

Oxidative and microbial stability of sea bass fillets containing *Padina gymnospora* extract stored under frozen conditions**Estabilidade oxidativa e microbiana de filés de robalo contendo extrato de *Padina gymnospora* armazenados sob congelamento**

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ABSTRACT

This study evaluated the antioxidant and antimicrobial activities of ethanolic extracts from five species of red and brown algae. The best algae were used to prepare an edible coating for sea bass (*Centropomus undecimalis*) fillets. The species *Padina gymnospora* was chosen for the application of the edible coating, as it presents greater efficiency in in vitro tests. The fillets were divided into four groups: SA1% + PG (1% sodium alginate solution + 20 mg mL⁻¹ of *Padina gymnospora*), SA1% + BHT (1% sodium alginate solution + 100 mg mL⁻¹ of butylated hydroxytoluene), SA1% (control) and uncoated fillets. The SA1% + PG group showed lesser lipid oxidation (0.80 ± 0.08 mg MDA kg⁻¹) than the uncoated group (2.10 ± 0.36 mg MDA kg⁻¹), without statistical difference from the value observed for containing BHT group (0.94 ± 0.07 mg MDA kg⁻¹). At 120 days, the group SA1% + PG reduced the count of psychrotrophic bacteria by 2-log cycles (p < 0.05). The colour of samples from SA1% + PG group was statistically different from that of other groups (p < 0.05), and influenced the acceptability of the coated fillets. The edible coating containing *P. gymnospora* extract may have potential application for the storage of perishable products such as fish, owing to its ability to reduce microbial load and lipid oxidation.

Keywords: Butylated hydroxytoluene. conservation of fish. edible coating. *Padina gymnospora*.

RESUMO

Este estudo avaliou a atividade antioxidante e antimicrobiana de extratos etanólicos de cinco espécies de algas vermelhas e marrons. A melhor alga foi usada para aplicação de um revestimento comestível em filés de robalo (*Centropomus undecimalis*). A espécie *Padina gymnospora* foi escolhida para o revestimento comestível, por apresenta maior eficiência nos testes in vitro. Os filés foram divididos em quatro grupos: SA1% + PG (solução de alginato de sódio a 1% + 20 mg mL⁻¹ de *Padina gymnospora*), SA1% + BHT (solução de alginato de sódio a 1% + 100 mg mL⁻¹ de hidroxitolueno butilado), SA1% (controle) e filés não revestidos. O grupo SA1% + PG apresentou menor oxidação lipídica (0,80 ± 0,08 mg MDA kg⁻¹) do que o grupo não revestido (2,10 ± 0,36 mg MDA kg⁻¹), sem diferença significativa do valor observado para o grupo BHT (0,94 ± 0,07 mg MDA kg⁻¹). O grupo SA1% + PG reduziu em 2 ciclos logarítmicos (p<0,05) a contagem de BP em 120 dias. A cor das amostras do grupo SA1% + PG foi estatisticamente diferente dos demais grupos (p<0,05), influenciando na aceitabilidade dos filés revestidos. O revestimento comestível contendo extrato de *P. gymnospora* apresenta potencial aplicação para o armazenamento de produtos perecíveis, como peixes, devido à sua capacidade em reduzir bactérias psicotróficas e a oxidação lipídica.

Palavras-chave: Hidroxitolueno butilado, conservação de peixes, revestimento edível, *Padina gymnospora*.

1 INTRODUCTION

Fish are valuable sources of proteins, lipids (mainly omega-3 and -6) and essential micronutrients for a balanced diet (Reverter et al., 2014). However, fish is highly susceptible to chemical and microbiological deterioration, presenting several risks to the

consumers health. In addition, the fish deterioration, due to its very short shelf life, may cause economic losses to producers (Alsaggaf et al., 2017).

Among fish conservation methods, freezing is an effective method of preservation as it minimizes undesirable chemical changes and keeps fresh fish characteristics. However, chemical and biochemical reactions that occur during prolonged freezing contribute to the fish deterioration. Fish and fish products lose their quality due to the loss of moisture in the environment and the oxygen diffusion in fish meat that increases lipid oxidation (Ambardekar, 2007).

The genus *Centropomus*, known commercially as sea bass, is a Brazilian native fish highly valued by the consumer market, occurring in the tropics and subtropics of the Americas from the Florida coast, in the United States, to Santa Catarina, Brazil (Rivas, 1986). The most economically important species are *C. undecimalis* and *C. parallelus*, with a production of 3,680.3 t in 2011 (Brasil, 2013). Fish are widely marketed whole and, when sold in fillet form, have a shorter shelf life. Thus, extending the shelf life of frozen fish fillets through edible coatings, minimizing biochemical changes that affect texture and flavour, increases the shelf life of the food.

Several studies have been conducted using natural compounds to extend shelf life and replace chemical preservatives in perishable foods such as fishery products (Choulitoudi et al., 2016; Alsaggaf et al., 2017). The use of macroalgae may be promising in the food industry, as the secondary compounds (around 1140, mainly of the terpenes classes and phenolic substances) produced during algal metabolism present themselves as potential natural antioxidant and antimicrobial compounds (Blunt et al., 2014; Peinado et al., 2014).

The extract of the genus *Padina* stands out for its antimicrobial and antioxidant effect on foods as well as high oxidative stability partially related to the presence of polyunsaturated fatty acids (Chander et al., 2014; Dussault et al., 2015). The immersion of fish meat in a seaweed coating solution acting as a semipermeable barrier may reduce moisture content, gaseous exchange, and oxidative reactions. In addition, the bioactive compounds may improve the colour and flavour of the meat and exert antimicrobial and antioxidant actions, thereby improving the shelf-life of the product (López-de-Dicastillo et al., 2012; Alsaggaf et al., 2017).

Although the application of edible coatings is ideal for foods that are not individually packed due to size, there are no reports on the use of edible coatings made from the ethanolic extract of *Padina gymnospora* for fish products. The objective of this work is to verify the antioxidant and antimicrobial activities of the ethanolic extracts of different seaweeds collected from natural banks and to evaluate the application of the edible coating from seaweed extracts in the frozen fillet of sea bass.

2 MATERIALS AND METHODS

2.1 CHEMICALS AND EQUIPMENT

Were used: Ethanol (95% v/v) made by Êxodo Científica, Hortolândia-SP, Brasil; 2,2-diphenyl-1-picrylhydrazyl, thiobarbituric acid, Potassium persulfate ($K_2S_2O_8$); 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) and Trolox, from Sigma-Aldrich, St. Louis, MO, USA; Hydrochloric acid (ProQuímicos, Rio de Janeiro, Brasil); Glycerin (ProQuímicos, ProQuímicos, Rio de Janeiro, Brasil); Chloramphenicol and Sodium resazurin (Cequímica-INLAB, Fortaleza-CE, Brasil), and Sodium alginate (Êxodo Científica, Hortolândia-SP, Brasil). In addition to the equipment: Spectrophotometer (Spectrum SP- 1105, Ningbo Hinotek Technology Co., Ltd., Zhejiang, China) and RE-52A Rotary Evaporator (Lanphan, Zhengzhou, Henan, China).

2.2 COLLECTION OF MACROALGAE

In the months of January to June 2016, two species of brown algae, *Sargassum vulgare* and *Padina gymnospora* (Phaeophyta) and three species of red algae, *Gracilaria birdiae*, *G. Cervicornis*, and *G. Caudata* (Rodophyta) were manually collected during low tide in the natural banks on the beach of Manguinhos in Itaparica, Bahia, Brazil. Samples of the algae were deposited in the Herbarium of the Federal University of the Recôncavo of Bahia, Cruz das Almas. During transportation, the species were placed in polyethylene bags packed in isothermal boxes containing ice.

2.3 PREPARATION OF EXTRACTS

For the preparation of algal extracts (in triplicates), the specimens were washed in running water and distilled water to remove soil, epiphytes, and salts. The ethanolic extracts were prepared using Brito et al. (2018) method with some modifications.

Extraction was carried out by adding 50 g of the algal powder to 500 mL of ethanol (92.8 °INPM) for 72 h at room temperature (24-25 °C), with no exposure to light. The solvent, 500 mL of ethanol, was added every 24 h. After 72 h, the ethanolic extracts were obtained under vacuum on a rotary evaporator (RE 52A) at 40 °C. The dried residue was stored in amber flasks at 5 °C until analysis. The yield of the extract was determined for each group of algae (Raymundo et al., 2004).

2.4 IN VITRO ANTIOXIDANT ACTIVITIES OF ALGAL ETHANOLIC EXTRACTS

The antioxidant activities of algal ethanolic extracts were evaluated by measuring the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radicals-scavenging activities in triplicates.

The Duan et al. (2006) method, with some modifications was used to performed the DPPH measurement. For this, about 300 µL of each of the algal extracts at different concentrations (1, 2,5, 5, 12,5, 15, and 20 mg mL⁻¹) were mixed in 2.7 mL of DPPH (6 x 10⁻⁵ mol L⁻¹). Blank for each concentration was prepared with 300 µL of the extract in 2.7 mL of methanol, owing to the colour of the extracts. The reaction was allowed to proceed for 2 h in the dark at room temperature, and the absorbance was read at 517 nm with a Genesys™ UV/Visible Spectrophotometer. Gallic acid (10 mM) was used as a positive control. The DPPH radical scavenging effect was calculated from the percentage of DPPH discoloration using the following equation: % scavenging effect = (1 - [A_{sample} - A_{sample white} / A_{control}]) × 100, where A_{sample} is the absorbance of the DPPH + sample solution, A_{sample white} is the absorbance of the sample without DPPH, and A_{control} is the absorbance of DPPH solution.

The ABTS method was performed as described by Re et al. (1999). ABTS + radical was generated from the chemical reaction with potassium persulfate (K₂S₂O₈), wherein 25 mL of ABTS (7 mM) was added to 440 µL of K₂S₂O₈ (140 mM). The solution was kept in the dark for 12-16 h at ambient temperature to generate radicals. An appropriate volume of the above solution was diluted in ethanol to obtain an absorbance value of 0.70 ± 0.02 at 734 nm wavelength. Upon formation of ABTS + radical, about 2 mL of this solution was mixed with 100 µL of each algal extract at different concentrations and the absorbance value was recorded at 734 nm. Trolox (10 mM) was used as the positive control. The ABTS radical scavenging effect was calculated from the percentage of

discoloration of ABTS using the following equation: % scavenging effect = $([A_{ABTS} - A_s] / A_{ABTS}) \times 100$, wherein A_s is the absorbance of the solution in the presence of the sample extract and A_{ABTS} is the absorption of the ABTS solution.

The antioxidant activity of samples was expressed as inhibitory concentration (IC_{50}), which was defined as the concentration ($mg\ mL^{-1}$) of the sample required to inhibit the formation of DPPH and ABTS radicals by 50%.

2.5 DETERMINATION OF DIFFERENT GROUPS OF PHENOLIC COMPOUNDS

The compositions of the ethanolic extracts of different algae were evaluated based on the different groups of phenolic compounds (Boulanouar et al., 2013). One milliliter of the extract was diluted in 1 mL of aqueous ethanol (95% v/v) containing 0.1% hydrochloric acid and 8 mL of 2% hydrochloric acid. To quantify the phenolic content, gallic acid solution was used as the standard and the absorbance was recorded at 280 nm wavelength. Caffeic acid was used as the standard for hydroxycinnamic acid derivatives and the absorbance was recorded at 320 nm, while quercetin solution was used as the standard for flavanols and the absorbance was recorded at 360 nm using a UV/Visible spectrophotometer, Genesys 10 mV model, Thermo Electron Corporation. The results were expressed as milligram of phenolic group per gram of extract.

2.6 IN VITRO ANTIMICROBIAL ACTIVITY OF ALGAL ETHANOLIC EXTRACTS

The minimum inhibitory concentration (MIC) of the five algal extracts were tested against Gram-negative bacteria, *Escherichia coli* ATCC 25922, *Salmonella enterica* serotype Enteritidis ATCC13076, and *Vibrio cholerae*, as well as Gram-positive *Bacillus cereus*, *Listeria monocytogenes* CERELA, *Enterococcus faecalis*, and *Staphylococcus aureus* ATCC 43300.

In order to choose the best inhibitory concentration, the microdilution technique was used in microplates (Santurio et al., 2007) with different concentrations of extracts (32, 16, 8, 4, 2, 10, 50, and $0.25\ mg\ mL^{-1}$). The negative control wells had no extracts, while the positive control included a solution of chloramphenicol ($0.10\ mg\ mL^{-1}$). About 20 μL of 0.01% sodium resazurin dye was added (Palomino et al., 2002), microbial multiplication was indicated by the change in the colour from blue to pink. The values of

MIC and minimal bacterial concentration (MBC) were obtained according to the Santurio et al. (2007) method. All assays were performed in triplicates.

2.7 ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *P. GYMNOSPORA* EXTRACT IN FOOD MATRIX

The fish, sea bass (*Centropomus undecimalis*), was purchased from the municipality of Canavieiras, Bahia, Brazil, and transported to the laboratory in thermal boxes containing ice. The fish were eviscerated, washed, and filleted. For edible coating, *P. gymnospora* extract was chosen due to its antioxidant and antimicrobial activities observed in vitro tests. The methodologies proposed by Oussalah et al. (2006) and Lu et al. (2009), with slight modifications, were used to obtain the coating solution. For this, 1% sodium alginate plasticizer was solubilized at room temperature in sterile distilled water (500 mL) by shaking at 1000 rpm. This was mixed with 5 mL glycerin (final concentration of 1%) and 10 g of the algal extract (concentration obtained from the results of antioxidant activity [DPPH and ABTS assays]).

About 100 g of fillets were immersed for 10 s in four treatment solutions as follows: group SA1% + PG (1% sodium alginate solution + 20 mg mL⁻¹ of *P. gymnospora*), group SA1% + BHT (1% sodium alginate solution + 100 mg mL⁻¹ of BHT), and SA1% (control) - 1% sodium alginate solution, and uncoated fillets. The samples were dried at room temperature for 2 min, packed in Styrofoam trays with polyethylene film, and stored at -18 °C for 120 days (Santos, 2014). Analysis was performed at different time points (0, 30, 60, 90, and 120 days) in triplicate. Fifteen fillets were prepared for each treatment; therefore, a total of 60 fillets were evaluated during the experiment. Frozen material analyses were performed after thawing obtained by storage at 4 °C, overnight.

2.8 MICROBIOLOGICAL AND PHYSICOCHEMICAL ANALYSES

During the storage period, psychrotrophic bacteria were calculated according to Silva et al. (2010).

Lipid oxidation analysis was carried out by the measurement of thiobarbituric acid reactive substances (TBARS), as described by Buege and Aust (1978), with some modifications. Ten grams of the sea bass fillet was homogenized in 50 mL of 7.5% solution of trichloroacetic acid (TCA) and filtered. The filtered solution was placed in a

50 mL flask and the final volume was adjusted with 7.5% TCA. Five mL of the filtered solution in triplicates was added to 5 mL of 0.02 M thiobarbituric acid (TBA), and the mixture was heated in a water bath at 100 °C for 15 min. The absorbance of the cooled mixture was read at 532 nm, with TCA solution as a blank. Quantification of TBARS levels in the samples was performed using a standard curve of 1,1,3,3-tetraethoxypropane (TEP 1.0×10^{-3} M). The results were expressed as milligram of malondialdehyde per kilogram of fish fillet sample.

2.9 SENSORY ANALYSIS

Fifty randomly selected members of academic community and untrained consumers participated in the sensory acceptance test. Of these, 60% were females and 40% were males, and 86% were between the age group 20 and 40 years; the remaining 14% were between 40 and 65 years of age. The project was approved by the Ethics Committee of Universidade Federal do Recôncavo da Bahia - UFRB with opinion no. 1,624,495. The fish fillets were thawed, seasoned with little salt and black pepper, grouped according to the groups treated, grilled, and served. To evaluate the sensory attributes (colour, visual appearance, aroma, texture, and flavour), a non-structured hedonic scale of 1-9 cm between the anchors was applied (weak, moderate, and strong) (Minim, 2006).

2.10 STATISTICAL ANALYSIS

The experiment was carried out in a completely randomized design in a simple 5 x 4 factorial scheme, with five evaluation periods (0, 30, 60, 90, and 120 days) and four treatments groups (Uncoated, SA1% (control), SA1% + PG, and SA1% + BHT), with three replicates. Moisture, pH, and TBARS were evaluated. Data was analyzed using analysis of variance followed by Tukey and Scott Knott tests. Regression analysis was used to evaluate the quantitative factor (evaluation periods). Shapiro-Wilks normality test was performed, and Spearman correlation coefficients and their significances were tested using the Student's *t*-test at 5% probability. For the sensory data, a biplot of the main components was generated representing a distribution of the data, related to the factorial loads of the attributes. The analyses were performed with the statistical program R (R Core Team, 2017).

3 RESULTS AND DISCUSSION

3.1. YIELD OF THE ALGAL EXTRACTS

The yield was higher for the Phaeophyta phylum species, *S. vulgare* and *P. gymnospora* (2.33% to 2.73%) compared to Rhodophyta species, *G. birdiae*, *G. cervicornis*, and *G. caudate* (0.27% to 0.80%). Specifically, the yield for *P. gymnospora* was 2.33%. Differences in the compositions may be attributed to the variation in yield values for the extracts from the same algal group.

3.2 PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY

Even though studies have shown that the content of phenolic compounds to be higher in brown and green algae than in red algae (Kumar et al., 2011), the chemical composition of brown seaweeds is still not completely elucidated. Recent studies have revealed at least part of the phenolic composition of this seaweed family (Agregán et al., 2017).

As shown in Table 1, significant differences ($p < 0.05$) were observed in the content of phenolic compounds among the species studied. Of the five ethanolic extracts analyzed, brown algae (*P. gymnospora* and *S. vulgare*) showed higher contents of flavonols, hydroxycinnamic acids, and phenols (descending order), whereas no significant difference ($p \leq 0.05$) was observed in red algae (*G. caudata* and *G. cervicornis*). *G. birdiae* presented the lowest concentration of all the three compounds.

Table 1. Different groups of phenolic compounds and antioxidant activity (DPPH and ABTS methods) obtained from ethanolic extracts of red and brown seaweed.

Phylum	Seaweed	Phenolic compounds ^a			Antioxidant activity ^b	
		Flavonols ¹	Hydroxycinnamic acids ²	Total phenols ³	DPPH IC ₅₀ ⁴	ABTS IC ₅₀ ⁴
Phaeophyta	<i>P. gymnospora</i>	104.78a	102.42a	206.68a	2.10b	2.06b
	<i>S. vulgare</i>	45.52b	47.56b	103.95b	4.75c	2.58bc
Rhodophyta	<i>G. cervicornis</i>	6.37c	7.95c	16.76c	5.48c	3.31c
	<i>G. caudata</i>	6.09c	7.84c	16.74c	6.40d	7.66d
	<i>G. birdiae</i>	4.33d	5.88d	13.02d	16.75e	17.28e

^aMeans followed by the same letter in the column do not differ significantly, at the 1% probability level by the Tukey Test. ¹mg EQ g⁻¹ (mg of quercetin equivalent per g extract); ²mg EAC g⁻¹ (mg of caffeic acid equivalent per g extract); ³mg EAG g⁻¹ (mg of gallic acid equivalent per g extract). ⁴Average IC₅₀ values (mg mL⁻¹). ^bMeans followed by the same letter in the column do not differ significantly, at the 5% probability level by the Tukey Test.

The concentration of total phenols in the *P. gymnospora* extract (206.68 ± 0.89 mg gallic acid equivalent [EAG] g^{-1}) was higher than that reported by Fellah et al. (2017). The value reported was higher than that observed for the extracts of *Sphaerococcus coronopifolius* (144.33 ± 1.76 mg EAG g^{-1}), *Halopteris scoparia* (105.40 ± 1.19 mg EAG g^{-1}), and *Zonaria tournefortii* (78.46 ± 0.79 mg EAG g^{-1}), and lower than the value observed by Uribe, Vega-Gálvez, Heredia, Pastén, and Di Scala (2018) (308.50 ± 19.08 mg EAG g^{-1}) for the extract of the red alga *Pyropia orbicularis*. The amount of phenolic compounds found among marine macroalgae may be related to intrinsic factors such as morphology, age and reproductive stage, interactions with the environment, like herbivory, irradiance, depth, salinity, temperature, and nutrients (Fellah et al., 2017), and the type of extraction solvent, which may influence the extraction efficiency of the bioactive compounds (Uribe et al., 2018).

The antioxidant activity evaluated by DPPH method showed significant differences in the reduction capacity of different extracts ($p \leq 0.05$). As show in Table 1, the IC_{50} values ranged from 2.10 ± 0.03 to 16.75 ± 0.8 mg mL^{-1} . No significant difference was observed between the activities of *S. vulgare* and *G. cervicornis* extracts ($p < 0.05$). However, *P. gymnospora* extract showed high antioxidant potential ($IC_{50} = 2.10 \pm 0.03$ mg mL^{-1}), while *G. birdiae* showed low IC_{50} activity (16.75 ± 0.8 mg mL^{-1}). Gallic acid which was used as a positive control showed significantly different IC_{50} value (0.05 mg mL^{-1}) (Table 1). The IC_{50} value of *P. gymnospora* and *G. birdiae* extract was 4.2 and 33.5 times higher than that of gallic acid (0.05 mg mL^{-1}), respectively.

The results of the ABTS method revealed the highest antioxidant activity for the *P. gymnospora*, *G. cervicornis*, and *S. vulgare* extracts; with no significant difference ($p \leq 0.05$) between the activities of these extracts (Table 1). Similar trend was observed in the DPPH analysis with the highest antioxidant activity for *P. gymnospora* (IC_{50} value of 2.06 ± 0.3 mg mL^{-1}) and *G. birdiae* extract (IC_{50} 17.28 ± 0.8 mg mL^{-1}). The IC_{50} value for trolox-positive control was 0.08 ± 0.002 mg mL^{-1} . The mean IC_{50} values of the algal extracts were higher than the value reported for the control. The IC_{50} value of the *P. gymnospora* and *G. birdiae* extracts were about 2.57 and 21.58 times higher than that of trolox.

The high DPPH free radical-scavenging capacity of brown algae (*P. gymnospora* and *S. vulgare*) may be related to the presence of phenolic compounds. Similar findings

were reported by Tenorio-Rodriguez et al. (2017), wherein the antioxidant activity of brown macroalgae was much higher than that of red and green macroalgae owing to the high polyphenolic content in brown macroalgae.

According to the Spearman correlation analysis, the content of flavonols, hydroxycinnamic acids, and total phenols showed significant correlations ($p \leq 0.05$) with DPPH and ABTS results. The values of Spearman correlation coefficient, r in all correlations were negative, indicating an inverse relationship between the phenolic contents and radical-scavenging abilities. The content of phenolic compounds and the low IC_{50} value of the extracts may highlight their antioxidant activities. According to Agatonovic-Kustrin et al. (2016), phenolic compounds are the main contributors of the antioxidant activity in algae.

3.3 IN VITRO ANTIMICROBIAL ACTIVITY OF ALGAL EXTRACTS

The macroalgae tested herein demonstrated antimicrobial activities against at least one tested microorganism. The highest antimicrobial activity was observed for the *G. caudate* extract, as observed from the inhibitory effect on the growth of four bacteria, followed by *G. cervicornis*, *G. birdiae*, and *P. gymnospora* that inhibited the growth of three bacteria, and *S. vulgare* (acting against single bacterium) (Table 2).

Table 2. Minimum inhibitory concentration (MIC) of the extracts from five species of macroalgae against gram-positive and gram-negative bacteria.

Microorganisms	Extracts of macroalgae (mg mL ⁻¹)					CLO
	<i>P. gymnospora</i>	<i>S. vulgare</i>	<i>G. birdiae</i>	<i>G. cervicornis</i>	<i>G. caudata</i>	
<i>E. coli</i>	>32.00	>32.00	32.00	>32.00	>32.00	0.24
<i>V. cholerae</i>	>32.00	>32.00	>32.00	>32.00	>32.00	0.24
<i>E. faecalis</i>	>32.00	>32.00	0.25	>32.00	32.00	0.12
<i>S. Enteritidis</i>	>32.00	>32.00	>32.00	32.00	32.00	0.12
<i>S. aureus</i>	8.00	>32.00	32.00	32.00	32.00	0.12
<i>L. monocytogenes</i>	0.25	0.50	>32.00	32.00	>32.00	0.24
<i>B. cereus</i>	8.00	>32.00	>32.00	>32.00	32.00	0.24

CLO - Chloramphenicol

Gracilaria species presented antimicrobial activity against both gram-positive and gram-negative bacteria at the highest concentration tested, except for *G. birdiae* that

presented an MIC value of 0.25 mg mL^{-1} for *E. faecalis*. The *P. gymnospora* extract was more efficient in inhibiting the growth of three microorganisms at low concentrations (Table 2). Among the gram-positive bacteria inhibited by *P. gymnospora*, *S. aureus* was the most sensitive, while inhibition of *E. faecalis* was not observed (Table 2). Algae extracts have antibacterial action, promoting the deformation of cytoplasmic protoplasm vacuolation in the cellular structure and distortion of outer cell boundary (El Shafay et al., 2016).

Enteric pathogens produce extracellular enzymes, toxins, flagella, and capsules and may undergo gene transfer; all of these factors may confer resistance to synthetic antimicrobials, as these drugs are widely used by industry to treat foodborne diseases (El Shafay et al., 2016).

These factors may have contributed to the high resistance of *Enterococcus* to the bioactive compounds present in *P. gymnospora* extract. El Shafay et al. (2016) reported that all extracts of *P. pavonia* isolated from the red sea inhibited most multi-resistant bacterial strains tested at MIC values ranging from 50 to 100 mg mL^{-1} , as observed in the present study. Baliano et al. (2016) analyzed the antimicrobial activity of *P. gymnospora* isolated from the coast of Espírito Santo, Brazil, and reported an MIC value of 0.5 mg mL^{-1} for *S. aureus*. Environmental variations in the region, the stage of algal growth, the solvent and extraction method employed may have contributed to the antimicrobial activity observed in the present work.

All the algal extracts presented bacteriostatic effect against at least three bacteria tested, except *S. vulgare* (one bacteria), validating the bioactive potential of the macroalgae, through the inhibition of the growth of pathogenic bacteria. However, it is believed that the synergistic action of two or more extracts may further increase the effects of the extracts. The antimicrobial activities of the five extracts studied revealed the presence of important secondary metabolites (Al-Saif et al., 2014) that may contribute to the development of useful alternatives to synthetic agents for food preservation.

3.4 EDIBLE COAT OF *P. GYMNOSPORA* IN THE FOOD MATRIX OF SEA BASS FILLETS

3.4.1 Determination of TBARS value

The effect of *P. gymnospora* extract on lipid oxidation after 120 days of storage observed in different groups was in the following order: SA1% + PG < SA1% + BHT < SA1% < uncoated (Figure 1). The SA1% + BHT group showed higher lipid oxidation rate than the SA1% + PG group, but no statistical difference was observed ($p > 0.05$), suggesting that the phenolic compounds present in the *P. gymnospora* extract (SA1% + PG) inhibited lipid peroxidation in fish fillets and may be used as an alternative to the synthetic antioxidant BHT (SA1% + BHT). In the edible coating, the phenolic compounds donate hydrogen atoms to the free radicals and may interrupt with the chain reaction during the process of lipid oxidation (Budilarto and Kamal-Eldin, 2015). Studies have reported the antioxidant effect of seaweed extracts on initial stages of lipid peroxidation (Gupta and Abu-Ghannam, 2011; Alsaggaf et al., 2017).

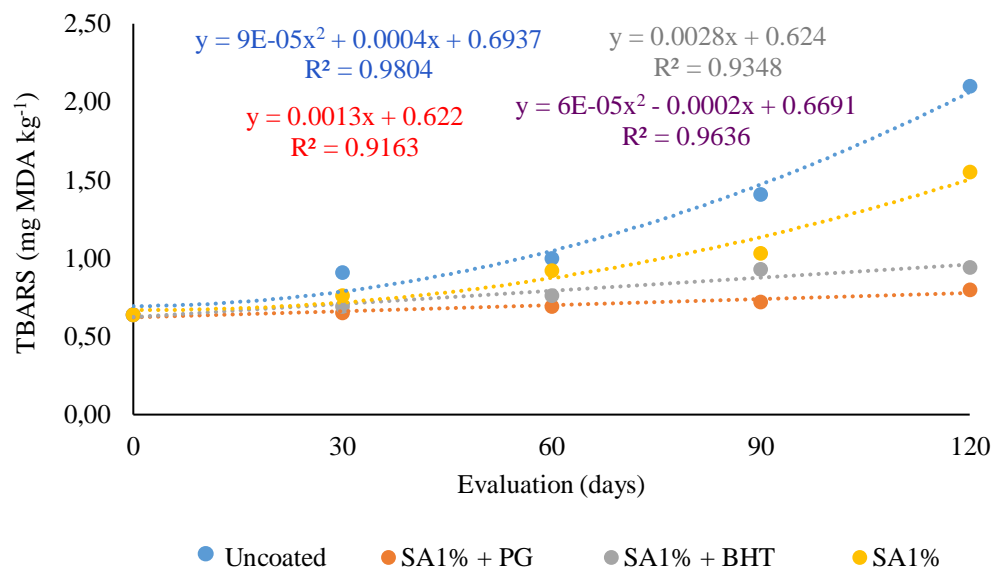


Figure 1. TBARS values (mg MDA kg⁻¹) in samples of sea bass fillets (*C. undecimalis*) stored at -18 °C for 120 days.

Uncoated: uncoated fillets; SA1% + PG: fillets coated with 1% sodium alginate solution + *P. gymnospora* extract; SA1% + BHT: fillets coated with 1% sodium alginate solution + BHT; SA1%: fillets coated with 1% sodium alginate solution.

The alginate control (S1%) coating may minimize the oxidative deterioration process (1.55 mg MDA kg⁻¹), and act as a semipermeable barrier, thereby reducing

moisture and gas exchange (Lu et al., 2009). Lipid oxidation value observed with alginate coating was almost double the value obtained with SA1% + PG (Figure 1). In the food industry, edible coatings have wide applications, as storage at low temperatures may be insufficient to prevent enzymatic processes such as lipid oxidation.

Lipid oxidation is an important phenomenon that determine the quality of foods. TBA value is one of the important indicators of oxidative rancidity (Ozogul et al., 2011). TBA value in the range of 1-2 mg MDA kg⁻¹ for fish samples is usually considered as the limit of acceptability (Lu et al., 2009). In the present study, the coated sea bass fillets had a TBA value below 2 mg MDA kg⁻¹, except for the uncoated group (2.1 mg MDA kg⁻¹). It was also observed that fish fillets coated with *P. gymnospora* extract may be stored for longer time, as the TBA value at 120 days of storage was only 0.80 mg MDA kg⁻¹ (Figure 1).

3.4.2 Total viable counts

Despite stalling microbial development the freezing process which makes them dormant, the microorganisms regain their activity during thawing. As the freezing process is faster than thawing, defrosting does not happen uniformly and exposes some parts of the meat immediately to temperatures favorable for bacterial growth. By favoring the exudate, thawing carries nutrients that will favor microbial growth thereby reducing the nutritional quality of the meat (Leygonie et al., 2012).

The count of cultivable psychrotrophic bacteria throughout the storage was lower ($p > 0.05$) in the SA1% + PG and SA1% + BHT groups. At 60 days storage, the population was reduced by 3-log cycles in the alga-treated group when compared to uncoated group and other treatments (Table 3), demonstrating the antimicrobial activity of the bioactive compounds in *P. gymnospora* extract. The bioactive compounds present in brown algae responsible for antibacterial activity may be indoles, terpenes, acetogenins, phenols, fatty acids, and volatile halogenated hydrocarbons compounds (El Shafay et al., 2016).

Table 3. Psychrotrophic count in sea bass fillets coated with macroalgal extract stored at -18 °C for 120 days.

Period of Storage (days)	Psychrotrophic counting log CFU g ⁻¹			
	Uncoated	SA1% + PG	SA1% + BHT	SA1%
0	4.33Ca	4.33Ca	4.33Ca	4.33Ca

30	4.30Ca	4.15Ca	4.15Ca	4.34Ca
60	3.48Bd	1.65Ba	2.86Bb	3.20Bc
90	3.13Ac	1.18Aa	2.74Bb	3.11Ac
120	3.07Ac	1.24Aa	2.09Ab	3.04Ac

Uncoated: uncoated fillets; SA1% + PG: fillets coated with 1% sodium alginate solution + macroalga extract *P. gymnospora*; SA1% + BHT: fillets coated with 1% sodium alginate solution + BHT; SA1%: fillets coated with 1% sodium alginate solution. Lower case letters compare treatments at each time and uppercase letters compare treatments over time. Different letters show significant differences as analyzed with Tukey's test ($p \leq 0.05$). CFU: Colony forming unit.

3.5 SENSORY ANALYSIS

The relative measure of the sensorial attributes in the performance variability of the treatments were 22.76% for texture, 21.25% for appearance, 20.08% for colour, 18.49% for aroma and 17.42% for flavour, demonstrating similarity between the evaluated parameters. According to the statistical analysis, it is evident that the tasters assigned scores of different magnitudes among the sensorial attributes proposed in this study. Considering the proximity of the positioning of the treatments groups in relation to the vectors, it was observed that (SA1% + PG) coating exhibited behavior opposite to the uncoated treatment. The SA1% + PG presented higher scores for appearance and colour, and lower for aroma and texture, while the uncoated received the highest scores for aroma and texture and lower for appearance and colour (Figure 2).

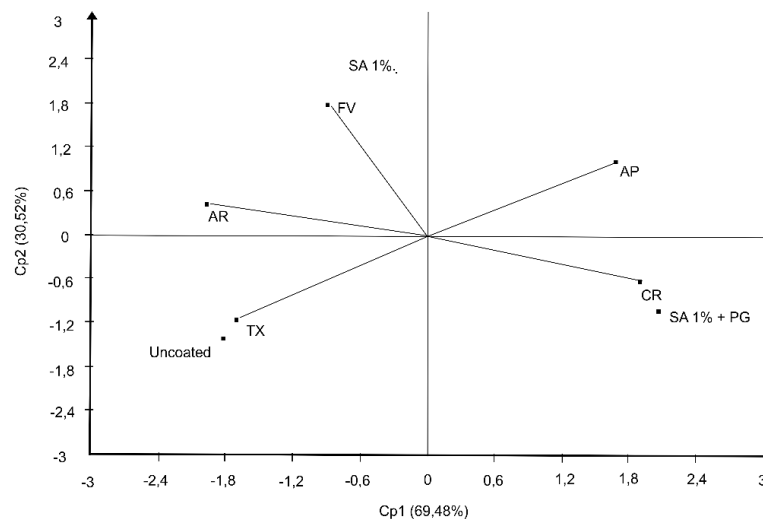


Figure 2. Sensory tests analysis by analyzing Principal Components. Biplot for the first and second principal components (PC1 and PC2 representing 69.48% and 30.52%, respectively).

Uncoated: uncoated fillets; SA1% + PG: fillets coated with 1% sodium alginate solution + *P. gymnospora* extract; SA1%: fillets coated with 1% sodium alginate solution.

The fillets coated with *P. gymnospora* received moderate sensorial attributes of (scores 5 to 7) for all evaluated parameters, i.e., texture (88%), taste (86%), aroma and colour (77% each) and appearance (74%). These results indicate strong influence of the algae pigment (greenish brown) on the visual appearance of the fillets. According to Monteiro et al. (2019), visual appearance of food is a determining attribute for the sensory acceptance of the product. When no artificial colourants are added to the fillets, the colour change is attributed only to the type and concentration of the treatments, as the algae is majorly composed of chlorophylls and carotenoids (Verma et al., 2017; Atitallah et al., 2019). In spite of the interference with the colour of the coating of sea bass fillets, when the order of preference of the samples was verified, it was observed that the treatment containing *P. gymnospora* was well accepted by the tasters, with an acceptance percentage of 44%, uncoated (32%) and control (SA1%), with 24%.

The tasters who opted for uncoated fillet justified their choice due to the colour and strong flavour. After the addition of algae to foods has a marked taste due to the presence of minerals, which, apart from functioning as pro-oxidant molecules, also produces undesirable metallic aromas. In addition to that, the content and type of fibers present in algae also contribute to both the colour and taste and the texture of the food (Sanz-Pintos et al., 2017; Atitallah et al., 2019)

The sea bass fillets that were treated with SA1% (control) were globally accepted by 24% of the tasters and were evaluated in the second component (Figure 3), as they scored highest for taste, while intermediate scores were recorded for other attributes. Despite the increase in the use of edible coatings on fruits and vegetables (Yosuf et al., 2018), the application of edible coatings for fish fillets is still limited. Thus, the use of edible alga-based coating may improve the nutritional value (the algae are rich in fibers, proteins, minerals, and vitamins) (Sanz-Pintos et al., 2017) and extend the shelf-life by minimizing oxidative reactions and growth of deteriorating microorganisms.

4 CONCLUSIONS

The results of this study indicate that the ethanolic extract of *P. gymnospora* is the best source of antioxidants among the five kinds of seaweeds analyzed. The antimicrobial and antioxidant activity obtained in the edible *P. gymnospora* is of great interest to the fishing industry, and researchers interested in methodologies that prolong the shelf-life

of sea bass and other marine fish. Being a safe, natural preservative, application of this edible alga maintains good microbiological, chemical and sensorial quality. Future work focusing on identifying bioactive *P. gymonospora* compounds are highly recommended.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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