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**Active exopolysaccharides based edible coatings enriched with red seaweed (*Gracilaria gracilis*) extract to improve shrimp preservation during refrigerated storage**

*Running Title:* Microalgae exopolysaccharides based edible coatings in seafood preservation

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**Abstract**

Active edible coatings from microalgal exopolysaccharides (EPS) enriched with different concentrations of red seaweed extract (RSE) (0.5, 1 and 1.5% (w/v)) were developed and their effects on shrimp quality with refrigerated storage ( $4 \pm 1$  °C) were studied over a period of 8 days. Untreated and treated shrimp were regularly evaluated bacteriologically (total viable bacteria, psychrotrophic bacteria and *Enterobacteriaceae* counts), chemically (pH, TVB-N, TMA-N, PV, TBARS), texturally and sensorially. The coating of a mixture of EPS and RSE, significantly reduced ( $p < 0.05$ ) TVB-N, TMA and TBARS values. With storage, EPS coatings containing RSE at 1 and 1.5% were most effective in the inhibition of the enumerated bacterial species, especially psychrotrophic bacteria. Also, EPS + RSE coated samples had lower polyphenol oxidase activity and better oxidative stability ( $p < 0.05$ ) until the end of shelf life. Generally, EPS coatings significantly improved hardness and color of shrimp. Moreover, sensory evaluation showed that the initial sensory attributes of shrimp were not significantly affected with any treatment. Overall, the results showed that the application of EPS-based coating treatments led to a quality improvement and extended shelf life of refrigerated shrimp.

**Keywords:**

White shrimp; *Penaeus vannamei*; Exopolysaccharides; Red seaweed; *Gracilaria gracilis*.

## **1. Introduction**

Shrimp are one of the most commercialized seafood products because of their high nutritional and economic value (Li et al., 2013). However, shrimp are highly perishable and they start quality deterioration immediately after death owing to their high water content and low-molecular-weight metabolites, such as free amino acids and nucleotides, resulting from hepatopancreas autolysis (Gokoglu & Yerlikaya, 2008; Nirmal & Benjakul, 2009a). During storage, several biochemical, microbiological and physical modifications have a direct influence on the nutritional quality of shrimp. In addition, microbial proliferation led to several sensory alterations. Moreover, pathogenic organisms that are naturally present in the environment or that result from cross-contamination can become established in shrimp and thus become serious health problems.

One of the biggest problems is melanosis (black spots) development, a dark pigment that is due to polyphenoloxidase that oxidizes phenolic compounds into quinones (Nirmal & Benjakul, 2011) even during cold storage. Shrimp usually have limited shelf life due to melanosis formation; although its presence is harmless to consumers, but it causes reduction in market value and consumer acceptance (Nirmal & Benjakul, 2009a).

To maintain product quality, the post-harvest procedures currently used, still include the addition of chemical preservatives such as metabisulfite and 4-hexylresorcinol (Galvão et al., 2017). Nevertheless, there are many concerns and safety issues about the use of sulfites as seafood additives and their consumption. Sulfites can trigger asthma and other symptoms of allergic responses such as skin rashes and irritations in sulfite-sensitive people (WHO, 1999). Therefore, the search for new alternatives for prolonging shrimp shelf life without the addition of non-natural additives might be beneficial.

Bioactive edible films and coatings containing natural antioxidants and antimicrobial agents have been successfully produced and used to enhance the quality of fresh food products

(Alotaibi & Tahergorabi, 2018; Alparslan & Baygar, 2017; Alparslan et al., 2016). Several natural biodegradable biopolymers from proteins, lipids, polysaccharides and their derivatives have been used to develop bioactive edible films and coatings. Particularly, polysaccharide-based edible films and coatings are considered to be amongst the most efficient for food preservation (Gennadios et al., 1997). Several recent studies have shown the feasibility of using these bioactive edible films and coatings to prolong the chilled storage of shrimp (Alotaibi & Tahergorabi, 2018; Alparslan & Baygar, 2017; Alparslan et al., 2016; Farajzadeh, et al., 2016).

Many studies have focused on the polysaccharides of plant and animal origins and only a few studies are based on hydrocolloids from algae. Bico et al. (2009) mentioned that sulfated polysaccharides extracted from red edible seaweeds called carrageenan are most commonly used in coating of fruits and vegetables. The application of this biopolymer as an active edible coating of minimally processed apples has shown a significant reduction in melanosis and effective color retention during storage (Lee et al., 2003). Also, microalgal exopolysaccharides (EPS) may be used as coatings for various food products because they are able to form films, and offer certain beneficial health effects, since they are distributed as biofunctional agents in foods and pharmaceuticals (Raposo et al., 2013).

*Porphyridium* sp. EPS can be characterized by their fluid-dynamic behavior and they can produce highly viscous solutions at relatively low polymer concentrations, with a wide range of pH and temperatures (Arad & Levy-Ontman, 2010). Besides their unique rheological properties, the red microalgal EPS showed numerous biological and physiological effects including antibacterial (Raposo et al., 2014), anti-oxidation (Tannin-Spitz et al., 2005), anti-inflammatory and anti-irritating (Matsui et al., 2003) activities. Bertrand et al. (2015) reported the positive impact of *Porphyridium cruentum* EPS coating on polyphenol oxidase activity

and color of banana slices during cold storage. But its use in edible coatings has not been reported.

Enrichment of edible packaging with bioactive phytochemicals is an effective modern method to maintain the highest fish quality during storage (Ojagh et al., 2010). Aquatic organisms have been identified as a good source for new value-added products, particularly in the health and nutraceutical sectors (Qi et al., 2005). Marine microalgae are a potential source of compounds that may be beneficial.

The red seaweed (*Gracilaria gracilis*) has several biological activities, especially strong anti-oxidation activity (Francavilla et al., 2013). These authors claimed that this marine biomass had a higher concentration of total phenols and had a high radical scavenging ability, similar to the synthetic food additive butylated hydroxytoluene (BHT), used as the reference. A mixture of *Porphyridium* EPS in combination with red seaweed bioactive molecules might be useful to preserve shellfish products. Therefore, the present study aimed to develop a microalgal EPS-based edible coating with red seaweed (*G. gracilis*) extract and to investigate the effect of coating treatments on the physico-chemical characteristics, bacterial contamination and sensory properties of white shrimp (*Penaeus vannamei*) during refrigerated storage.

## **2. Material and methods**

### **2.1. EPS recovery and accelerated red seaweed extraction**

The *P. cruentum* microalga (strain 161 from the culture collection of algae at the University of Texas, Austin, TX, USA) were grown in batch mode within a tubular photo-bioreactor (PBR) with a capacity of 110 L at 25 °C inoculated from a prior culture in an airlift PBR (10 L). The PBR 110 L consists of a transparent polycarbonate cylindrical chamber (204 cm high × 33 cm in diameter) illuminated using a set of 11 white fluorescent light tubes (Sylvania Luxline plus F58W/860, Feilo Sylvania, Gennevilliers, France) placed parallel to the front

side of the PBR with the same height as the PBR. The incident photon flux was equal to 120  $\mu\text{mol}/\text{m}^2/\text{s}$  determined using a flat cosine quantum sensor (LI-250Q, LI-COR, Lincoln, NE, USA). A modified Bold's basal medium (BBM) was used for the cultivation. The fresh medium contained 15 g/L NaCl and 3.4 g/L of other nutrients (Table 1). The pH was regulated at 7.5 with automatic injection of pure ( $\geq 99.7\%$ )  $\text{CO}_2$  (Air Liquide, Paris, France). Directly after harvesting, the culture was stored for 24 h in the dark at ambient temperature ( $20 \pm 1$  °C), to release a part of the bound EPS. Then, microalga cells were separated from the culture medium using a Sorvall RC6 Plus centrifuge (Thermo Fisher Scientific Inc, Illkirch-Graffenstaden, France) at  $17,600 \times g$  for 25 min at 4 °C.

EPS from the crude cell-free medium of the marine red microalga was concentrated and diafiltrated at the Génie des Procédés-Environnement-Agroalimentaire (GEPEA) Laboratory located at Saint Nazaire in France (Balti et al., 2018). Cross-flow microfiltration was used to concentrate the cell-free medium. The operation was done using a Microlab40 pilot plant provided by VMA Industries (Meung-sur-Loire, France) equipped with a 0.032  $\text{m}^2$  tubular ceramic membrane (Inside CéRAM, Cell 60, 7 channels, TAMI Industries Co., Nyons, France) having a mean pore diameter and water permeability equal to 0.14  $\mu\text{m}$  and  $75 \pm 6$   $\text{L}/\text{h}/\text{m}^2/\text{bar}$ , respectively. The filtration was carried out at 20 °C using a fixed transmembrane pressure equal to 4 bars according to the procedure described by Balti et al. (2018).

A different red seaweed, *G. gracilis* was collected from the Bizerte Lagoon, in the North of Tunisia. About 3 kg of wet biomass were sampled in November and December 2017. The collected samples were packed in polyethylene bags and transported to the laboratory within 2 h. Upon arrival, algal biomass was washed with tap water and their epiphytes were removed by hand. Then, the fresh seaweed was placed in a freezer (-20 °C) for a maximum of one wk immediately after collection. The cleaned seaweed was lyophilized in a Christ Alpha 2-4

LDplus freeze-dryer (Martin Christ Co., Osterode am Harz, Germany) for 2 days under 0.05 mbar vacuum and then ground to a fine powder using a blender (Knife Mill Grindomix GM 200, Retsch, Haan, Germany) and stored in airtight glass jars in a refrigerator at 4 °C for a maximum of two wk. Polyphenol compounds were extracted from this red macroalga using an accelerated solvent extractor (ASE 200, Dionex, Sunnyvale, CA, USA) according to the procedure described by Balti et al. (2017). Absolute ethanol ( $\geq 99.8\%$ ) was used as the solvent and the ethanolic extract was obtained with optimal conditions (115 °C and 15 min). The red seaweed extract (RSE) was covered with aluminum foil and stored at -18 °C for a maximum of one wk.

## **2.2. Preparation of edible coating solutions**

EPS coating solutions were prepared using the method of Bertrand et al. (2015) with slight modifications. Aqueous solutions of 0.5% (w/v) were prepared by dissolving 0.5 g EPS in 100 mL sterilised deionised water. Deionized water was obtained from a commercial deionizer (Culligan Co., Northbrook, IL, USA) with specific resistivity of 17.25 M $\Omega$ ·cm at 25 °C. A mixture of 1.5 mL glycerol (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and 50  $\mu$ L sunflower oil (Rewe Bio, Rewe Markt GmbH, Köln, Germany) were added as plasticizers. After stirring for 5 min, red seaweed extracts (RSE) were also incorporated into the prepared coating solution at different concentrations (0.5-1.5% (w/v)).

## **2.3. Application of active EPS and EPS/RSE coating solutions**

Whole white shrimp (*P. vannamei*) (50-55 shrimp/kg) were purchased within one day of harvest from a local seafood market in Beja, Tunisia and identified using the FAO species identification key (Isabel & Kensley, 1997). The shrimp were kept in ice with a shrimp/ice ratio of 1:2 (w/w) and transported to the laboratory in iceboxes within 30 min. Upon arrival, shrimp were washed in tap water and stored in ice until used (not more than 3 h).



Five treatment groups consisting of one uncoated control (Group 1) and 4 groups of whole shrimp treated with EPS or EPS/RSE coating solutions enriched with different concentrations (0-1.5%) of RSE were dipped for 15 min at 4 °C: Group 2 (coated with EPS), Group 3-5 (coated with EPS + 0.5, 1 and 1.5%, respectively of RSE). After dipping, they were allowed to drain for 5 min on a pre-sterilized metal net under a biological containment hood. After draining, all treated shrimp were placed into open sterile Petri dishes (one/Petri dish) and stored at 4 °C for 8 days.

## **2.4. Chemical analyses**

### *2.4.1. Determination of pH value*

The pH of shrimp were determined using the method of Lopez-Caballero et al. (2007) with slight modifications. Shrimp meat (5 g) were homogenized with 50 mL of deionized water for 1 min using an Ultra-Turrax (IKA T25-Digital Ultra-Turrax, Staufen, Germany) at maximum speed and pH of the homogenate was measured using a digital pH-meter (Sartorius, Gottingen, Germany).

### *2.4.2. Determination of total volatile base (TVB-N) and trimethylamine (TMA-N) contents*

For determination of TVB-N and TMA-N, shrimp extracts were prepared by homogenizing 100 g with 200 mL 7.5% (v/v) trichloroacetic acid (TCA) (Merck, Darmstadt, Germany) in the laboratory homogenizer for 1 min at maximum speed. The homogenate was centrifuged at 946 x g (3000 rpm in a angle rotor 1016, MIKRO 22R refrigerated centrifuge, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) at 4 °C for 5 min and the supernatant was then filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, Kent, UK). TVB-N content was measured using steam-distillation of the TCA-shrimp extract, using the modified method of Malle and Tao (1987). Twenty-five mL of the filtrate were loaded into a Kjeldahl-type distillation tube, followed by 5 mL 10% (w/v) NaOH solution. Steam-distillation was done using a vertical steam-distillation unit (Vapodest 30s, Gerhardt,

Königswinter, Germany), and the distillate was received into a beaker containing 15 mL of 4% (v/v) aqueous boric acid solution brought to a final volume of 50 mL. The titration was done using 0.05 M sulphuric acid using an automatic titrator (DL 25 Titrator, Mettler-Toledo AG, Greifensee, Switzerland) equipped with stirrer and pH electrode. The same experimental procedure was used for the TMA-N content measurement (Malle & Poumeyrol, 1989). The only difference was the addition of 20 mL 35% (v/v) formaldehyde to the distillation tube to block the primary and secondary amines, whilst leaving only the tertiary amines to react. The amount of TVB-N and TMA-N were calculated from the volume of 0.05 M sulphuric acid used for titration and the results were reported as mg N/100 g of shrimp.

#### *2.4.3 Determination of thiobarbituric acid reactive substances (TBARS) and peroxide value (PV)*

TBARS values as mg of malonaldehyde (MDA)/kg was determined colorimetrically using the method reported by Tarladgis et al. (1960). Briefly, 10 g of sample was added to 97.5 mL distilled water and 2.5 mL 4 N HCl followed by homogenization and distillation as above. Subsequently, 5 mL of the distillate was added to 5 mL 0.02 M thiobarbituric acid (TBA) (Sigma-Aldrich Corp., St. Louis, MO, USA), heated in a water bath at 100 °C for 35 min and cooled. The color was measured at 528 nm using a UV mini-1240 spectrophotometer (Shimadzu Corp., Kyoto, Japan). Prior to PV analysis, lipids were extracted using the Bligh and Dyer method (Bligh & Dyer, 1959). Briefly, the PV were measured from the lipid extracts using AOCS official method Cd 8-53 (AOCS, 1994) and the results were reported as meq O<sub>2</sub>/kg of lipids.

#### **2.5. Texture and color analyses**

Texture analysis was done using a texture analyzer TA-XT2 (Texture Technologies Corp., Scarsdale, NY, USA). Measurements were done using the TPA test mode as described by Tahergorabi et al. (2012). Samples of a single raw coated shrimp were subjected to two-cycle

compression to 50% compression using the texture analyzer with a 70-mm TPA compression plate attachment moving at a speed of 127 mm/min. From the resulting force-time curves, hardness, springiness, cohesiveness, gumminess, chewiness, and resilience were determined according to the definitions of Bourne (2002) using the software provided with the instrument.

The color parameters ( $L^*$ ,  $a^*$  and  $b^*$ ) were evaluated using a Minolta Chroma Meter CR-400/410 colorimeter (Konica Minolta Co. Ltd., Osaka, Japan) with an 8 mm diameter measuring area. The instrument was standardized using the “C” illuminant condition according to the CIE (Commission International de l’Eclairage of France) using a white calibration plate ( $L^* = 97.57$ ,  $a^* = -1.08$  and  $b^* = 1.25$ ) supplied by the manufacturer. Three readings for three different places along the surface, one each from the head, middle and tail of each sample were measured, and the average of these measurements was obtained.

## **2.6. Microbiological analyses**

Microbial quality was evaluated by enumerating three indicators of microbial contamination: total viable counts (TVC), psychotropic bacteria counts (PBC) and *Enterobacteriaceae* (EB). For each shrimp sample, 10 g was aseptically diluted with 90 mL 0.1% peptone water solution (Merck) and then homogenized for 1 min using a Stomacher (BagMixer 400, Interscience Co., Saint-Nom-la-Bretèche, France). The same diluting solution was used to prepare the respective serial decimal dilutions of each homogenate prior to microbiological analysis.

For TVC enumeration, 1 mL of the respective decimal dilution was spread on triplicates sterile plates of plate count agar (PCA, Merck) and incubated at 37 °C for 48 h. PBC were enumerated using the same procedure and culture medium after incubation at 7 °C for 10 days (FDA/BAM, 2009). Finally, EB were enumerated using the double layer technique on violet red bile agar (VRB Agar, Merck). The plates after streaking were overlaid with a new layer of

the same growth medium before incubation at 37 °C for 24 h (ICMSF, 1982). Microbiological counts were expressed as log<sub>10</sub> CFU/g, after averaging before the log conversion.

### **2.7. Measurement of polyphenol oxidase (PPO) activity**

The PPO enzyme extraction from shrimp was carried out similar to the protocol reported by Nirmal and Benjakul (2009a). Briefly, the enzyme activity was measured using the method described by Qian et al. (2014). Briefly, the reaction mixture was prepared containing 480 µL 0.03 M L-proline (Sigma Aldrich), 480 µL 0.03 M catechol (Sigma Aldrich) and 40 µL PPO enzyme extract. All reagents were prepared in 5 mM sodium phosphate buffer (pH 7). PPO activity was measured in triplicate at 530 nm after 5 min at 45 °C (Giménez et al., 2010). One unit of PPO activity was the amount of enzyme able to increase the absorbance 0.001/min of reaction (Wang et al., 1992). The results were shown as relative activity (%), i.e., the ratio of the PPO activities for each time of storage and the initial enzyme activity (440 ± 10 U/mL) expressed as a percentage.

### **2.8. Sensory analysis**

The sensory quality of the coated and uncoated shrimp was evaluated by >60 untrained male and female volunteer panelists (29 men, 43 women) from 24 to 38 years old, from the staff of the laboratory and the Higher Institute of Biotechnology of Beja at the University of Jendouba. The coated and control samples were identified with random three-digit codes and presented to panelists on clean stainless steel trays at room temperature. During sensory test, each panelist was asked to score each group (including 10 shrimp) of raw coated shrimp for several sensory attributes such as color, odor, texture (firmness) and overall acceptability. The scores of these parameters were based on a 9-point hedonic scale, in which 9 means “like extremely”, 5 means “neither like nor dislike” and 1 means “dislike extremely” (Tsironi et al., 2009).

## 2.9. Statistical analysis

Each experiment was replicated at least three times for three independent samples purchased at different times and a completely randomized design (CRD) was used. All data were statistically analyzed using the professional edition of the Statistical Package for the Social Sciences (SPSS) version 16.0 software package (SPSS Inc., Chicago, IL, USA) and expressed as means  $\pm$  standard deviation (SD). Analysis of variance and Duncan's multiple range tests were used to estimate the significant differences between means among various treatments. The value of  $p < 0.05$  was considered significant.

## 3. Results and discussion

### 3.1. pH changes

As shown in Fig. 1, shrimp initially had a pH value of  $\sim 7$ , which was close to the values reported for deep-water rose shrimp (*Parapenaeus longirostris*) (7.25) and whiteleg shrimp (*Litopenaeus vannamei*) (7.30) (Alparslan et al., 2016; Huang et al. 2012). But, it was markedly lower than those previously obtained with frozen shrimp (8.94-9.35) (Alotaibi & Tahergorabi, 2018). Group, feeding, fishing period, degree of stress and a possible phosphate treatment of shrimp before freezing can be factors responsible for this difference in pH.

pH is often used for the freshness evaluation of marine organisms since it is modified by microbial or enzymatic activities (Varlik et al., 2000). In general, pH values of all samples increased during storage, and the rate of increase of pH varied with different treatments ( $p < 0.05$ ) (Fig. 1). Post-mortem changes and degradation of shrimp quality is generally the result of the combined action of endogenous and microbial enzymes. These autolytic mechanisms produce a large amount of basic compounds and their accumulation leads to a significant increase in shrimp pH values during refrigerated storage (Nirmal & Benjakul, 2009b). The pH values of all the samples determined on the first day of refrigerated storage had no significant differences ( $p \geq 0.05$ ).

Shamshad et al. (1990) mentioned that pH increased as a function of duration and temperature and confirmed a positive correlation between this parameter and overall acceptability. The pH of crustaceans is generally used as a suitable indicator of freshness and its critical limit was fixed at 7.8 (Chung & Lain, 1979). Three levels of quality can be distinguished according to the pH value as follows: prime quality (<7.7), poor but acceptable quality (7.70-7.95) and unacceptable quality (>7.95) (Marshall & Wiese-Leigh, 1997).

A pH value of  $7.70 \pm 0.04$  was obtained for untreated shrimp after 4 days of cold storage. Also, the pH value of the same sample after 6 days of storage was  $7.80 \pm 0.02$  and it can be considered that the end of the shelf-life of shrimp was reached. Moreover, significantly lower pH values were measured for coated shrimp with EPS at several levels of RSE, compared to those obtained for the control group. Especially, during storage the use of 1.5% RSE led to the greatest decrease of pH values of the coated samples. The mixture of EPS and RSE had a significant impact on the shrimp freshness compared to EPS alone. This may be because both natural compounds (EPS and RSE) could significantly slow down or block bacterial growth, and, therefore prevent protein hydrolysis, which helps to extend the shelf life of shrimp samples.

### **3.2. Variation in TVB-N and TMA-N**

The TVB-N level is still considered a useful index of the microbiological quality deterioration of different fresh and lightly preserved seafoods. The volatile bases are usually formed from the post-mortem enzymatic degradation (decarboxylation) of amino acids (Botta, 1994; Ozoğul & Ozoğul, 2000). TVB-N levels of treated shrimp through 8 days of storage are shown in Fig. 2a. The initial value of TVB-N in shrimp was  $8.3 \pm 0.6$  mg N/100 g which was close to the values reported for *L. vannamei* (8.8 mg N/100 g) (Li et al., 2016) and (7.9 mg N/100 g) (Huang et al., 2012), but lower than those reported for *P. longirostris* (17.1 mg N/100 g) (Alparslan et al., 2016), *L. stylirostris* (29.1 mg N/100 g) (Dalila et al., 2015) and

*P. borealis* (33.5 mg N/100 g) (Zeng et al., 2005). Some other factors such as catching season and region, species, growth stage and handling process or storage prior to analysis influence the TVB-N contents of seafood products may partly explain the differences in TVB-N values observed in this study.

As shown in Fig. 2a, higher TVB-N values were obtained in all shrimp samples during storage. However, this variation differed greatly across the coating treatments used and also the untreated shrimp. From the second day of storage, a significant increase of TVB-N contents was obtained in untreated shrimp.

The limit of TVB-N values with seafood products of good sensory quality has been reported to be 30 mg N/100 g (Zeng et al., 2005). On the other hand, this limit is sometimes ignored for shrimp species since the majority of the reported TVB-N contents of fresh crustaceans far exceed this limit (Aşık & Candoğan, 2014; Oehlenschläger, 1997). Compared to control samples, a slower increase of TVB-N of shrimp coated with EPS at different concentrations of RSE was observed. Also, lower TVB-N were found in all coated shrimp than in the uncoated samples during storage ( $p < 0.05$ ), whereas TVB-N of the shrimp treated with EPS was very comparable to those obtained for EPS + 0.5% RSE group ( $p \geq 0.05$ ).

Smaller amounts of TVB-N were measured in EPS + 1.5% RSE group. The current study showed that EPS coatings enriched with RSE effectively reduced ( $p < 0.05$ ) the levels of TVB-N during refrigerated storage. This was possibly due to their antimicrobial effect and subsequently to their preventing protein decomposition by bacteria and hindering their capacity for oxidative deamination of nitrogenous compounds from non-protein sources (Fan et al., 2008). Recently, Alparslan et al. (2016) reported that an edible gelatin coating mixture with orange essential oil significantly decreased TVB-N value of deep water pink shrimp.

Generation of TMA in seafood during refrigerated storage is frequently used as an index of bacterial growth and its content provides some informations about the quality degradation of

product. Essentially, this substance is produced through the decomposition of trimethylamine-oxide by bacteria or enzymes during unsuitable storage conditions (Botta, 1994). Fig. 2b shows TMA-N changes of treated shrimp during cold storage period. Initially, a TMA-N of  $0.32 \pm 0.12$  mg N/100 was found which is similar to the value (0.33 mg N/100 g) reported for Pacific white shrimp (*L. vannamei*) (Okpala et al., 2014). No TMA-N was found at day 0 in the control samples of shrimp stored in crushed ice (Nirmal & Benjakul, 2009a).

A significant increase ( $p < 0.05$ ) in TMA levels was observed for all shrimp groups during the storage period. The TMA-N of untreated shrimp differ significantly ( $p < 0.05$ ) from those obtained for other treatments. The results showed a slight increase ( $p < 0.05$ ) of TMA-N in samples coated with EPS enriched with higher concentrations (1 and 1.5%) of RSE during the storage period (Fig. 2b). For TMA-N content, 5 mg N/100 g in shrimp has been used as an acceptable limit (Cobb et al., 1976). In this study, TMA-N values for coated shrimp did not exceed the fixed limit during the cold storage period. The ammonia is generally the major degradation product in crustaceans and the trimethylamine oxide is present in small quantities (Yeh et al., 1978).

### **3.3. Change in TBARS and PV**

Generally, lipid oxidation is induced during processing and storage of crustaceans (Chaijan, 2011; Morrissey et al., 1998). PV is the parameter commonly used for evaluating the initiation of oxidation process and the quantification of hydroperoxides of fatty acids as primary products of lipid oxidation (Chaijan, 2011). Usually, TBA values are used as important quality parameter for seafood oils for lipid peroxidation monitoring with malondialdehyde (MDA) concentration. MDA is an end product of the primary hydroperoxides during the oxidative decomposition of unsaturated lipids (Fernandez et al., 1997).



Fig. 3 shows the changes of PV and TBARS values of treated shrimp (*P. vannamei*) with different coating solutions through a refrigerated storage period of 8 days. The initial PV was  $1.4 \pm 0.2$  meq O<sub>2</sub>/kg which was consistent with the  $1.6 \pm 0.3$  meq O<sub>2</sub>/kg by Okpala et al. (2014). A significant ( $p < 0.05$ ) increase with the storage time of PV was measured in coated and uncoated shrimp. Furthermore, all PV values measured for untreated shrimp were significantly higher ( $p < 0.05$ ) than those for treated groups. No PV changes ( $p \geq 0.05$ ) were measured within the uncoated shrimp at the beginning of the storage period. A rapid and continuous increase of this quality index was observed from the second day to reach its maximum ( $9.4 \pm 0.4$  meq O<sub>2</sub>/kg) at the end of the refrigerated storage period ( $p < 0.05$ ). The release of degradation products of polyunsaturated fatty acids such as peroxide or hydroperoxide of lipid peroxidation over the course of cold preservation of shrimp can explain the apparent increase in PV values. Autoxidation is a direct reaction of molecular oxygen with organic compounds, especially unsaturated fatty acids. Initiation of oxidation corresponds to the formation of fatty acid free radicals by homolytic disruption of a hydrogen atom adjacent to a double bond to generate hydroperoxides (Nirmal & Benjakul, 2009b).

Among all samples, shrimp coated with EPS + 1.5% RSE significantly showed the lowest PV throughout the storage ( $p < 0.05$ ). Based on the determination of peroxide values, Ludorff and Meyer (1973) suggested a simple classification of the freshness of seafood oils: very good (PV < 2 mmol of O<sub>2</sub>/kg), good (PV 2–5 mmol of O<sub>2</sub>/kg), acceptable (PV 5–8 mmol of O<sub>2</sub>/kg) and spoiled (PV 8–10 mmol of O<sub>2</sub>/kg).

Untreated shrimp had an estimated level of PV of  $8.1 \pm 0.2$  meq O<sub>2</sub>/kg on the seventh day of storage, indicating that these samples reached the alteration stage. However, all peroxide values obtained for coated shrimp (*P. vannamei*) with EPS + RSE remain below the spoilage limit mentioned above at all the refrigerated storage times.

The results showed that the association between microalgae EPS and seaweed bioactive compounds is indeed capable of delaying the generation of certain oxidation products such as peroxides in refrigerated shrimp. Also, the formation of the EPS films on the shrimp surface reduces the transfer of gases as well the antioxidant potentialities of both EPS and RSE could explain the protective effect during storage.

A gradual increase in TBARS levels of uncoated shrimp was obtained from the second day until the end of the storage period ( $p < 0.05$ ). Initially, the level of TBARS was estimated to be  $0.3 \pm 0.0$  mg MDA/kg and then this value increased significantly ( $p < 0.05$ ) in untreated shrimp to reach  $1.8 \pm 0.1$  mg MDA/kg on the eighth day of storage (Fig. 3b). The loss of some water quantities of samples as well the increase of the oxidation degree of polyunsaturated fatty acids are probably the two main factors responsible for the dramatic increase in TBARS values during storage. Thereafter, EPS + RSE coated samples had a significantly lower ( $p < 0.05$ ) TBARS values compared to those measured in the shrimp batch used as a control, whereas EPS and EPS + 0.5% RSE groups showed no significant difference ( $p \geq 0.05$ ) of this freshness index. The application of an active coating blocks or delays the rate of passage of oxygen, water molecules and other volatile compounds, thereby slowing the spread of the lipid peroxidation process.

Among all samples, EPS + 1.5% RSE coated samples showed lower TBARS values, compared to other coated shrimp groups for all cold storage days ( $p < 0.05$ ) (Fig. 3b). Also, TBARS and PV levels changed in the same way during the cold storage period (Fig. 3a). The results showed that the use of EPS coating solutions enriched with 1 or 1.5% RSE for shrimp induces more better oxidative stability compared to other solutions.

Generally, seafood products with a TBARS content that does not exceed 5 mg MDA/kg are considered of good quality, except that this limit is commonly extended to 8 mg MDA/kg and these products are still considered safe and appropriate to eat (Yildiz, 2015). The results

showed that TBARS levels for uncoated and coated shrimp did not exceed in any case the lower reference (5 mg MDA/kg) (Alotaibi & Tahergorabi, 2018; Sharaf Eddin & Tahergorabi, 2017). Indeed, the lowest TBARS levels were found in shrimp treated with EPS with or without RSE.

These results were consistent with those reported by Li et al. (2016), who mentioned that using an edible coating rich with polyphenols and polysaccharides from coldwater seaweed (*Porphyra yezoensis*) could significantly protect the oil and could prolong the shelf-life of shrimp by 3-4 days compared with the untreated group. The application of potato starch-based edible coating solutions containing different concentrations of thyme essential oils were able to preserve the sensory quality and to extend the shelf-life of refrigerated shrimp (Alotaibi & Tahergorabi, 2018).

#### **3.4. Change in PPO activity**

Undesirable enzymatic browning mediated by PPO on the shrimp shell post harvest, which greatly reduces the market value and consumers acceptability of these seafood products. Indeed, crustaceans and particularly shrimp are prone to develop quickly melanosis at the cephalothorax as soon as the cell integrity is affected, especially during processing and storing. The melanosis process is mainly catalyzed by an enzymatic complex called PPO, which oxidizes phenolic compounds to quinones in the presence of molecular oxygen. Subsequently, these quinones polymerize and give dark pigments (Nirmal et al., 2015).

Fig. 4. shows the variation of the PPO activities in the uncoated and coated shrimp groups through cold storage period. On the fourth day of storage, the relative PPO activity of untreated shrimp reached its maximum ( $92 \pm 2\%$ ) and then decreased progressively until the end of the storage period ( $74 \pm 3\%$ ). PPO activity of EPS coated shrimp showed a tendency to decrease significantly through refrigerated storage (Fig. 4). As shown in Fig. 4, the PPO enzymatic activities measured in the EPS group and uncoated shrimp (control group) did not

show any significant differences ( $p \geq 0.05$ ). Whereas, the inhibition of enzyme activities in the EPS with 1 and 1.5% RSE had greater PPO activities of 56.3 and 45.6% at the end of the storage period, respectively. PPO activities levels were significantly weaker ( $p < 0.05$ ) than those determined in untreated shrimp, which shows the effectiveness of these bioactive coatings in the inhibition of the melanosis formation.

The results are consistent with those of Li et al. (2016) who showed that active coating solution containing algae polysaccharides and ethanol extract from coldwater seaweed (*P. yezoensis*) could effectively inhibited PPO activities of the refrigerated shrimp (*L. vannamei*). Several factors are involved in the enzymatic browning development: PPO enzyme which catalyzes the reaction, substrates, oxygen, phenolic compounds and finally the products of the primary (quinones) and secondary (polymerization products) reactions that are responsible for the coloration. Therefore, the methods of inhibition or prevention of enzymatic browning involve essentially three categories depending on whether they affect the PPO activity, the substrates or the products of the reaction (Gokoglu & Yerlikaya, 2008). Other methods that can simultaneously affect two factors of melanosis are still possible.

The EPS from marine red microalga (*P. cruentum*) and carrageenan coatings are able to effectively inhibit PPO activities and therefore delay the melanosis formation on the surface of banana slices (Bertrand et al., 2015). Also, Basiri et al. (2015) showed that the immersion of shrimp in pomegranate peel methanolic extract significantly reduced the enzymatic browning during the refrigerated storage.

### **3.5. Color changes**

Color is among the most useful criteria in assessment of the quality level of fishery products. Haard (1992) reported that the initial color of fishery products changed during cold storage affecting the quality. In general, the color of the outer edible coating or film has a great influence on consumer decision-making processes. The effect of coating on color parameters

of shrimp during storage is shown in Table 2. A significant decrease ( $p < 0.05$ ) in lightness values ( $L^*$ ) during storage time was observed mainly in untreated shrimp compared to other treatments. A decrease in  $L^*$  value may be considered as indicative of browning. The lightness value of uncoated shrimp was estimated to be 18.4 at the end of storage time, resulting in a loss of commercial value and sensory properties of seafood product.  $L^*$  intensities obtained on coated samples with EPS + 1 and 1.5% RSE were 25.7 and 28.7, respectively, after 8 days of storage at 4 °C. The use of bioactive coatings based on an association between EPS and RSE affects the lightness of seafood product. These results showed an effect of EPS + RSE coating on the inhibition of PPO activity of white shrimp during cold storage. Alparslan et al. (2016), indicated that the  $L^*$  values of shrimp (*P. longirostris*) coated using a preparation of gelatin and essential oils of orange leaves were found to be higher than those of control sample during storage.

During storage, a significant increase ( $p < 0.05$ ) in redness ( $a^*$ ) values was also measured in shrimp treated with EPS and RSE coatings (Table 2). The results highlighted that the change in the red color of the white shrimp cephalothorax occurs simultaneously with the process of formation of melanosis. Redness intensity of untreated shrimp increased significantly ( $p < 0.05$ ) and reached 3.3 on the eighth day of storage. From point of view melanosis inhibition, EPS coatings combined with RSE had a weaker capacity to avoid the development of red color in white shrimp.

As shown in Table 2, yellowness variation of treated white shrimp by various coating solutions showed several fluctuations during storage. In addition, there is a drastic drop in the yellowness value of control sample and it goes from 7.4 (day 1) to 1.4 (day 8). These values differ significantly ( $p < 0.05$ ) to those measured for others treatments groups. In comparison to uncoated group, the  $b^*$  value of shrimp coated only with EPS decreased slowly and reached 1.9 at the end of the storage period. Nevertheless, when coated with EPS containing 1.5%

RSE, the  $b^*$  value increased to reach a high intensity estimated to 8.6 after 5 days of storage. Finally, yellowness level was diminished to 5.4. This rapid decrease of yellowness intensities could be explained by the accelerated formation of dark browning pigments towards the end of the storage time. Similarly, Alotaibi and Tahergorabi (2018) observed a significant increase in the  $b^*$  value of coated shrimp during refrigerated storage when using an active starch-based edible coating solution.

### **3.6. Bacteriological evaluation**

Seafood products are the source of bacterial growth which, without being a health risk for the consumer, often leads to premature deterioration (sensory defects) even before the expiry date. In addition, the microbiological risk of fishery products is mainly reflected in their ability to cause food poisoning. Changes in microbial flora of coated and uncoated shrimp during refrigerated storage are shown in Fig. 5.

A relatively low level of TVC ( $2.4 \pm 0.2 \log_{10}$  CFU/g) was obtained for the starting raw material (Fig. 5a). This value is very close to that observed by Carrión-Granda et al. (2016) who suggested that this is correlated to a correct handling of the product onboard. Generally, TVC levels of all shrimp groups increase significantly ( $p < 0.05$ ) during a cold storage period of 8 days. The International Commission on Microbiological Specifications for Foods (ICMSF, 1986) recommended that the microbiological limit of TVC level is  $7 \log_{10}$  CFU/g. As mentioned with Fig. 5a, a significant increase ( $p < 0.05$ ) in TVC level was obtained in the batch of untreated shrimp and from the sixth day of cold storage this value ( $7.3 \pm 0.2 \log_{10}$  CFU/g) slightly exceeds the damage limit. Increase of the TVC in samples coated with EPS containing RSE at different concentrations was slower compared to uncoated sample for all sampling days. TVC levels in coated samples with EPS + 1 and 1.5% RSE were 4.5 and 5.3  $\log_{10}$  CFU/g, respectively, at the last day of cold storage period. Also, TVC levels noted in uncoated shrimp differ significantly ( $p < 0.05$ ) from these two types of treatments throughout

the storage period. However, the TVC levels in the untreated lot showed no significant difference ( $p \geq 0.05$ ) compared to those with EPS and EPS + 0.5% RSE groups during storage. The results suggested that the association between *Porphyridium* EPS and RSE effectively inhibited bacterial proliferation, especially TVC. Recently, several studies on the evaluation of bacterial quality of shrimp during refrigerated storage were consistent with TVC levels being decreased with the use of polysaccharides based coating solutions (Alotaibi & Tahergorabi, 2018; Alparslan & Baygar, 2017; Li et al. 2016).

The most active microorganisms that alter refrigerated seafood products are psychrotrophic bacteria and sometimes these may be responsible for digestive disorders. At the beginning, PBC level of raw material is estimated to  $2.6 \pm 0.1 \log_{10}$  CFU/g (Fig. 5b). Similar psychrophilic bacterial counts have been reported by Alparslan & Baygar (2017) for deepwater pink shrimp (*P. longirostris*;  $2.5 \log_{10}$  CFU/g) and Dalila et al. (2015) for blue shrimp (*L. stylirostris*;  $2.6 \log_{10}$  CFU/g). In addition, a significant increase ( $p < 0.05$ ) in PBC level was obtained in the uncoated group and at the end of cold storage period, it went to  $7.3 \pm 0.2 \log_{10}$  UFC/g. This value exceeds  $7 \log_{10}$  CFU/g limit (ICMSF, 1986). PBC levels determined for shrimp only coated with EPS changed significantly ( $p < 0.05$ ) in the same manner as previously mentioned and did not exceed the allowed limit (Fig. 5b). However, PBC values of the EPS + RSE coated groups increased insignificantly ( $p \geq 0.05$ ) without exceeding the fixed limit at all cold storage times. Thus, the incorporation of RSE in EPS coatings can retard the psychrotrophic bacteria growth compared to untreated shrimp or coated only with EPS. Carrión-Granda et al. (2016) reported that PBC levels were reduced by about  $1 \log_{10}$  CFU/g when white shrimp (*P. vannamei*) were coated with chitosan with oregano essential oils compared to the control.

EB, in some food sectors, can be a useful index of the hygienic state of the product but they are not an indicator of the presence or absence of pathogenic microorganisms. Fig. 5c shows

the variation in EB levels of different treatment groups of refrigerated shrimp for a storage period of 8 days. Initially, EB level of raw material was  $1.6 \pm 0.1 \log_{10}$  CFU/g. EB level in the untreated sample increased with storage time (Fig. 5c). For the entire storage period, EPS + RSE groups had the lowest EB levels ( $p < 0.05$ ) in comparison with those determined for the untreated group. Shrimp treatment with EPS + 1.5% RSE effectively slowed the growth of this bacteria, followed by the coating containing EPS + 1% RSE ( $p < 0.05$ ). However, EPS alone showed a limited effect on inhibition of *Enterobacteriaceae*. All results obtained suggested that the coating of EPS and RSE effectively inhibits EB. Carrión-Granda et al. (2016) reported that coated whiteleg shrimp with chitosan based hydrogels enriched with plant aroma compounds were also able to significantly decrease EB.

Overall, the application of EPS enriched with RSE as active edible coatings is a promising alternative to extend the storage period of refrigerated seafood by minimizing the growth rate of several bacteria.

### **3.7. Texture properties**

Texture is an substantial physical parameters of shellfish, and modification of this characteristic can lead to possible alteration of product quality. Six textural parameters regarding coated and uncoated samples were evaluated using the TPA test and the results of this analysis are shown in Table 3.

Various hardness values were obtained within the different groups during the cold storage period ( $p < 0.05$ ). Generally, samples treated with an EPS-based coating solution had the highest hardness values compared to untreated shrimp ( $p < 0.05$ ). Shrimp coated with EPS + RSE showed the greatest hardness during the storage time. Higher hardness values were found in shrimp coated with EPS + 1.5% RSE, compared with those treated with EPS + 0.5 and 1% RSE, on the last day of storage ( $p < 0.05$ ). The results were consistent with the shrimp



coating with a mixture of EPS and RSE effectively contributed to the reduction of muscle softening during cold storage.

Benjakul et al. (1997) suggested that the deterioration of muscle texture is mainly due to the presence of active proteolytic enzymes in muscle and viscera or microbial proteases, especially those with collagenolytic activity. Most important hardness values of samples coated with EPS and RSE had the lowest levels of enumerated bacteria (Fig. 5). Degradation of muscle proteins is catalyzed by the proteolytic enzymes produced by spoilage organisms. The inhibition of the growth of these microorganisms by the RSE decreased with the consequent of increased muscular proteolysis process in the refrigerated shrimp. On the other hand, other textural parameters determined for untreated or treated shrimp did not differ significantly during the cold storage period (Table 3). The active coating based on EPS and RSE appears to have only a positive impact on the hardness of refrigerated shrimp. These results were consistent with previously results of Alotaibi and Tahergorabi (2018), who confirmed that the incorporation of thyme essential oil in shrimp coatings only enhanced hardness during refrigerated storage.

### **3.8. Sensory evaluation**

Changes in organoleptic attributes of different treatment groups before and at the end of storage period at 4 °C are shown in Table 4. Before storage, all groups had about the same levels ( $p \geq 0.05$ ) of quality based on color, odor, texture and acceptability.

Generally, the highest quality scores ( $p < 0.05$ ) were obtained in treated groups with active coating solutions containing EPS and RSE. Compared to other groups, the uncoated shrimp usually showed the lowest scores for all attributes on the last day of storage. These results could be explained by the appearance of a slight black pigmentation on the shrimp surface. Less browning was detected in all treated lots during cold storage. The use of the two concentrations 1 and 1.5% of RSE in EPS-based coating formulations resulted in the highest

sensory scores for all attributes on the eighth day of storage. The better sensory quality assessed for these groups correlated with a fairly low microbial level compared to uncoated shrimp. Furthermore, organoleptic evaluation data of the coated groups with only EPS and EPS + 0.5% RSE did not differ significantly between them ( $p \geq 0.05$ ). Therefore, the coating of white shrimp with EPS containing RSE better retained the organoleptic attributes of coated samples during refrigerated storage.

Similarly, Li et al. (2016) suggested that the application of coating solutions containing polysaccharides + polyphenols from *P. yezoensis* can preserve organoleptic quality more than the control leading to an extension of the storage period.

#### **4. Conclusion**

An active coating based on a combination of EPS + RSE was prepared and its inhibition of lipid peroxidation, protein decomposition and bacterial proliferation of shrimp stored in refrigerator measured. In addition, the possibility of organoleptic quality preservation of chilled shrimp using the EPS + RSE coating solution has been shown. Overall, shrimp coating with EPS + RSE mixture effectively extended the shelf life of shrimp and retained all sensory attributes while keeping bacterial levels relatively low. Therefore, microalgal EPS coating together with RSE may lead to an active coating that can be used as a safe preservative for refrigerated shrimp.

#### **Conflict of interest statement**

We confirm no conflicts of the interest concerning publication of this research.

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## Figure caption

**Fig. 1.** Changes in pH of coated and uncoated white shrimp during refrigerated storage. Each number is the mean of three samples taken from different experiments. Bars represent the standard deviation from triplicate determinations.

**Fig. 2.** Changes in TVB-N (a) and TMA-N (b) values of coated and uncoated white shrimp during refrigerated storage. Each number is the mean of three samples taken from different experiments. Bars represent the standard deviation from triplicate determinations.

**Fig. 3.** Changes in PV (a) and TBARS (b) values of coated and uncoated white shrimp during refrigerated storage. Each number is the mean of three samples taken from different experiments. Bars represent the standard deviation from triplicate determinations.

**Fig. 4.** Changes in PPO activity of coated and uncoated white shrimp during refrigerated storage. Each number is the mean of three samples taken from different experiments. Bars represent the standard deviation from triplicate determinations.

**Fig. 5.** Changes in total viable count (a), psychrotrophic bacteria (b) and *Enterobacteriaceae* (c) of coated and uncoated white shrimp during refrigerated storage. Each number is the mean of three samples taken from different experiments. Bars represent the standard deviation from triplicate determinations. Different capital letters on the bars within the same treatment indicate significant differences ( $p < 0.05$ ). The different lower case letters on the bars within the same storage time indicate significant differences ( $p < 0.05$ ).

## Figures

Fig. 1.

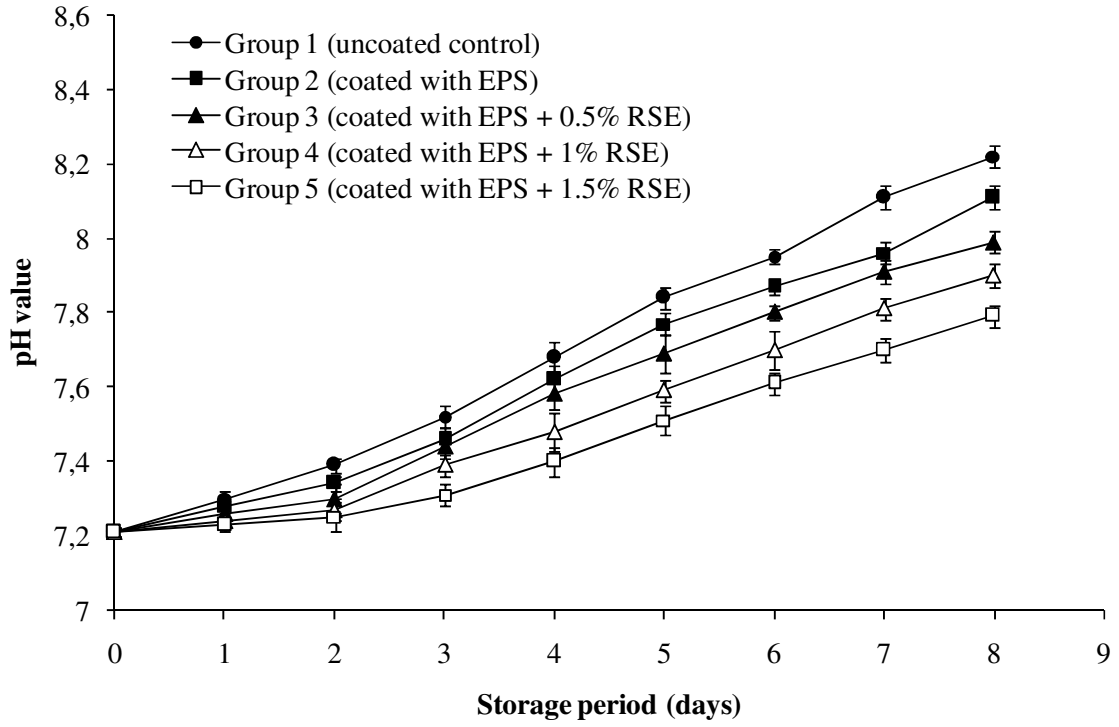
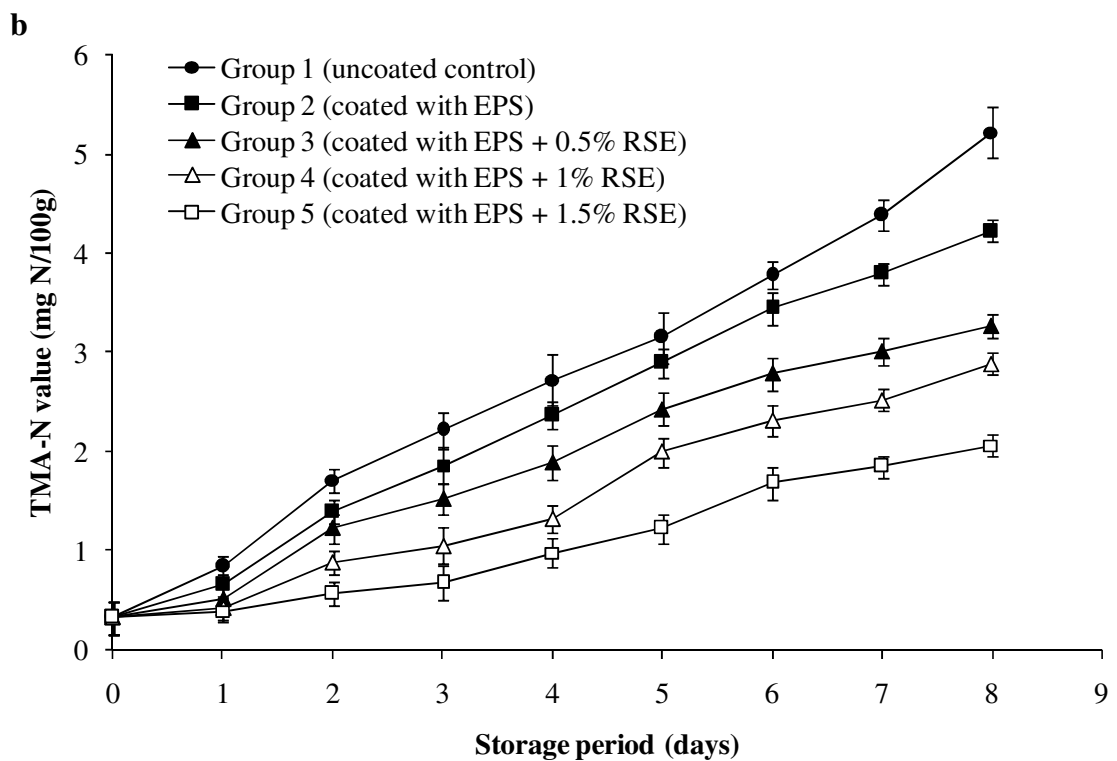
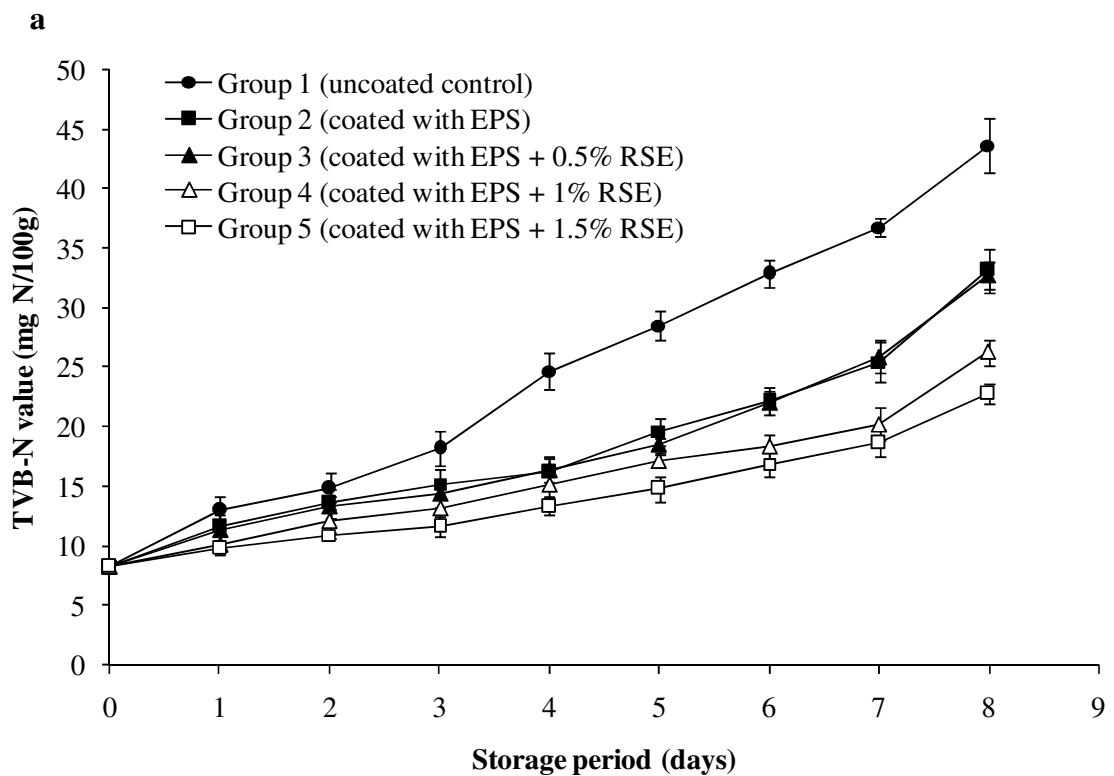
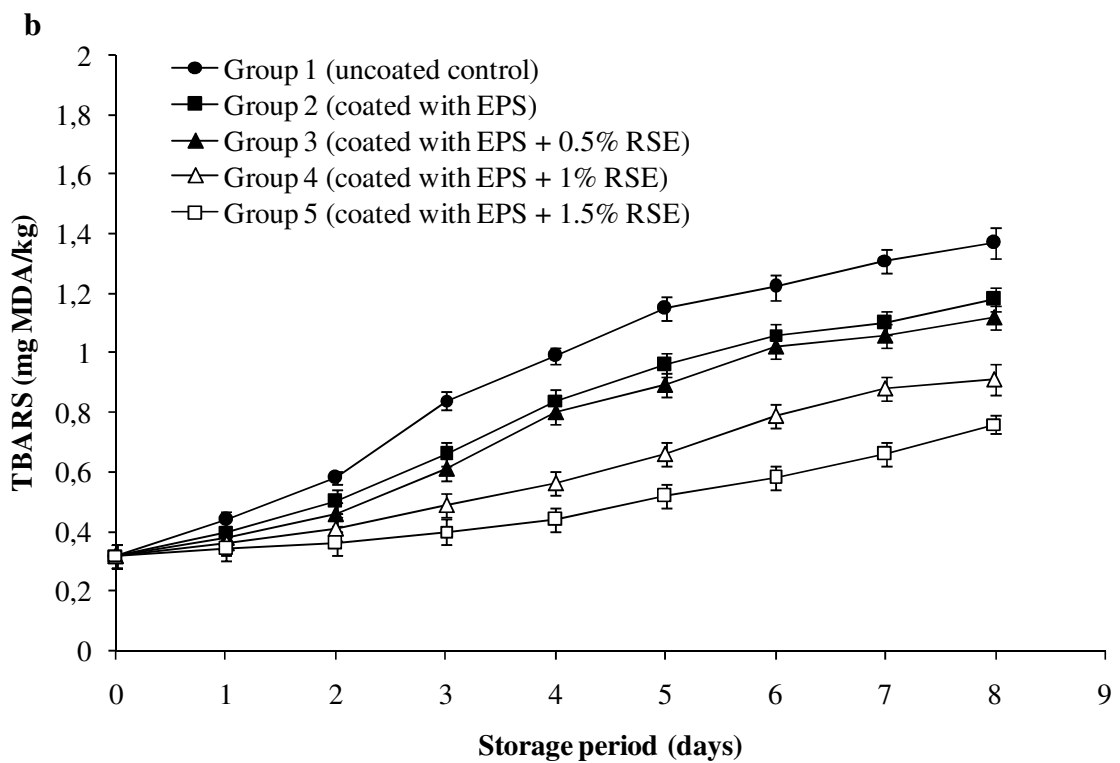
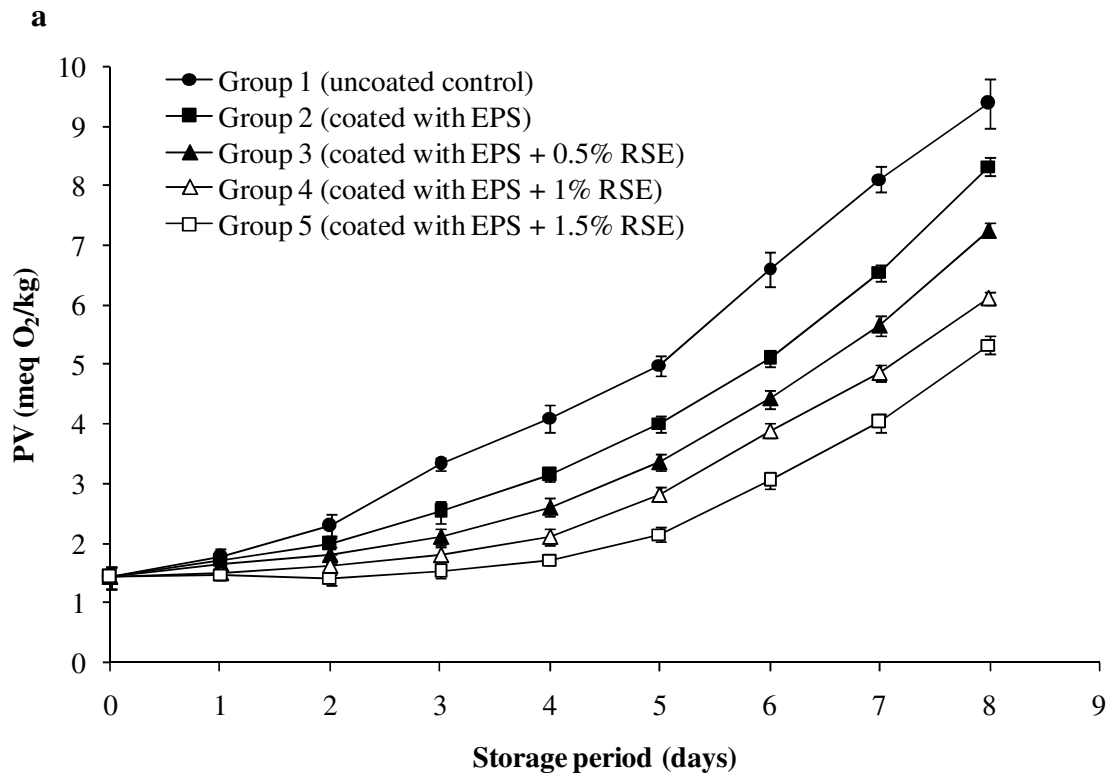


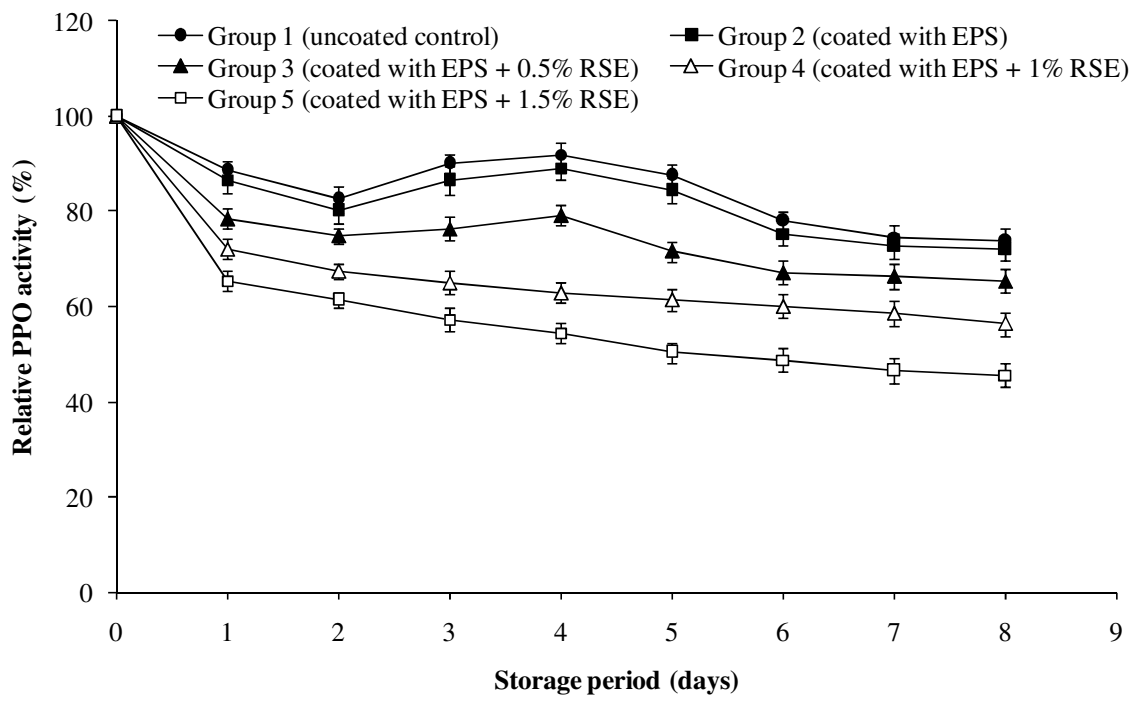
Fig. 2.



**Fig. 3.**

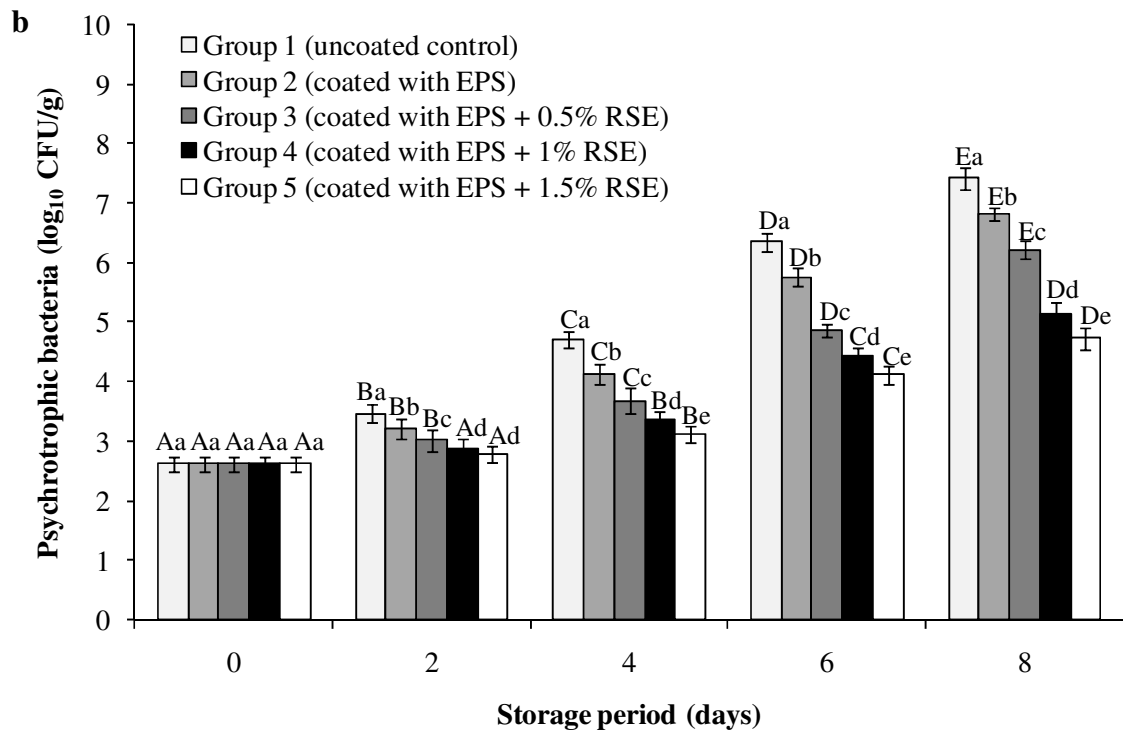
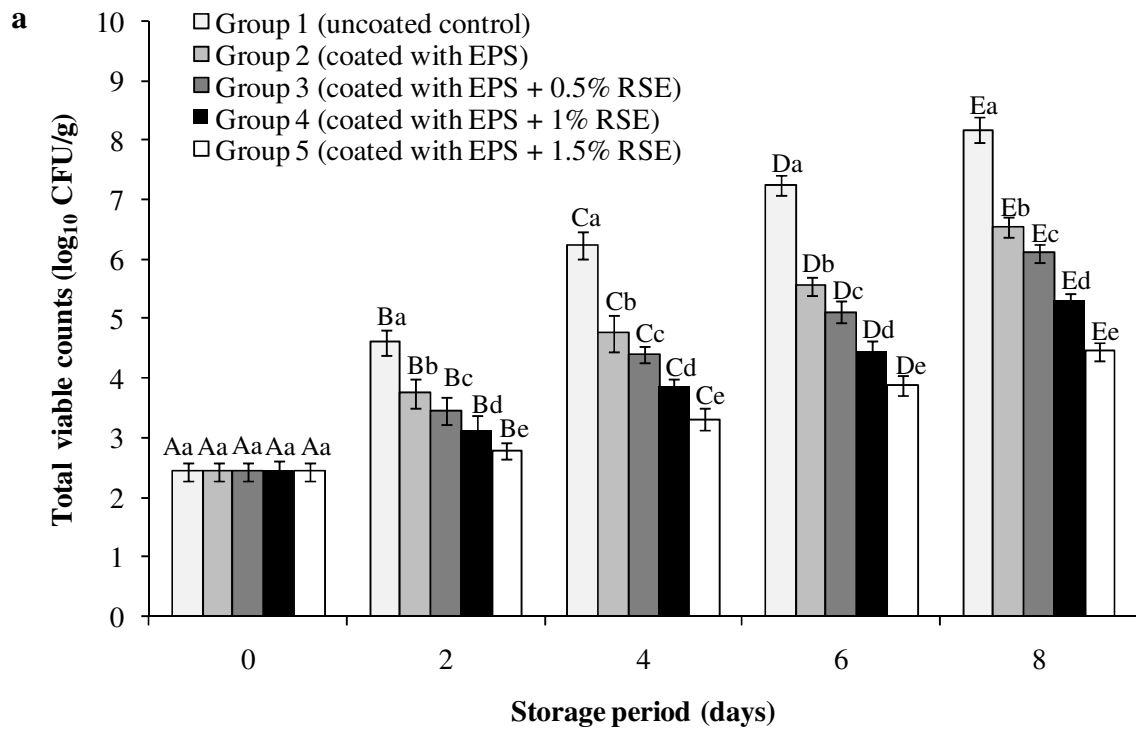


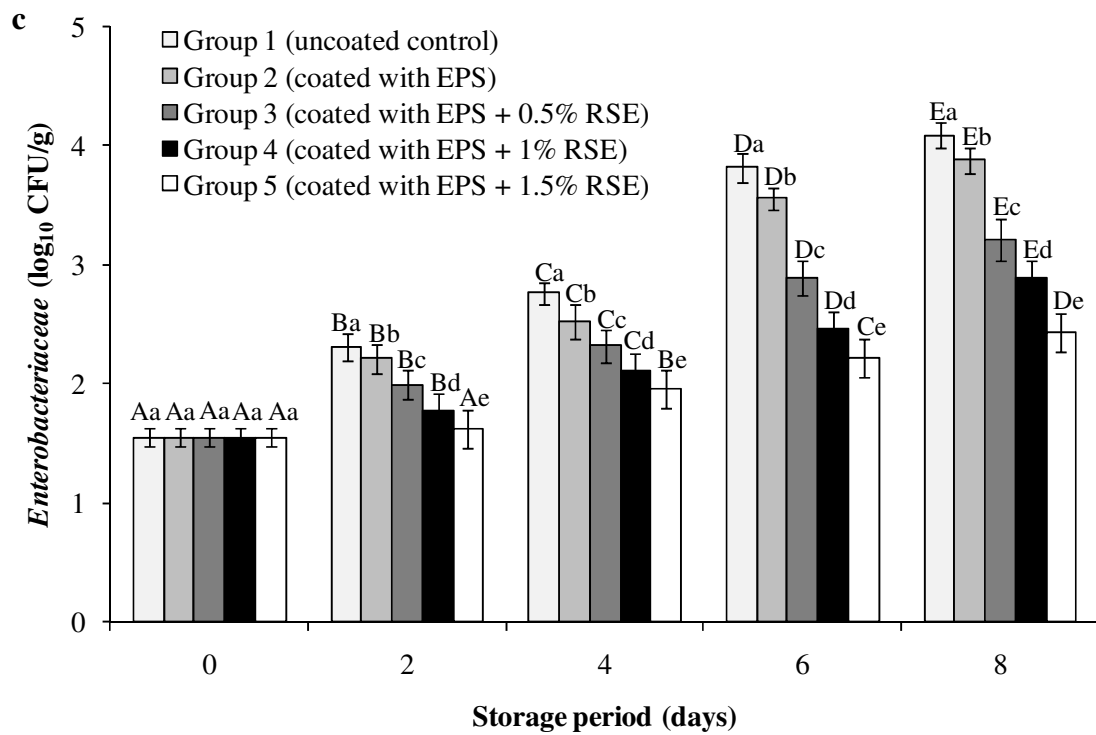
**Fig. 4.**





**Fig. 5.**





**Table 1.** Composition of the modified BBM culture medium.

Composition of modified BBM medium (g/L)			
NaNO <sub>3</sub>	1.5		
MgSO <sub>4</sub> . 7H <sub>2</sub> O	0.225	Composition of additive 1	
CaCl <sub>2</sub> . 2H <sub>2</sub> O	0.025	ZnSO <sub>4</sub> . 7H <sub>2</sub> O	0.2
Na <sub>2</sub> EDTA. 2H <sub>2</sub> O	0.05	Co(NO <sub>3</sub> ) <sub>2</sub> . 6H <sub>2</sub> O	0.044
FeSO <sub>4</sub> . 7H <sub>2</sub> O	0.014	CuSO <sub>4</sub> . 5H <sub>2</sub> O	0.1
K <sub>2</sub> HPO <sub>4</sub>	0.15	H <sub>3</sub> BO <sub>3</sub>	2.9
KH <sub>2</sub> PO <sub>4</sub>	0.123	MnCl <sub>2</sub> . 4H <sub>2</sub> O	1.8
NaCl	15		
NaHCO <sub>3</sub>	1.26	Composition of additive 2	
+ 1 mL/L of additive 1 and 1 mL/L of additive 2		MoO <sub>3</sub>	0.2

**Table 2.** Changes in the color parameters of coated and uncoated white shrimp samples stored at refrigerated temperature ( $4 \pm 1$  °C) for 8 days.

Day	Treatments				
	Control	EPS	EPS + 0.5% RSE	EPS + 1% RSE	EPS + 1.5 % RSE
	Lightness (L*)				
1	40 <sup>a,A</sup> ± 2	40 <sup>a,A</sup> ± 1	40 <sup>a,A</sup> ± 1	41 <sup>a,A</sup> ± 1	41.4 <sup>a,A</sup> ± 0.3
3	25 <sup>a,B</sup> ± 1	30 <sup>a,B</sup> ± 1	36 <sup>b,B</sup> ± 1	38 <sup>c,A</sup> ± 1	40 <sup>c,A</sup> ± 1
5	21 <sup>a,C</sup> ± 1	26 <sup>b,C</sup> ± 1	31 <sup>c,C</sup> ± 1	35 <sup>d,B</sup> ± 1	36.9 <sup>d,B</sup> ± 0.2
8	18 <sup>a,D</sup> ± 1	21.9 <sup>b,D</sup> ± 0.2	23 <sup>c,D</sup> ± 1	26 <sup>c,C</sup> ± 1	28.7 <sup>d,C</sup> ± 0.3
	Redness-greenness (a*)				
1	-0.8 <sup>a,A</sup> ± 0.1	-0.9 <sup>b,A</sup> ± 0.0	-1.2 <sup>c,A</sup> ± 0.0	-1.7 <sup>d,A</sup> ± 0.1	-1.9 <sup>d,A</sup> ± 0.0
3	1.6 <sup>a,B</sup> ± 0.2	1.3 <sup>b,B</sup> ± 0.1	0.9 <sup>c,B</sup> ± 0.1	0.63 <sup>d,B</sup> ± 0.1	0.2 <sup>e,B</sup> ± 0.0
5	2.8 <sup>a,C</sup> ± 0.4	2.2 <sup>b,C</sup> ± 0.2	1.9 <sup>c,C</sup> ± 0.1	1.5 <sup>e,C</sup> ± 0.1	1.2 <sup>f,C</sup> ± 0.2
8	3.3 <sup>a,D</sup> ± 0.5	3.1 <sup>b,D</sup> ± 0.3	2.9 <sup>b,D</sup> ± 0.3	2.8 <sup>b,D</sup> ± 0.5	2.5 <sup>c,D</sup> ± 0.4
	Yellowness-blueness (b*)				
1	7.4 <sup>a,A</sup> ± 0.3	7.7 <sup>a,A</sup> ± 0.3	7.9 <sup>b,A</sup> ± 0.9	8.0 <sup>b,A</sup> ± 0.3	8.1 <sup>b,A</sup> ± 0.2
3	4.1 <sup>a,B</sup> ± 0.2	5.7 <sup>b,B</sup> ± 0.7	7.1 <sup>c,B</sup> ± 0.3	7.7 <sup>d,B</sup> ± 0.2	8.4 <sup>e,A</sup> ± 0.2
5	2.2 <sup>a,C</sup> ± 0.2	3.9 <sup>b,C</sup> ± 0.3	4.8 <sup>c,C</sup> ± 0.3	5.7 <sup>d,C</sup> ± 0.2	8.6 <sup>e,A</sup> ± 0.1
8	1.4 <sup>a,D</sup> ± 0.1	1.9 <sup>b,D</sup> ± 0.1	2.9 <sup>c,D</sup> ± 0.1	4.5 <sup>d,D</sup> ± 0.1	5.4 <sup>e,B</sup> ± 0.2

Data are given as mean values ± standard deviation (n = 3). The different lower case letters in the same row within the same storage time indicate significant differences ( $p < 0.05$ ) between mean values. Different capital letters in the same column within the same treatment indicate significant differences ( $p < 0.05$ ).

**Table 3.** Changes in the textural parameters determined from texture profile analysis (TPA) of coated and uncoated white shrimp samples stored at refrigerated temperature ( $4 \pm 1$  °C) for 8 days.

	Treatments				
	Control	EPS	EPS + 0.5% RSE	EPS + 1% RSE	EPS + 1.5 % RSE
Day	Hardness (N)				
1	43 <sup>a,A</sup> ± 1	45 <sup>a,b,A</sup> ± 1	46 <sup>b,A</sup> ± 1	48 <sup>c,A</sup> ± 1	50 <sup>d,A</sup> ± 2
4	27 <sup>a,B</sup> ± 1	38 <sup>b,B</sup> ± 1	41 <sup>c,B</sup> ± 1	43 <sup>d,B</sup> ± 1	43.8 <sup>d,B</sup> ± 0.4
8	35 <sup>a,C</sup> ± 3	37 <sup>a,B</sup> ± 1	42 <sup>b,B</sup> ± 1	44 <sup>b,C</sup> ± 1	47 <sup>c,C</sup> ± 1
	Resilience				
1	0.30 <sup>a,A</sup> ± 0.02	0.30 <sup>a,A</sup> ± 0.03	0.20 <sup>a,A</sup> ± 0.02	0.20 <sup>a,A</sup> ± 0.04	0.30 <sup>a,A</sup> ± 0.03
4	0.20 <sup>a,A</sup> ± 0.01	0.30 <sup>a,A</sup> ± 0.01	0.20 <sup>a,A</sup> ± 0.05	0.30 <sup>a,A</sup> ± 0.03	0.30 <sup>a,A</sup> ± 0.02
8	0.20 <sup>a,A</sup> ± 0.02	0.20 <sup>a,A</sup> ± 0.02	0.20 <sup>a,A</sup> ± 0.04	0.30 <sup>a,A</sup> ± 0.01	0.30 <sup>a,A</sup> ± 0.05
	Cohesiveness				
1	0.40 <sup>a,A</sup> ± 0.10	0.20 <sup>b,A</sup> ± 0.05	0.40 <sup>a,A</sup> ± 0.02	0.40 <sup>a,A</sup> ± 0.03	0.40 <sup>a,A</sup> ± 0.10
4	0.40 <sup>a,A</sup> ± 0.02	0.40 <sup>a,B</sup> ± 0.01	0.40 <sup>a,A</sup> ± 0.05	0.40 <sup>b,A</sup> ± 0.04	0.40 <sup>a,A</sup> ± 0.04
8	0.40 <sup>a,A</sup> ± 0.04	0.40 <sup>a,B</sup> ± 0.01	0.40 <sup>a,A</sup> ± 0.05	0.40 <sup>b,A</sup> ± 0.04	0.40 <sup>b,A</sup> ± 0.02
	Springiness				
1	0.50 <sup>a,A</sup> ± 0.02	0.70 <sup>b,A</sup> ± 0.04	0.50 <sup>a,A</sup> ± 0.10	0.60 <sup>a,A</sup> ± 0.10	0.50 <sup>a,A</sup> ± 0.02
4	0.50 <sup>a,A</sup> ± 0.03	0.70 <sup>b,A</sup> ± 0.10	0.60 <sup>b,A</sup> ± 0.01	0.60 <sup>b,A</sup> ± 0.04	0.70 <sup>b,B</sup> ± 0.02
8	0.50 <sup>a,B</sup> ± 0.02	0.60 <sup>b,A</sup> ± 0.05	0.60 <sup>b,A</sup> ± 0.03	0.70 <sup>b,A</sup> ± 0.04	0.70 <sup>b,B</sup> ± 0.05
	Gumminess				
1	17 <sup>a,A</sup> ± 3	11 <sup>b,A</sup> ± 1	12 <sup>b,A</sup> ± 2	12 <sup>b,A</sup> ± 1	12 <sup>b,A</sup> ± 1
4	17 <sup>a,A</sup> ± 2	12 <sup>b,A</sup> ± 1	14 <sup>b,A</sup> ± 1	12.5 <sup>b,A</sup> ± 0.2	13 <sup>b,A</sup> ± 0.4
8	15 <sup>a,B</sup> ± 1	14 <sup>b,A</sup> ± 1	12 <sup>c,A</sup> ± 1	12.5 <sup>d,A</sup> ± 0.3	13 <sup>d,A</sup> ± 1
	Chewiness				
1	6.5 <sup>a,A</sup> ± 1.2	6.7 <sup>a,A</sup> ± 0.5	7.5 <sup>b,A</sup> ± 0.3	7.7 <sup>b,A</sup> ± 0.2	7.7 <sup>b,A</sup> ± 0.6
4	8.2 <sup>a,B</sup> ± 1.3	6.7 <sup>b,A</sup> ± 0.9	7.4 <sup>c,A</sup> ± 0.5	7.9 <sup>d,A</sup> ± 0.5	7.8 <sup>d,A</sup> ± 0.2
8	6.8 <sup>a,A</sup> ± 0.3	6.1 <sup>a,A</sup> ± 0.3	7.3 <sup>b,A</sup> ± 0.2	7.9 <sup>c,A</sup> ± 0.6	7.9 <sup>c,A</sup> ± 0.3

Data are given as mean values ± standard deviation (n = 3). The different lower case letters in the same row within the same storage time indicate significant differences ( $p < 0.05$ ) between mean values. Different capital letters in the same column within the same treatment indicate significant differences ( $p < 0.05$ ).

**Table 4.** Changes in the sensory properties of coated and uncoated white shrimp samples before and after 8 days of refrigerated storage.

Storage time (days)	Treatments	Color	Odor	Texture	Acceptability
0	Control	8.2 <sup>a,A</sup> ± 0.6	7.9 <sup>a,A</sup> ± 0.9	7.6 <sup>a,A</sup> ± 0.9	8.1 <sup>a,A</sup> ± 0.8
	EPS	8.3 <sup>a</sup> ± 0.2	8.2 <sup>a,A</sup> ± 0.4	7.6 <sup>a,A</sup> ± 0.5	8.2 <sup>a,A</sup> ± 0.6
	EPS + 0.5% RSE	8.4 <sup>a</sup> ± 0.4	8.1 <sup>a,A</sup> ± 0.3	7.5 <sup>a,A</sup> ± 0.3	8.2 <sup>a,A</sup> ± 0.3
	EPS + 1% RSE	8.5 <sup>a</sup> ± 0.1	8.2 <sup>a,A</sup> ± 0.6	7.7 <sup>a,A</sup> ± 0.1	8.2 <sup>a,A</sup> ± 0.7
	EPS + 1.5% RSE	8.6 <sup>a</sup> ± 0.2	8.2 <sup>a,A</sup> ± 0.3	7.8 <sup>a,A</sup> ± 0.2	8.1 <sup>a,A</sup> ± 0.7
8	Control	6.2 <sup>c,B</sup> ± 0.4	5.7 <sup>c,B</sup> ± 0.3	5.9 <sup>c,B</sup> ± 0.8	6.6 <sup>c,B</sup> ± 0.5
	EPS	6.8 <sup>b,B</sup> ± 0.3	6.2 <sup>b,B</sup> ± 0.2	6.8 <sup>b,B</sup> ± 0.5	7.2 <sup>d,B</sup> ± 0.1
	EPS + 0.5% RSE	7.0 <sup>b,B</sup> ± 0.1	6.5 <sup>b,B</sup> ± 0.2	6.9 <sup>b,B</sup> ± 0.3	7.4 <sup>d,B</sup> ± 0.2
	EPS + 1% RSE	7.7 <sup>d,B</sup> ± 0.2	7.7 <sup>a,B</sup> ± 0.2	7.1 <sup>a,B</sup> ± 0.4	8.3 <sup>a,B</sup> ± 0.3
	EPS + 1.5% RSE	8.2 <sup>a,B</sup> ± 0.3	7.8 <sup>a,B</sup> ± 0.2	7.4 <sup>a,B</sup> ± 0.7	8.5 <sup>a,B</sup> ± 0.2

Data are given as mean values ± standard deviation (n = 3). The different lower case letters in the same column within the same storage time indicate significant differences ( $p < 0.05$ ). Different capital letters in the same column within the same treatment indicate significant differences ( $p < 0.05$ ).