4and 8°C, respectively (p<0.05). The lowest PV and pH were seen in the shrimp samples covered with 3-5% saffron nanoemulsions stored at 4°C (p<0.05).It is concluded that nanoemulsions of saffron 3% or 5% can enhance the shelf life of shrimp,*P. semisulcatus* stored at 4°C and 8°C.

Keywords: Saffron extract, Nanoemulsion, Ultrasonic, Shelf life, *Penaeus semisulcatus*

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Introduction

Seafood is an essential source of nutrients for population of the world (Sikorski, 2012). Therefore, preservingthe quality of aquatic products via increasing shelf-life would be of crucial and industrial importanceto meet theoptimum requirements of consumers(Aboutorab *et al.*, 2021).

Shrimp, one of the most perishable commodities, regrettably is caught in unhygienic circumstances and sold to consumers (Abdirad *et al*., 2022). Hence, seafood (fish and shrimp) that is caught or farmed is subject to a variety of security issues (Ahmed *et al*., 2014).As one of the most sold and consumed aquatic products, shrimps are more sensitive to deterioration, in terms of their highly soluble non-protein nitrogenous substances(Abbasvali *et al.*, 2016). Shrimp is known as one of the rich sources of nutrients. Compared to other food, it has relatively fewer calories and provides a lot of protein and healthy fats, as well as various vitamins and minerals (Mehrzadeh and Roomiani 2021).

Green tiger shrimp, *P. semisulcatus* is an indigenous wild-raised species in the Persian Gulf environment. In the global seafood market, shrimp is an important commercial commodity that, despite its high number of consumers, has a rapid spoilage rate. This food source is prone to spoilage like other food products (Zaini *et al.*, 2009). The highest portion, 65.6%, was accounted for by the export of white shrimp. Other marine shrimp made up 11.6%, while black tiger shrimp made up 23%. In terms of the white leg shrimp's nutritional makeup, *Litopenaeus vannamei*, is an excellent source of protein, carbs, fat, moisture, and ash. Many studies reported this issue focusing on the species in the Persian Gulf environment (Sharifian *et al.*, 2022).

Some chemical preservatives, such as sorbate, boric acid, borax, and metabisulfite are utilized for processing aimed at extending shelf-lifeand improving sensory features and texture (Anand and Sati, 2013). Consequently, it is vital to utilize a biological preservative with antibacterial and antioxidant properties that has no negative effects (Martin Xavier *et al*., 2019). Using natural additions such as plant phenolic compounds is a safer technique to improve the shrimp's storage stability from a food safety standpoint (Abbasvali *et al*.,2016). Among those plant-derived substances with antimicrobial compounds (regarding phenolic groups), pine bark extract (Pycnogenol), and garlic can increase the shelf-life of fresh and cooked meat products (Sallam *et al.*, 2004; Ahn *et al.*, 2007), showing antimicrobial or antioxidant properties, playing a crucial role in the host defense systems against microbial diseases (Shahi *et al.*, 2016; Kaur and Srinivasa Rao, 2018).

The potential of herbal in the form of nanoemulsion in covering the sea foods like shrimp has attracted the attention of scientists. For example, the nanoemulsion of extracted corn zein

with garlic extract as a biodegradable and edible coatingcould enhance the shelf life of Vannamei prawn (*L.vannamei*) (Rahnama *et al.*, 2021). In another study, researchers prepared an edible coating based on the nanoemulsion of Aloe vera and eugenol to control the physicochemical properties of shrimp during cold storage (Sharifimehr *et al.*, 2019).

The costliest spice in the world, saffron is the dried stigma of the *Crocus sativus* flower, which has a long, orange color (Alonso *et al*., 2012). Distilling saffron with distilled water, under the flow of carbon dioxide gas, yields the volatile oils (monoterpene aldehyde in Safranal), which are responsible for the spice's distinctive scent (Akowuah and Htar, 2014). As food preservatives, using these aromatic plants has increased significantly in nanotechnology (Paramanya *et al.*, 2020). Currently, nanoparticles are used as sensors to ascertain food quality (Ameta *et al.*, 2020).

Nanoemulsion-based edible coatings technology (20 to 200 nanometers), is recognized as a worthwhile alternative to developing seafood quality, and physical stability, meetingthe criteria for the healthy minimally-processed (Hasan *et al.*, 2020), increases bioavailability and absorption conditions(Jafari *et al.*, 2017). The primary components of the herbal extract and essential oils, which are antibacterial and antioxidant, have this ability. Moreover, providing them at nanoscale sizes boosts their potential (Durmuş *et al*., 2020).The ultrasonic homogenization method is a technique to produce nanoemulsions (Chang *et al.*, 2020) via the mechanical vibrations, making micro-bubbles formed by cavitation with improved stability (Nirmal and Benjakul, 2009; Aboofazeli, 2010). Moreover, using high-pressure homogenizers enhances their efficiency in stabilizing emulsions (Aboutorab *et al.*, 2021). Whereas other processes need more time or greater energy, our method produces nanodroplets quickly and with less energy. Also, it is straightforward and does not need more intricate methods. Regarding high nutritional value and increasing demands for aquatic food, it was tried to produce a saffron-based nanoemulsion in different concentrations of saffron using an ultrasonic technique. The final saffron-nanoemulsion was used to cover *P. semisulcatus* products with the aim of shelf life Extensionvia physicochemical, and microbial evaluations.

Material and methods

Nanoemulsion preparation

Essential oil

To take the highest amount of active substance the combination of water and acetonitrile (1:19 v/v) was selected as a suitable solvent for essential oil extraction (Larson *et al.*, 1999). To make it, 250 mL of the solvent and acetonitrile were added for every 50 g of crushed saffron. The mixture was then put into an ultrasonic bath for 30 minutes, left at room temperature for 6 hours, filtered using Whatman No. 42

filter paper, and centrifuged to extract the clear solution. A rotary movement was used to concentrate the resultant solution to a mass of 5 g.

Sample Preparation

Twenty-four shrimp sampleswere purchased from fish-market in Tehran. After de-heading, and cleaning the shrimp, they were washed with cold water and then transferred to the laboratory (Islamic Azad University lab)close to ice. All samples were thoroughly dried before being placed in polyethylene bags for both short-term and long-term storage at either 4 or 8°C for 0, 3, and 7 days. Samples underwent sensory evaluation as well as chemical quality testing for pH, PV, TBARS, and microorganisms.

Nanoemulsion preparation

To synthesize saffron nanoemulsion with the concentration of 3 and 5 %, 3 and 5 mL of saffron essential oil s first mixed with span 80 as surfactant separately. The resulting solution was then added dropwise to the 100 mL aqueous phase under vigorous stirring (containing 10% tween 80 and 5% tween 40) to form the final emulsion. To produce nanoemulsion, the final emulsion was sonicated using an Ultrasonic machine (100 W, 15 min). the final nanoemulsion was stored in a refrigerator(Carpenter and Saharan, 2017).

DLS analysis

Firstly, emulsions were diluted up to 10% with deionized water to prevent multiple scattering of particles. About 5 mL of the nanoemulsion colloidal solution was poured into the specific cells of the device and placed in the DLS instrument. After passing the light through the sample, the mean droplet size distribution was measured three times, and based on the results nanodroplets with a mean size of 100 nm were synthesized (Sugumar *et al.*, 2014).

Microbial analysis

Disk Diffusion

Standardized bacterial suspensions were inoculated into Mueller-Hinton Agar (MHA) plates. 0.1 of each bacterial suspension with a turbidity of 0.5 McFarland (1.5×10^8 CFU/mL) cultured on MHA media. Then, sterile disks immersed with 4, 8, 16, and 32 microliters of nano-essential oil were placed on culture media of *E. coli*, *Staphylococcus aureus*, and *Salmonella bacteria*, incubated at 37°C for 24h. The diameter of inhibition zones (in millimeters) was measured by a ruler (Özogul *et al.*, 2022).

Bacterial count tests

To determine the antimicrobial properties of saffron nanoemulsion on *P. semisulcatus*, bacterial count tests, including total mesophilic bacteria, psychrophilic bacteria, *S. aureus*, and *Escherichia coli* were performed. Salmonella and Vibrio levels were examined. The cleaned and prepared

shrimp samples were cut into 10g pieces and dipped into saffron essential oil-based nanoemulsions at 3% and 5% concentrations. The samples in the Control group weren't exposed to the essential oil-based nanoemulsions. Shrimp samples were placed on sterile trays under the UV-radiated biological hood to dry, and packed separately in polyethylene bags (Ozen, 2000).

To prepare physiological serum, 8.5 g of sodium chloride was first weighed, poured into a volumetric flask of 1000 mL, made up to volume with distilled water, and then stirred well to dissolve the salt particles in the water. A 90 mL of physiological serum was poured into the funnel. Also, 9 mL of serum was poured into the test tubes. Then, they were autoclaved at a temperature of 121°C and a pressure of 115 Pa for 15 min.

Total bacterial count

For total bacterial count, Plate Count Agar (PCA) culture medium was prepared based on the manufacturer`s instructions. After sterilization of the medium, it was poured into the plates. The cultured plates were kept at 37° for 24 h, and then the number of bacteria was counted on the plate (He *et al.*, 2021).

Psychrophilic count

For *psychrophilic*count bacteria, instruction was followed like the previous stage. Cultured plates were placed into the refrigerator (7-10°C) for 7 days and then counted (Khanzadi *et al.*, 2020).

Staphylococcus aureus count

According to the manufacturer's instructions, Baird Parker Agar (BPA) culture media was made. Tellurized egg yolk was added and then placed onto a plate after autoclaving. For counting, the cultured plates were used (Sugumar *et al.*, 2014). Bacterial staining was followed usingGram staining as a method of bacterial determination.A colony was removed from the plate and spread on the slide.It was then stained with crystal violet, Lugol, alcohol, and Fuchsin. After drying, the slide was examined using light microscopy (Olympus DSX1000 Digital Microscope).

S. aureus count was the next evaluation so that, black and shiny colonies were cultured using Gram staining, and coagulase test on Mannitol Salt Agar (MSA) medium. In lecithinase test process, lecithin was degraded by lecithinase, therefore, a zone was formed around the *S. aureus* colonies in the BPA plate.

Coagulase test

The 250 microliters of citrated rabbit plasma, 750 microliters of saline, and bacterial colony were poured into the plastic tube and incubated at 35-37°C. Then it was examined for clot formation at 4°C (Elsherif *et al.*, 2022).

Mannitol salt agar test

A colony of bacteria was removed and a streaking culture was performed on the MSA medium.

Total coliform count

First, 1 mL of the desired dilution was transferred to the center of each plate, and immediately 15 mL of Violet Red Bile Agar (VRBA) medium at 45°C was transferred to each of them (pour plate). The plates were then positioned on a level surface until the culture medium solidified. After adding 5 mL of medium to the top of the first layer and allowing it to harden, the plates were incubated at 37°C for 24 hours. The method of counting the maximum possible number was used to estimate the probable number of microorganisms per gram of sample. Suspected colonies were inoculated into Brilliant Green Bile (BGB) broth medium with Durham tube, and the Nutrient Broth (NB) medium, then incubated for 24 h at 44.5°C. Gas-producing colonies in BGB and NB-positive endolysins confirmed *E. coli* growth in the medium.

Salmonella count

The lactose broth medium was made in accordance with the directions provided by the manufacturer. 15 mL of the necessary dilutions were added to the aforementioned culture medium after it had warmed to room temperature, and they were then incubated at 37°C for 18 hours. It was then inoculated in the selective culture medium of Rappaport Vassiliadis Soy peptone (RVS) broth and enriched, then incubated at 37°C for 24 h. Xylose Lysine Deoxycholate Agar (XLD), and *Salmonella*, *Shigella* Agar (SS agar) were poured onto a plate after sterilization and reached room temperature. Then, RVS broth was cultured by streaking method on those two media.

Diagnosis of Salmonella

Suspicious colonies were cultured on XLD, and PCA plates until pure colonies were obtained and then cultured in differential Triple Sugar Iron Agar (TSI) culture medium in a deep and zigzag pattern on a slant surface. It was then incubated for 24h at 37°C. Subsequently, colonies were deep grown on SIM and streaked on urea agar medium to test for sugar fermentation, gas generation, and H2S production. Afterwards, an ONPG disc was put in a sterile tube, 1mL of sterile saline was added, and a sterile colony loop was used to dissolve it in the saline. The tube was then cultured for 1 to 6 hours at 35 to 37°C. After *Salmonella* diagnosis. *serogrouping* was specified by slide agglutination with O antiserum.

Diagnostic test for Vibrio

To examine *Vibrio* from the broth enrichment culture medium Alkaline Saline Peptone Water (ASPW), NaCl, peptone, and distilled water were poured into the container in the specified amount and 0.1 N KOH was added until the pH reached 8.5. Then the culture medium of Thiosulfate Citrate Bile-Salt sucrose (TCBS) agar was prepared according to the manufacturer's instructions and sterilized in an autoclave at 121°C for 15 min. Suspiciouscolonies were evaluated by culture in urea and TSI

media, and an oxidase test. To prepare dilution 1, 10 g of shrimp samples were placed inside Stomacher bags and 90 mL of sterile physiology serum was added to it and homogenized by the stomacher. Then 1 mL of the suspension was poured into 9 mL of physiological saline tubes, then homogenized by the shaker, thus the desired dilutions were prepared.

Chemical analysis

Peroxide Value (PV)

After impregnating *P. semisulcatus* shrimp samples with prepared saffron nanoemulsions, samples were packed in Low-density polyethylene (LDPE) bags with two concentrations of 3%, and 5% and stored at 4 and 10°C.5gr of sample oil extracted from shrimps was weighed in 250 mL of Erlenmeyer sandpaper, and about 30 ml of chloroformic glacial acetic acid solution (Acetic Acid/Chloroform, 3:2) was added which was mixed for 5 min to dissolve the oil in the solvent.Then, 500 µL of saturated potassium iodide solutionwas added and mixed, after 1 min, 30 mL of distilled water was added and was titrated with 0.01 N sodium hyposulfite solution. Until the yellow hue vanished, the titration procedure was maintained. The complex was then given 500µL of the starch solution, and the titration was maintained until the color vanished entirely. A control sample was considered and no more than 0.1 mL of sodium hyposulfite solution was used in the control titration. The peroxide value (meq g O2/kg Fat) was calculated based on following equation;

Thiobarbituric acid reactive substances (TBARS)

TBARS method was used to assess the lipid oxidation of shrimp samples. The amount of TBA was determined by the colorimetric method. 5g of shrimp samples were placed in tubes with lids and the specifications of each were written on the tubes. Then, 8 mLof 5% HCl was added to each tube. In the next step, 5 mL of initial BHT was added and mixed with a mixer. It was then centrifuged at 4,000 rpm for 20 min. After the centrifugation, the contents of plastic tube were divided into two phases, the liquid part of which was poured into the microtube by the sampler, and then the microtubes were placed in the centrifuge for 10 min at a speed of 13,000 rpm. Then, 400 µL of each was poured into the Tube, and then 400 µL of TCA, secondary BHT (Butylated hydroxytoluene), EDTA, SDS, and 2500 µL of TBA were added, respectively. For the standard sample, 200 microliters of the principal HCl and BHT were put into the plastic tube rather than 400 microliters of each. After that, a boiling pan held the plastic tubes for a whole hour. After that, it was cooled for 10 minutes in an ice bucket. The plastic tubes were then placed in a centrifuge at 8,000 rpm, at which point the amount of light absorbed at a wavelength of 532 nm was read by a spectrophotometer.

The amount of TBARS (miligram per kg shrimp tissue) and malondialdehyde (MDA) were calculated based on the equation 2.

pH value analysis

First, the samples weighed 1 gram and were placed inside the plastic tube, and 10 mL of distilled water was added under homogenization in a blender for the 30s. Then, it was passed bio filter paper. After calibration, a pH-Meter probe was inserted inside the container. The instrument showed the pH value. After each measurement, the probe was cleaned with distilled water and dried.

Sensory evaluation

Before each experiment, the samples were examined for color, odor, and texture changes by 3 panelists on a 5-point score.

Statistical analysis

GraphPad Prism 5 and ANOVA statistical test were used to analyze data. Data were presented as mean±standard error and the experiment was done in triplicate. P-values less than 0.05 were statistically considered as significant.

Results

Disk Diffusion

The disk diffusion method was used to measure the antibacterial activity of saffron nano-emulsions against *S. aureus*, *E. coli*, and *Salmonella*, and the results are shown in Figure 1-A, B. The resulting data showed the positive effects of saffron nanoemulsions on inhibiting the growth of three studied bacteria. Considering *S. aureus* and *E. coli*, for each volume of used Nanoemulsion, no significant difference was observed from 3% to 5% Saffron treatments (p>0.05). In all instances, an increase in concentration often led to increased inhibition. Plates treated with the 3% saffron nano-emulsion at a volume of 4 microliters produced the lowest inhibition zone (16 mm for S. aureus and 21 mm for *E. coli*), while the 5% saffron nano-emulsion produced the biggest inhibition zone (25 mm for S. aureus, 30 mm for *E. coli*, and 17 mm for *Salmonella*) (Fig. 1).

Figure 1: Synthesis of saffron nanoemulsion.

Therefore, 5% saffron nano-emulsion prepared by ultrasonic homogenizer method showed significant activity against *S. aureus*, *E. coli*, and *Salmonella*. Moreover, no significant difference was observed between the 8

& 16-microliter volumes of 3% and 5% Saffron Nanoemulsion (p>0.05).

Microbial tests

Total count

According to the results shown in Figure 2, thetotal bacterial count in the control group significantly increased compared to other groups during 14 days of storage at 4 and 8°C. Nano-emulsion of saffron with the concentration of 5% and 3% increased during storage time at 4 and 8°C. On the third day at 4 & 8°C, microbial growth was observed for all samples with no significant difference (p>0.05). Days 7 and 14 of storage at 4°C resulted in a considerably lower total bacterial count in the 5% and 3% saffron nano-emulsion than days 7 and 14 of storage at 8°C. The samples stored in 3% saffron nano-emulsion at 4°C and the samples stored in 5% saffron nano-emulsion at 8°C had the lowest total count on day 14.

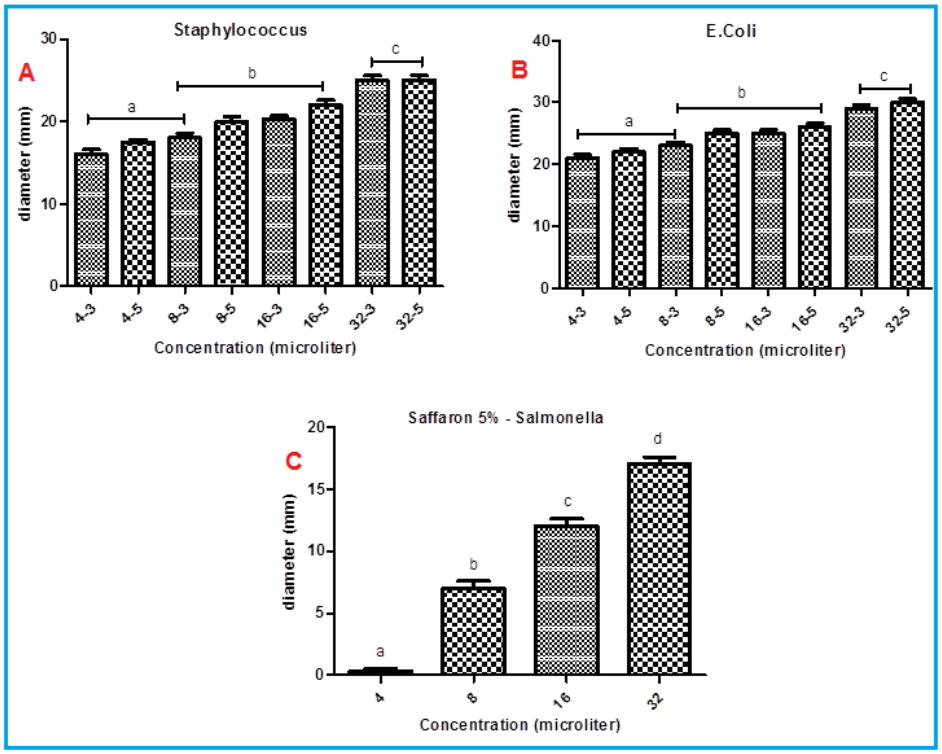

Figure 2: The measurement of inhibition zone diameter in millimeters for saffron nano-emulsion per concentration (microliter) against A) *Staphylococcus aureus* (3% and 5%), B) *Escherichia coli* (3% and 5%), and C) Salmonella (5%). The same letters show no significant differences, and different letters mean significant differences.

Coliform count

Total coliforms include a group of bacteria discovered in the intestines of animals and humans. They are specified as gram-negative, anaerobic, and non-spore-forming bacteria. *E.coli* is the only member of the total coliform group of bacteria which exclusively discovered in the intestines of humans and mammals(Jorgensen *et al.*, 1979; Ogunleye *et al.*, 2021).

The changes in coliform count of shrimp samples during 14 days of storage are presented in Figure 3. As can be seen, up to 3 days, no significant difference was observed for all groups (p>0.05). The coliform count of the control and treated samples significantly increased from day 7 to day 14 of storage. At 4 and 8°C, the lowest coliform count was observed for the samples kept in 5% saffron nano-emulsion during storage. On day 14, both 3% and 5 % saffron Nanoemulsion showed a significant difference in comparison with the control group at 4°C and 8°C. A Similar trend was observed for 5% saffron Nanoemulsion at 4°C on day 7 (Fig. 3-A), while no significant difference was observed between 3% and 5 % saffron Nanoemulsionon day 7 at 8°C (p>0.05) (Fig. 3-B). Accordingly, our results proved that 3% and 5% saffron nanoemulsions could inhibit microbial growth in shrimp samples.

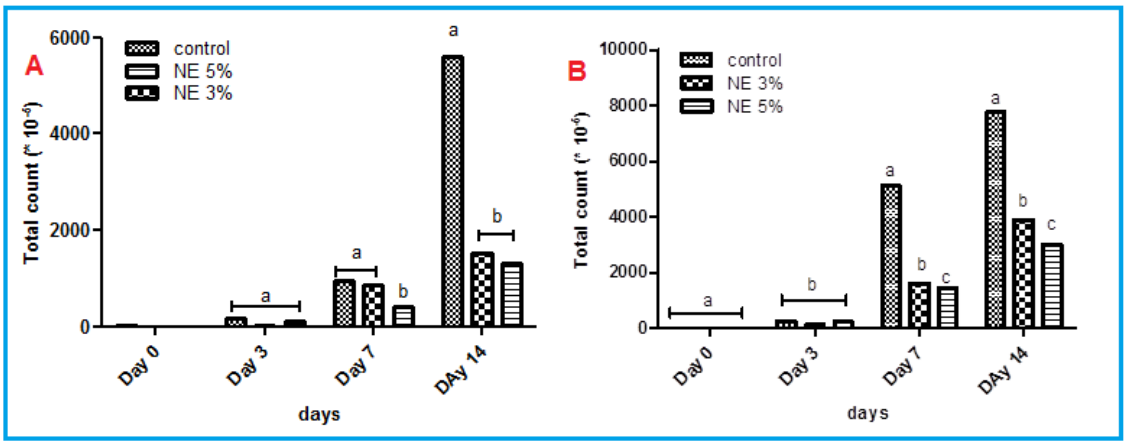

Figure 3: The results of the total bacterial count for treated samples by 3 and 5% saffron nano-emulsion at A)4°C and B) 8°C for 14 days of storage. The same letters show no significant differences, and different letters mean significant differences.

Staphylococcus aureus count

Figure 4 displayed the findings of the S. aureus counts. At 8°C, neither the 3% nor the 5% saffron Nanoemulsions exhibited any signs of microbial development, but at 4°C, they did, but not significantly (p<0.05) in contrast to each other. The control group in all periods showed significant differences compared with the other groups (p<0.05) meaning that the control group showed the highest amount of *S. aureus* count during 14 days of storage at 4 and 8°C while other groups increased slightly, especially at 8°C. The lowest *S. aureus* count was seen for the 5% saffron nano-emulsion at 4°C and 8°C.On day 14, no significant difference was seen between 4% and 5% saffron Nanoemulsion at 8°C (p>0.05) (Fig. 4-B).

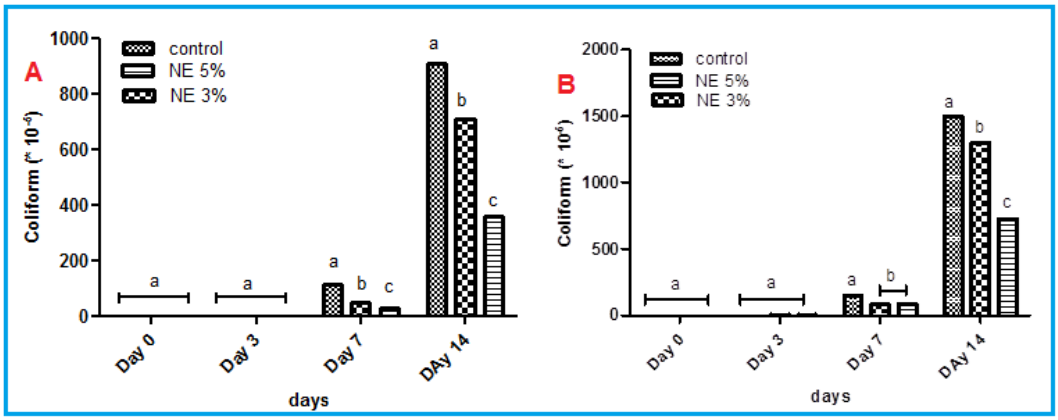

Figure 4: The results of the coliform count for treated samples by 3% and 5% saffron nano-emulsion at A) 4°C and B) 8°C for 14 days of storage.

Psychrophilic count

Owing to high water activity in the 0.95-1 range, shrimp is food that spoils quickly. The International Committee on Food Microbiology has stated the acceptable total count of live and durable psychrophilic aerobic bacteria in fresh marines is 10^6 CFU/g (Mejlholm *et al.*, 2008; Bahrami *et al.*, 2019). Saffron is a dry substance, and its microbial load naturally reduces during the course of storage. Nonetheless, you should take care to keep the package from becoming damp or becoming too hot (Eslami *et al.*, 2016). The findings shown in Figure 5 indicate that there was no significant difference between 3% and 5% of saffron Nanoemulsion on day 3 of storage at 8 °C (p>0.05).At 4°C on day 3, 3% saffron Nanoemulsion showed no significant difference compared to the control group (p>0.05) while during storage time 3% and 5% showed similar efficacy (p>0.05).The *psychrophilic* count of the control and treated groups increased continuously during storage time until the end of the storage period (day 14). The Control group showed the highest amount of *psychrophilic* count through 14 days of storage at 4 and 8°C. On day 14, the lowest *psychrophilic* count was seen for the 3% and 5% saffron nano-emulsion at 4°C. After 14 days, the best results belonged to 5% saffron nano-emulsion at 8°C.

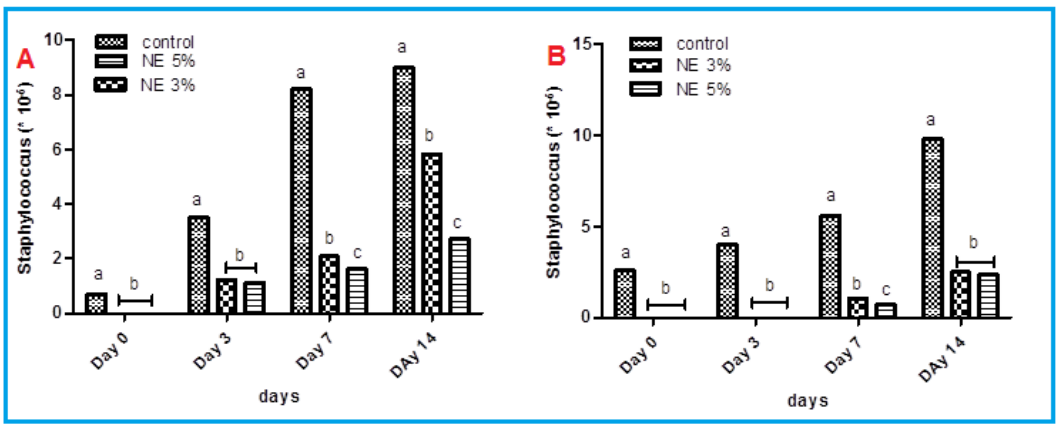

Figure 5: The results of the *S. aureus* count for treated samples by 3% and 5% saffron nano-emulsion at A) 4 and B) 8°C for 14 days of storage.

Chemical analysis

Peroxide value

Based on Figure 6-A,B, PV values were significantly affected (P<0.05) from days 3 and 7 and raised in all groups. The PVs of control and treated groups revealed a significant elevation (p<0.05)along with storage time. At 4 °C, no significant difference was observed between the control and treated groups on day 3 (p>0.05) (Fig. 6-A), while at the same time during storage at 8°C, all groups showed a significant difference in comparison with each other (p<0.05) (Fig.6-B). However, by the end of storage time (day 7), significant differences (p<0.05) were observed in the PV between the controls and samples treated with the nano-emulsion for nano-emulsification methods at 4 and 8°C, which indicated lower values. PV increased significantly (p<0.05) in all treatments along with the storage time. Significantly lower PVs were seen for treated samples, especially for treated samples storage at 4°C (nano-emulsion, 3% saffron). The highest PV values were seen for the control samples at 8°C (Fig. 6).

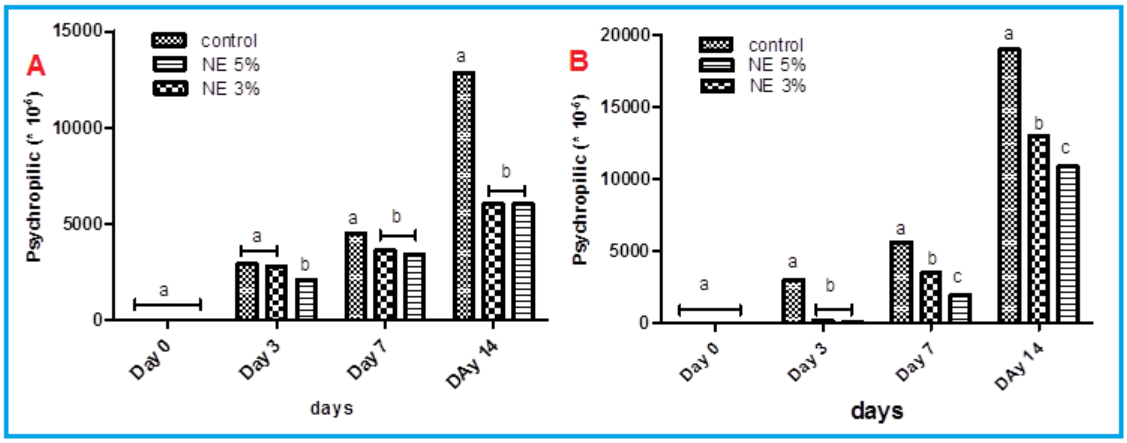

Figure 6. The results of the *psychrophilic* bacteria for treated samples by 3% and 5% saffron nano-emulsion count at A) 4 and B) 8°C for 14 days of storage.

pH value

The changes in the pH of shrimp samples as a function of the treatments and storage time are shown in Figure 6-C, D. The initial pH of the control samples on day 0 was 7, indicating the freshness of the shrimp samples kept in nano-emulsions prepared by ultrasonic homogenizer techniques. After 14 days of storage at 4°C, the pH levels of all the samples rose. Three days of storage in 3% saffron resulted in a rise in pH value. The samples stored in a 5% saffron nano-emulsion at 4°C showed the lowest pH values. Compared to this time, all treatments on days 7 and 14 showed pH values of ~8, but there were no significant differences between the different treatments (p>0.05).

Thiobarbituric Acid Reactive Substances (TBARS)

The changes in the TBARS of shrimp samples during the storage are shown in Figure 7-A,B. The initial TBARS value was 0.2 and 0.3 mg malonaldehyde

equivalents/kg (MDA eq/kg) of tissue for the shrimp samples during storage at 4 and 8°C respectively for all saffron concentrations. The TBARS values of the control and treated samples increased continuously during storage until the end of the storage period (day 14). 5% nanoemulsion saffron resulted in a significantly (p<0.05) lower TBARS value in comparison with 3% nanoemulsion saffron, after 14 days of treatment. Control groups showed the highest amount of TBARS on the last day of the experiment (day 14).

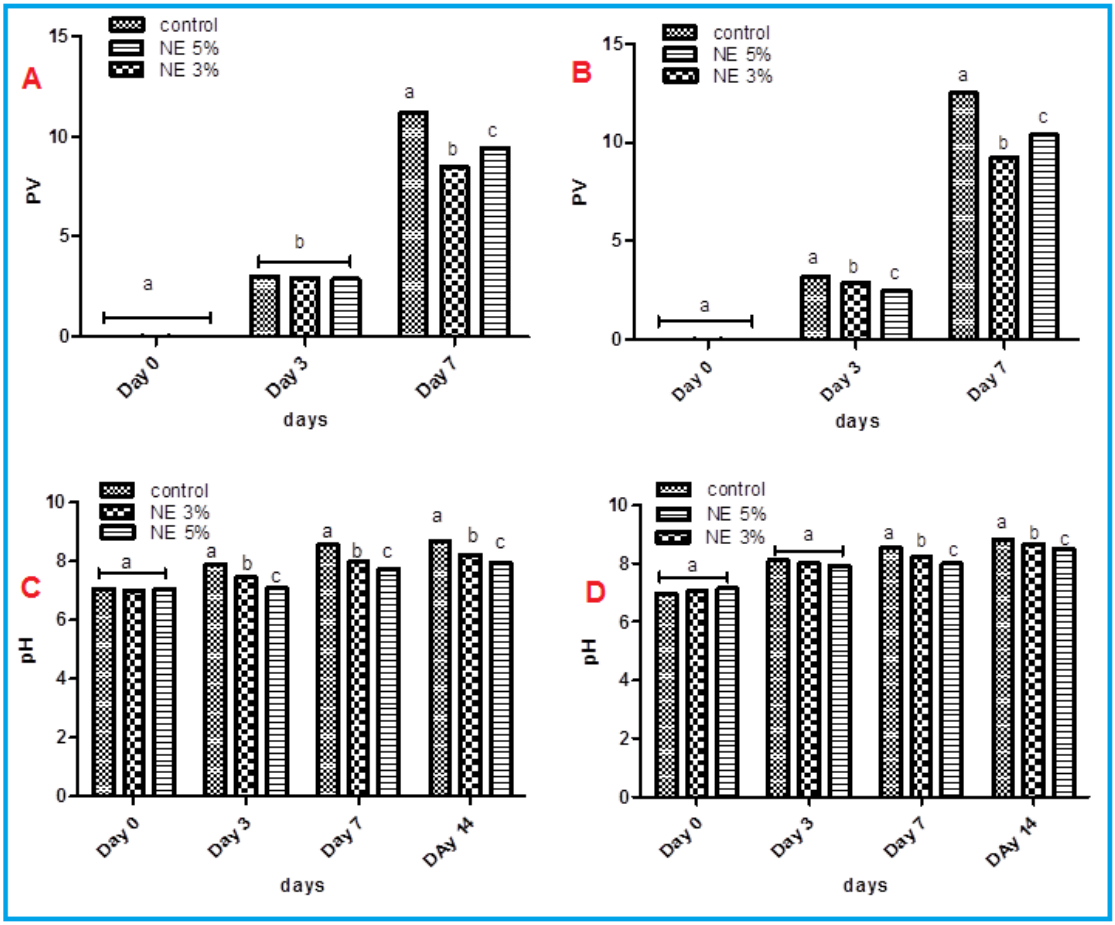

Figure 7: A, B) The measurement of Peroxide values (meq/kg) (A, B) and pH (C, D) of shrimp samples treated by nano-emulsion of saffron during storage at 4 and 8°C. Data are shown as three independent experiments. Each one contains three samples.

Sensory evaluation

Over 150 volatile and nonvolatile chemicals are found in the dried stigma of saffron (*Crocus sativus* L.), a member of the Iridaceae family. These derivative compounds may improve food flavor and fragrance (Ahmed *et al*., 2021). Figure 7-C shows the changes in sensory characteristics ofGreen Tiger Shrimp, or *P. semisulcatus*, with and without various treatments throughout 14 days of storage at 4 and 8°C.On day 0 the scores of color, odor, and texture of treated shrimps were the same in all three groups, control, 3%, and 5% nano-emulsion saffron (p<0.05). During 14 days of storage, the

decreases in likeness for all attributes in all samples were noticeable (p<0.05). On day 3, the lower scores were found in odor and texture at 8°C, compared with the control group (p<0.05). On day7, scores of the color of all samples were significantly lower than the control (p<0.05). For all three groups at 4 and 8°C, ratings for color, odor, and texture on day 14 were considerably lower than on earlier days. As a result, saffron nano-emulsion treatment of *P. semisulcatus* shrimp may enhance their sensory qualities even after prolonged storage.

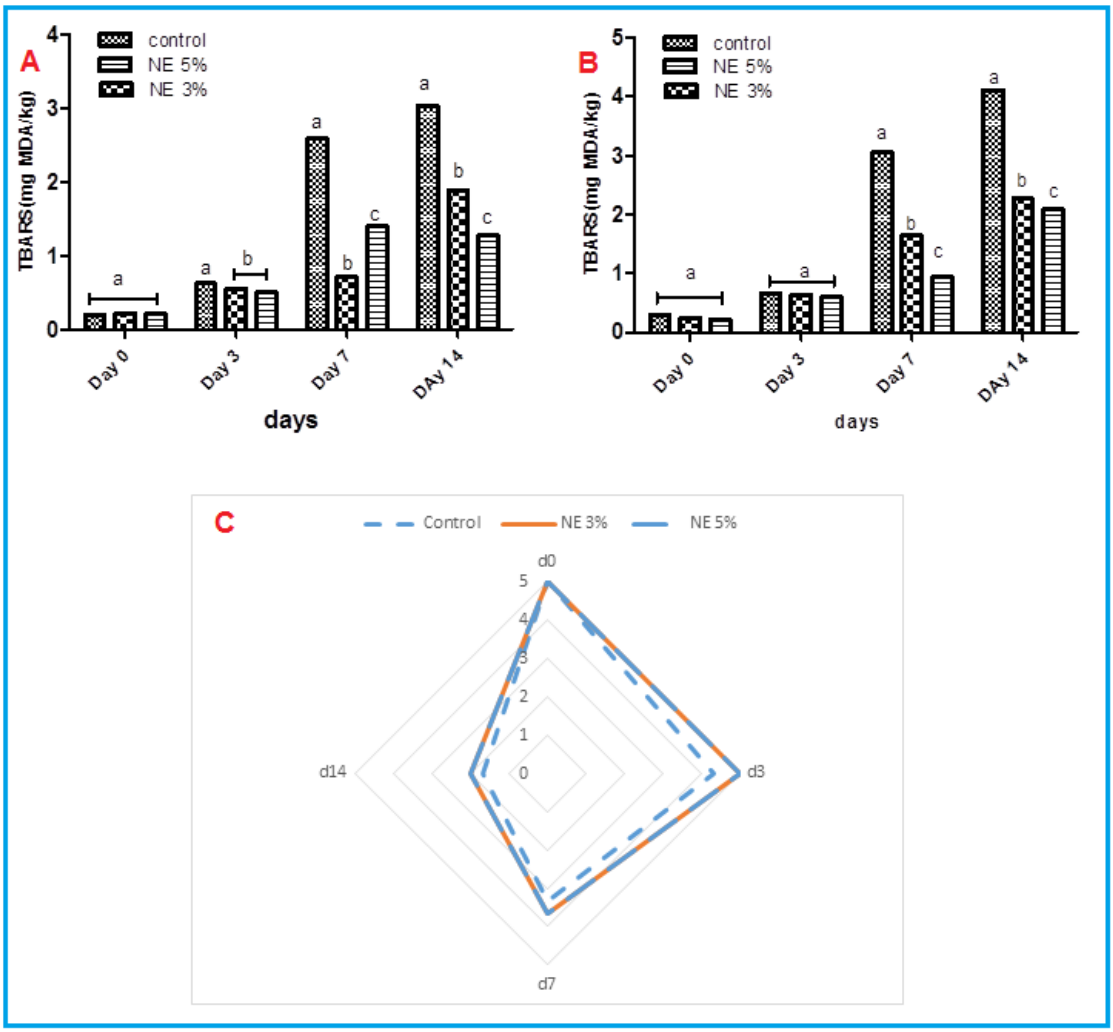

Figure 8: A,B) The measurement of Thiobarbituric Acid Reactive Substances values of shrimp samples treated by nano-emulsion of saffron during storage at 4 and 8°C (mg of malonaldehyde/ kg of tissue). C) The effect of saffron nano-emulsion treatment on the likeness score of *Penaeus semisulcatus* shrimp during 14 days of storage at 4 and 8°C Data are presented as three independent experiments. Each one contains three samples.

Discussion

Using nanoemulsion as a coating to protect foods specifically marine foods has attracted the attention of scientists and industries. Thereby, numerous research was performed to evaluate the potential of distinct sources, including essential oils from herbal sources to

enhance the shelf life of foods.Thenanoemulsion of saffron was prepared to prolong the shelf life of *P. semisulcatus*.

The final nanoemulsion showed notable antibacterial activity. Considering the results from disk diffusion, the final saffron nanoemulsion could prevent the growth of both positive and negative microorganisms.Aboutorab indicated that samples treated with saffron nano-emulsion exhibited more antibacterial activities against E. coli and S. aureus compared to untreated groups (Aboutorab *et al.*, 2021).

Abbasvali *et al.* (2016), analyzed the shelf life of pacific white shrimp treated with saffron tepal extracts stored in ice for up to 9 days. They indicated that the extracts of saffron tepal with a concentration of 5 mg/mL raised the lag phase time of *S. aureus* growth curve compared to the positive control but did not demonstrate any repressive effect on the growth of *E. coli*. The inhibitory effect of water extract (WEST) against *S. aureus* was 76.57% which was higher than ethanolic extract (EEST) and methanolic extract (MEST) (p<0.05). This suggested that the studied extracts' antibacterial activity was strongly tied to their phenolic components (Abbasvali*et al*., 2016). Methanolic, ethanolic, and aqueous extracts of saffron petals demonstrated more antibacterial action against S. aureus than E. coli, according to research done in 2012 by GandomiNasrabadi *et al*. (2012). The higher sensitivity of *S. aureus* is probably because of its cell wall structure and outer membrane (Gandomi Nasrabadi *et al.*, 2012).

In another study, Khoshbouy Lahidjani *et al.* (2020) examined the effects of curcumin-loaded nano-emulsions on the shelf life of Oncorhynchus mykiss and revealed that the prepared 5% curcumin nano-emulsions by emulsion phase inversion method could retard the bacterial growth and improve the shelf life of the fish fillets of Oncorhynchus mykiss (Khoshbouy Lahidjani *et al.*, 2020).Many studies have shown the influence of nanoemulsion of plant essential oils on the preservation of fish and shrimp. In a study, the results of the microbial test showed that the film containing curcumin has strong antimicrobial properties against gram-negative and gram-positive bacteria (with a halo diameter of 6.1 and 4.8 mm, respectively, against Staphylococcus aureus and Escherichia coli bacteria) (Jafarian *et al.*, 2021)

Abbasvali *et al*. (2016) showed that microbial growth in shrimp could be delayed using saffron tepal extract as an antimicrobial agent (Abbasvali *et al.*, 2016). Similarly, our results indicated that 3% and 5% saffron nano-emulsion could inhibit the microbial growth in shrimp and can reduce the total bacterial count.

Prepared saffron samples were positive for *Clostridium perfringens, A. flavus,* Enterobacteriaceae, *F. oxysporum, Bacillus cereus,* and *S. aureus* (Eslami *et al.*, 2016). In 2009, *Cosano et al.* also revealed the presence

of *E. coli*, *B. cereus* species, *Enterobacter*, and spore-forming bacteria separated from the samples while they were without *Salmonella* species (Cosano *et al.*, 2009). In another study reported the antimicrobial activity of the Safran (Rezaee and Hosseinzadeh, 2013).

Similarly, in 2020, Aboutorab *et al.*(2021) showed that samples treated with saffron nano-emulsion showed more antibacterial activities against *E. coli* compared to untreated groups (Aboutorab *et al.*, 2021). According to earlier research, none of the conventional methods, such as cooling, refrigeration, and freezing, have succeeded in keeping shrimp in good condition for an extended period of time (Odeyemi *et al.*, 2020). Nowadays, there is a trend toward using nanoparticles made in various ways across many industries, including the preservation of seafood (Aboutorab *et al*., 2021). Microbial growth is the main concern because of some microorganisms such as *S. aureus* and *E. coli*, which can potentially lead to foodborne illness (Bahrami *et al.*, 2019). Based on a research, herbal essential oils and extracts, such as saffron nano-emulsion have antioxidant, anti-fungi, and antibacterial properties and also prevent unwanted interactions in food products (Bolhassani, 2018; Khorshidian *et al.*, 2018).*Crocin, picrocrocin,* and *Safranal* are the main bioactive compounds of saffron and contain several health-beneficial features (Saroglu *et al.*, 2021).

In 2009, Nirmal and Benjakul (2009) indicated that the least *psychrophilic* bacterial count was observed in shrimps that were treated with 2% ferulic acid (Nirmal and Benjakul, 2009). Moreover, the extract's phenolics may chelate certain metal ions necessary for microbial development. Results from Abbasvali *et al.* (2016) showed that, in comparison to EEST and MEST, WEST had the highest total phenolic content. Bahrami *et al*. (2019) reported that using 10% essential oil nanoemulsion slows the rate of increase in the number of psychrophilic bacteria. Antioxidants are the molecules that scavenge free radicals caused by oxidative stress. Most of the antioxidants taken exogenously are phytochemicals achieved from plants (Sarangarajan *et al.*, 2017). They belong to several classes of secondary metabolites including flavonoids, carotenoids, isothiocyanate, and alkaloids (Dinkova-Kostova, 2008). These antioxidants are examined for their antioxidant potential. For determining the antioxidant capacity of natural substances, several in-vitro and in vivo methods have been developed. The choice of in vitro technique relies on whether an antioxidant molecule has a functional group and if the solvent can extract this molecule and demonstrate its antioxidant ability (Nanda and Madan, 2021). PV is another index for the lipid oxidation measurement of primary oxidation products. Shrimp are highly susceptible to microbiological and chemical damage due to their chemical composition (Goulas and

Kontominas, 2005). The presenceof phenolic compounds in natural products, as free radicals scavengers, declines the PV value.

In 2020, researchers investigated nanoemulsion of saffron (*Crocus sativus* L.) essential oil by ultrasonic homogenization and spontaneous emulsification for extending the shelf life of shrimp. Results revealed that the peroxide value in all samples rose over the study period. Nevertheless, samples treated with a 5% saffron nano-emulsion made by ultrasonic homogenization at 4°C had the lowest PV (Aboutorab*et al*., 2021). This finding validated the preceding work (Ahmadian *et al.*,2019). Scientists in 2020, revealed that the pH values of control shrimp were significantly higher than those treated with nano-emulsion of saffron essential oil either those produced by ultrasonic homogenization or spontaneous emulsification methods (Aboutorab *et al.*, 2021). However, the dissolution of CO_2 in shrimp samples may lead to an initial decrease in pH (Shamshad *et al.*, 1990). Additionally, compared to control groups, samples treated with nanoemulsion saffron exhibited superior qualities in our investigation. Several fish species, including red mullet and gold band goatfish, exhibited comparable findings under ice storage (Ozyurt et al.,2009). Results of TBA by *Aboutorab et al.* proved that the elevation of lipid peroxidation in samples depended on time which complied with data of PV (Aboutorab *et al.*, 2021). TBA is one of the general indicators to determine oxidative stress due to lipid oxidation (Jouki *et al.*, 2014). It has been shown that fish with a TBA value of 8 mg of malonaldehyde equivalents per kilogram of tissue is also healthy to ingest. The highest TBA value that indicates a good grade of fish that was cooled, frozen, or maintained with ice is 5 mg of malonaldehyde equivalents per one kg of tissue (Sallam, 2007). Our results described a maximum TBARS level of 3 mg MDA/kg. These consequences were similar to those reported previously (Arashisar *et al.*, 2004; Sallam, 2007; Ojagh *et al.*, 2010; Song *et al.*, 2011; Angiş and Oğuzhan, 2013).

Researchers showed that saffron includes antioxidant components, most notably crocin, as a unique carotenoid (Ahmed *et al.*, 2021). These compounds can decrease free radicals and therefore may have antioxidant activity (Assimopoulou *et al.*, 2005). Our results revealed that the concentration of saffron nano-emulsions was important in their antimicrobial or antioxidant activity. In a similar study, researchers (Aboutorab *et al.*, 2021) indicated that 5% saffron nano-emulsion had the best impact on the maintenance of shrimp quality and growth inhibition of *E. coli* and *S. aureus* after 14 days of storage. This value was attained in accordance with the 6.17 Log cfu/g equivalent, which is a suitable level for human consumption, demonstrating the considerable effect of 10% essential oil nano-emulsion in controlling the psychrophilic bacteria (Bahrami *et al.*,2019). This research is

in line with those of other researchers (Ojagh *et al.*, 2010). In our result, the lowest psychrophilic count was observed for the 3% and 5% saffron nanoemulsions. By increasing the concentration of Shirazi thyme essential oil nanoemulsion in carp fillet, the amount of chemical indicators (pH, N-TVB, TBA, PV, FFA) decreased (p<0.05). Also, the microbial load in all treatments (except in the nanoemulsion treatment 1.5%) on the 15th day, the percentage of essential oil of Shirazi thyme exceeded 10 Log cfu/g (permissible limit of 7). In terms of smell and texture, two nanoemulsion treatments of 1% and 1.5% of essential oil of Shirazi thyme had higher quality. In all four treatments, the color index was in the condition It was bright and brown (Taheri *et al.*, 2013).

Abbasvali *et al.* (2016) investigated the effects of saffron tepal extracts on the sensory properties of pacific white shrimp during iced storage. More acceptable odor and texture of samples treated with saffron tepal extracts or sodium metabisulfite (SMS) were connected with the lower microbial load in those samples, improving the sensory properties of treated shrimps after extended storage (Abbasvali *et al.*, 2016).

This research demonstrated the effectiveness of saffron essential oil nano-emulsions in protecting shrimps for 14 days, particularly those produced at 5% concentration and using the ultrasonic homogenizer method. From the perspective of food safety control, and the consumer health viewpoint, using natural additives, such as saffron extract or other plant phenolic compounds offers a safer way to optimize the storage stability of shrimp, compared to synthetic additives. Because of the proper antibacterial and protective features of essential oil and nano-emulsions of saffron, it could be applied as a natural protective resource in the food industry.

Based on previous studies, by increasing the concentration of Shirazi thyme essential oil nanoemulsion in carp fillets, the level of chemical parameters (pH, N-TVB, TBA, PV, FFA) decreased (p<0.05). Furthermore, the microbial load in all treatments except in the nanoemulsion treatment 1.5 on the 15th day, and the percentage of essential oil of Shirazi thyme exceeded 10 Log cfu/g (permissible limit of 7). In terms of smell and texture, two nanoemulsion treatments of 1% and 1.5% of essential oil of Shirazi thyme had higher quality. In all four treatments, the color index was in the condition It was bright and brown.

To sum up, the size of nanodroplets was observed in a significant range (less than 100 nanometers). With the help of nanoemulsion, the shelf life of *P. semisulcatus* increased up to 32 days. Moreover, saffronnanoemulsion was able to keep organoleptic properties at a high level. Also, with the conversion of saffron essential oil into nanoemulsion, a conversion factor of 4 was reported.

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