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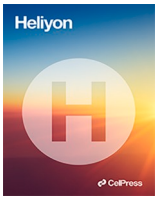

Research article

Physicochemical, structural, and biological properties of novel water-soluble polysaccharide derived from the Tunisian *Hammada scoparia* plant and its application on beef meat preservation

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ABSTRACT

This work aims to characterize a novel water-soluble polysaccharide from *Hammada scoparia* leaves named PSP. The Infrared (FT-IR) and nuclear magnetic resonance (NMR) spectra confirmed the presence of different polysaccharide functional bands. The High-Performance Liquid Chromatography (HPLC) analysis identified a heteropolysaccharide composed of two monosaccharides. A semi-crystalline structure of PSP was proved using the X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM) analysis. The evaluation of the antioxidant activity revealed an interesting potential to prevent oxidative stress.

Additionally, PSP showed interesting functional propreties such as good oil and water retention abilities, higher foaming stability, and higher emulsifying capacity and stability. However, the effect of PSP on the oxidation of lipids in the ground beef meat was established during nine days at 4 °C. Obtained data revealed a significant decrease in malondialdehyde levels, inhibition of metmyoglobin (MetMb) accumulation, and significant inhibition of microbial growth compared with the control sample during storage. Moreover, incorporating PSP in minced meat proved color pH and moisture stability. Overall, the findings in the present study confirmed that PSP could be considered a natural bioactive polymer for food applications.

1. Introduction

Polysaccharides are heterogeneous compounds of the carbohydrate class composed of monosaccharide units with glycosidic linkage [1]. They are extensively found throughout microorganisms, fungi, plants, and seaweed [2]. Like proteins and polynucleotides, polysaccharides represent a necessary macromolecules in life activities and have significant roles in molecular identification, cell adhesion, and cell-cell communication in the immune system [3].

Recently, polysaccharides derived from natural sources have become one of the most attractive biopolymers in the food and

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pharmaceutical sectors due to their numerous merits, notably natural abundance, low manufacturing costs, water solubility, bioactivity, biocompatibility, and biodegradability, non-toxicity [4]. These polymers have been praised for their multi-functional bioactivities, such as anti-viral, antioxidant, anti-inflammatory, immune-stimulatory, antitumor, prebiotic, and hypoglycemic activities [5]. Furthermore, increasing attention has been attributed to plant polysaccharides in the food industry due to their safer use compared with synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) [6], which have been reported to induce a carcinogenic effect after a long-term intake [7]. Recently, food industries have had to consider incorporating natural alternative products, which have become recommended by worldwide consumers and food processors [8]. In this way, government agencies and consumers are concerned about the food's safety and the impact of synthetic ingredients on their health. It is becoming crucial necessity to generate alternative natural antioxidant to address these concerns. In this context, several studies have outlined the importance of polysaccharides as an additive to food due to their non-toxic and biocompatible characteristics and their varied rheological and antioxidant properties [9].

Hammada scoparia (*H. scoparia*) is a halophyte plant (known in Tunisia as Rimth) that belongs to the Chenopodiaceae family. This plant exhibits several physiological effects because of pharmacologically active molecules. The leaves have been widely employed in traditional medicine in the prevention of several diseases such as cancer, hepatitis, inflammation, and obesity [10]. Several studies have also shown that *H. scoparia* leaf extract possesses a molluscicidal effect of the primary alkaloids against *Galba truncatula* and a strong anti-tumor activity [11].

The novelty of the present research lies in investigating the spectroscopic and biological activities of a novel polysaccharide extracted from *H. scoparia* leaves, named PSP, and exploring their structural features, chemical composition, biological activities, and functional properties. The effect of the incorporation of PSP into minced beef meat during 9 days of refrigerated storage was also assessed. The importance of the current study is that it explores a novel alternative of bio-preservation which can solve several problems of meat deterioration in food industry at a low cost while preventing the use of synthetic antioxidant compounds.

2. Materials and methods

2.1. Material and reagents

H. scoparia was sampled from Chorbane City (Mahdia, Tunisia) in September 2019 (Fig. 1). The leaves were gently removed from the fresh plants and thoroughly washed and are left in air dried at ambient temperature in the shade for 7 days in the laboratory, then crushed using Molinex LM 241 to a fine powder. Fine particles were selected using sieve with size of particle less than 100 μm. The plant powder was kept in a sterile limp container.

2.2. Polysaccharide fraction extraction

The polysaccharide, named PSP, was extracted using the procedure outlined by Liu et al. (2015) [12]. The dried plant material was pre-extracted with 95 % ethanol to remove pigments. For the extraction, 50 g of dry residue was extracted twice with 1000 ml of distilled water at 90 °C while continuously stirring for 4 h. The extracts were combined, filtered, and then evaporated under vacuum. The concentrated solution was precipitated with 95 % (v/v) ethanol at 4 °C for 24 h and centrifuged using a refrigerated centrifuge. The final residue was re-dissolved in double distilled water and freeze-dried using a freeze dryer (Bblock Scientific Christ ALPHA 1–2, IllKrich-Cedex, France) to obtain the polysaccharide. The latter was stored at −20 °C for further use. The yield was expressed as a percentage (%) of the mass (g) of PSP against the initial group (g) of plant powder.

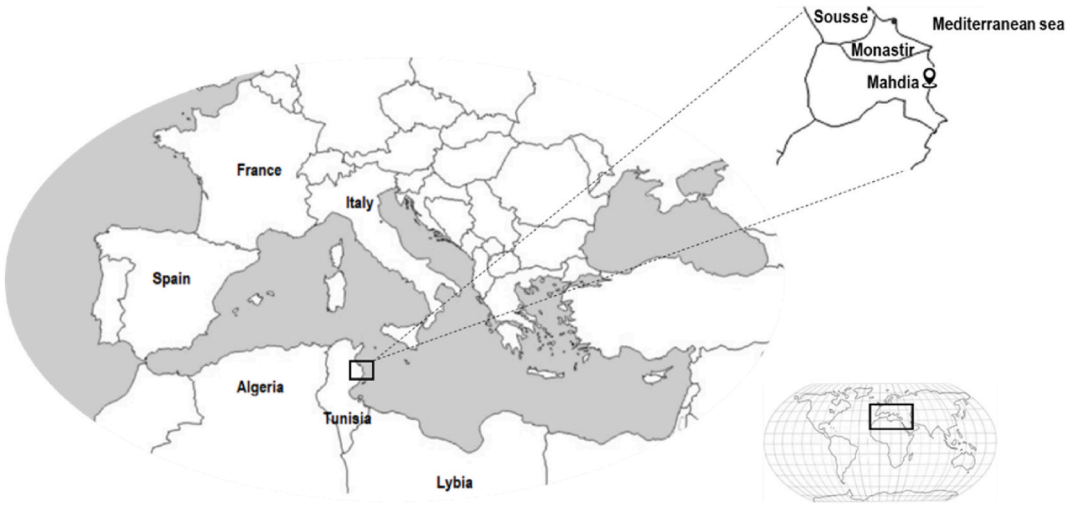

Fig. 1. Fig. 1: Location of the collection zone (the coastal area of Chorbane city, Mahdia, Tunisia).

2.3. Chemical compositions

Protein content was determined by a colorimetric assay using bovine serum albumin (BSA) as standard [13]. The ash content was measured according to the AOAC standard method [14]. Sugar content was determined by the phenol–sulfuric acid method, using galactose as a standard [15]. After acid hydrolysis (1 mL of HCl for 5 h at 100 °C), free sulfate content in the sample was estimated by the barium chloride ($BaCl_2$)/gelatin method, using potassium sulfate (K_2SO_4) as a standard. Total uronic acid content was determined by [16] Filisetti-Cozzi and Carpita, using >

2.4. Spectroscopic analysis of PSP

2.4.1. Monosaccharide analysis by HPLC-FID

The HPLC-FID assay was conducted as described by Bayar et al. (2016) [17] using an Aminex HPX-87H column with (H_2SO_4) as the mobile phase of (0.001 N), a flow rate of 0.4 mL/min and a column temperature of 60 °C. Glucose (LOD = 0.1 μg/mL, LOQ = 0.5 μg/mL, $R^2 = 0.998$), fructose (LOD = 0.2 μg/mL, LOQ = 0.8 μg/mL, $R^2 = 0.999$), sucrose (LOD = 0.05 μg/mL, LOQ = 0.15 μg/mL, $R^2 = 0.999$), gluconic acid (LOD: 0.02 μg/mL LOQ: 0.08 μg/mL, $R^2 = 0.999$) galacturonic acid (LOD: 0.03 μg/mL LOQ: 0.10 μg/mL, $R^2 = 0.999$), mannose (LOD = 0.15 μg/mL, LOQ = 0.7 μg/mL, $R^2 = 0.998$), arabinose (LOD = 0.04 μg/mL, LOQ = 0.15 μg/mL, $R^2 = 0.999$), galactose (LOD = 0.1 μg/mL, LOQ = 0.5 μg/mL, $R^2 = 0.997$), and xylose (LOD = 0.2 μg/mL, LOQ = 0.8 μg/mL, $R^2 = 0.999$) were used as standard pure monosaccharides (Sigma Aldrich). The percentage of recovery of standards used was 100 %.

2.4.2. FT-IR spectrometry analysis

A Nicolet Nexus spectrometer determined the I.R. spectroscopy of PSP. At room temperature, the measurement range was 4000-500 cm−1, and the spectra was recorded at a resolution of 4 cm−1. The OPUS 3.0 data gathering program (Bruker, 100 Ettlingen, Germany) was used to analyze the spectrum data.

2.4.3. Solid-state ^{13}C NMR spectroscopy analysis

^{13}C NMR (nuclear magnetic resonance) assay was obtained using a BRUKER-ASX300 instrument. The NMR spectrum was revealed at the 8000 Hz frequency, and results were established using the MestRe Nova 5.3.0 (Mestrelab Research S.L.) software.

2.4.4. U.V. Absorption peak detection

A final concentration of 0.1 % of PSP was prepared. The sample's UV absorption spectra was captured between 200 and 800 nm in wavelength [18].

2.4.5. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was conducted using a Zeiss DMS 960 SEM microscope operating at 25 kV. Before analysis, the PSP was frozen and freeze-dried to minimize sample charging in the electron beam, and then examined under a scanning electron microscope.

2.4.6. X-ray diffraction (XRD)

The PSP's X-ray diffraction (XRD) patterns were obtained on an instrument (Bruker, Kappa APEX II, USA). Data were collected in the 2ɵ range of 5- 80° with a step size of 0.05° and a counting time of 5 s/step.

2.5. In vitro antioxidant properties of PSP

2.5.1. DPPH free radical scavenging assay

The DPPH radical scavenging activity was estimated and assayed following Lopes-Lutz et al. (2008) [19]. The absorbance was determined at 517 nm with a spectrometer. The percent of inhibition (P.I.) was calculated using the equation below:

$$PI(\%) = \frac{Ac + Ab - As}{Ac} * 100$$

where Ab, Ac, and as represent the optical densities of the blank, control, and sample, respectively. All experiments were conducted in triplicate, using gallic acid as a positive control.

2.5.2. Ferrous iron chelating activity

The chelating antioxidant activity of P.S. toward ferrous ions (Fe^{2+}) was determined according to Carter (1971) [20]. The decrease in the red color of the (Fe^{2+}-ferrozine) complex was measured at 562 nm. The percent of ferrozine-Fe^{2+} complex formation was calculated as follows:

$$\text{Ferrous ion chelating activity } (\%) = \frac{(A \text{ control} + A \text{ blank} - A \text{ sample})}{A \text{ control}} * 100$$

2.5.3. Reducing power assay

The capacity of PSP to reduce iron (III) was determined according to Oyaizu et al. (1986) [21]. Amounts of Fe^{2+} complex were determined by measuring the formation of Perl's Prussian blue at 700 nm, where higher absorbance indicates higher reducing power.

2.5.4. DNA nicking assay

The DNA nicking assay was estimated according to the method described by Lee et al. (2002) [22] using pUT57 plasmid DNA and Fenton's reagent (40 mM H_2O_2, 50 μM L-ascorbic acid, and 80 μM $FeCl_3$).

2.6. Functional properties

2.6.1. Oil-holding (OHC) and water-holding (WHC) capacities

WHC and OHC were assessed using the method of Lin et al. (1974) [23]. 0.5 g of PSP was dissolved in 10 mL of corn oil to evaluate OHC and 50 mL of distilled water to evaluate WHC. The mixture was then stirred every 15 min for 5 s during their incubation of 1 h at room temperature, and then centrifuged at 8000 rpm for 20 min. The liquid phase was eliminated, and the tube was drained for 30 min on a filter paper after tilting to a 45° angle. The ratio between the weight of the tube content after draining and the weight of PSP was determined, and the capacity (%) was reported as grams of water or oil bound per gram of PSP on dry weight (D.W.).

2.6.2. Foaming properties

Foam capacity (F.C.) and foam stability (F.S.) were evaluated according to Shahidi et al. (1995) [24]. Volumes of 10 mL of PSP solution at different concentrations (0.25 %, 0.5 %, and 1 %; w/v) were homogenized using a Moulinex-R62 homogenizer at room temperature. The F.C. and F.S. were estimated as follows:

$$FC\ (\%) = \frac{(VT - V0)}{V0} * 100$$

$$FS\ (\%) = \frac{(VT - V0)}{V0} * 100$$

V. T. is the volume of PSP solutions just after homogenization, Vt is the volume after 30 min, 60 min, and 24 h, and V0 is the initial volume.

2.6.3. Emulsifying activities

PSP emulsifying activities were evaluated as described by Freitas et al. (2009) [25]. A volume of 6 mL (corn oil or olive oil) was added to 4 mL of an aqueous preparation of PSP at different ratios (0.25, 0.5, 1 and 2 %) in a test tube and then stirred in the vortex at 2400 rpm for 2 min (at room temperature). After 1, 24, and 168 h, respective emulsification indexes were calculated as follows:

$$ht = \frac{he}{ht} * 100$$

where he (mm) is the emulsion layer height, and ht (mm) is the overall mixture height after t hours.

2.7. Application of PSP on minced meat beef preservation

2.7.1. Beef meat sample preparation

Fresh beef meat was obtained from a local market in Sfax city (Tunisia) and ground after excessive fats were removed. Four equal formulations of 25 g of meat were placed separately in sterile plastic bags and stored at 4 °C for nine days.

Control: meat without the addition of polysaccharides.
BHA: meat with the addition of BHA (0.5 %).
PSP 0.5 %: meat with adding 0.5 % of crude polysaccharide PSP.
PSP 1 %: meat with the addition of 1 % of PSP.
PSP 2 %: meat with the addition of 2 % of PSP.

2.7.2. Beef meat characterization

2.7.2.1. pH evaluation and moisture analysis. The pH values of each sample were determined with a pH meter according to the method of Hidayat et al. (2017) [26]. The moisture content was determined according to Hamzaoui et al. (2020) [27] after drying at 105 °C for 24 h.

2.7.2.2. Color analysis. The color was determined using a Color Flex spectropolarimeter (Hunter Associates Laboratory Inc., Reston, VA, USA) and expressed as L*, a, and b* values, referring to the measuring parameters of lightness, redness/greenness, and yellowness/blueness, respectively.

2.7.2.3. Metmyoglobin evaluation (MetMb). The contents of MetMb in beef meat were determined according to Smaoui et al. (2017) [28]. Briefly, 5 g samples were mixed in 25 mL ice-cold 40 mM phosphate buffer (pH 6.8) for 10s and kept for 1 h at 4 °C. After centrifugation at 4500*g* for 30 min. The supernatant was filtered through Whatman filter paper, and the absorbance was recorded at 572, 565, 545, and 525 nm. The percentage of MetMb were calculated as follows:

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

2.7.2.4. Heme iron determination. The minced meat samples were tested for heme iron content, according to Clark et al. (1997) [29]. Briefly, 2 g of meat and 9 mL of acidified acetone (90 % acetone, 8 % deionized water and 2 % HCl) were homogenized and incubated for 1 h at 25 °C in the dark, then filtered. The absorbance was recorded at 640 nm. The heme iron content was determined using the equation below:

$$Heme\ iron\ (\mu g\ iron\ /\ g\ sample) = A640 \times 680 \times 0.0882.$$

2.7.2.5. Conjugated dienes content. The conjugated dienes were determined according to the method described by Srinivasan et al. (1996) [30]. The values were expressed as μmol/mg of meat.

2.7.2.6. Determination of thiobarbituric acid reactive substance (TBARS). TBARS is the most widely used method for measuring lipid peroxidation levels in various meat samples Malondialdehyde (MDA), the final product of lipid peroxidation reaction, reacts with TBA to produce TBA-reactive substances (TBARS), which can be detected at 530 nm. Briefly, a sample (0.5 g) of meat at different storage times (1, 3, 6, and 9 days) was homogenized with 625 μL of TBS (50 mM Tris containing 150 mM NaCl, pH 7.4) and 375 μL of TCA-BHT (TCA 20 %, BHT 1 %) to precipitate proteins, and then centrifuged (1000 g, 10 min, 4 °C). A volume of 400 μL of the supernatant was mixed with 80 μL of HCl (0.6 M) and 320 μL of Tris-TBA (Tris 26 mM; TBA 120 mM). The mixture was incubated for 10 min at 90 °C. The absorbance was recorded at 530 nm. TBARS values were calculated from a standard curve of MDA and expressed as mg MDA/kg of meat. The tests were carried out in triplicate.

2.7.2.7. Microbial analysis. Microbial count was assessed following the method of Hajji et al. (2021) [31]. 0.5 g sample of minced meat was homogenized in a 9 mL peptone water solution (0.1 %, w/v) at room temperature. Psychrophilic bacteria (P.B.) were identified using a PCA medium after incubation at 4 °C for 7 days. The total mesophilic flora (TMF) was measured after incubation for 72 h at 30 °C using a Plat Count Agar (PCA) medium. The microbiological count was expressed as log CFU/g.

2.8. Statistical analysis

All experiments were carried out in triplicate, and average values are reported with standard errors. Mean separation and significance were analyzed via the SPSS software package ver. 17.0 professional edition (SPSS, Inc., Chicago, IL, USA), using ANOVA analysis based on Duncan's multiple range test to estimate the significance among the main effects at the 5 % probability level.

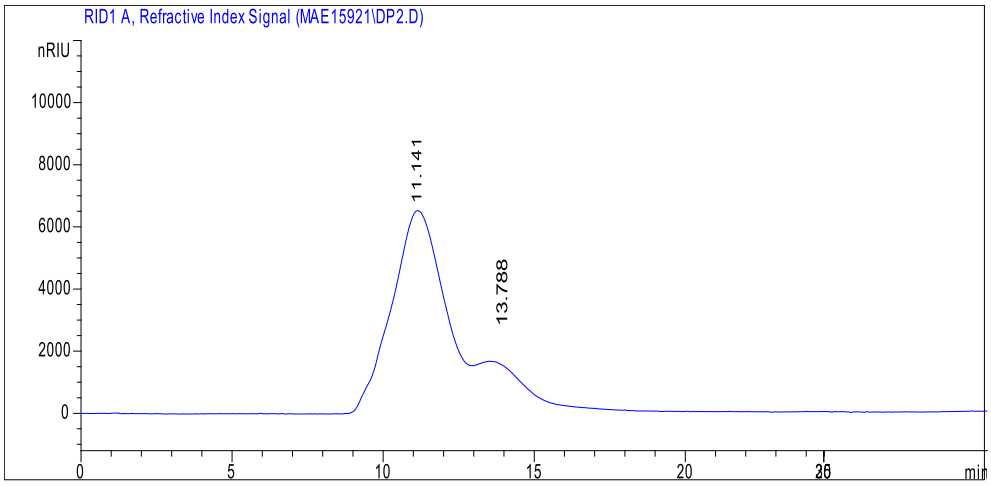

Fig. 2. Monosaccharide composition analysis by HPLC-FID of PSP.

3. Results and discussion

3.1. Chemical compositions

The PSP extraction yield from *H. scoparia* leaves was calculated to be 4.89 % ± 0.67 based on dry weight. This yield was higher than the polysaccharide recovered from *Morchella esculenta* (3 %) [32] and red algae *Chondrus canaliculatus* (2.05 %) [33], but lower than other polysaccharides like *Cucurbita moschata* (48.6 %) [34]. Furthermore, the total sugar content was found to be approximately 60.88 % ± 0.825, which is greater than polysaccharides isolated from *Tiliacora triandra* (59.5 %) [35]. The projected values for proteins, uronic acids, and sulfated groups were 3.95 % ± 0.318, 13.5 ± 0.18 %, and 2.87 % ± 0.33, respectively. These values appear greater than those discovered in *Cyclocarya paliurus* polysaccharides [6].

3.2. Spectroscopic analysis of PSP

3.2.1. Monosaccharide analysis by HPLC-FID

HPLC-FID is widely used to determine composition of monosaccharide in polysaccharides. The monosaccharide analysis (Fig. 2) revealed that PSP composition consisted in two monosaccharide units, mannose, and glucuronic acid at retention times of 11.141 and 13.788 min, respectively, based on the elution times of monosaccharide standards. These findings demonstrated the heterogeneity of PSP, with mannose being the most abundant unit. A similar study by Eljoudi et al. (2022) [36] on polysaccharides extracted from *Malcolmia triloba* leaves showed a heterogeneous composition with glucuronic acid as the most abundant monosaccharide unit. The difference in polysaccharide composition may be due to several external factors such as extraction temperature, water concentration and extraction process [37].

3.2.2. FT-IR spectrometric analysis

FT-IR spectrometry was employed to identify the functional groups of PSP, measuring absorbance in the range of 4000 to 400 cm^{-1}. As shown in Fig. 3, the FT-IR spectrum revealed a prominent peak at 3352 cm^{-1} and a weak peak at 2920 cm^{-1}. These two peaks are referred to as the stretching vibration of the O-H and C-H groups, respectively [38]. An additional peak of 1629 cm^{-1} was assigned to C=O stretching vibration of carboxylic groups [39]. Moreover, the absorption band at around 1370 cm^{-1} suggested the presence of sulfated esters (S=O) [40]. The absorption band at 1020 cm^{-1} could be assigned to the vibration of the (COH) groups and the (COC) glycosidic bond vibration [8]. The peak detected at around 820 cm^{-1} indicated the presence of 6-sulfate galactose [40]. Finally, the small peak at 652 cm^{-1} reflected the pyranose structure of PSP [41]. Finally, these infrared data confirmed the presence of typical bonds characteristic of polysaccharides derived from plants.

3.2.3. Solid-state ^{13}C NMR spectroscopy analysis

Solid-state ^{13}C NMR spectroscopy is among the most widely used techniques for analyzing the structures of polymers [42]. As shown in Fig. 4, The ^{13}C NMR spectrum revealed an intense signal around 70.61 ppm, which could be assigned to osidic CH_2-O and CH-O groups [43]. Another signal at 100.48 ppm enveloping various peaks corresponds to anomeric carbon in carbohydrates [33,43]. Furthermore, the spectra revealed a low signal at 167.38 ppm assigned to the carboxyl group in glucuronic acids [43].

3.2.4. U.V. Absorption peak detection

The U.V. spectrum is presented in Fig. 5. PSP showed a peak of maximum absorption at. around 201 nm, indicating the sample as a polysaccharide [44,45]. The absorption peak at 280 nm indicates a trace amount of protein in PSP [46].

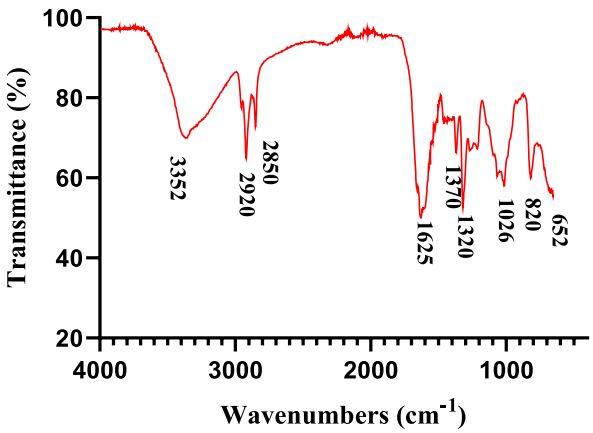

Fig. 3. FT-IR spectra of PSP.

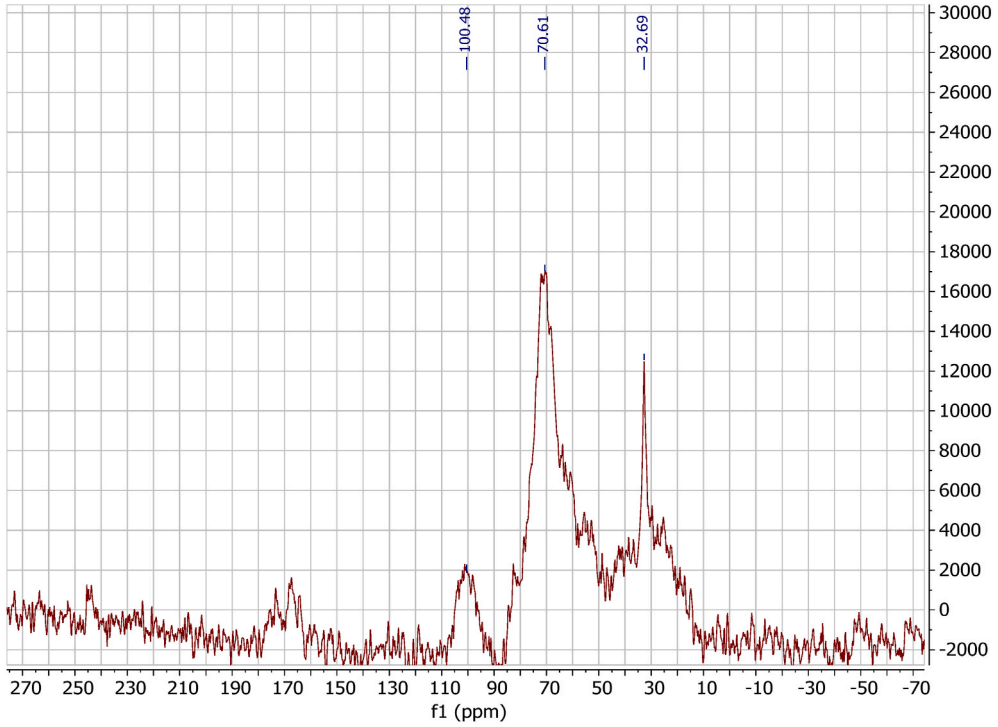

Fig. 4. Solid-state ^{13}C NMR spectroscopy of PSP.

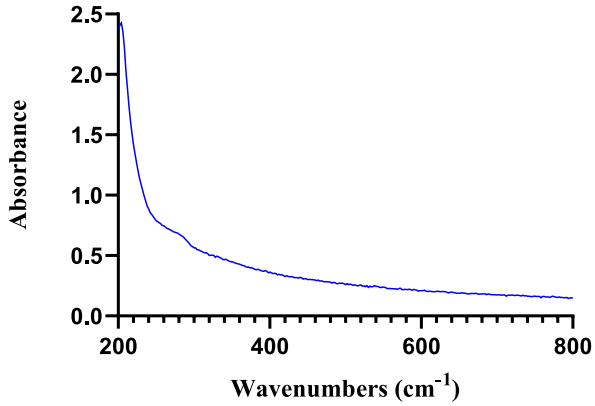

Fig. 5. UV–visible absorption spectrum of PSP.

3.2.5. X-ray diffraction (XRD)

The X-ray diffraction (XRD) pattern was utilized to assess the microstructural changes and the degree of crystallinity of PSP. As shown in Fig. 6, PSP showed a characteristic XRD pattern for a semi-crystalline polymer. Based on these findings, the polysaccharide PSP has a semi-crystalline nature due to the coexistence of crystalline and non-crystalline regions [47]. In fact, crystalline and semi-crystalline structures are directly affected by a number physical characteristics, including tensile strength, flexibility, solubility, swelling, viscosity or opacity of the bulk polymer [41].

3.2.6. Scanning Electron Microscopy (SEM)

The PSP's surface morphology was analyzed using SEM, which revealed the microstructure of polysaccharides (Fig. 7). It was identified that PSP appeared as a fibrous sheet structure with a rough surface and a granular, massive cluster structure characterizing the porous morphology. These findings might be explained by the intense attraction between the functional groups on the surface of the polysaccharide, that induce polysaccharide chain aggregation by destroying inter-molecular interactions [48]. The porous surface appeared to be similar to the polysaccharide from the green alga *Codium bernabei* [49]. Consequently, both XRD and microscopic analysis support the semi-crystalline structure of PSP, which is highly related to its bioactivity [47].

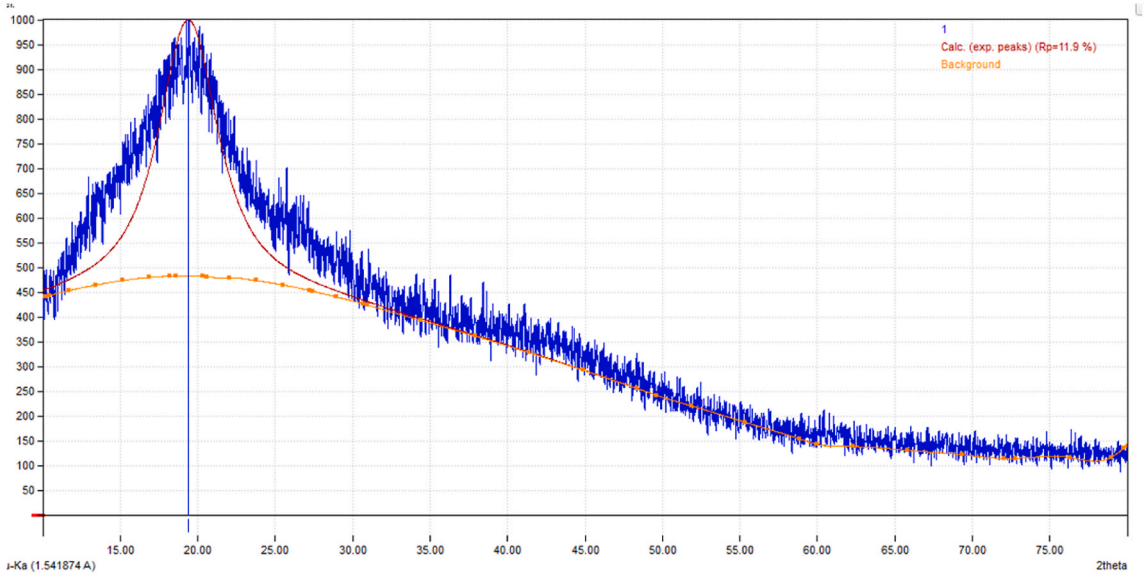

Fig. 6. X-ray diffraction of PSP.

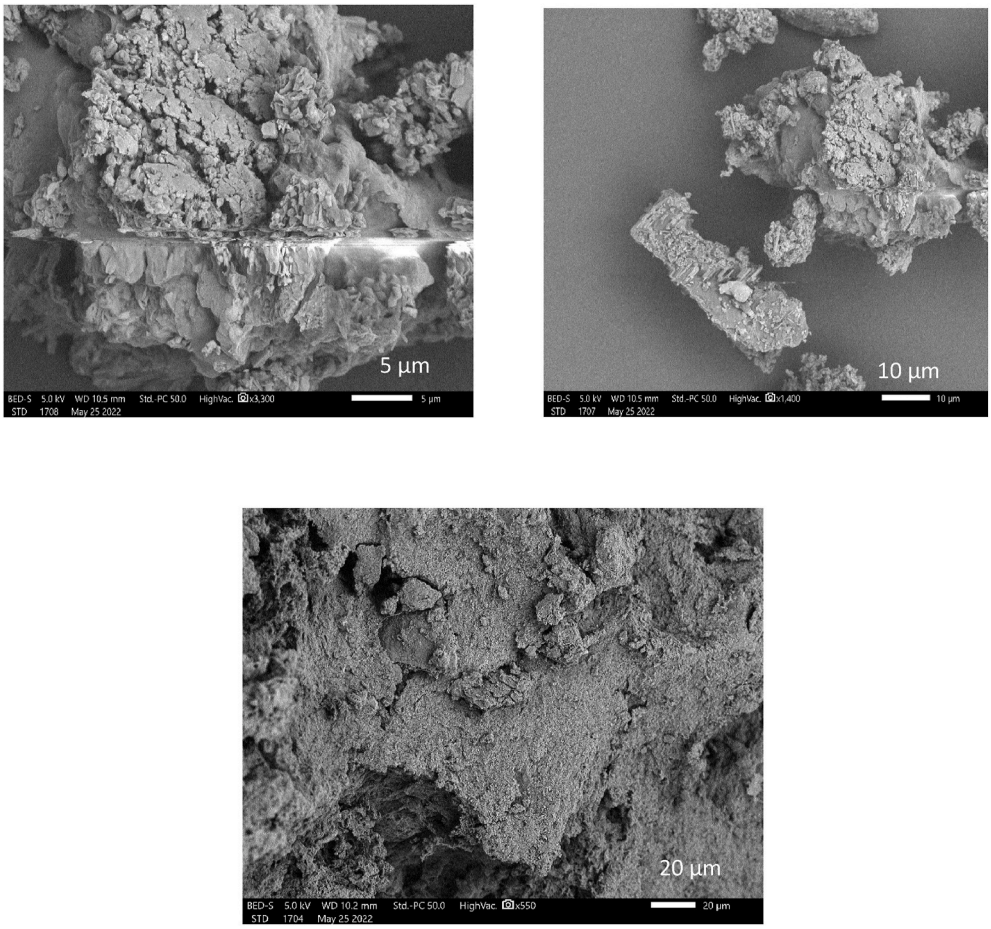

Fig. 7. Scanning electron Microscopy of PSP (5, 10 and 20 µm).

3.3. 3. In vitro antioxidant properties of PSP

3.3.1. DPPH free radical scavenging assay

DPPH is a free radical compound. It is often applied to assess the capacity for scavenging free radicals. Our research showed that PSP has a remarkable antioxidant capacity that rises with PSP concentration. The highest DPPH inhibition observed was 66.5 % ± 0.05 (Fig. 8 (A)) at a concentration of 5 mg/mL of PSP. In this context, several reports indicated that the antioxidant activity of polysaccharides is highly related to their monosaccharide compositions, molecular weight, and configuration [28,50]. Similarly, the radical scavenging capacity of PSP could be could result from the presence of hydroxyls and carboxyls in the polysaccharide, which acts as a hydrogen donator for neutralizing the DPPH free radical, thereby decreasing the impact of oxidative stress [51].

3.3.2. Ferrous iron chelating activity

Metal chelating ability is claimed to be one of the antioxidant mechanisms. It reduces the concentration of the catalyzing transition metal in lipid peroxidation [52]. This work calculated the metal chelating activity with BHA used as a positive control. Fig. 8 (B) shows that PSP exhibited considerable activity, which increased with their levels. The highest level of activity around 88 % ± 0.02, was signaled at 5 mg/mL of PSP concentration. Comparing this chelating activity to others polysaccharides, it was greater than the polysaccharide extracted from *Epimedium folium* (64.40 % at 5 mg/mL) [53].

3.3.3. Reducing power assay

The reduction of the Fe^{3+}/ferricyanide complex significantly indicates a potent antioxidant capacity [33]. Fig. 8 (C) shows the reductive effects of PSP using BHA as a standard positive. Like the iron chelating activity and DPPH free radical scavenging, PSP's reducing power increased with increasing doses. The maximum reducing activity was observed at 1.3 ± 0.03 (OD 700 nm) with a concentration of 5 mg/mL of PSP, but it was still lower than BHA. The reducing capacity of polysaccharides is generally due to bioactive compounds such as uronic acids, sulfates, and monosaccharide compositions [54].

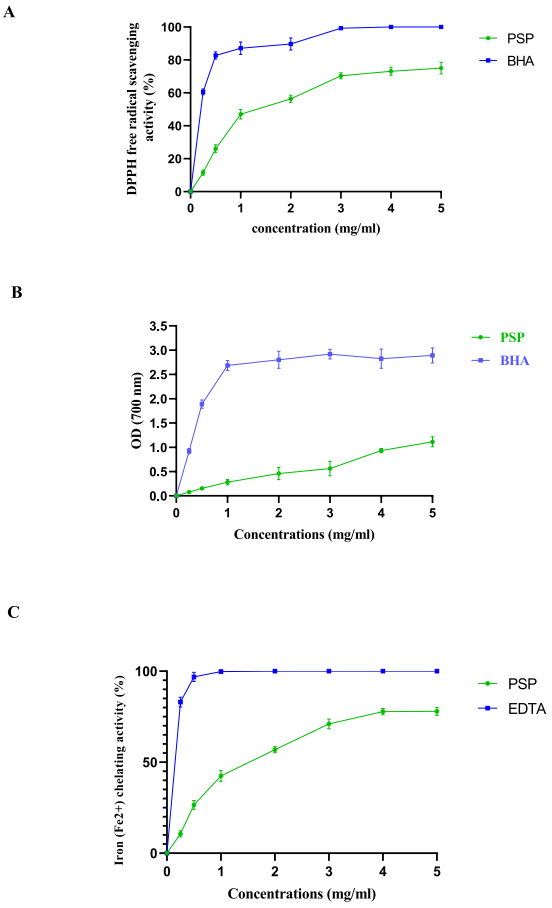

Fig. 8. DPPH free radical scavenging assay **(A),** ferrous iron chelating activity **(B),** and Reducing power assay **(C).**

3.3.4. DNA nicking assay

One of the most reactive free radicals in biological systems is the hydroxyl radical. This radical can severely damage biomolecules including DNA, lipids, and proteins [33,54]. In Fig. 9, The untreated plasmid (native DNA) in lane 1 split into three different forms: supercoiled, linear, and nicked circular. Lane 2 shows how DNA is incubated with Fenton's reagent, which caused DNA degradation. Adding PSP at two doses (2 and 5 mg/mL) (lanes 3 and 4) revealed higher DNA protection against oxidative degradations. It is indicated that molecules could chelate iron ions and change them, being inactive in a Fenton reaction [54].

With these findings, PSP could be classified as a strong candidate to prevent oxidative stress damage of biomolecules (DNA, proteins, and lipids). Many research have reported that the antioxidant activities of polysaccharides are generally associated with the frequency of double bonds and the presence of functional groups such as sulfated groups [45,47]. Additionally, the composition of monosaccharides and the contents of uronic acid strongly influence the increase of the antioxidant activity of polysaccharides [55].

3.4. Functional characteristics

3.4.1. Oil-holding (OHC) and water-holding (WHC) capacities

The WHC and OHC of polysaccharides are very interesting parameters in the food industry, which represent the most useful functional characteristics in food formulation [56] and depend on the capacity of polysaccharides to absorb oil and water [27]. However, WHC represents the capacity of a moist material to hold water when exposed to external forces such as centrifugal gravity or compression [8]. As shown in Table 1, the WHC of the PSP was around 1.372 ± 0.57 g water/g D.W. This value is lower than the WHC of polysaccharides extracted from Chickpea flour (5.14 g water/g D.W.) from hemoproteins of meat during the storage time [57]. Meanwhile, OHC was around 2.239 ± 0.25 g oil/g D.W. which is higher than OHC polysaccharide extracted from Chickpea flours (2.12 g oil/g D.W.) [57]. These properties are influenced by different factors, such as polysaccharide origin and experimental conditions [58].

3.4.2. Foaming properties

Polysaccharides are typically recognized as thickeners and stabilizers of foams [53]. The Foam Capacity (F.C.) and Foam Stability (F.S.) of PSP at different concentrations (0.5 %, 1 %, and 2 % w/v) are shown in Table 2. However, F.C. increased PSP concentrations with an interesting F.C. of 50.3 % at 2 % concentration. This character indicates the ability of PSP to enhance the viscosity of the aqueous phase and to develop a system that stabilizes the interface film (air-water) [8,27]. In addition, F.S. decreased with time at all tested doses. At 2 % concentration, F.S. after 30 min, 60 min, and 24 h were 44.6 %, 40.3 %, and 10.5 %, respectively. It is interesting to indicate that PSP foam remained stable after 24 h. However, the increasing in polysaccharide concentration might offered more surface for foam formation and enhancing the intermolecular interactions that stabilize the foam structure. This result is higher than the data obtained with polysaccharides extracted from leek (20 % after 5 min at a 2.5 % concentration) [59]. These interesting data prove that PSP could be used as a natural stabilizing system and ameliorate functional characteristics in food and biomedical industries [56].

3.4.3. Emulsifying activities

Nowadays, emulsifiers commonly utilized in the food industry, such as sucrose esters and fatty acid monoglycerides, are mainly synthetic [54]. Nevertheless, with the growing apprehension regarding food health, the advancement of natural emulsifiers has attracted much attention [60]. Many recent studies focused on polysaccharides as natural emulsifiers [8,61]. The emulsifying activity index (EAI) and emulsion stability index (ESI) are illustrated in Fig. 10. The EAI and ESI increased proportionally with the increasing PSP dose. Interestingly, PSP displayed a significant ability to emulsify corn oil and olive oil after 1 h, with index values at 52.9 % and 61.7 %, respectively, at a concentration of 2 % PSP with a strong capacity in emulsion stability after 336 h with index values being 35.7 % in corn oil and 46.8 % in olive oil at a 2 % concentration. The emulsifying properties of polysaccharides derived from plants could be due to proteins covalently branched in polysaccharide structure [62], which can act as hydrophobic groups [63]. Several polysaccharides have good surface activity and are typically employed as stabilizers for food emulsions [64]. Various studies have reported that emulsifying ability is mainly related to functional groups such as carboxyl, sulfate, and hydroxyl groups, as demonstrated by FT-IR

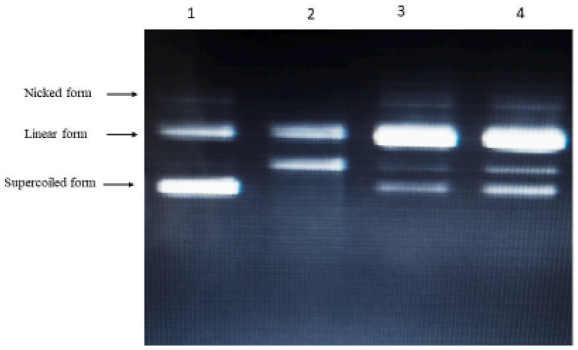

Fig. 9. DNA nicking assay of PSP.

Table 1
Water-holding and oil-holding capacities of PSP.

Properties	Capacities
WHC (g/g DW)	1.37 ± 0.57[a]
OHC (g/g DW)	2.23 ± 0.25[b]

Values are given as mean of three determinations (X ± SD); SD: standard deviation. a,b designate significant differences between values.

Table 2
Foaming properties of PSP.

Concentrations (g/100 ml)	FC (%)	FS (%)	
		30 min	60 min
0.5	20.5 ± 0.192[a]	10.5 ± 0.92[a]	5.3 ± 0.25[a]
1	31 ± 0.348[b]	26.33 ± 0.64[b]	25 ± 0.38[b]
2	50.33 ± 0.33[c]	44.66 ± 0.25[c]	40.3 ± 0.25[c]

Values are given as mean of three determinations (X ± SD); SD: standard deviation.
Values in the same column with different letters (a-c) designate significant differences between concentrations.
FC, foam capacity; FS, foam stability.

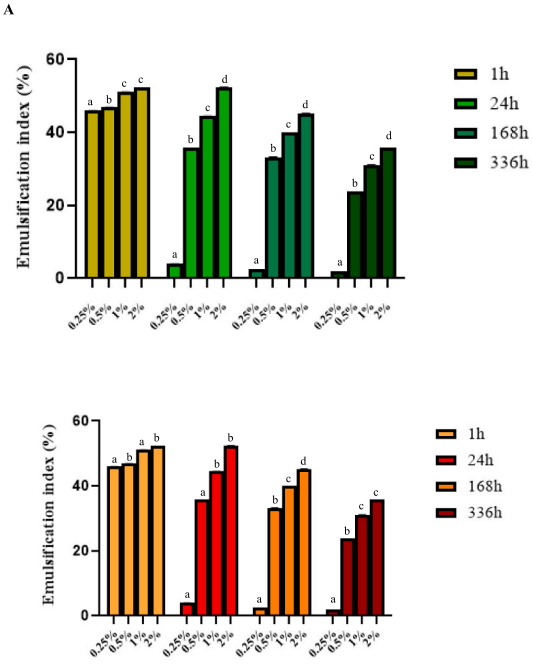

Fig. 10. Emulsification activity in mais oil (A), Emulsification activity in olive oil (B). Values with different letters (a–d) designate significant differences between concentrations in the same time ($p < 0.05$).

and chemical composition analysis [8,64].

3.5. Application of PSP on minced meat beef preservation

3.5.1. pH evaluation and moisture content

The pH value is a key parameter for evaluating the freshness of meat [65]. As mentioned in Table 3, the pH of all minced meat samples ranged between 5.05 and 5.28 on the first day of storage. Nevertheless, pH values of all samples increased gradually during the 9 days storage duration, which is associated with bacterial development and the liberation of alkali compounds such as ammonia, trimethyl, and dimethyl amines [27]. At the last day of storage, a significant increase in samples' pH values ($p < 0.05$) ranged between 5.27 and 6.01. a significant difference was signaled between all formulation samples ($p < 0.05$). Ben slima et al. (2017) [66] reported

Table 3
Evolution of pH values during the storage time of minced meat.

Samples	Storage time (days)			
	1	3	6	9
Control	5.05 ± 0.04^{bC}	5.13 ± 0.03^{bD}	5.18 ± 0.03^{cB}	5.27 ± 0.02^{aA}
Standard	5.15 ± 0.03^{aA}	5.20 ± 0.01^{dA}	5.28 ± 0.01^{cA}	5.38 ± 0.01^{bB}
PSP 0.5 %	5.1 ± 0.01^{aB}	5.15 ± 0.02^{cB}	5.29 ± 0.04^{bA}	5.9 ± 0.06^{bA}
PSP 1 %	5.03 ± 0.01^{aB}	5.17 ± 0.03^{dB}	5.45 ± 0.04^{cC}	5.78 ± 0.01^{bC}
PSP 2 %	5.28 ± 0.03^{bD}	5.38 ± 0.02^{dC}	5.56 ± 0.03^{cD}	6.01 ± 0.01^{aD}

Values in the same row with different letters (a-c) indicate significant differences for the same formulation within different days of conservation ($p < 0.05$).
Values in the same column with different letters (A-D) designate significant differences between formulations in the same storage day ($p < 0.05$).

that the elvation in pH values in meat resulting from the production of lactic acid by lactic acid bacteria, including Lactobacillus and Enterococci. These results follow those of other previous studies [27,36].

Moisture is a crucial factor that influences the quality of meat, such as visual appearance, texture and flavor [36]. As shown in Table 4, the moisture content decreased significantly ($p < 0.05$) in all formulation meat between the day 1 and the day 9 of storage. PSP 0.5 % sample showed a marked decrease by day 6 and continues to decrease by day 9. This indicates that a lower concentration of PSP is less effective over extended storage periods. The PSP 1 % sample demonstrated significantly a more stable moisture content compared to PSP 0.5 %. The slight increase by day 3 and subsequent stability suggest that PSP at this concentration can help in maintaining moisture better than lower concentrations. The PSP 2 % sample exhibits the best moisture retention among all samples, with an overall increase by day 3 and minimal loss thereafter. This indicates that higher PSP concentrations are more effective in retaining moisture during storage period. Nevertheless, there was no significant difference ($p > 0.05$) in moisture content in same formulation meat incorporated with PSP 1 % and PSP 2 % between day 6 and the last day of the storage time. This result might be due to the richness in components with higher water retention capacities, such as proteins [8]. These findings demonstrate a greater capacity of the polysaccharide PSP in moisture retention compared to BHA, suggesting their potential application in meat preservation. This stability in pH and moisture levels can inhibit the growth of pathogenic bacteria, thereby enhancing safety of meat [61].

3.5.2. Color analysis

Color is among the most interesting physical parameters in influencing consumer acceptance of the product [67]. As shown in Table 5, the redness (a*) and lightness values decreased significantly ($p < 0.05$) in all samples of minced meat during the storage period. The* parameter was regarded as the primary factor influencing the meat color. In addition, reducing redness affects the acceptability of the meat product to consumers [61,68]. Interestingly, the color of different samples incorporated with PSP was not affected by the progression of storage time ($p > 0.05$). The control sample shows significant changes in color values over the storage period. The increase in lightness (L*) and slight increase in redness (a*) may indicate oxidative changes and moisture loss, which can make the meat appear paler and slightly redder due to concentration of pigments. The decrease in yellowness (b*) suggests the presence of pigment degradation. The incorporation of PSP decreased slightly ($p < 0.05$) L* values compared to the control group during storage. The decreased lightness levels can be the consequence of Heme pigments interruption during the storage processing [67]. The yellowness (b*) for all samples showed a significant decrease ($p < 0.05$). Kallel et al. (2015) [68] reported that polysaccharides could diminish metmyoglobin formation when incorporated into meat. Probably, the reduction in the redness of treatments made with PSP is most likely caused by the conversion of pink oxymyoglobin to brown metmyoglobin [61]. These data follow the results of Hamzaoui et al. (2020) [27] who reported that the reduction of color parameters might be attributed to a browning reaction.

3.5.3. Metmyoglobin evaluation (MetMb)

Myoglobin is a water-soluble protein that affects the color of meat. The color change of meat and the development of metmyoglobin result from the reduction of heme redox stability by the liberation of an electron during oxidation processes [8]. As shown in Fig. 11,

Table 4
Evolution of moisture values during the storage time of minced meat.

Samples	Storage time (days)			
	1	3	6	9
Control	25.76 ± 0.01^{aA}	25.90 ± 0.26^{aA}	25.78 ± 0.31^{aA}	25.48 ± 056^{aA}
BHA	33.46 ± 3.74^{bC}	32.84 ± 2.32^{aC}	26.76 ± 0.61^{aD}	26.21 ± 0.78^{aC}
PSP 0.5 %	29.86 ± 0.20^{dB}	30.87 ± 1.60^{cB}	26.58 ± 0.73^{bC}	25.66 ± 0.74^{aB}
PSP 1 %	25.92 ± 0.09^{cA}	27.27 ± 0.88^{bA}	26.77 ± 0.82^{bB}	26.38 ± 0.23^{aA}
PSP 2 %	26.72 ± 0.04^{bA}	29.58 ± 0.87^{bA}	26.47 ± 2.93^{aB}	26.89 ± 0.61^{aA}

Values in the same row with different letters (a) indicate significant differences for the same formulation within different days of conservation ($p < 0.05$).
Values in the same column with different letters (A-D) designate significant differences between formulations in the same storage day ($p < 0.05$).

Table 5
Evolution of color values during the storage time of minced meat.

Samples		Storage time (days)	
		1	9
L*	**Control**	30.74 ± 0.38^{a}	49.57 ± 0.77^{b}
	BHA	40.35 ± 0.35^{b}	51.32 ± 0.17^{d}
	PSP 0.5 %	40.25 ± 1.48^{b}	49.10 ± 0.13^{a}
	PSP 1 %	44.05 ± 0.63^{c}	50.25 ± 0.07^{c}
	PSP 2 %	48.71 ± 0.69^{d}	54.20 ± 1.14^{e}
a*	**Control**	10.05 ± 0.06^{e}	11.01 ± 0.01^{d}
	BHA	8.45 ± 0.49^{c}	10.09 ± 0.14^{c}
	PSP 0.5 %	7.75 ± 0.07^{a}	9.47 ± 0.07^{a}
	PSP 1 %	8.15 ± 0.07^{b}	9.87 ± 0.03^{b}
	PSP 2 %	9.65 ± 0.65^{d}	10.12 ± 0.1^{c}
b*	**Control**	9.09 ± 0.01^{a}	7.17 ± 0.10^{a}
	BHA	10.12 ± 0.10^{b}	8.23 ± 0.02^{c}
	PSP 0.5 %	9.33 ± 0.15^{a}	7.48 ± 0.05^{b}
	PSP 1 %	10.35 ± 0.21^{b}	8.07 ± 0.03^{c}
	PSP 2 %	10.90 ± 0.14^{c}	8.68 ± 0.02^{d}

Values in the same column with different letters (a-e) designate significant differences.
between formulations in the same storage day ($p < 0.05$).

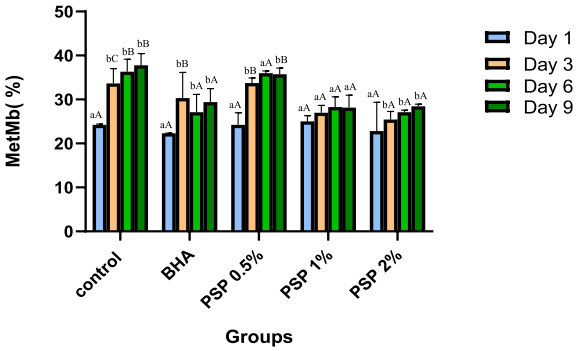

Fig. 11. Fig. 11: Effects of PSP on metmyoglobin MetMb in minced beef meat during storage at4°C. Values with different letters (a–b) indicate significant differences for the same formulation within different days of conservation ($p < 0.05$). Values with different letters (A–C) designate significant differences between formulations in the same storage day ($p < 0.05$).

the MetMb rate increased with the storage time in all minced meat samples, which could be due to the denaturation of myoglobin [61]. MetMb percentage increases significantly during storage time from around 24 % on day 1 to approximately 64 % on day 9 in the control sample. This indicates oxidation of myoglobin to metmyoglobin, which is associated with the browning of meat, a sign of quality deterioration. Overall, the oxidation of myoglobin could conduce to the formation of free radicals such as peroxyl radicals and oxidized iron [69]. Sample incorporated with BHA, a synthetic antioxidant, shows a slower increase in metmyoglobin percentage compared to the control, starting at about 20 % on day 1 and reaching around 55 % in the day 9. These data provided a delay in the oxidation process in meat. The addition of 0.5 % of PSP Starts at approximately 21 % on day 1 and increases to about 58 % by day 9. This increase seems to be slower than the control sample. The addition of 1 % of PSP in meat demonstrated a more significant inhibitory effect on metmyoglobin formation, starting at around 18 % on day 1 and reaching about 52 % on day 9. This indicates better preservation of meat color compared to PSP 0.5 % and the control. On day 9 of the storage period, PSP 2 % exhibited the lowest MetMb amount, with 28.15 %, when compared with PSP 1 %, PSP 0.5 %, control, and the standard samples, with about 28.44 ± 0.34 %, 36.85 ± 0.0016 %, 37.76 ± 1.88 % and 29.41 ± 2.15 %, respectively. These data could be proportionally linked to the reduction in lipid peroxidation [61]. A similar study by Eljoudi et al. (2022) [36] showed that a crude polysaccharide derived from *Malcolmia triloba* plant could protect myoglobin against oxidation by preventing oxygen from entering. These data proved the ability of PSP to protect myoglobin by inhibiting the oxidation process.

3.5.4. Heme iron determination

Heme iron is characterized as a part of hemoglobin and is also found in other hemoproteins, including myoglobin, catalases, heme peroxidase, and endothelial nitric oxide synthase [70]. Fig. 12 reveals that storage time significantly affected the variation in Heme iron rate in beef minced meat of all samples stored at 4 °C. The heme iron content decreases over the storage period from around 9 μg/g on day 1 to approximately 5 μg/g by day 9 in the control sample. This decline indicates the degradation of heme proteins and loss of meat color quality over storage time. The PSP 0.5 % sample exhibits a moderate initial heme iron content (around 11 μg/g) which

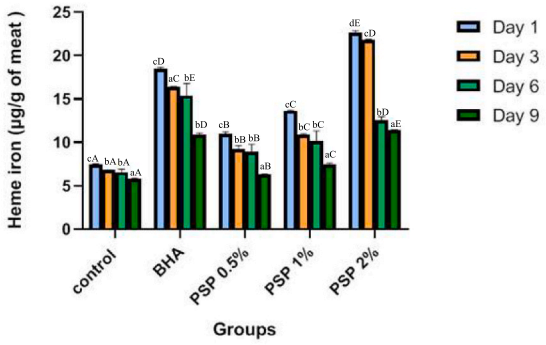

Fig. 12. Effects of PSP on heme iron in minced beef meat during storage at 4 °C. Values with different letters (a–d) indicate significant differences for the same formulation within different days of conservation ($p < 0.05$). Values with different letters (A–E) designate significant differences between formulations in the same storage day ($p < 0.05$).

decreases to about 8 µg/g by day 9. This suggests that PSP 0.5 % has some protective effect on heme iron. The PSP 1 % sample starts with heme iron content around 16 µg/g and shows a gradual decline to about 10 µg/g on the day 9. This indicates a more better preservation of heme iron compared to PSP 0.5 % and the control. Indeed, the lowest reduction in iron values ($p < 0.05$) was obtained for the PSP 2 % as well as the standard about 11.25 ± 0.042 and 10.88 ± 0.21 µg/g of meat at the last day of storage, respectively, compared with PSP 1 %, PSP 0.5 %, and the control which are 7.43 ± 0.16, 6.32 ± 0.042 and 5.78 ± 0.12 µg/g of meat, respectively. This protection is associated with the antioxidant effect of PSP or BHA in the meat [61]. The mechanism through which the inhibition occurred could be associated with the chelation of free iron, released from hemoproteins of meat during the storage time [71]. These findings prove the role of PSP in maintaining the stability of meat during the storage period.

3.5.5. Conjugated dienes content

The conjugated dienes, identified as the primary oxidation products, are presented in Fig. 13. The present study revealed that the conjugated dienes content of differently prepared samples increased the duration of storage, likely due to the production of hydroperoxide substances [72]. The incorporation of PSP at different concentrations induced significantly ($p < 0.05$) inhibition in the oxidation process and the formation of conjugated dienes in comparison to the control and BHA samples. After 9 days of storage, meat with the addition of PSP displayed the values PSP 0.5 % (OD233 = 0.305), PSP 1 % (OD233 = 0.296), and PSP 2 % (OD233 = 0.29) significantly ($p < 0.05$) lower than the control (OD233 = 0.323) and BHA (OD233 = 0.315).

3.5.6. Determination of thiobarbituric acid reactive substance (TBARS)

The TBARS method is commonly employed to assess the extent of lipid oxidation. TBARS is generated during the second stage of autooxidation, where peroxides are converted into aldehydes and ketones [68]. Meat is influenced by oxidation processes, which can lead to quality deterioration during food manufacturing, storage, and distribution [27]. In this context, PSP was assayed for its antioxidant activity against lipid oxidation in minced meat, as presented in Fig. 14, by measuring the changes of TBARS as an indicator

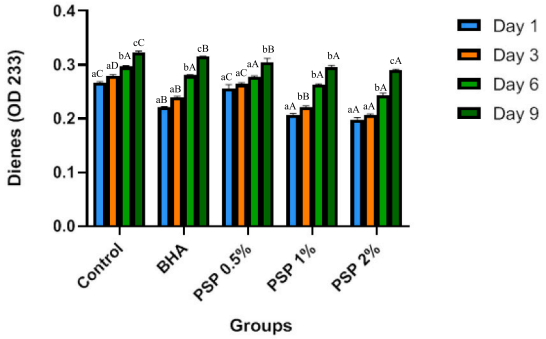

Fig. 13. Effects of PSP on conjugated diene content in minced beef meat during storage at 4 °C. Values with different letters (a–c) indicate significant differences for the same formulation within different days of conservation ($p < 0.05$). Values with different letters (A–D) designate significant differences between formulations in the same storage day ($p < 0.05$).

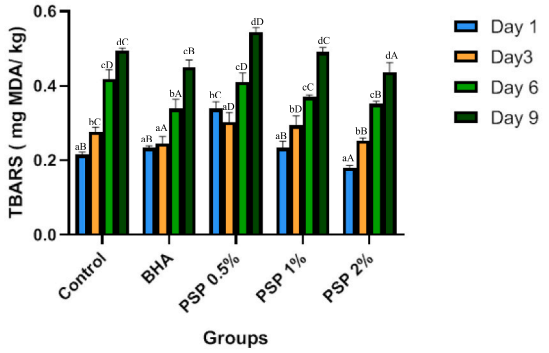

Fig. 14. Effects of PSP on TBARS content content in minced beef meat during storage at 4 °C. Values with different letters (a–d) indicate significant differences for the same formulation within different days of conservation ($p < 0.05$). Values with different letters (A–D) designate significant differences between formulations in the same storage day ($p < 0.05$).

of the lipid oxidation degree. Results revealed that MDA levels in the different samples of meat increased significantly ($p < 0.05$) during the storage period but varied among the different samples. On the last day of storage, the control displayed a higher level of TBARS value (0.57 mg MDA/kg) when compared to other samples. However, the sample reinforced with 2 % of PSP exhibited significantly ($p < 0.05$) the lowest TBARS value (0.43 mg MDA/kg) compared with PSP 0.5 %, PSP 1 %, and standards which were recorded at 0.46, 0.44, and 0.45 mg MDA/kg, respectively. These findings reflect the interesting effect of PSP in beef meat preservation, which might be due to the antioxidant capacity of polysaccharides [36].

3.5.7. Microbial analysis

Evaluation of the microbial flora of meat on psychrophilic bacteria (P.B.) and total mesophilic flora (TMF) during 9 days of storage are presented in Table 6. Data revealed that microbial count increased proportionally with the storage period in all meat samples. Additionally, among the samples, meat incorporated with PSP showed significantly ($p < 0.05$) lower counts than the other samples, which could be due to bioactive compounds of polysaccharides that inhibit microbial growth [61]. Concerning mesophilic flora, all meat samples incorporated with PSP at 0.5 %, 1 %, and 2 % revealed lower values (3.18, 3.12, and 3.08 log CFU/g, respectively) compared with the control group (3.48 log CFU/g) and BHA sample (3.33 log CFU/g) at the end of storage period. These findings align with those reported in the literature [27,36]. Concerning psychrophilic bacteria, obtained data showed that the samples with the addition of PSP indicated lower count (3.80, 3.78, and 3.72 log CFU/g) compared with the control group (3.12 log CFU/g) and BHA group (3.84 log CFU/g) at the end of storage. These results could be due to the interesting antimicrobial activity of PSP, which controls microbial growth over the 9 days of storage. Previous research mentioned that bioactive polymers could interact with lipid bilayers of the microbes through various mechanisms involving disrupting the integrity and functionality of cell membranes and suppressing bacterial enzymes responsible for transcription, ATP synthesis, and genetic material replication [1].

4. Conclusion

The current study was conducted to identify a new water-soluble polysaccharide. named PSP extracted from *H. scoparia* leaves.

Table 6
Microbiological characteristics (log $CFUg^{-1}$) during storage of minced meat.

		Storage time (days)			
		1	3	6	9
Total mesophilic flora	Control	3.09 ± 0.01^{aD}	3.41 ± 0.00^{bE}	3.45 ± 0.01^{cD}	3.48 ± 0.00^{dE}
	BHA	2.97 ± 0.01^{aA}	3.28 ± 0.01^{bA}	3.29 ± 0.00^{cA}	3.33 ± 0.01^{cA}
	PSP 0.5 %	2.94 ± 0.01^{aD}	3.006 ± 0.01^{bD}	3.13 ± 0.01^{cC}	3.18 ± 0.01^{cD}
	PSP 1 %	2.86 ± 0.01^{aC}	2.97 ± 0.00^{bC}	3.11 ± 0.00^{cB}	3.12 ± 0.01^{dC}
	PSP 2 %	2.84 ± 0.01^{aB}	2.91 ± 0.01^{bB}	3.06 ± 0.01^{cA}	3.08 ± 0.01^{cB}
Psychrophilic bacteria	Control	2.75 ± 0.01^{aD}	2.85 ± 0.00^{bD}	3.04 ± 0.02^{cD}	3.12 ± 0.00^{dC}
	BHA	2.52 ± 0.02^{aE}	2.64 ± 0.01^{bE}	2.69 ± 0.01^{bE}	2.71 ± 0.01^{bD}
	PSP 0.5 %	2.74 ± 0.02^{aC}	2.78 ± 0.02^{bC}	2.81 ± 0.01^{cC}	2.84 ± 0.01^{dB}
	PSP 1 %	2.67 ± 0.01^{aB}	2.70 ± 0.01^{bB}	2.79 ± 0.01^{cB}	2.80 ± 0.00^{dB}
	PSP 2 %	2.63 ± 0.02^{aA}	2.68 ± 0.01^{bA}	2.71 ± 0.01^{cA}	2.72 ± 0.05^{dA}

Values in the same row with different letters (a-d) indicate significant differences for the same formulation within different days of conservation ($p < 0.05$).
Values in the same column with different letters (A-E) designate significant differences between formulations in the same storage day ($p < 0.05$).

Structural characteristics, biological activities, and functional properties of PSP were investigated. HPLC analysis revealed that PSP was a heteropolysaccharide composed of mannose and glucuronic acid monosaccharide units. The FTIR spectra showed the presence of sulfated groups, and a semi-crystalline pattern was proved with the XRD analysis. Data revealed that PSP possesses good antioxidant activities *in vitro*. In addition, our results showed that PSP exerted higher water and oil-holding capacities along with emulsification and foaming capacities.

Interestingly, adding PSP to minced beef meat prevented lipid oxidation and undesirable bacterial growth during 9 days of storage at 4 °C. Data obtained in this study, namely TBARS, metmyoglobin, and Heme stability, as well as pH values, proved the effectiveness of PSP as a potent food additive and, consequently, as a functional compound to enhance their shelf life and microbiological safety during refrigeration. These findings highlighted the potential use of the polysaccharide PSP as natural and effective alternatives to synthetic additives in meat preservation in food industry, offering advantages such as improved food safety, extended shelf life, and reduced reliance on synthetic chemicals.

CRediT authorship contribution statement

Malek Eleroui: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Amal Feki:** Formal analysis, Data curation. **Marwa Kraiem:** Methodology. **Asma Hamzaoui:** Methodology. **Zakaria Boujhoud:** Methodology. **Ibtissem Ben Amara:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Conceptualization. **Hatem Kallel:** Validation, Supervision.

Data availability statement

Data will be made available on request.

Funding statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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