



## Effect of the incorporation of polysaccharides from green alga *Bryopsis plumosa* on beef sausages quality

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### ABSTRACT

Maintaining the quality and stability of functional meat products during storage is one of the major challenges of these products. The aim of this study was to evaluate the potential of polysaccharides extracted from green alga *Bryopsis plumosa* as a new natural additive in the formulation of beef sausages. In order to evaluate the impact of the incorporation of polysaccharides in beef sausages formulation, the physico-chemical, microbiological, and antioxidant properties were investigated during 12 days of storage at 4°C. Obtained data illustrated that the addition of this polymer in the formulation of beef sausages leads to a distinct antioxidant activity during 12 days of storage (4°C) with lower values in terms of lipid peroxidation compared to untreated samples. In addition, samples formulated with polysaccharides reduced the oxidation of Myoglobin, which consequently improved the color stability of meat during refrigerated storage. Furthermore, as compared to standard formulation, the addition of polysaccharides appears to have interesting antimicrobial potential that maintains sausage quality within a shelf life of 12 days. In conclusion, our results prove the efficiency of polysaccharides in providing more hygienic and safer meat products, which may suggest that PS could be used as a natural additive in functional foods.

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### Introduction

In recent years, intensive research is currently involved in maintaining the quality and stability of meat functional foods by avoiding lipid peroxidation and the growth of microorganisms during their storage is among the main challenges of these products (1). This is commonly done by incorporating important amounts of nitrate salts and vitamin C, which are also used as synthetic additives to control flavor, color and shelf life. However, frequent consumption of these substitutes can increase the risk of colorectal cancer, among other diseases. Due to health concerns, the demand for processed meat products could be affected (2). Thus, as a safe alternative, incorporating polysaccharides as hydrocolloids in food formulation could be an effective strategy for developing healthier products. Such substances are the most favored in the functional food industry to improve the nutritional functions of numerous food stuffs, as well as for colorant and even therapeutic purposes (3). Among these, marine resources like seaweeds have received special attention as a new source of bioactive and carbohydrate compounds (4).

Seaweeds produce a variety of biologically active components with different structures and interesting functional properties, including polyphenols, peptides, flavonoids, and polysaccharides(5,6). Among these, algal polysaccharides are commonly praised for their biological merits (7), including antiviral (8), antioxidant (9) and anticoagulant activities (10). Therefore, they seem to supply healthier, safer and better quality meat products with a natural flavor and taste and a fresh appearance(3,11). In fact, algal

polysaccharides have been used in the food industry as hydrocolloids, as they displayed interesting textural, thickening, gelling, antioxidant and anti-microbial properties (10,12-14), which favor their use as food additives (15). In the lab, several researchers have sought functional meat products that include algal polysaccharides (15). *Bryopsis plumosa* is one of the most common marine green macroalgae (Class: Bryopsidophyceae; Order :Bryopsidales; Family : Bryopsidaceae). *Bryopsis plumosa* is an epilithic species that is found in deep lower shore pools or in low littoral rock pools and in the sublittoral(16). Despite its abundance in Tunisia, it is poorly valued, and there is very little information on its bioactive compounds. To the best of our knowledge, this is the first time to use this alga and its biomolecules, particularly as food additives.

This work was undertaken to characterize the polysaccharides extracted from green alga *Bryopsis plumosa* (PS) harvested from Sfax coasts, Tunisia and to evaluate the impact of its incorporation in beef sausages formulation. Physico-chemical, microbiological and antioxidant properties were investigated during 12 days of storage at 4°C and compared to beef sausage formulated without PS supplementation.

### Materials and Methods

#### Sample harvesting and polysaccharides extraction

The seaweed *Bryopsis plumosa* was harvested from the coast of Sfax, Tunisia. The polysaccharides extraction procedure was determined following Zhang et al. (17). The algal powder was pre-extracted with 95% ethanol and

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deionized water at 90°C for 4 hours under stimulation. The dried PS was then stored for further studies.

### Yield and chemical properties

The extraction yield was expressed as the percentage (%) of the mass (g) of PS against alga dry mass (g). The ash content was determined according to the AOAC standard methods (18). Total sugar content of each fraction was determined according to the method of DuBois et al. (19). Proteins content assay was performed according to Lowry's method (20) using bovine serum albumin (BSA) as the standard.

### Molecular weight analysis by gel permeation chromatography

The PS was analyzed by HPLC-RID to determine the retention time of standards and sample. The average molecular weight (Mw) of PS was calculated by constructing a calibration curve in which Dextrans were used as standards with known molecular weight.

### Functional properties

#### Water -holding (WHC) and oil -holding (OHC) capacities

WHC and OHC were determined according to the method of Lin et al (21). 0.5g of PS was dispersed in 50 mL of distilled water and 10 mL of corn oil to evaluate WHC and OHC, respectively. The mixture was stirred every 15 min for 5 s during the 1h incubation period at room temperature, and then centrifugated at 8000 rpm for 20 min. Subsequently, the upper phase was eliminated and the tube drained for 30 min on filter paper. The ratio between the content weight after drainage and dried sample weight of PS was determined and the WHC and OHC capacities were expressed as the gram of water or oil bound per gram of the PS on dry weight (DW).

#### The Emulsifying capacity

Emulsifying capacity (EC) and its stability were determined according to Freitas et al (22), with some modifications. A volume of 4 mL of PS aqueous solution at different concentrations (0.25; 0.5 and 1%) was mixed with 6 mL of corn oil and stirred in the vortex for 2 minutes at room temperature. After 1, 2, 24 and 48 hours, respectively, emulsification indices EC1, EC2, EC24, and EC48 were calculated as follows:

$$EC (\%) = \frac{He}{Ht} \times 100$$

where He (mm) is the height of emulsion layer, and Ht (mm) is the overall height mixture after t hours. All samples were stored at room temperature.

### Effect of polysaccharides incorporation on beef sausage quality

#### Beef sausage preparation

Mechanically separated meat (MSM) was obtained from the local processor CHAHIA, Sfax, Tunisia. The beef sausages were prepared based on the standard formulation of the CHAHIA industry: meat (64%), fat (12%), cold water (14%), sodium chloride (NaCl) (1.3%), sodium nitrite (NaNO<sub>2</sub>) (0.047%), sodium tripolyphosphate (0.31%), modified starch (8.45%), carrageenan (0.7%), and vitamin C (0.125%). The same basic formulation was used to make

five different preparations:

T (+): Standard reference was prepared according to the basic formulation of the company CHAHIA.

T (-): Negative control was prepared using the basic formulation of the company with no addition of vitamin C «E300».

T1, T2, T3: Group of beef sausages prepared using the commercial formula of CHAHIA company, with the substitution of vitamin C «E300» with PS (0.05%, 0.125% and 0.25%, respectively).

MSM, water, additives, ingredients and PS at different concentrations were homogenized. The mixtures were stuffed into the collagen-reconstituted casing of 100 g manually. Cooking was performed in a controlled water bath at a constant temperature of 95°C with an internal temperature of 74 °C. The preparations were then cooled using tap water and stored at 4 °C for 12 days. The microbiological stability and antioxidant parameters of samples were sought during storage.

### Physico-chemical properties

#### Measurement of pH value

pH values were measured using a pH meter (Systronics Instruments, India) with complete immersing of the glass electrode into the homogenate as described by Verma et al. (23). Sausages (10 g) were homogenized with 100 mL distilled water (pH 7) using a blender. pH values were conducted in the homogenous paste on days 1, 3, 6, 9 and 12.

#### Moisture

The dry matter content consists of all the organic and inorganic substances. It is determined by evaporation of the water contained in 5 g of the sample at 105°C for 24 h until a constant weight is obtained (18).

#### Color investigation

Color is an important factor in the consumer acceptability of fresh and processed meat. Color of sausages was measured using a Color Flex spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA, USA) with the CIE L\* a\* b\* color system by measuring lightness (L\*), redness (a\*), and yellowness intensities (b\*). The colorimeter was calibrated against a standard Minolta reflector plate before each actual color measurement. The colors of both cut-end sides of the sample pieces were calculated on days 1, 6 and 12.

#### Measurement of metmyoglobin (MetMb)

Metmyoglobin (MetMb) analysis was performed as described by Singh et al. (24). Three grams of each sample was mixed with 30 mL cold phosphate buffer (40 mM, pH 6.8). After incubation of the mixture for 1 hour in ice and centrifugation at 4500 rpm for 30 min at 4 °C, the supernatant was filtered through a Whatman filter paper. MetMb, expressed in percentage, was calculated on the basis of these absorbance values using the following formula:

$$MetMb (\%) = [-2.51(A572/A525) + 0.777(A565/A525) + 0.8(A545/A525) + 1.098] \times 100$$

Where, A572, A565, A545 and A525 are the respective absorbance at 572, 565, 545 and 525 nm.

#### Measurement of Heme iron

Heme iron levels were determined following Clark et

al. (25). Minced sausage (2 g) was mixed with 9 mL of acidified acetone. After incubation for 1 hour at 25 °C in the dark, the homogenate obtained was centrifuged at 2200 g for 10 min. Then, the supernatant was filtered by the wool glass. The absorbance of the filtrate was determined at 640 nm and the levels of Heme iron were calculated by the equation below:

$$\text{Heme iron } (\mu\text{g g}^{-1} \text{ sausages}) = A_{640} \times 680 \times 0.0882$$

### Measurement of thiobarbituric acid reactive substances (TBARS)

The thiobarbituric acid (TBA) method is the most commonly used method for the determination of secondary compounds of lipid oxidation. It was estimated by measuring malondialdehyde (MDA) levels using thiobarbituric acid reactive substances (TBARS). TBARS determination was based on the method adapted from Salih et al. (26). Briefly, 2 g of the minced sausage was homogenized with 16 mL of a mixed solution containing 5% trichloroacetic acid (w/v) and BHT solution (1 mg/mL). The mixture was homogenized 3 times for 15 seconds using a homogenizer at a rate of about 3500 rpm. The ground material was filtered on pleated filter. To 2 mL of filtrate was added 2 mL of TBA. After incubation at 70 °C for 30 minutes, the closed tubes were cooled in a cold-water bath. An absorbance spectrum was made at 532 nm, 508 nm and 600 nm. TBARS value was expressed as mg MDA/kg sample.

### Microbiological analysis

The microbiological stability of beef sausage samples was monitored during sample storage at 4 °C for 12 days (samples were taken every 3 days). 0.5 g of each sausage sample was diluted in a vial containing 4.5 mL of physiological saline to prepare the  $10^{-1}$  dilution. The diluted samples (0.1 mL) were placed into a Petri dish with medium. The dishes were then incubated under favorable conditions (temperature/incubation time) for multiplication for each seed. Colonies were counted after incubation and results were expressed as CFU/g of the sample.

### Statistical analysis

Statistical analyses were performed with the ANOVA method with the SPSS program (V20.0) by Tukey's post-hoc test. All values were expressed as mean $\pm$ SD. Differences were considered significant at  $p < 0.05$ . All tests were performed in triplicate.

## Results and Discussion

### Yield and Chemical properties of PS

Yield and ash content are illustrated in Table 1. Results showed that the yield of PS was estimated to be 4.77% $\pm$ 0.15. This yield is lower than that extracted from *Monostroma oxyspermum* 18.39%(27) and from *Ulva rotundata* (21.5%) (28). Polysaccharides extraction yield can be influenced by time, temperature, and the ratio of water to raw material (29). Moreover, PS low ash content (1.39% $\pm$ 0.02) seems to be related to the low ash content of the raw material. It was lower than that found in *Monostroma oxyspermum* (16.7%)(27) and in *Ulva lactuca* (26%) (30).

The chemical composition of PS is also shown in Table 1. Sugar contents of PS were found to be 61.70% $\pm$ 0.40, which seems to be higher than that of polysaccharides ex-

**Table 1.** Yield and Chemical composition of PS.

Parameters	(%)
Yield	4.77 $\pm$ 0.15
Ash	1.39 $\pm$ 0.02
Proteins	0.26 $\pm$ 0.03
Sugars	61.70 $\pm$ 0.4

tracted from *Ulva lactuca* (29.12%) (31).

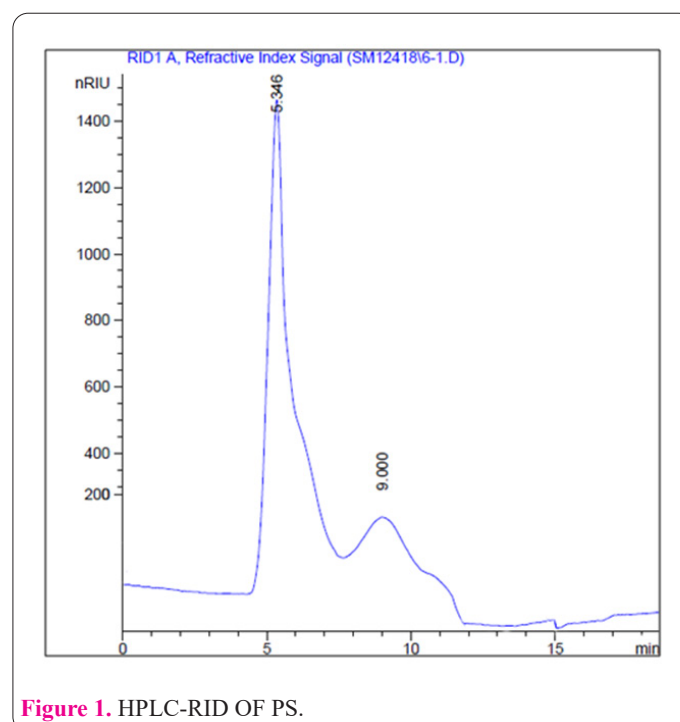
The protein content of the PS was estimated to be 0.26% $\pm$ 0.03. It was lower than that of two polysaccharides isolated from marine green algae *Monostroma nitidum* (0.66 and 0.99%) (32). According to Audrey Robic et al. (33), proteins have usually been considered as potential cell wall polysaccharide contaminants, mainly because they are part of cell wall structure and are closely associated with polysaccharides. In fact, these results were mostly attributed to the efficiency of the extraction method.

### Molecular weight analysis by gel permeation chromatography

The molecular weight of polysaccharides was among the most important factor responsible for biological activity (34). Therefore, the HPLC system was used to determine the molecular weight of the polysaccharides. The exclusion chromatography spectrum of the PS is shown in Figure 1. The average molecular weight of the PS was calculated as 598.63k Da and 72.36 kDa, with the retention times at 5.611 min and 9.00 min, respectively. These peaks are the perfect sign of sugars in these polysaccharides. The obtained Mw distribution of PS was higher than those of ulvan and its fractions purified from *Ulva pertusa* ranged from 151.6 to 28.2 kDa (35). This large distribution of molecular weight in polysaccharides could be caused by the acid hydrolysis during the extraction process as well as the high temperature used for their dissolution, which could stand for potent bioactivity (36).

### Functional properties

The WHC and OHC of polysaccharides were largely explored in food applications. These proprieties are affect-



**Figure 1.** HPLC-RID OF PS.

ted by water interaction polysaccharides and its capacity to absorb oil. According to the previous studies, WHC was the ability of a moist material to retain water when subjected to an external centrifugal gravity force or compression. Results indicate that WHC of PS was  $4.36 \pm 0.18$  g water /g DW, which is lower than WHC of polysaccharides extracted from *Chaetomorpha linum* seeds ( $7.33 \pm 1.2$  g water/g DW) (37) (Table2).

While OHC was recorded about  $1.84 \pm 0.01$ g oil /g DW, which is in accordance with results obtained by kolsi et al from *Cymodocea nodosa* sulfated polysaccharide ( $1.56 \pm 0.08$  g oil /g DW) (38). This finding implied the possibility of its use as a potential ingredient in different food products.

For emulsifying properties, PS exhibited a high emulsifying activity with corn oil ( 87.71 % at 1% of concentration), and this activity was maintained at 48h. In view of this, it could be an acceptable emulsifier because it has the capacity to maintain 50% of the original volume of the emulsion after 24h (39). Overall, the results suggest that PS has the required properties to be used as a surface-active compound, which are suitable for food processing (37).

### Physico-chemical properties of sausages

The pH value of sausages was significantly affected by the storage duration and the addition of PS (Table 3), as they increased from pH 5.6 to 6.2 ( $p < 0.05$ ). Such data probably resulted from protein denaturation coming from the protonation of some basic amino acid residues present in the side chains(40).

**Table 2.** Functional properties of PS.

WHC	4.36±0.18 (g water/g DW)			
OHC	1.84±0.01(g oil/g DW)			
Concentrations (g/100ml)	EC1	EC2	EC24	EC48
1%	87.71±0.52%	84.21±1.12%	63.15±0.11%	59.70±0.50
0.5%	78.94±1.91%	68.42±1.40%	55.78±0.20%	48.20±0.60
0.25%	81.25±0.15%	68.75±0.82%	50.00±0.71%	34.32±0.75

OHC, oil holding capacity; WHC, Water-holding capacity; EC, emulsifying capacity.

Values are given as mean of three determinations ( $X \pm SD$ )

**Table 3.** Physico-chemical properties of sausages.

Physico-chemical properties	DAYS	T+	T -	T1	T2	T3
Moisture(%)	1	26.67±0.06 <sup>aA</sup>	31.67±0.01 <sup>abA</sup>	32.22±0.05 <sup>abA</sup>	33.33±0.11 <sup>abA</sup>	38.89±0.11 <sup>bA</sup>
	3	38.33±0.01 <sup>aAB</sup>	52.22±0.04 <sup>bA</sup>	38.89±0.06 <sup>aAB</sup>	36.11±0.12 <sup>aA</sup>	42.78±0.07 <sup>aA</sup>
	6	57.78±0.13 <sup>abC</sup>	52.22±0.20 <sup>aA</sup>	50.00±0.1 <sup>abC</sup>	42.22±0.12 <sup>aAB</sup>	52.78±0.13 <sup>aA</sup>
	9	65.56±0.26 <sup>aC</sup>	55.00±0.16 <sup>aA</sup>	58.33±0.07 <sup>aC</sup>	56.67±0.03 <sup>aB</sup>	58.33±0.24 <sup>aAB</sup>
	12	66.67±0.01 <sup>abC</sup>	51.67±0.33 <sup>aA</sup>	61.11±0.1 <sup>abC</sup>	72.22±0.15 <sup>bc</sup>	72.22±0.25 <sup>bB</sup>
pH	1	5.65±0.07 <sup>bA</sup>	6.05±0.07 <sup>dB</sup>	5.90±0.10 <sup>cA</sup>	5.88±0.04 <sup>cAB</sup>	5.51±0.03 <sup>aA</sup>
	3	5.91±0.01 <sup>bcB</sup>	6.01±0.04 <sup>cB</sup>	5.93±0.12 <sup>bcAB</sup>	5.83±0.11 <sup>bA</sup>	5.56±0.05 <sup>aA</sup>
	6	5.92±0.02 <sup>ab</sup>	5.96±0.04 <sup>aAB</sup>	5.96±0.05 <sup>aABC</sup>	5.95±0.10 <sup>aABC</sup>	6.14±0.02 <sup>bB</sup>
	9	6.06±0.01 <sup>bcC</sup>	5.85±0.06 <sup>aA</sup>	6.02±0.05 <sup>Bbc</sup>	6.04±0.01 <sup>bBC</sup>	6.20±0.10 <sup>cB</sup>
	12	6.11±0.01 <sup>abC</sup>	5.98±0.02 <sup>aAB</sup>	6.04±0.10 <sup>abC</sup>	6.06±0.06 <sup>abC</sup>	6.21±0.16 <sup>cB</sup>

T1, T2, T3 sausages manufactured supplemented with different concentrations of PS (0.05%, 0.125% and 0.25% respectively); T (+) positive control; T (-) negative control without Vit C.

Values are ± standard deviation of three replicates. Different letters (a-c) in the same row are significant differences between formulations on the same storage day ( $p < 0.05$ ) and different letters (A-C) in the same column indicate significant differences during the storage period ( $p < 0.05$ ) for the same sample.

Moisture levels of the beef sausages measured during refrigerated storage are shown in Table 3. In fact, no significant differences on moisture in the treated and the control samples were noted ( $p > 0.05$ ).

The changes in the color parameters of the studied sausages are expressed in Table 4 in terms of L\*, a\*, and b\*. In fact, a pronounced decrease in the lightness (L\*) level of the treatments was observed during storage. The formation of brownish MetMb due to protein oxidation is probably the cause behind this variation (41).The red intensity (a\*) is the most sensitive parameter for color measurement, red color characterization, and color stability( 42). The redness (a\*) values of sausages were decreasing along with the period of storage. On the first day of treatment, the sausages (T3) showed a lower intensity of the yellow color (b\*) compared to the control sample. All treatments showed a decrease in yellowness towards the end of the process, with no significant difference between control and T1, T2 and T3( $p > 0.05$ ). The changes in (b\*) found during storage are probably due to the oxygen consumption by microorganisms during their exponential growth phase and the decrease in oxymyoglobin, which contributes to the (b\*) values (43).

### Effect on myoglobine oxidation

The potential effect of PS on sausage myoglobin oxidation was investigated during storage. Obtained data revealed no significant difference in the control's MetMb amounts of all formulations (Figure 2). Remarkably, sausage without Vit C (T-) showed from the first day the highest MetMb oxidation with 40.75% compared with

**Table 4.** Effect of PS on the color traits during the shelf life of 12 days at 4 °C.

Parameters	Days	T+	T -	T1	T2	T3
<b>L*</b>	1	59.52 ± 0.33 <sup>bcB</sup>	58.23 ± 0.21 <sup>aB</sup>	59.12 ± 0.22 <sup>bcC</sup>	59.77 ± 0.05 <sup>cC</sup>	58.52 ± 0.14 <sup>bB</sup>
	6	58.09 ± 0.11 <sup>cA</sup>	55.44 ± 0.33 <sup>aA</sup>	58.13 ± 0.15 <sup>cB</sup>	58.48 ± 0.15 <sup>cB</sup>	56.95 ± 0.14 <sup>bA</sup>
	12	57.78 ± 0.92 <sup>bA</sup>	55.05 ± 0.07 <sup>aA</sup>	57.32 ± 0.14 <sup>bA</sup>	57.60 ± 0.43 <sup>bA</sup>	57.13 ± 0.24 <sup>bA</sup>
<b>a*</b>	1	31.59 ± 0.04 <sup>aB</sup>	32.37 ± 0.13 <sup>bB</sup>	31.66 ± 0.12 <sup>aC</sup>	31.55 ± 0.24 <sup>aC</sup>	32.11 ± 0.14 <sup>bC</sup>
	6	31.19 ± 0.07 <sup>bA</sup>	32.65 ± 0.14 <sup>eB</sup>	30.80 ± 0.07 <sup>aB</sup>	32.25 ± 0.10 <sup>dB</sup>	31.55 ± 0.17 <sup>cB</sup>
	12	32.65 ± 0.13 <sup>cC</sup>	31.51 ± 0.07 <sup>bA</sup>	30.44 ± 0.05 <sup>aA</sup>	33.50 ± 0.14 <sup>dA</sup>	30.64 ± 0.27 <sup>aA</sup>
<b>b*</b>	1	15.24 ± 0.07 <sup>bB</sup>	13.77 ± 0.005 <sup>aA</sup>	15.30 ± 0.10 <sup>bB</sup>	16.12 ± 0.13 <sup>cB</sup>	13.96 ± 0.18 <sup>aA</sup>
	6	15.22 ± 0.07 <sup>bB</sup>	14.11 ± 0.09 <sup>aB</sup>	15.25 ± 0.12 <sup>bB</sup>	14.24 ± 0.22 <sup>aA</sup>	13.92 ± 0.14 <sup>aA</sup>
	12	14.02 ± 0.14 <sup>aA</sup>	14.49 ± 0.02 <sup>bc</sup>	14.15 ± 0.10 <sup>aA</sup>	14.05 ± 0.11 <sup>aA</sup>	14.11 ± 0.10 <sup>aA</sup>

T1, T2, T3 sausages manufactured supplemented with different concentrations of PS (0.05%, 0.125% and 0.25% respectively); T (+) positive control; T (-) negative control without Vit C. Values are ± standard deviation of three replicates. Different letters (a-e) in the same row are significant differences between formulations on the same storage day ( $p < 0.05$ ) and different letters (A-C) in the same column indicate significant differences during the storage period ( $p < 0.05$ ) for the same sample.

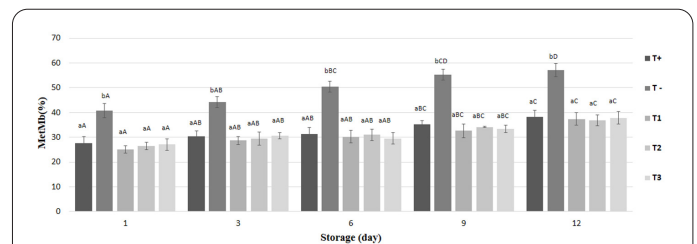
other sausages group ( $p < 0.05$ ). Interestingly, PS incorporation seemed to induce the stabilization and the protection of myoglobin to be efficient as recorded by the standard formulation (T+). From day 1<sup>st</sup> to the 12<sup>th</sup> day of storage, sausages formulated with PS showed lower oxidation values and deemed effective in limiting oxymyoglobin (OxyMb) oxidation of beef sausage. The results confirm the ability of polysaccharides to create a barrier to oxygen entry to protect Oxy Mb against oxidation, probably due to a decrease in MetMb activity or a delay of myoglobin oxidation (44). Our results are in agreement with those obtained by Hamzaoui et al. (37) who assigned the lowest values of MetMb recorded to 0.05% of a preparation based on polysaccharides extracted from the green alga *Chaetomorpha linum*.

### Measurement of Heme iron

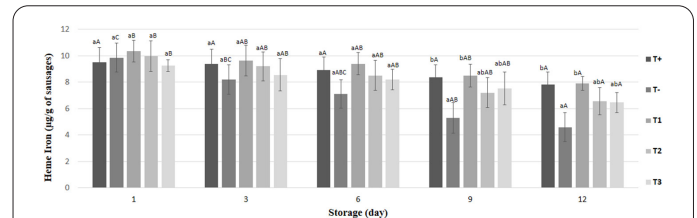
Heme iron, which is present mainly in meat, poultry and fish, represents more than 95% of functional iron in the human body (45). The antioxidant potential of PS, as reflected by the Heme iron contents in the different prepared sausages, was assessed (Figure 3). Heme iron value variation among different treatment groups proves that the storage time governs the variation of its contents in sausages. In fact, the Heme iron content was influenced in all sausage samples during the storage period at 4°C. Remarkably, the treatment with PS exhibited a potent antioxidant activity established by the Heme iron content. Thus, sausages treated with PS was found similar to the standard formulation and more effective than sausages without VitC. Moreover, an improvement in the transformation of the Heme iron was shown at the end of the storage at standard formulation (T+) as well as PS-treated sausages. These findings could prove the role of PS in the stability of beef sausages.

### Measurement of 2-thiobarbituric acid value

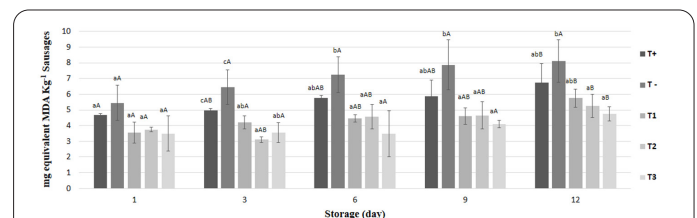
Lipid peroxidation leads to undesirable flavors, rancid odor, color change, and formation of toxic compounds, including as 4-hydroxynonenal(46). Therefore, the effect of PS on the rate of lipid peroxidation in beef sausages formulation was assayed by MDA analysis during 12 days of storage at 4 °C. Indeed, MDA assessment is widely used as a marker of oxidative stress and lipid peroxidation index (3,41). As illustrated in Figure 4, the MDA values constantly increased in all samples during storage. After



**Figure 2.** Effect of PS incorporation on Metmyoglobin (MetMb) in sausages during refrigerated storage. T: untreated control; T-: negative control; T1: 0.05%PS; T2: 0.125% PS; T3: 0.25% PS. Values with different letters (a–b) indicate significant differences for the same formulation within different days of conservation ( $p < 0.05$ ). Values with different letters (A–D) designate significant differences between formulations on the same storage day ( $p < 0.05$ ). Values are means ± SE.



**Figure 3.** Effect of PS incorporation on Heme iron in sausages during refrigerated storage. T: untreated control; T-: negative control; T1: 0.05% PS; T2: 0.125% PS; T3: 0.25% PS. Values with different letters (a–b) indicate significant differences for the same formulation within different days of conservation ( $p < 0.05$ ). Values with different letters (A–C) designate significant differences between formulations on the same storage day ( $p < 0.05$ ). Values are means ± SE.



**Figure 4.** Effect of PS incorporation on Thiobarbituric-acid reactive substances (TBARS) in sausages within a shelf life of 12 days of storage at 4 °C. T: untreated control; T-: negative control; T1: 0.05%PS; T2: 0.125% PS; T3: 0.25% PS. Values with different letters (a–c) indicate significant differences for the same formulation within different days of conservation ( $p < 0.05$ ). Values with different letters (A–B) designate significant differences between formulations in the same storage day ( $p < 0.05$ ). Values are means ± SE.

**Table 5.** Microbiological analysis of sausages treated during the shelf life of 12 days at 4 °C.

	Days	Colonies nombre (log UFC/mL)				
		T+	T -	T1	T2	T3
<b>Mesophilic flora</b>	1	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>
	3	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>
	6	0 <sup>aA</sup>	1.00±0.04 <sup>bb</sup>	0 <sup>aA</sup>	0 <sup>aA</sup>	0 <sup>aA</sup>
	9	2.40±0.06 <sup>cB</sup>	3.25±0.05 <sup>dC</sup>	1.98±0.12 <sup>bb</sup>	2.11±0.12 <sup>bb</sup>	1.55±0.10 <sup>aB</sup>
	12	2.75±0.01 <sup>bC</sup>	3.78±0.10 <sup>dD</sup>	2.30±0.04 <sup>aC</sup>	3.04±0.05 <sup>cC</sup>	2.45±0.12 <sup>aC</sup>
<b>Total Coliform</b>	1	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>
	3	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>
	6	2.15±0.34 <sup>cB</sup>	2.26±0.12 <sup>cB</sup>	1.05 ±0.50 <sup>bb</sup>	0 <sup>aA</sup>	0 <sup>aA</sup>
	9	2.00±0.50 <sup>abB</sup>	2.56±0.60 <sup>bbC</sup>	2.00 ±0.50 <sup>abC</sup>	1.14±0.18 <sup>aB</sup>	1.25±0.10 <sup>aB</sup>
	12	2.75±0.42 <sup>bcB</sup>	3.14±0.18 <sup>cC</sup>	2.14±0.15 <sup>abC</sup>	2.10±0.13 <sup>aC</sup>	2.18±0.15 <sup>abC</sup>
<b>Listeria monocytogenes</b>	1	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>
	3	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>
	6	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>	0 <sup>A</sup>
	9	3.10±0.10 <sup>bcB</sup>	3.35±0.15 <sup>cB</sup>	2.54±0.08 <sup>abB</sup>	2.00±0.50 <sup>aB</sup>	2.80±0.01 <sup>bcB</sup>
	12	3.40±0.10 <sup>bc</sup>	3.54±0.50 <sup>bb</sup>	2.68±0.05 <sup>abC</sup>	2.50±0.50 <sup>aB</sup>	3.20±0.02 <sup>abC</sup>

T1, T2, T3 sausages manufactured supplemented with different concentrations of PS (0.05%, 0.125% and 0.25% respectively); T (+) positive control; T (-) negative control without Vit C. Values are ±standard deviation of three replicates. Different letters (a-d) in the same row are significant differences between formulations in the same storage day ( $p<0.05$ ) and different letters (A-D) in the same column indicate significant differences during the storage period ( $p<0.05$ ) for the same sample.

12 days of storage period, sausages reinforced with PS exhibited significantly lower values in T2 ( $p=0.001$ ) (T3) ( $p=0.003$ ) compared to sausages treated without Vit C (T-). According to these results, PS displayed an efficient inhibitor effect on lipid peroxidation during storage.

### Microbiological analysis

The level of total mesophilic flora (TMF) in a product is an indicator of meat microbiological quality and its life stability(48). In the present work, samples of all beef sausage formulations were investigated for the microbial count. The results are reported in Table 5. Regarding the storage time, data demonstrated a significant increase in total bacterial count upon storage time, in which they increased more slowly with samples supplemented with PS compared to the control samples. On day 6, we observed 2.15 CFU/g and 1.05 CFU/g of total coliforms on control and T1 (0.05%) sausage, respectively. The result suggests that PS significantly inhibited bacterial growth ( $p<0.05$ ), especially at an amount of T2 and T3 (0.125% and 0.25%, respectively). These results are in accordance with feki et al.(49) which the addition of polysaccharides extracted from *Falkenbergia rufolanosa* on beef sausages even at low concentration (0.05%) induced a reduction in the microbial load during the 12 days of storage period.

Hamdi et al.(50), assign this anti-bacterial aspect to the antimicrobial bioactive compounds that are inherent to biopolymers. This proves the efficiency of PS in providing more hygienic and safer meat products, compared to control. Based on microbiological results, no growth of *salmonella* and *clostridium perfringens* were detected for all treatments during storage.

### Conclusion

This study aimed to determine the antioxidant and antimicrobial activities of polysaccharides extracted from green alga *Bryopsis plumosa* through its incorporation into beef sausage formulations. Interestingly, the addition

of PS into sausages inhibited lipid peroxidation and reduced the OxyMb oxidation, which enhanced meat color stability during refrigerated storage. Moreover, PS maintained microbiological properties and reduced the counts of widely pathogenic bacteria on sausages upon storage. Based on these data, PS could be used as a safe natural food additive.

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### Interest conflict

The authors report that they have no conflict of interests with respect to the work described in this manuscript.

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