Rheological and structural properties of *Hemiramphus far* skin gelatin: Potential use as an active fish coating agent

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A B S T R A C T

The present study aims to characterize black-barred (*Hemiramphus far*) skin gelatin (BG) gel enriched or not with its hydrolysate (BGH) in terms of their rheological and micro-structural properties, as well as to evaluate their effect on smooth hound fillet quality during chilled storage. The results of the rheological properties showed that peptides addition influenced elastic and loss modulus as observed from temperature sweep. Based on the microstructure, BG gel had a fine network with small voids that were filled by the presence of BGH peptides. On the other hand, when used as coating agents for smooth hound fillets, BG and BG-BGH showed that, in comparison to control group, fish spoilage was significantly delayed in coated samples. In fact, after 8 days of storage, samples coated with BG and BG + BGH showed the lowest weight loss levels, and preserved their initial water activity (a_w) values. In addition, fillet coating was able to significantly reduce TVB-N content and oxidation process, as reflected by the low malondialdehyde and carbonyl contents. Furthermore, compared to the control group, all the treatments were found to reduce the degree of microbial deterioration of the fillets, leading to low the free amino acids and nucleotides contents in wrapped fish samples. Therefore, gelatin coating helps to prevent fish fillets from deterioration and oxidation processes.

1. Introduction

Fish gelatin is a protein obtained by partial hydrolysis and thermal denaturation of fish collagen (Jridi, Lassoued, Nasri, Ayadi & Nasri, 2014). Gelatins from various marine raw materials generally have different rheological and structural properties (Sinthusamran, Benjakul, Swedlund, & Hemar, 2017), which are directly related to their biochemical characteristics such as amino acid composition and molecular weight, etc. (Jridi, Lassoued, Nasri, Ayadi & Nasri, 2014). Gelatin has a wide range of applications, in pharmaceutical and cosmetic industries, as food additive, biopolymer, and photographic agent (Das, Suguna, Prasad, Vijaylakshmi, & Renuka, 2017). In addition, gelatin is known by its film-forming capacity giving transparent films, which make it an excellent edible film component that can fulfill commercial packaging needs (Arvanitoyannis, 2002; Hosseini & Gómez-Guillén, 2018). Gelatin-based packaging film may be also used as a carrier of antimicrobial and/or antioxidant agents (Benbettaïeb, Karbowiak, & Debeaufort, 2017), in order to improve the quality and the shelf life of packaged foods.

The stability of fresh fish fillets during chilled storage is used to be perishable after a limited period of time. In fact, fresh fish products are very sensitive due to the presence of huge number of factors that can cause their deterioration including neutral pH, high water activity, presence of endogenous autolytic enzymes, and the high content in proteins and unsaturated fatty acids (Duan, Jiang, Wang, Yu, & Wang, 2011). Spoilage bacteria causing-sensory deterioration, lipid and protein oxidation and nucleotides degradation are the most common problems that usually affect the quality of fresh seafood products.

The increasing demand for fresh chilled fish with an extended shelf life has intensified the search of technologies that support fresh fish quality. One of the main developments is the modification of the atmosphere packaging (Viji et al., 2016), or the use of edible coatings (Fan et al., 2009; Jian, Wenyi, & Wuyong, 2011) and active bio-based packaging (Heydari, Alemzadeh, & Vossoughi, 2013). Gelatin edible coating can act as a barrier to the permeability of oxygen and, thereby it can reduce the oxidation reactions and food products deterioration. Numerous studies have been focused on using edible coatings to preserve food quality (De Oliveira & Silva, 2017; Soares, Mendes, & Vicente, 2013). Gelatin properties, such as non-toxicity, biocompatibility, and biodegradability increased its use in food processing

industries. In fact, gelatin coatings have been found to decrease moisture loss and control oxidation during chilled storage (Feng, Bansal, & Yang, 2016).

Unlike conventional edible coating technique, bioactive packaging is considered a very promising technique for shelf-life extending of food products (Hosseini & Gómez-Guillén, 2018). In fact, gelatin matrix allows controlling the release of the bioactive substances from the packaging film to the coated food during the storage period and thereby slowing down the deterioration of food products. Bioactive peptides from collagen and gelatin with biological properties have become a topic of great interest for healthy food and processing/preservation industries. For instance, gelatin hydrolysates from tuna and squid skin gelatins were found to be potential antibacterial agents (Gómez-Guillén et al., 2010). Furthermore, gelatin hydrolysates from thornback ray (*Raja clavata*) skin (Lassoued et al., 2015), cuttlefish (*Sepia officinalis*) skin (Jridi, Lassoued, Nasri, Ayadi & Nasri, 2014) and giant squid (*Dosidiscus gigas*) tunics (Alemán et al., 2011) have been reported to exhibit antioxidant activity.

Black-barred halfbeak (*Hemiramphus far*) is among the actinopterygii fish species, which are geographically widespread and numerically abundant around the world, besides their low market value. So, the utilization of halfbeak species for bioactive molecules extraction could be a good way for their valorization. In our previous study, *H. far* skin had been used to extract gelatin and bioactive gelatin peptides with antioxidant and antibacterial properties (Abdelhedi et al., 2017). Limited studies have been conducted on the utilization of gelatin gels containing gelatin hydrolysate as bioactive agent for maintaining fish quality and safety. Therefore, the purpose of this study is to evaluate the effect of black-barred halfbeak gelatin (BG) coating enriched with its hydrolysate (BGH) in preserving the quality of fresh fish fillets during 8 days of cold storage. Particularly, weight loss, color evolution, lipid oxidation, carbonyl content, total volatile basic nitrogen (TVB-N) content, free amino acids and nucleotides degradation as well as microbial counts of coated and uncoated fish samples were determined during 8 days of storage. These parameters were studied in order to evaluate the specific effect of gelatin coating.

2. Materials and methods

2.1. Raw material preparation

Black-barred halfbeak (*Hemiramphus far*) skin was obtained from fish market located in Sfax City, Tunisia. The samples were packed in polyethylene bags, placed in ice and immediately transported to the laboratory. The skin was washed with tap water to eliminate pigments and then stored in sealed plastic bags at −20 °C until use for gelatin extraction.

2.2. Gelatin extraction and hydrolysate preparation

The gelatin extraction procedure was performed as previously detailed in Abdelhedi et al. (2017). After freeze-drying, the obtained powder, referred as black-barred halfbeak gelatin (BG), was stored at 4 °C until use.

BG powder was dissolved in distilled water (2%; w/v) and subjected to enzymatic hydrolysis using Purafect® with an enzyme/substrate ratio of 30/1 (U/mg) (pH 10.0, 50 °C). The gelatin solutions were allowed to equilibrate for 30 min before hydrolysis were initiated. During the reaction, the pH of the mixture was maintained constant by continuous addition of 2 M NaOH solution. Finally, the hydrolysate mixtures were centrifuged at 8000 × g for 20 min and the soluble fraction was freeze-dried and stored at −20 °C for further use. The degree of gelatin hydrolysis (DH) was determined according to Adler-Nissan (1986, pp. 57–131). The obtained powder after freeze-drying was referred as black-barred halfbeak gelatin hydrolysate (BGH).

2.3. Gelatin gel characterization

2.3.1. Scanning electron microscopy

Gelatin-based gel microstructure was visualized using a scanning electron microscope (SEM, Cambridge Scan-360 microscope) at an accelerating voltage of 3.0 kV, as previously described by Jridi et al. (2015). The sample was frozen under liquid nitrogen. Prior to visualization, sample was mounted on brass stub and sputtered with gold in order to make the sample conductive. Samples were photographed with an angle of 90° to the surface to allow observation of the films cross section. Photos were taken at 1000 and 3000 magnifications.

2.3.2. Viscoelastic properties

Dynamic studies were performed on an AR1000 Rheometer (Physica MCR-301, Anton Paar, Germany) using a cone-plate geometry (cone angle 2°), as previously detailed in Abdelhedi et al. (2017). The viscosity measurement was performed at a scan rate of 1 °C/min, frequency 1 Hz, oscillating applied stress of 3.0 Pa and gap 0.15 mm. During the experience, the gel was heated from 5 to 50 °C, cooled from 50 to 5 °C and then kept at 5 °C for 10 min. The tan δ was calculated from the ratio of G' and G" obtained from temperature sweep tests.

2.4. Characterization of coated of smooth hound fillets

2.4.1. Coating preparation

Fresh smooth hound fillets, with an average weight of 1000–1200 g each, were obtained after removing the head, bone, skin and viscera, from the local fish market (Sfax City, Tunisia). Fillets were then cut into pieces with dimensions of 10 cm × 5 cm x 2 cm (length x width x height), divided into four groups, with 50 pieces in each, and immediately coated. Bovine commercial gelatin (CG) was used as control. CG and BG coating solutions were prepared by mixing 4 g of each dried gelatin in 100 ml of distilled water at 40 °C for 30 min. In parallel, the gelatin hydrolysate was dissolved in black-barred halfbeak gelatin solution at 1 mg/ml to prepare active gelatin solution. Fish samples were assigned into four treatment groups, consisting of non coated control samples (C), samples coated with bovine commercial gelatin solution for 30 s (CG), samples dipped into black barred halfbeak gelatin solution during 30 s (BG) and samples dipped, for 30 s also, into BG solution enriched with gelatin hydrolysate (BG + BGH).

After treatment, all samples were weighed and stored at 4 °C without further external packaging (overwrap) to accentuate oxidation process in simulated fresh conditions. Groups were sampled at 0, 3, 6 and 8 days of storage for physicochemical and microbial analysis.

2.4.2. Weight loss

The weight loss of the fish samples on days 3, 6 and 8 was calculated using the following equation, according to Sathivel (2005):

$$\text{Weight loss (\%)} = [(W0 - Wi) / W0] \times 100$$

where W_0 is the initial weight of sample (day 0) and W_i is the weight of the same sample after 3, 6 and 8 days of chilled storage.

2.4.3. Color, pH and water activity measurements

The color of fish samples was determined using a CR-410 colorimeter (Minolta Chroma Meter Measuring Head, Osaka, Japan) with D65 illuminant. The instrument was standardized using a standard white plate. An average value of color was determined by taking observations from three different fish samples from each group on days 0, 3, 6 and 8 of storage. CIE lightness (L*), redness (a*) and yellowness (b*) were recorded. Chroma (C*) was calculated based on the following equation:

$$C^* = (a^{*2} + b^{*2})^{1/2}$$

To measure the pH of fish from each group during the storage period, the fillet sample (2 g) was first homogenized in 20 ml of

bidistilled water for 2 min. The pH was then measured using a pH-meter (Hanna, 211, Mauritius).

Water activity (a_w) was determined using FAst-lab water activity meter (Gbx, Romans Isère Cédex, France) at 25 °C. The equipment was previously calibrated with sodium chloride and potassium sulfate.

2.4.4. Total volatile basic nitrogen

The total volatile basic nitrogen (TVB-N) value was estimated by the micro-diffusion method (Abelti, 2013) following the distillation of the perchloric acid of the homogenized fish samples. The distillate was collected in a flask containing aqueous solution of boric acid and methyl red as an indicator. Afterward, the boric acid solution was titrated with sulfuric acid solution. The TVB-N value (mg N/100 g of fish) was determined according to the volume of sulfuric acid consumed for titration.

2.4.5. Lipid per-oxidation measurement

Thio-barbituric acid reactive substances (TBARS) of fish samples were evaluated using the method proposed by Witte, Krause and Bailey (1970). Briefly, a fish sample (5 g) was dispersed in 5% trichloroacetic acid solution (20 ml) and homogenized in a Polytron homogenizer (PT 2100, Kinematica AG, Switzerland) for 5 min. The homogenate was centrifuged at 10,000 × g for 10 min at 4 °C. The supernatant (4 ml) was reacted with 0.8 ml of chlorhydric acid (HCl 0.6 M) and 3.2 ml of tris-thiobarbituric acid (TBA) solution (Tris 26 mM, TBA 120 mM) and then incubated in a water bath at 85 °C for 10 min. The absorbance of each mixture was measured at 532 nm. TBARS values were calculated from a standard curve of malonaldehyde (MDA) and expressed as mg of MDA/kg of fish sample.

The lipid oxidation was as well evaluated by the conjugated diene content, as previously described by Esterbauer, Cheeseman, Dianzani, Poli, and Slater (1982).

2.4.6. Carbonyl content

Carbonyl content was measured using the method described by Vuorela et al. (2005). Fish sample (1 g) was homogenized with 10 ml of 0.15 M of potassium chloride solution (KCl) using a Polytron homogenizer (Brinkman Inc., Westbury, NY, USA) for 60 s. Homogenate (100 µl) was then mixed with 10% trichloroacetic acid (TCA) solution and centrifuged for 5 min at 5000 × g. The supernatant was removed and the pellet (bottom layer) was used for carbonyl content measurement. A volume of 1 ml of 2 M HCl containing 0.2% (w/v) dinitrophenyl hydrazine was thereafter added. After incubation for 1 h (shaken periodically), 1 ml 10% TCA was added. The sample was mixed and centrifuged again for 5 min at 5000 × g and the supernatant was removed without damaging the pellet. A volume of 1 ml of ethanol/ethyl acetate solution (v/v, 1:1) was used to wash the pellet. This step was repeated twice to ensure purity. Thereafter, the pellet was dissolved in 1.5 ml of 20 mM sodium phosphate buffer with 6 M guanidine hydrochloride. The solution was shaken and centrifuged for 2 min at 5000 × g. The carbonyl content was measured by reading the absorbance of each sample at 370 nm (Abs_{370nm}). A bovine serum albumin solution was used to plot the standard curve for protein quantification and carbonyls (µmol/g) were calculated as the following:

$$\text{Carbonyl content} = Abs_{370nm} / 21.0\ mM^{-1}\ cm^{-1}$$

where $21.0\ mM^{-1}\ cm^{-1}$ is the molar extinction coefficient of carbonyls.

2.4.7. Microbiological analysis

Bacteriological counts were determined by mixing 1 g of fish sample in 9 ml of 0.9% NaCl solution. Decimal dilutions were prepared and plated in the appropriate media. The inoculated plates were incubated at 37 °C for 2 days for total viable counts (TVC), and at 4 °C for 7 days for total psychrotrophilic counts (TPC). Total viable and psychrotrophilic bacterial counts were determined by the pour plate method, using plate count agar (PCA) medium. All microbial counts were converted to logarithms of colony-forming units per gram of fish sample (log_{10} CFU/g) (Nowzari, Shábanpour, & Ojagh, 2013).

2.4.8. Nucleotide content analysis

The extraction and quantification of the nucleotides were carried out as described by Hernández-Cázares, Aristoy, and Toldrá (2011). 5 g of each sample were homogenized with 15 ml of 0.6 M of perchloric acid for 10 min at 4 °C in a Stomacher (IUL Instrument, Barcelona, Spain). The extract was centrifuged at 10,000 × g for 20 min at 4 °C and the supernatant was filtered through glass wool. Then, 12 ml was neutralized (pH 6.5) with potassium carbonate in an ice bath. The neutralized solution was centrifuged and the supernatant was used for nucleotides determination.

Nucleotides content was analyzed by reverse phase-HPLC (RP-HPLC) with a 1200 Agilent liquid chromatography (Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector. The separation was performed at 30 °C using a Zorbax Eclipse plus C18 column (4.6 × 250 mm, 5 µm) (Agilent Technologies). The eluents used were (A) containing 0.1 M potassium phosphate buffer (pH 6.0) mixed with Pic A reagent (Paired-Ion Chromatography, Waters), while (B) contained the mobile phase A: methanol mixture (75:25). The flow rate used was 1 ml/min. Nucleotide peaks were identified by comparison of their retention times and spectra between 200 and 350 nm with those of standards (Sigma, St Louis, MO). The ratio of the area of the nucleotide peak was used as the y-axis variable to prepare a calibration curve and subsequently used to determine the nucleotide concentration in each sample. Results were expressed as µmol of nucleotide per g of fish.

2.4.9. Free amino acids

Free amino acids (FAA) were analyzed according to Jurado, García, Timón and Carrapiso (2007). Five grams of the sample were homogenized with 20 ml of 0.01 M HCl in Stomacher (IUL Instrument, Barcelona, Spain) at 4 °C for 8 min. The homogenate was centrifuged at 10,000 × g for 20 min at 4 °C and supernatant was filtrated through glass-wool and served for FAA analysis. 250 µl of supernatant were mixed with 50 µl of internal standard, norleucine (10 mM) and 750 µl of acetonitrile. The mixture was centrifuged at 10,000 × g and supernatant (500 µl) was dried without heating in a centrifugal evaporator (Jouan RCT 90 cold trap). Then, 15 µl of drying reagent (methanol: sodium acetate 1 M: TEA, 2:2:1) were added to the dried samples and the mixtures were dried again. The derivatization was then carried out with phenyl isothiocyanate (PITC) by adding 15 µl of PITC solution (methanol: water: TEA: PITC, 7:1:1:1). Solutions were kept at room temperature for 20 min and then dried again. Standard solutions (1 mM) of amino acids (Sigma Chemical Co., USA) were similarly treated and used for amino acid identification.

Before analysis, samples and standards were reconstituted in 300 µl of a dilution reagent solution composed of 5 mM sodium phosphate buffer and 5% acetonitrile (pH 7.4). The PITC derivates were quantified by RP-HPLC with a 1200 Agilent liquid chromatography (Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector, using a PicoTag® column (300 mm × 3.9 mm, Waters). The temperature was set at 52 °C and the detection was carried out at 254 nm. The eluents used were (A) 0.07 M sodium acetate adjusted to pH 6.55 and containing 2.5% acetonitrile and (B) 45:40:15 acetonitrile: water: methanol, with a flow rate of 1 ml/min. Results were expressed as mg of each amino acid per g of fish fillet sample.

2.5. Statistical analysis

Results were expressed in mean ± SEM (Standard Error Mean) and analyzed using the statistical software SPSS ver. 18.0, professional edition. A one-way and two-way analysis of variance (ANOVA) was

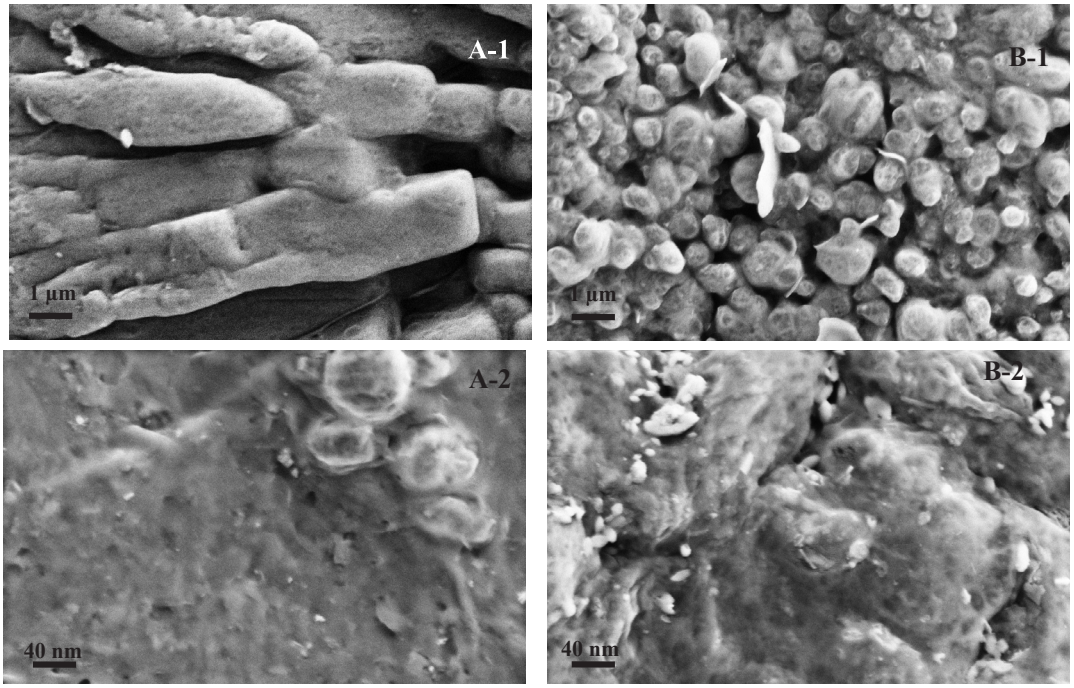

Fig. 1. Scanning electron microscopy micrographs of gelatin gel (A) and gelatin gel enriched with its hydrolysate (B). Magnifications: A1 and B1 (1000×), A2 and B2 (3000×).

then performed and followed by Duncun test to estimate the significance among the main effects at the 5% probability level.

3. Results and discussion

3.1. Gelatin gel characterization

3.1.1. Scanning electron microscopy analysis

The structure of gelatin-based gel enriched or not with its hydrolysate, was evaluated by scanning electron microscopy analysis (Fig. 1). The microstructure of BG gel showed a typical morphology of gelatin gel materials (Fig. 1A). Gelatin-based gel contained a dense network of short and thick fibers organized in sheets, along with heterogeneous voids. Additionally, the morphology of BG gel showed a low porosity microstructure with visibly very small pores, similar to previous gelatins extracted from other sources such as cuttlefish skin gelatin (Jridi et al., 2015), commercial acid-soluble collagen skin (Potorac, Popa, Maier, Lisa, & Verestiuc, 2012) and horse mackerel bones (Jansson, Haegerstrand, & Kratz, 2001). Wang et al. (2014), reported in their study, that the collagen gel extracted from Amur sturgeon skin, showed an irregular dense fibrillar microstructure with high interconnection network and massive and porous nature. Generally, such hydrogel structure is useful for a large variety of applications, especially for food coating, or as a support material for bioactive substances. In fact, gelatin gel with small pore sizes leads to absorb a large amount of water and serve as texturing, gelling, stabilizing and emulsifying agent. On the other hand, Fig. 1 (B1 and B2) showed that the addition of peptides into the gelatin gel network had influence on the microstructure of BG gel leading to decrease the porosity, by occupying the empty pores observed in the initial structure. These results suggested that the secondary structure of gelatins was affected by the addition of peptides.

3.1.2. Rheological properties

Viscoelastic properties refer to the changes of viscoelastic modulus in sol-hydro gel conversion. Gelling properties of gelatin solutions (6.67%, w/v) were measured through the programs of cooling and heating. The temperature at which the curves of G′ and G″ joined is considered as the gelling point and is close to the sol–gel transition temperature. From the graph (Fig. 2), the gelling and melting temperatures of control gelatin were about 19.5 °C and 25 °C, respectively. The observed behavior against the temperature is typical to previously studied fish skin gelatin extracted from *Catla catla* (Chandra & Shamasundar, 2015). Similar to our findings, the cod skin gelatin showed gelling/melting temperatures of 21.2 and 27.4 °C, respectively (Cai et al., 2018). However, after peptides addition, these values significantly changed and the resulting bioactive gelling solution acquired new gelling and melting points of 20.5 and 21.9 °C, respectively. The slight decrease observed in the gelling temperature of fish gelatin added with BGH may be due to the fact that hydrolysate addition increased the penetration pores and thus the gelatin gel would be weaker. Similar observation was obtained by Surowka (1997), who demonstrated that, even in the range of the lowest hydrolysate concentrations, peptides addition affected negatively the gelatin network maintaining.

3.2. Gelatin coated smooth hound fillets

3.2.1. Moisture loss

The weight loss of fish samples was evaluated by measuring the weight of samples, coated or not, during the storage period at 4 °C (Fig. 3A). The results indicated that the weight loss was rapid in control samples (uncoated), whereas it slowed down in coated fillets. In fact, in the control group, the weight loss was estimated as 27%, 47% and 68% after 3, 6 and 8 days of storage, respectively. This abusive increase may be due to the absence of further external packaging (overwrap) to accentuate oxidation process in simulated fresh conditions. However, gelatin coating contributed to the decrease of moisture loss, which was significantly reduced to 46%, 44%, and 47% in CG, BG and BG + BGH groups, respectively, after 8 days of storage. There were no significant differences in preventing weight loss between the fish samples coated in the different treated groups ($p > 0.05$). Previous studies showed that gelatin coatings act as barrier to water and reduce exudates that result in water loss in fresh fish fillet. In fact, Herring, Jonnalongadda, Narayanan, and Coleman (2010) reported moisture loss values ranged from 30% to 60% for coated and uncoated meat samples, respectively,

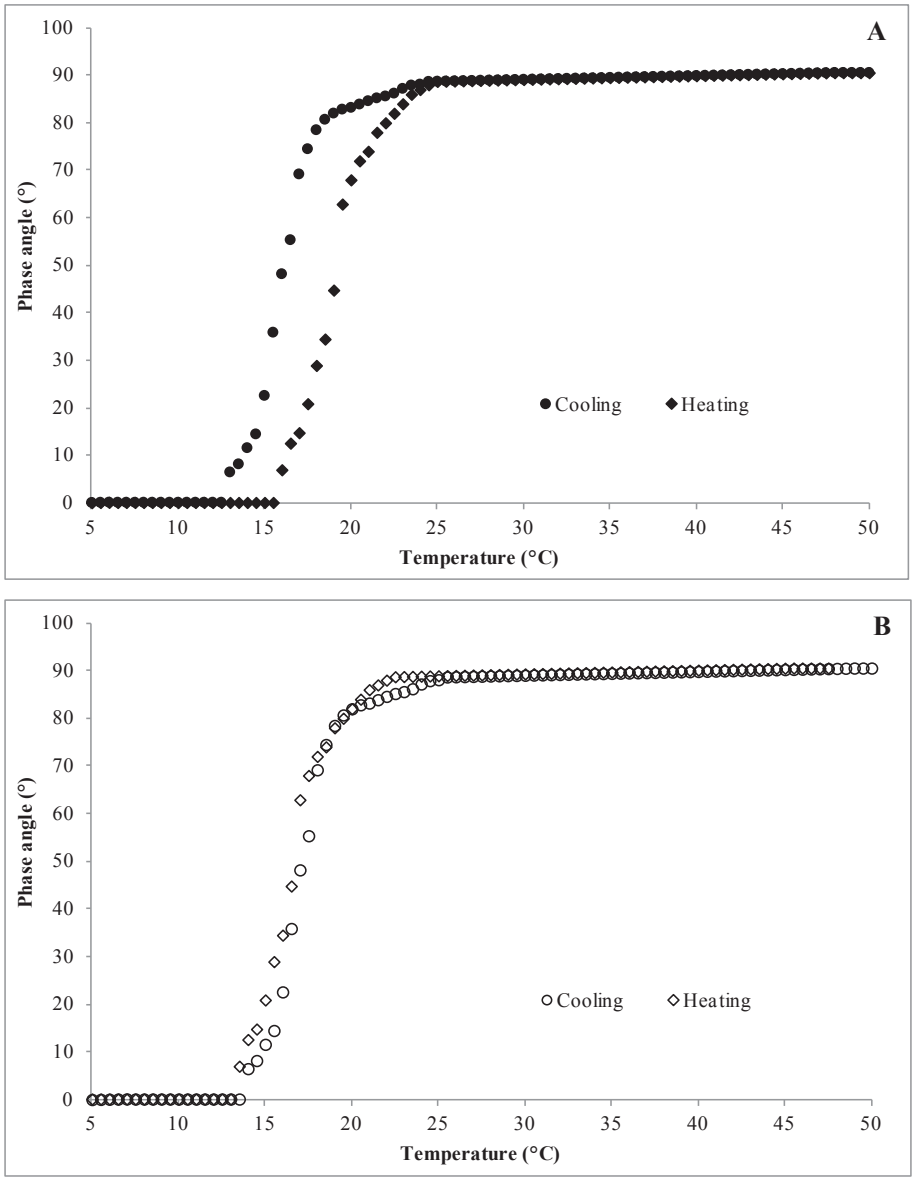

Fig. 2. Evolution of the phase angle during cooling from 50 °C to 5 °C and heating from 5 °C to 50 °C of black-barred skin gelatin gel alone (A) and enriched with its hydrolysate (B).

throughout the 7 days of storage period. In addition, Feng et al. (2016) reported the efficacy of gelatin coating in reducing moisture loss and proved that gelatin can reduce the purge of fish fillet during 17 days of chilled storage. Also, Farajzadeh, Motamedzadegan, Shahidi, and Hamzeh (2016) reported that chitosan-gelatin coating act as a barrier to water and reduce exudates resulting in the water loss of coated shrimps.

It is well known that gelatin is rich in hydrophobic amino acids, such as proline and leucine, capable to retain water, resulting in the prevention from moisture loss outside the fish fillet. Furthermore, the water holding capacity and water barrier properties of gelatin could explain the reduction of the weight loss in gelatin-coated samples (Limpisophon, Tanaka, Weng, Abe & Osako, 2009).

3.2.2. Changes in pH, water activity and color parameters

The effects of gelatin coating on changes in water activity (a_w), pH and color parameters of fish fillet during storage are shown in Table 1. Initially, the pH of all samples was close to neutral and then increased over time. In control samples, the pH increased to 8.72 after 6 days and then decreased to 7.35 at the end of the experimental period (8^{th} day). However, in coated fish samples, the pH continued to increase to reach 8.34 and 8.31 in CG and BG groups, respectively, which still significantly lower than the control group ($p \leq 0.05$). Data showed no significant differences during the 8-days of storage in all the treated samples. The decrease in pH value after 8 days may be due to the production of lactic acid and dissolution of carbon dioxide into the fish aqueous phase (Lorenzo et al., 2014). Interestingly, high pH values recorded during the first period of storage may be due to the fish spoilage state and the production of free amino acids leading to the formation of NH_3 groups caused by microorganisms' hydrolytic effect (Kakaei & Shahbazi, 2016). However, the pH variation of coated fish fillets was slower than that of the control group, proving the beneficial effect of gelatin coating in maintaining the quality of fish during chilled storage. Lower pH value observed in coated fish samples is associated to the weight loss decrease in fresh fish, as previously reported by Alemán et al. (2016), when evaluating the effect of chitosan-gelatin-shrimp concentrate, as coating or film packaging, on the quality of fish sausages during chilled storage during 42 days.

Moreover, water activity (a_w) is an important parameter in controlling microorganisms' growth of stored meats, and it may be defined as the amount of free water, available for bacterial growth, present in a

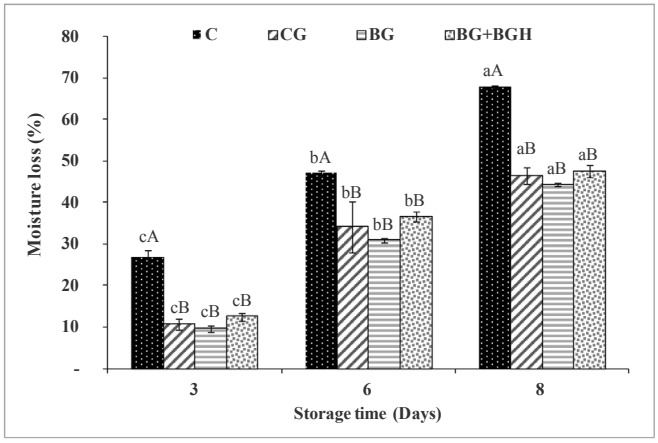

Fig. 3. Weight loss of the different fish samples measured at 0, 3, 6 and 8 days of storage. C represents the uncoated control group; CG and BG indicate commercial gelatin and Black-barred halfbeak gelatin, respectively; BG + BGH represent black barred halfbeak gelatin gel enriched with its hydrolysate. A,B: means in each treatment with different letters are significantly different ($P \leq 0.05$); a,b,c: means in different days of storage with different letters are significantly different ($P \leq 0.05$).

product. Water activity is an important criterion for the evaluation and control of food safety and quality of foodstuffs, since the 1950s when it became obvious that water content is strongly related to the microbial growth limitations. Results of a_w among all samples did not reveal any significant difference during the first 6 days of chilled storage. Contrary to uncoated group, in which a_w values decreased to 0.83 on the 8th day, treated fillets showed a constant a_w evolution, that remains over 0.97, indicating that coated meat conserve its initial freshness even after 8 days of storage. The obtained results are similar to those reported by Jridi et al. (2018). The steady a_w values observed in coated groups may be due to the water vapor barrier property of gelatin material (Limpisophon, Tanaka, Weng, Abe & Osako, 2009).

On the other hand, color is a crucial parameter in food quality control that influences consumers' demand. Table 1 illustrates color parameters changes of different fish samples coated or not with gelatin. During 8 days of chilled storage, results of uncoated samples clearly indicated that lightness values (L*) significantly decreased, while they remained invariable in all the coated groups. In addition, based on visual observation, color of uncoated sample changed from yellow, dark yellow to brown, at the end of the experimental period. The observed brown color of fish muscle is essentially caused by oxygen, responsible to the oxidation of myoglobin to form metamyoglobin, which imparts a brownish color (Bekhit & Faustman, 2005). However, colors of coated fish samples were slightly affected and the fish fillet still visually acceptable. In fact, according to Table 1, the redness (a*) of uncoated fish increased with increasing storage time, and values were higher than those of treated one. Similarly, after 3, 6 and 8 days of storage, yellowness (b*) values of uncoated fish were dramatically decreased and they were estimated at 4.00, 2.89 and 0.26, respectively. However, in CG and BG groups, b* values were moderately decreased to reach 2.83 and 2.51, respectively, in the last day. Particularly, BG and BGH coating was more effective than BG on preserving the color of the stored fish, where the best results were recorded for the BGH group when compared to the gelatin coated fish samples, showing the lowest a*, b* and C* values (20.04, 4.45, and 20.53, respectively).

The obtained results are in agreement with previous studies dealing with turbot (*Psetta maxima*) fillets packaging (Santos et al., 2013) and Alaska Pollock surimi (Sell, Beamer, Jaczynski & Matak, 2015). The

Table 1
Changes in pH, a_w and color parameters of fish samples at 0, 3, 6 and 8 days of storage.

Attribute		Treatments	Storage time (Days)			
			0	3	6	8
pH		Control	6.69 ± 0.02^{Da}	8.30 ± 0.02 ^{Ba}	8.72 ± 0.04 ^{Aa}	7.35 ± 0.02 ^{Cb}
		CG	6.68 ± 0.04 ^{Da}	7.25 ± 0.00 ^{Cb}	8.03 ± 0.05 ^{Bb}	8.34 ± 0.01 ^{Aa}
		BG	6.65 ± 0.03 ^{Aa}	7.10 ± 0.02 ^{Ac}	8.00 ± 0.04 ^{Ab}	8.31 ± 0.02 ^{Aa}
		BG + BGH	6.70 ± 0.01 ^{Da}	7.13 ± 0.02 ^{Cd}	8.00 ± 0.04 ^{Bb}	8.31 ± 0.02 ^{Aa}
a_w		Control	0.99 ± 0.00 ^{Aa}	0.98 ± 0.01 ^{Aa}	0.90 ± 0.01 ^{Ba}	0.83 ± 0.00 ^{Cb}
		CG	0.99 ± 0.00 ^{Aa}	0.98 ± 0.01 ^{Aa}	0.99 ± 0.00 ^{Aa}	0.99 ± 0.05 ^{Aa}
		BG	0.98 ± 0.00 ^{Ab}	0.99 ± 0.01 ^{Aa}	0.98 ± 0.01 ^{Aa}	0.97 ± 0.01 ^{Aa}
		BG + BGH	0.99 ± 0.00 ^{Aa}	0.99 ± 0.00 ^{Aa}	0.98 ± 0.00 ^{Aa}	0.97 ± 0.02 ^{Aa}
Color parameters	L*	Control	27.32 ± 1.02 ^{Ba}	29.00 ± 0.00 ^{Aa}	21.04 ± 0.36 ^{Cb}	12.01 ± 0.95 ^{Db}
		CG	28.42 ± 0.96 ^{Aa}	29.20 ± 0.13 ^{Aa}	28.91 ± 1.00 ^{Aa}	27.35 ± 1.46 ^{Aa}
		BG	27.95 ± 0.46 ^{Aa}	29.25 ± 1.43 ^{Aa}	28.36 ± 1.30 ^{Aa}	26.05 ± 1.96 ^{Aa}
		BG + BGH	27.30 ± 0.75 ^{Aa}	27.48 ± 0.94 ^{Ab}	28.00 ± 1.31 ^{Aa}	27.61 ± 1.01 ^{Aa}
	a*	Control	20.05 ± 1.40 ^{Da}	32.96 ± 0.96 ^{Ca}	35.48 ± 0.72 ^{Ba}	49.36 ± 0.09 ^{Aa}
		CG	21.03 ± 1.00 ^{Ba}	23.97 ± 2.24 ^{Bc}	25.74 ± 3.34 ^{Bb}	35.50 ± 1.68 ^{Ab}
		BG	21.23 ± 1.29 ^{Ca}	27.69 ± 0.16 ^{Bb}	34.89 ± 1.27 ^{Aa}	32.75 ± 0.75 ^{Ab}
		BG + BGH	20.95 ± 0.59 ^{Aa}	20.65 ± 0.16 ^{Ad}	21.65 ± 1.27 ^{Ac}	20.04 ± 0.70 ^{Ac}
	b*	Control	4.24 ± 0.40 ^{Aa}	4.00 ± 0.10 ^{Aa}	2.89 ± 0.10 ^{Bb}	0.26 ± 0.03 ^{Cc}
		CG	4.36 ± 0.30 ^{Aa}	4.17 ± 0.00 ^{Aa}	4.55 ± 0.25 ^{Aa}	2.83 ± 0.03 ^{Bb}
		BG	4.26 ± 0.24 ^{Aa}	4.08 ± 0.25 ^{Aa}	4.47 ± 1.1 ^{Aa}	2.51 ± 0.67 ^{Bb}
		BG + BGH	4.33 ± 0.14 ^{Aa}	4.08 ± 0.45 ^{Aa}	4.77 ± 1.1 ^{Aa}	4.45 ± 0.09 ^{Aa}
	C*	Control	20.49 ± 0.59 ^{Da}	32.91 ± 1.24 ^{Ca}	35.60 ± 0.02 ^{Ba}	49.36 ± 2.68 ^{Aa}
		CG	21.48 ± 1.26 ^{Ca}	24.33 ± 0.68 ^{Bc}	26.18 ± 0.63 ^{Bb}	35.61 ± 0.23 ^{Ab}
		BG	21.65 ± 0.75 ^{Ca}	27.99 ± 0.15 ^{Bb}	25.18 ± 0.63 ^{Bb}	32.85 ± 0.65 ^{Ab}
		BG + BGH	21.39 ± 0.57 ^{Ba}	21.05 ± 0.02 ^{Bd}	22.17 ± 0.22 ^{Ac}	20.53 ± 0.79 ^{Cc}

A,B,C,D: For each attribute, means in the same line with different letters are significantly different ($P < 0.05$); a,b,c,d: For each attribute, means within a column, with different letters are significantly different ($P < 0.05$).
CG and BG indicate bovine commercial gelatin and black-barred halfbeak gelatin, respectively; BG + BGH represent black-barred halfbeak gelatin gel enriched with the gelatin hydrolysate.

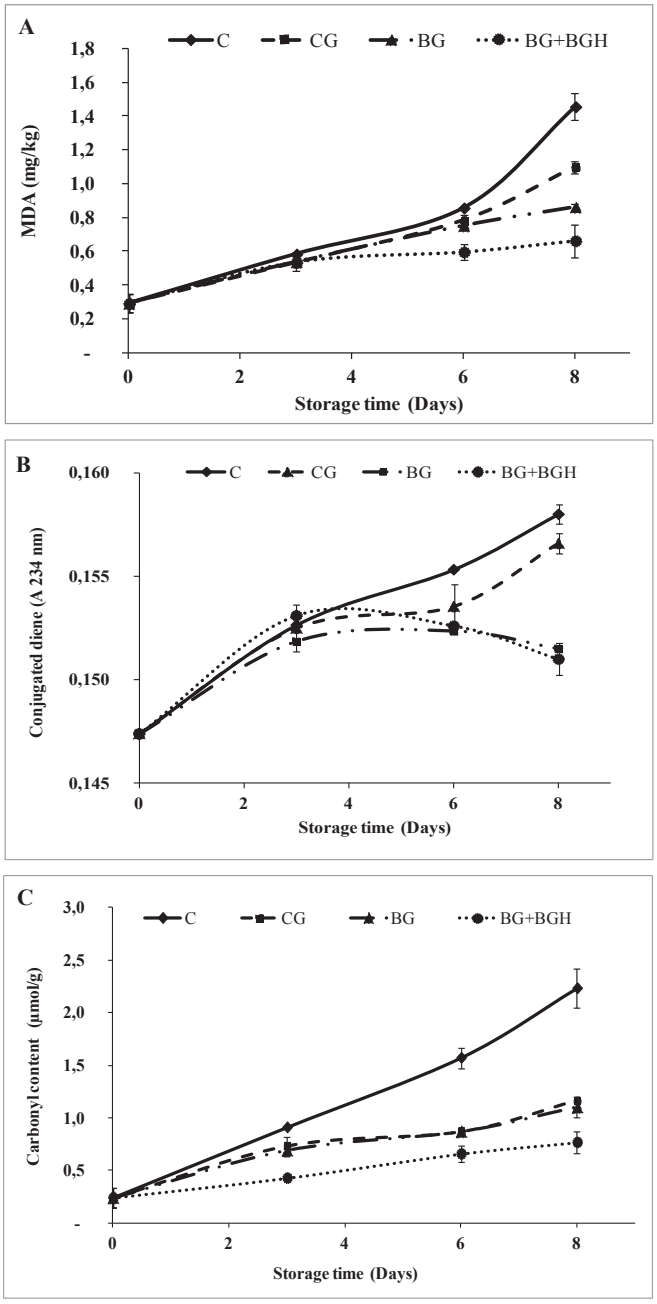

Fig. 4. Lipid oxidation (mg MDA/kg of fish fillet) (A), conjugated diene (A_{234nm}) (B) and carbonyl content (µmol/g) (C) of fish samples measured at 0, 3, 6 and 8 days of storage.

application of a gelatin coating did not affect the initial color parameters of fish fillet, which encourage its use for food products packaging without affecting consumers' desires. In fact, the coating formed a very fine layer that could drain away after application, giving less coloring to the fillets. In addition, gelatin coating offer an excellent barrier to avoid oxygen penetration involving in preserving fish fillet against myoglobin oxidation and therefore color loss. In their study, Feng et al. (2016) demonstrated that the use of gelatin as edible coating form a barrier against oxygen and protect golden pomfret fillet during cold storage. In this study, the protective effect of gelatin was considerably improved by the addition of BGH, previously showing great antioxidant activity (Abdelhedi et al., 2017).

3.2.3. Lipid oxidation

Lipid oxidation is a main factor limiting the shelf-life of marine products. Lipid peroxidation during fish storage was measured in terms of thiobarbituric reactive substances (TBARS) (mg of malondialdehyde (MDA) per kg of fish sample) and conjugated dienes (Fig. 4A and B). Results showed that MDA and specific extinctions coefficients of different samples increased during storage period. Up to the 3rd day of chilled storage, there was no significant difference in MDA content and conjugated dienes between uncoated and gelatin-coated samples. Nevertheless, the control group showed the highest MDA content (1.46 of MDA/kg of fish) after 8 days of storage, while BG + BGH coated fillets exhibited the lowest MDA content (0.66 mg of MDA/kg), among all studied samples ($p \leq 0.05$). In fact, the MDA content has been reduced by 25%, 41% and 54%, respectively, after CG, BG and BG + BGH coatings (Fig. 4A). Similarly, for the conjugated diene level, BG + BGH-coated samples displayed the lowest values all over the storage period (Fig. 4B). Gelatin hydrolysates are known by their antioxidant potential involved in the inhibition of the early stages of lipid oxidation as well as the delay of the oxidation process propagation (Jridi et al., 2014). These results suggest that gelatin hydrolysate contains bioactive peptides, which could react with free radicals to convert them to more stable products and terminate the radical chain reaction, resulting in the protection from lipid peroxidation. In this context, Jridi et al. (2014) demonstrated that gelatin hydrolysis generated peptides, with electron donating potential, to terminate lipid peroxidation reaction.

In this context, Nowzari, Shábanpour and Ojagh (2013) demonstrated that gelatin-chitosan coating reduced TBARS level in refrigerated rainbow trout fillets during 16 days of storage. Similarly, Nikoo, Benjakul and Xu (2015) showed that Amur sturgeon skin gelatin hydrolysates were effective in preventing lipid oxidation as evidenced by the lower MDA formation in unwashed fish mince. Farajzadeh et al. (2016) reported also that both antioxidant and oxygen barrier properties of gelatin contributed to control the lipid oxidation of shrimp under refrigerated condition. Chicken breast fillets, likewise, wrapped in starch-gelatin exhibited very slow lipid oxidation throughout storage (Moreno, Atarés, Chiralt, Cruz-Romero, & Kerry, 2018).

A gelatin coat on fresh meat products should reduce lipid oxidation because the hydrogen bonds present in gelatin act as a barrier to oxygen (Antoniewski, Barringer, Knipe, & Zerby, 2007). On the other hand, Marggrander and Hofmann (1997) studied the lipid oxidation of gelatin coated fish and found lower level of MDA compared to uncoated sample. These authors attributed the protective effect of gelatin films to the presence of ionic functional groups, which create strong polymer-fish interaction, resulting in good oxygen barrier properties. Thus, it could be concluded that gelatin is a good barrier to oxygen diffusion and its potential could be strongly enhanced by the addition of bioactive molecules, such as gelatin hydrolysates.

3.2.4. Protein oxidation

Protein oxidation of the fish samples was analyzed by the quantification of carbonyl compounds (µmol) per g of protein extracted. As shown in Fig. 4C, carbonyl level increased for all the groups as the storage time progressed through 8 days of storage, indicating that the proteins would be more oxidized with the progression of time. A significant difference ($p \leq 0.05$) was recorded between carbonyl content in control and coated fish fillets. However, no significant difference ($p > 0.05$) was observed between the two gelatin (bovine and that from black-barred halfbeak skin) coating treatments on the carbonyl compounds level during all the days of storage. Nevertheless, BG + BGH-coated group showed the lowest oxidized proteins level estimated at 0.43, 0.66 and 0.77 µmol/g of protein on day 3, 6 and 8, respectively. This result leads to suggest that the amount of antioxidants (gelatin hydrolysate) in fish was enough to prevent from fish

Table 2
Free amino acid and nucleotide contents of fish samples measured at 0, 3, 6 and 8 days of storage.

		Day 0	Control « un-coated»			CG			BG			BG + BGH		
			Day 3	Day 6	Day 8	Day 3	Day 6	Day 8	Day 3	Day 6	Day 8	Day 3	Day 6	Day 8
Free amino acids (μg/g of sample)	Asp	91.75	75.73	71.26	178.32	80.26	80.98	80.36	86.85	96.65	75.15	90.32	90,25	90.33
	Glu	115.04	273.91	288.13	337.92	115.20	200.00	200.63	201.76	177.35	188.97	103.65	179,36	180.39
	Ser	42.68	108.68	105.33	171.64	31.02	100.20	103.25	66.56	150.00	160.13	46.03	50,3	50.16
	Gly	242.96	262.36	301.42	682.36	258.36	248.63	335.65	276.35	337.56	412.60	225.36	260,5	262.85
	His	241.36	–	–	–	221.38	32.44	–	242.63	33.47	30.32	251.36	159,7	149.50
	Thr	73.50	73.27	61.48	103.16	62.16	64.74	65.60	79.20	79.20	68.26	73.25	76,3	79.36
	Ala	63.97	–	–	–	48.93	46.53	43.67	64.64	51.87	52.68	63.90	55,5	55.89
	Arg	42.27	–	–	–	49.36	43.78	38.11	64.22	38.63	31.00	46.72	45,34	44.33
	Pro	128.32	100.30	101.50	90.83	113.22	101.50	99.47	105.60	105.62	109.60	105.32	100,78	98.36
	Tyr	225.67	456.35	1421.25	1475.00	222.20	215.36	527.84	375.46	354.00	335.60	387.20	458	457.80
	Val	1308.20	1528.40	1206.80	2960.6	1363.25	1614.16	1754.83	1466.93	1429.35	1426.80	1400.00	1270,3	1249.2
	Ile	24.70	45.12	41.39	178.60	22.02	44.99	44.44	25.96	38.73	40.53	30.21	35,79	34.34
	Leu	141.74	191.17	163.23	270.25	148.20	150.63	149.21	140.02	145.68	149.65	155.25	156,1	160.01
	Phe	–	–	–	11.07	3.78	–	–	19.83	–	8.33	8.12	10,2	9.94
	Trp	4.99	5.59	6.51	13.44	5.71	5.97	6.00	5.00	6.01	5.36	4.78	5,31	5.57
	Lys	3.35	65.24	67.03	62.07	4.28	8.35	7.87	3.48	4.48	6.57	5.24	5,59	5.62
	Total (mg/g)	2.75	3.19	6.84	6.54	2.75	2.96	3.46	3.22	3.04	3.10	3.00	2.95	2.93
Nucleotide (μmoles/g)	ATP	199.21	106.64	0.02	0.03	199.79	81.34	73.37	188.82	87.47	76.77	180.72	123.73	76.62
	ADP	48.50	20.79	17.47	0.27	17.69	17.87	14.90	26.92	15.11	15.04	21.15	21.96	19.45
	AMP	265.68	268.60	59.73	14.58	200.68	207.19	195.30	210.39	179.20	157.90	259.30	253.30	260.32
	IMP	1697.59	1176.69	899.03	667.93	980.34	967.17	691.29	1053.82	1760.20	904.00	1176.30	1547.9	1495.32
	Hx	661.61	775.82	501.25	423.19	821.06	507.29	412.21	674.91	443.98	538.77	767.71	538.34	700.24
	Ino	1402.72	1058.81	749.21	584.55	1033.79	780.04	607.14	1147.46	900.01	800.48	975.37	584.3	235.90
	Ud	24.15	152.34	297.56	487.36	103.20	251.42	356.20	92.35	105.96	204.38	22.65	15.20	8.76

Values are the mean of the different determinations.

meat proteins' oxidation during storage at 4 °C. Herring et al. (2010) showed that the carbonyl compounds increased for all the samples as the days progressed through 7 days of storage, and values were significantly lower after gelatin coating treatment.

Proteins are attacked by reactive oxygen species generated by diverse external factors, such as manipulation, slaughter, or the presence of metals (iron). The interaction can lead to the formation of carbonyl groups, affecting structural, functional, and nutritional properties of fish muscle. Furthermore, protein oxidation induces the decrease of water holding capacity as the protein network would be more friable, once proteins were oxidized (Feng et al., 2016). Morachis-Valdez et al. (2017) demonstrated also that chitosan coating prevented common carp (*Cyprinus carpio*) fillet from protein oxidation, during its storage in the freezer.

3.2.5. Free amino acids quantification

The free amino acid (FAA) contents in all fish samples were determined and results are presented in Table 2. The total content of FAA in uncoated fish fillets was 2.75 μg/g and 6.84 μg/g of fish meat before and after 6 days of storage, respectively. By contrast, this rise was significantly lower in coated fish groups, and no significant increase was noted in fish samples coated with BG + BGH after 8 days of storage (2.93 μg/g of fillet). The increase in the FAA quantities is mainly due the hydrolysis of meat proteins by the microorganisms developed during storage. Nevertheless, during the last two days, total FAA decreased (from 6.84 to 6.54 μg/g) in uncoated fillets, which may be explained by the consumption of available amino acids by endogenous bacteria during their growth. Particularly, His, Ala, and Arg were completely degraded since the first 3 days of storage at 4 °C, while this degradation was delayed by 3 additional days after CG or BG coating.

It has been reported that the content of FAA in fish muscle depend on the activation of neutral amino-peptidases at high pH value (Kakaei & Shahbazi, 2016). This decrease was, however, absent in coated smooth hound fillets, thanks to the barrier role played by the gelatin against bacteria proliferation. As the same, Jridi et al. (2018) showed that the uncoated control meat had the highest FAA content, while it was significantly reduced in gelatin coated ones after 8 days of storage.

3.2.6. Nucleotides quantification

Post-mortem degradation of ATP in fish muscle occurs due to the hydrolytic effect of endogenous enzymes. This degradation goes through the intermediate products ADP, AMP, inosine mono-phosphate (IMP), inosine (Ino), and hypoxanthine (Hx). Most of the adenosine nucleotides disappear quickly because they are degraded into IMP within the first days after fish capture, and as the degradation continues, Ino and then Hx will be produced (Souza et al., 2010). Thus, the high inosine and hypoxanthine levels indicated about the development of fish spoilage. In this study, the quantification of nucleotides in the different groups was carried out in order to evaluate fish freshness (Table 2).

Results showed that adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) levels decreased with time in all the groups, and their degradation rate was faster in control samples than in coated ones ($p \leq 0.05$). On the other hand, the decrease of IMP was accompanied with a proportional increase of uridine (Ud). In coated samples, however, the nucleotides degradation was slower, where BG and BG + BGH treated samples showed the highest ATP, ADP and AMP contents and the lowest Ud contents after 8 days ($p \leq 0.05$). Özogul, Taylor, Quantick, and Özogul (2000) reported that freshness indicators related to the nucleotides degradation are mainly based on the autolysis of ATP in the muscle. Additionally, the rapid rise of degradation is entirely due to the sharp decline of IMP in the fish flesh. The loss of IMP through degradation to Hx and Ino would cause the loss of fish freshness.

In this context, several works have studied the changes in the nucleotides metabolism and they found, similarly, increased levels in Ino and Hx during fish processing (Souza et al., 2010). In addition, Fan et al. (2009) showed that chitosan coating was effective in inhibiting the degradation of ATP and extending the frozen storage life of fish samples, showing a reduction of 45% of nucleotide degradation after 7

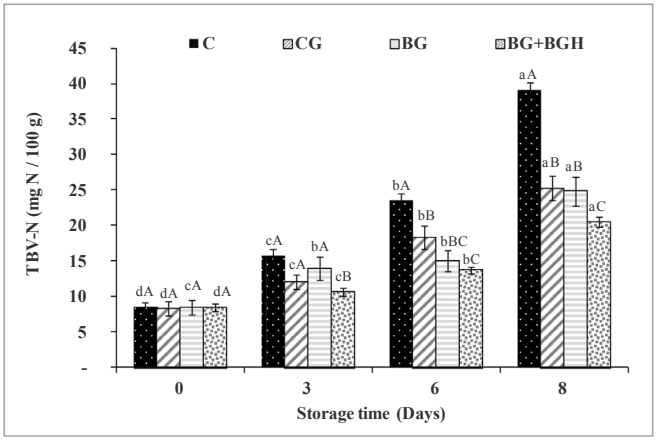

Fig. 5. Total volatile basic nitrogen (TVB-N) (mg N/100 g) at 0, 3, 6 and 8 days of storage. A,B,C: means in each treatment with different letters are significantly different (P ≤ 0.05); a,b,c,d: means in different days of storage with different letters are significantly different (P ≤ 0.05).

days of frozen storage. The oxygen barrier property of gelatin helps to prevent fillets against oxidation, implied in ATP degradation, resulting therefore in extending the shelf-life of fish fillets (Andevari & Rezaei, 2011).

3.2.7. Total volatile basic nitrogen

As the fish spoilage progresses, total volatile basic nitrogen (TVB-N) compounds develop and their increasing levels are regularly used as an indicator of the spoilage of fish products (Santos et al., 2013). Fig. 5 shows the variation of TVB-N contents during the chilled storage of smooth hound fillet. The initial TVB-N was about 8.5 mg N/100 g of sample in all the groups. Thereafter, this value increased gradually with the time of storage and it was evident that the most rapid rate of increase was found in the control group. In fact, after 6 days of storage, TVB-N value reached 23.57, 18.30, 15.0 and 13.73 mg N/100 g in control, CG, BG and BG + BGH groups, respectively. The protective effect of gelatin coating was further recorded after 8 days of storage, and the BG + BGH treatment was able to reduce the TVB-N content to 20.49 mg N/100 g, whereas it was equal to 39.10 mg N/100 g in the uncoated fillet. At the end of the storage, TVB-N value didn't reach the acceptable limit of 25–35 mg N/100 g of fish (EC Commission Regulation 1022/2008), except for control sample (39.10 mg N/100 g), which proves that coated fillets still preserve their freshness. Furthermore, there was no significant difference between CG and BG ($p > 0.05$), but a clear difference was recorded between BG and BG + BGH-coating ($p \leq 0.05$). The increase in TVB-N content may be attributed to ammonia produced from bacterial catabolism of nitrogen-containing compounds (Arancibia, López-Caballero, Gómez-Guillén, & Montero, 2015). The catabolism of amino acids in fish muscle results in the accumulation of different products such as, ammonia, monoethylamine, dimethylamine, trimethylamine, and other volatile bases, which affect sensory properties of fish (Huang et al., 2012).

Our results were similar to those of Ojagh, Rezaei, Razavi, and Hosseini (2010) who demonstrated that coated and uncoated rainbow trout fillets contained 22.86 and 42.93 mg N/100 g, respectively, after 8 days of refrigerated storage. Likewise, Andevari and Rezaei (2011) found that gelatin coating incorporated with cinnamon oil significantly lowered the TVB-N content of rainbow trout fish. In addition, Farajzadeh et al. (2016) reported that chitosan-gelatin coating of shrimp (*Litopenaeus vannamei*) caused a reduction of 35% in the formation TVB-N after 14 days of storage period.

It can be concluded that coating with gelatin/hydrolysate is more effective than the gelatin applied alone. These effects may be attributed to the inhibitory activity of microbial growth exerted by BGH. Overall,

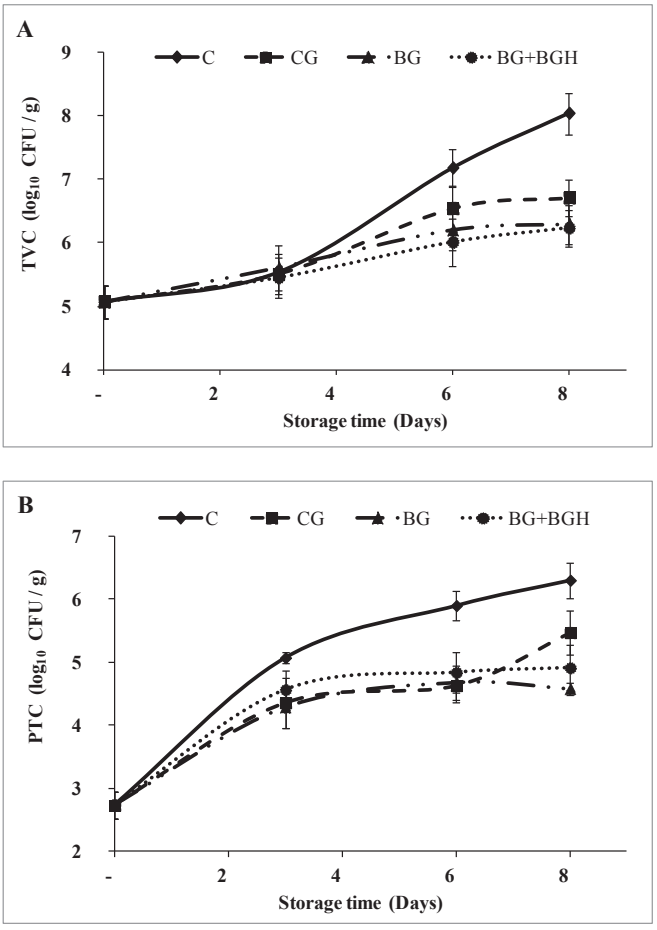

Fig. 6. Total viable counts (TVC) (A) and psychrotrophic counts (PTC) (B) of fish samples at 0, 3, 6 and 8 days of storage.

the TVB-N results are in accordance with the lipid and protein oxidation as well as fish color. This study revealed, therefore, that gelatin coating helps to prevent color deterioration and meat oxidation of fish fillets and its effect may be improved by the addition of peptides as bioactive agent.

3.2.8. Microbiological quality during storage

The total viable count (TVC) of bacteria is a key indicator of the quality of fish fillets. Changes in microbial populations in fish fillets during storage were determined and the results are shown in Fig. 6. On the first day, the total viable (TVC) and psychrotrophic (PTC) counts were about 5 and 3 $\log_{10}$ CFU/g, respectively, thanks to hygienic conditions used during transport, slaughter and manipulation of fish fillets. During storage time, these levels trend to be markedly increased in all samples and reached, at the last day, 8 $\log_{10}$ CFU/g (TVC) and 6.3 $\log_{10}$ CFU/g (PTC) in control fillets. Nonetheless, as compared to control samples, gelatin coating significantly reduced TVC and PTC ($p < 0.05$), delayed and restricted the growth of microorganisms. Particularly, BG + BGH lead to 1.77 $\log_{10}$ CFU/g reduction in TVC and 1.45 $\log_{10}$ CFU/g in PTC, after 8 days of storage. Further, there was no significant difference between BG and BG + BGH treatments ($p > 0.05$). According to the International Commission on Microbiological Specifications for Foods (ICMSF, 1986), the limit established for a bacterial count in fish is 7 log CFU/g. This limit can be applied for both TVC and PTC. Thus, the gelatin-coating could be an effective way to extend the storage time of fish meat up to 8 days.

The effect of edible gelatin coating may be due to its barrier role against oxygen diffusion and thus bacterial proliferation, by forming a protein bio-film around the fish sample. Particularly, black-barred skin

gelatin could be a potential agent for better preservation of fish fillet during chilled storage. It has been reported that gelatin hydrolysates are known by their antibacterial effect against specific spoiler organisms of chilled fish, including *Shewanella putrefaciens* and *Photobacterium phosphoreum* (Gómez-Guillén et al., 2010). As the same, Jridi et al. (2018) have shown the antibacterial effect of fish gelatin coating on the protection of meat against bacterial spoilage during cold storage. In another study, Hosseini, Rezaei, Zandi, and Ghavi (2015) coated rainbow trout fillet with fish gelatin containing oregano essential oil (bioactive molecules) and observed an enhanced reduction of psychrotrophilic bacterial counts after bioactive gelatin coating in comparison with control and gelatin-coated samples. In addition, Wu et al. (2014) demonstrated that grass carp muscle covered with fish gelatin-chitosan film containing oregano oil showed the lowest total aerobic bacteria and TVB-N values, compared to the other groups.

4. Conclusions

In summary, this study emphasizes the use of black-barred halfbeak gelatin enriched with its Purafect-hydrolysate in the enhancement of smooth hound fillets quality and in the delay of their spoilage during conservation at 4 °C. In addition, gelatin coating helps to preserve the initial color of fresh fillets, slow the lipid and protein oxidation, microbial growth and nucleotides and proteins degradation. Moreover, BG coating was more efficient than bovine commercial gelatin, which was further improved by the addition of BGH in the gel of gelatin before fish dipping. Therefore, black-barred gelatin plays a beneficial role, as an edible coating agent, in extending the shelf life of the fish meat products.

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