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Polysaccharide from Garlic Straw: Extraction, Structural data, Biological properties and Application to beef meat preservation

Fatma Kallel*a, Dorra Drissa, Fatma Bouaziza, Lilia Belghitha, Soumaya Zouari-Ellouzi,a
Fatma chaaría, Anissa Haddara, Semia Ellouz Chaabouniaux, Raoudha Ghorbela,b

*aEnzyme Bioconversion Unit (04/UR/09-04), National School of Engineering P.O. Box 1173-3038, Sfax University, Tunisia

bCommon Service Unit of Bioreactor coupled with an ultrafilter, National School of Engineering P.O. Box 1173-3038, Sfax University, Tunisia

*Corresponding author: E-mail address: F11fatma@yahoo.fr
Tel.: +216 74 274 418; Fax: +216 74 275 595.
Abstract

A novel polysaccharide (GSP) was isolated from garlic straw (Allium sativum L.) by hot water technique. The structural characterization, antioxidant and antimicrobial activities were investigated. The results showed that GSP was mainly composed of glucose, mannose, galactose and xylose and the major functional groups identified from FT-IR spectrum includes 1631.38 cm⁻¹ (-COO⁻) and 3193 cm⁻¹ (-OH). In addition, GSP had high DPPH radical scavenging activity, a strong reducing power and inhibited the peroxidation of linoleic acid. The antimicrobial activity of GSP was evaluated against a panel of 7 bacteria and 2 fungal strains using agar diffusion method. Results have shown that GSP exhibited moderate to strong antimicrobial activity against the tested species. These interesting results incite the experimental inoculation of GSP in minced beef meat preservation amended with different concentrations of the GSP and stored at 4°C for 9 days. The obtained results showed significant inhibitions (p ≤ 0.05) of lipid oxidation over 9 days of aerobic storage and also improvement of meat colour stability while differences in total aerobic cell populations did not change noticeably over storage. Finally, sensory characteristics, e.g. colour, odour and texture, of treated meat with GSP, were higher than the control.

Keywords: Garlic straw polysaccharide; Extraction; Antioxidant activity; Antimicrobial activity; Meat preservation.
1. Introduction

Food researchers and industry overseers continued to seek more and better tools/agents to maintain food safety especially by the use of natural preservatives as antimicrobial and antioxidant agents. They are the added or supplemented agents in foods that lead to a retardation of spoilage, extension of shelf-life, and maintenance of quality and safety (Devatkal and Naveena, 2010). Meat processing industry is regularly facing many serious challenges regarding the safety and hygiene of its products (Davidson, 2001). In fact, meat products typically spoil during refrigeration due to two major causes: microbial growth and oxidative rancidity (Sebranek et al., 2005).

For this reason, efforts to reduce oxidation have been increased. Most often, the best strategy is the addition of antioxidants (Brewer, 2011). Moreover, some antioxidants may additionally exhibit antibacterial activities (Puupponen-Pimiä et al., 2001). To deal with lipid oxidation issues and microbial growth in meat products; either synthetic or natural food additives are commonly used in the meat industry (Mielnik et al., 2003; Sallam et al., 2004; Estevez and Cava, 2006). Many synthetic preservatives, such as BHT, butylated hydroxyanisole (BHA) and propyl gallate (PG), are typically used to protect foods from spoilage, although their use is restricted due to possible carcinogenic effects. Thus, it is believed that natural preservatives and antimicrobial agents will have more efficiency and safety regarding consumers’ health and preference (Venkatesan et al., 2011). As opposed to synthetic compounds, natural preservatives are safe, can protect the human body from free radicals and delay the progress of many chronic diseases (Kinsella et al., 1993; Singha and Rajini, 2004).

In recent years, polysaccharides from plants, animals, and microorganisms have piqued the interest of many researchers, owing to their many biological activities. Plant-based polysaccharides could be effective antioxidants against lipid peroxidation by scavenging
initiating radicals, breaking chain reaction, decomposing peroxides and binding chain
initiating catalysts, such as metal ions (Trommer and Reinhard, 2005). They are advantageous
for this task as well because they are also naturally occurring substances with no or minor
adverse effects (Tombs and Harding, 1998).

Many researchers have investigated the process of utilizing food wastes for the
extraction of natural preservatives, mainly from plant by-products such as vegetable
processing waste, potato starch waste, onion (Allium cepa) solid waste and garlic (Allium
sativum L.) husk waste (Rusendi and Sheppard, 1996; Kiassos, et al., 2009; Kallel et al.,
2014). Garlic (Allium sativum L.) has been used throughout its history for both culinary and
medicinal purposes (Rivlin, 2001). During harvesting period garlic bulb yields a considerable
amount of straw, consists of two major parts the leaf and the stem, which is simply thrown or
disposed (Banerjee and Maulik, 2002). Garlic straw is one of the numerous examples of
grossly underutilized by-products. Recently, the phenolic compounds from garlic by-product
have been investigated (Kallel et al., 2014). However, there are, so far, no reports on the
chemical composition and the extraction of polysaccharide from this industrially disposed
waste.

The objectives of this study were to determinate the chemical composition and to
evaluate the antioxidant and antimicrobial activities of GSP. Based on these in vitro results,
GSP was applied to raw beef patties as a natural preservative, we thus investigated the
influence of GSP on lipid oxidation and microbial growth, as well as instrumental and sensory
colour and odour characteristics of minced beef patties.

2. Materials and methods

2.1. Plant material and chemicals
The garlic straw (GS) was discharged from the manufacturing process of conservation of garlic. It was first cleaned, dried in sunlight and then cut into small pieces (1–3 cm). The cut straw was ground to pass a 1-2 mm size screen and stored at 4°C. Gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Tween 20, linoleic acid, potassium ferricyanide, ferric chloride, and BHA were obtained from Sigma–Aldrich (St. Louis, USA). Trichloroacetic acid, Folin–Ciocalteu’s reagent, sodium carbonates anhydrous, di-sodium hydrogen and phosphate dehydrates were obtained from Fluka (Steinheim, Switzerland). All other chemicals and solvents were of analytical grade.

2.2. Chemical analyses

Moisture was determined according to the AOAC (1997). Total nitrogen content was determined by Kjeldahl’s method. Protein was calculated using the general factor (6.25) (Vandercook et al., 1979). Fat content was determined according to the AFNOR (1986). Ash was determined by sample combustion in a muffle furnace at 550°C for 4 h. Atomic Absorption Spectrophotometer (Analytic Jena ZEEnit 700 spectrometer, USA) was used using the filter corresponding to each mineral element. Phosphorus was determined calorimetrically using the vanado molybdate method (AOAC, 1999). Ethanol extractive was obtained after successive extractions with ethanol (95%) for 24 h. The mass of extractive solubilized was determined by the difference between the initial mass of dry garlic straw sample and the mass of the solid residue obtained after extraction dried at 105°C (Pujol et al., 2013). Dietary fibre was determined by the enzymatic-gravimetric AOAC method (Prosky et al., 1988). Lignin was isolated from GS as residual precipitate after total hydrolysis of cellulose and hemicellulose by sulfuric acid according to the method TAPPI T 222 om-88 (1988). Acid-soluble lignin was determined according to TAPPI UM 250 standards.
The carbohydrate content was determined as a weight difference according to the formula: Carbohydrates = 100 - (% moisture + % protein + % fat + % ash + % lignin).

2.3. Extraction of garlic straw polysaccharide

The extraction of garlic straw polysaccharide (GSP) was conducted by the method of Yao et al. (2005). The GS was defatted with 95% ethanol for 24 h to remove impurities and small lipophilic molecules. The defatted powders were diluted with distilled water (ratio of water to raw material, 10 to 40 in distilled water (mL/g) and incubated in thermostat-controlled water-bath (60 to 100°C) for 90 to 180 min. The aqueous extract was filtered through Whatman no. 4 paper and then the supernatant was concentrated by rotary vacuum evaporator (Shanghai, China) at 50°C, and precipitated by addition of a 4-fold volume of 95% ethanol and then incubated at 4°C for 24 h. Finally, the precipitate from centrifugation (5000 rpm, 15 min) was dissolved in deionized water, dialysis (cut off range 1 KDa) and lyophilized to afford the GSP. The polysaccharide yield (% w/w) is calculated as follows:

\[
\text{Polysaccharide yield} = 100 \times \frac{\text{Polysaccharide content of extraction (g)}}{\text{Weight of garlic straw (g)}}
\]

2.4. Analysis of polysaccharide characterization

2.4.1. Analysis of contents of total sugars, reducing sugar, protein and total phenol

The obtained GSP under the optimum condition was stored in a desiccator prior the preliminary characterization and antioxidant activities experiments. Sugar content was determined according to Dubois et al. (1956). The protein contents were measured by the method of Bradford using bovine serum albumin (BSA) as the standard (Bradford, 1979). Reducing sugar content was analyzed by dinitrosalicylic (DNS) colorimetric method (Miller,
1959), using D-glucose as a standard. The total phenolic content was determined by the
Folin–Ciocalteau assay (Singleton & Rossi, 1956) using gallic acid as calibration standard.

2.4.2. Monosaccharide composition of GSP

GSP (10 mg) was dissolved in 2 M trifluoroacetic acid solution (TFA, 2 mL) and
hydrolyzed at 120°C for 3 h in a sealed glass tube. The hydrolysate of GSP was evaporated to
dry under reduced pressure at 45°C. Then, GSP was removed by washing with methanol (3
ml) four times in order to remove TFA absolutely. The final residue was dissolved in 2 mL
deionized water and used for further analysis. HPLC (Agitant 1260) was used for the
identification and quantification of monosaccharide. Experiment was performed on an ion-
exchange column (HPX-87H) (300 x 7.8 mm) with a refractometer index detector (IR). The
temperature was kept at 30°C and the injection volume was 20 µL. The mobile phase was
0.004 M H\textsubscript{2}SO\textsubscript{4} at a flow-rate of 0.5 mL/min. D-Gal A, D-Glu A, D-Man, D-Xyl, D-Rib, D-
Glu, D-Gal, D-Fru, L-Rha, and L-Ara were used as references.

2.4.3. UV, Infra-Red and NMR analysis

The GSP was applied to ultraviolet (UV) spectrum, infrared spectrum (IR) and nuclear
magnetic resonance (NMR) analysis. UV spectrum was recorded by scanning the GSP
solution (2 mg/mL) in a UV-V spectrophotometer (Shimadzu, Japan) with wavelength of
200–400 nm.

The FT-IR spectrum of GSP was recorded between 400 and 4000 cm\textsuperscript{-1} in a NICOET
spectrometer. The transmission spectra of the samples were recorded by using the KBr pallet
containing 0.1% of sample.

GSP structural analysis was carried out by \textsuperscript{13}C NMR with CP/MAS technique (cross-
polarization, magic-angle-spinning) using a BRUKER-ASX300 instrument. NMR spectra
were recorded at a $^{13}$C frequency of 75.5 MHz (field of 7.04 T). CP/MAS sequence was used with the following parameters: the $^{13}$C spin lattice relaxation time was 5 s, powdered samples were placed in an alumina rotor used for the double air-bearing-type MAS system and spun as fast as 8 kHz. Contact time was 8 ms.

2.5. Antioxidant activity

2.5.1. Assay of DPPH radical scavenging activity

DPPH radical-scavenging activity of the polysaccharide (0.1-5 mg/mL) was determined as described by Bersuder et al. (1998). Sample was measured for absorbance at 517 nm. The percent radical scavenging activity is determined from the difference in absorbance of DPPH between the control and samples. BHA was used as positive standard. The extract concentration providing 50% inhibition (IC$_{50}$) was calculated from the graph of scavenging effect percentage against extract concentration in the solution.

2.5.2. Conjugated diene method

The antioxidant activities (AOAs) of the polysaccharide were evaluated according to the conjugated diene method, as described in literature (Lingnert et al., 1979). The polysaccharide at different concentrations (0.1-5 mg/mL) was mixed with 2 mL of 10 mM linoleic acid emulsion stabilized with Tween-20 in 0.2 M sodium phosphate buffer (pH 6.5). These were put onto test tubes and placed in darkness at 37°C in order to achieve oxidation. After incubation for 15 h, 8 mL of 80% methanol in de-ionized (DI) water was added onto each tube and mixed thoroughly. The absorbance of the mixture at 234 nm was re-measured against a blank. AOA was calculated as:

$$AOA (%) = \left[ \frac{\Delta A_{234} \text{ of control} - \Delta A_{234} \text{ of sample}}{\Delta A_{234} \text{ of control}} \right] \times 100$$
The blank comprises DI water and the control, which consisted of DI water and reagent solution without the solvent extract. IC\textsubscript{50} (mg/mL) is the effective concentration at which the AOA was 50\% and was calculated from the graph of antioxidant activity percentage against extract concentration in the solution.

2.5.3. Reducing power assay

The reducing power was determined according to the method of Yildirim et al. (2001). An aliquot of 1 mL sample (1-5 mg/mL) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1\%). The mixture was incubated at 50°C for 30 min. After incubation, 2.5 mL trichloroacetic acid (10\%) was added and the reaction mixtures were centrifuged for 10 min at 3000 rpm. Finally, the supernatant solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl\textsubscript{3} (0.5 mL, 0.1\%). After 10 min, the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated reducing power. Higher absorbance of the reaction mixture indicated higher reducing power. Extract concentration providing 0.5 of absorbance (RP\textsubscript{0.5AU}) was calculated from the graph of absorbance at 700 nm against extract concentration in the solution.

2.6. Antimicrobial activity

Bacteria (Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Micrococcus luteus, Enterococcus faecalis, Bacillus thurengensis, Staphylococcus aureus), and fungi (Aspergillus niger and Rhizopus oryzae) were selected as test organisms in this study.

Antimicrobial activity assays were performed according to the method described by Berghe and Vlietinck (1991). Sterile nutrient agar medium was prepared and distributed into Petri plates of 90 mm diameter. A suspension of the previously prepared test microorganism (0.1 mL of 10\textsuperscript{6} UFC/mL) was spread over the surface of agar plates (LB medium for bacteria.
and Potato Dextrose Agar (PDA) medium for fungi). Then, bores (3 mm depth, 5 mm
diameter) were made using a sterile borer and loaded with 50 µL and 100 µL of GSP. Before
incubation, all petri dishes were kept in the refrigerator for 2 h to enable pre-diffusion of the
substances into the agar. After that, they were incubated at 37°C for 24 h for bacteria and at
30°C for 72 h for fungi. Ciprofloxacin and Amphotericin B were used as positive references
and distilled water as negative control for bacteria and fungi activities, respectively. The
diameters of the inhibition zones were measured using a ruler, with an accuracy of 0.5 mm.
Each inhibition zone diameter was measured three times (in two different plates) and the
results were expressed as an average of the radius of the inhibition zone in mm.

2.7. Application of GSP on minced meat storage

2.7.1. Meat sample preparation

Beef steaks from the longissimus dorsi muscle part were purchased from local market in
Sfax City (Tunisia) and were ground, using a kitchen mixer, after eliminating the excessive
fats and connective tissues. Salt (70% NaCl and 30% KCl) was added at a rate of 1.5% to the
minced meat. The minced meat was subdivided into four treatments: A negative control (NC)
(not added GSP). Two formulations were blended with 2, and 4% of GSP and a positive
control (PC) (A synthetic antioxidant BHA instead of GSP). Patties of 25 g were shaped by
hand (5.5 cm diameter, 1.5 cm thickness) and placed in plastic foam meat trays, wrapped with
polyethylene and kept at 4 °C for nine days. Four samples were taken at the 1st, 3rd, 6th and
9th day in order to evaluate their oxidation, colour stability and the potential microbial
contamination. A strict sanitation procedure was followed during the preparation of meat
samples to avoid microbial contamination.
2.7.2. Evaluation of lipid oxidation

Lipid oxidation was evaluated using the thiobarbituric acid-reactive substance (TBARS) as previously performed (Witte et al., 1970). Four samples taken at the 1st, 3rd, 6th and 9th day of the chilled storage were used. Briefly, 20 g from each prepared formula were homogenized with 50 mL reagent solution containing 20% TCA in 2 M phosphoric acid, at 4°C. The mixture was then adjusted to 100 mL distilled water, shaken and filtered using Büchner funnel through Whatman paper. 5 mL of the filtrate was then transferred into a glass test tube and an equal volume of 2-thiobarbituric acid (5 mM), freshly prepared, was added at 4°C. The test tube was then well shaken and incubated at room temperature for 17 h. The absorbance of the mixture was measured at 530 nm (A530) and the results were expressed, as mg malondialdehyde (MDA)/Kg meat as follows: TBA (mg (MDA)/Kg meat) = A530 × 5.2.

2.7.3. Microbial analysis

Microbial count was carried out at days 0, 1st, 3rd, 6th and 9th of storage at 4°C according to the following procedure. 225 mL sterilized peptone solution (25.5 g/L) was added to the 25 g minced meat, with and without GSP, then homogenized. Decimal dilutions up to 10^8 prepared from the initial concentration (100 mg/mL) and aliquots of the appropriate dilutions were placed on PCA media. Total viable count (TVC) were determined using plate count agar (PCA) after incubation at 30°C for 72 h. Microbiological count was expressed as the log_{10} of colony-forming units per gram of patty (log CFU/g of minced beef patties).

2.7.4. Colour changes in minced beef patties

Colour changes in the patties during storage were monitored with a tristimulus colorimeter (model DP-400 with chroma meter model CR-400, Konica Minolta Sensing, Inc., Osaka, Japan). Colour was expressed with L* (100 = white, 0 = black), a* (positive = redness,
negative = greenness), and b* (positive = yellowness, negative = blueness) values. A standard white plate with reflectance values of \( L^* = 93.68, a^* = -0.69, b^* = -0.88 \), was used as reference. Colour readings were measured on five randomly chosen spots on the minced beef patties at ambient temperature and were utilized as an estimate of meat discoloration.

### 2.7.5. Sensory Evaluation

The samples were presented in a perfectly homogeneous way, i.e. identical conditions of conservation, preparation and presentation. The samples were put in white, opaque containers and presented in an anonymous way with a simple coding of three numbers. The meat samples were evaluated for texture, colour and odour. The mean value of these sensory properties was evaluated as overall acceptability. Hedonic evaluation was done by an untrained panel consisting of 34 subjects (12 males and 22 females) from the students and the staff members of the National School of Engineer (Sfax, Tunisia). Their ages ranged from 23 to 50 years. The panelists were asked to evaluate change of the meat after 9 days of storage with or without GSP and BHA. The samples were evaluated based on a five point hedonic scale, where one represented “disliked extremely” and five represented “liked extremely”.

### 2.8. Statistical analysis

All analytical determinations were replicated in triplicate. Values of each parameter are expressed as the mean ± standard deviation (x± SD). Duncan’s multiple range tests provided mean comparisons with the level of statistical significance set at \( P < 0.05 \). Statistical analyses were performed using SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA) using Duncan test performed after analysis of variance (ANOVA).
3. Results and discussion

3.1. Chemical composition of garlic straw

The chemical compositions of garlic straw (GS) is shown in Table 1. The moisture content of the GS was about 13.18 ± 0.45%. The protein content of the sample was 4.38 ± 0.21%. The lipid content (2.66 ± 0.13%) of the GS was higher than lipid content of 0.68% in garlic as reported by Nwinuka et al. (2005). The ethanol extractives and ash in GS were 1.2 ± 0.06% and 10.08 ± 0.3%, respectively. The higher content of extractives and ash in straw is may be caused by the higher content of epidermal cell, which is mainly composed by suberized cells and silica cells. The ash content was higher than the ash content of 4.06% in garlic and of 9% in Alfalfa (Alvo et al., 1996; Romano and Zhang, 2011). The summary of the results of mineral composition of GS is presented in Table 2. The result showed that GS is a very good source of minerals. From the seven mineral elements investigated, calcium had the highest concentration (246.15 mg/100g). GS contained high concentration of phosphorus (382.6 g/100g). The results are in agreement with previously published data for other by-product/co-products from the food and agro industries, as onion residue (Romano and Zhang, 2011). GH also contains noticeable fractions of lignin (6.32% ±0.36%). Similar content of lignin where found in other lignocellulosic materials such as coconut husk (3.54%) (Adyey, 2010).

The total dietary fibre (TDF) content, which consists of cellulose and hemicellulose, was 20.1 ± 1.7% of the garlic straw. It was relatively higher than crude fibre content of 10% in onion residual (Romano and Zhang, 2011). In fact, fibre-rich by-products, rich in dietary fibre and bioactive compounds, are a prize to food processors, especially since consumers prefer natural supplements, fearing that synthetic ingredients may be the source of toxicity. They possess many beneficial nutritive and protective effects (Elleuch et al., 2010). Besides, the carbohydrate content of GS was 56.55%. It was higher than carbohydrate content of 32%...
in corn (Buenaventura et al., 1986). Garlic straw was particularly of interest due to its recognized potential as a source of biomass such as polysaccharide; it is an environmentally friendly and cost-effective alternative.

3.2. Extraction of garlic straw polysaccharide GSP

The extraction time, temperature and ratio of water to raw material have an impact on the yield of GSP (Fig. 1). As depicted in Fig. 1A, the effects of temperature on extraction yield of GSP were investigated. Then, the extraction process was carried out using different extraction temperature of 60, 70, 80, 90 and 100°C, respectively, while the other two extracting parameters were set as follows: extracting time 120 min, extracting ratio of water to raw material 30 mL/g. Fig. 1A shows that the extraction yield increased as the extraction temperature ascended from 60 to 90°C, and the maximum yield of polysaccharide (15% ± 0.74) was observed when the extraction temperature was 90°C, after this point, the extraction yield of polysaccharide started to decrease and no longer increased when the extraction time exceeded 90°C (Fig. 1A). This tendency was in agreement with other reports in extracting polysaccharides (Vinogradov et al., 2003). These results indicated that, the temperature has enhanced the GSP extraction leaving particles into the water to a certain level, followed by their possible loss due to decomposition at a higher temperature (Guo et al., 2010). Extraction time was another factor that would influence the extraction efficiency. It has been reported that a long extraction time favored the production of polysaccharides (Liu et al., 2006). On the other hand, excessive lengthening of extraction time may induce the change of polysaccharides molecule structure. The yield of GSP affected by different extraction time is seen in Fig. 1B, when other parameters (extraction temperature and ratio of water to raw material) were fixed at 90°C and 30 mL/g. The extraction time displayed a positive effect on the yield of polysaccharides when the time ranged from 90 to 120 min, and then the yield
decreased with increasing the extraction time. The extraction yield of GSP reached a maximal value of (15% ± 0.75) at 120 min, and no longer changed as the extraction time prolonged, as seen in Fig. 1B. Ratios of water to raw material were set at 10, 20, 30 and 40 mL/g in order to investigate the effect of different extracting ratio of water to raw material on the yield of GSP (Fig. 1C). The extraction yield of GSP firstly increased with the ratio of water to materials and then decreased and the highest value was obtained with the ratio 20 mL/g. This is probably due to the increase in the driving force for the mass transfer of polysaccharides (Bendahou et al., 2007). The optimum extraction conditions were as follows: extraction time was 120 min, extraction temperature at 90°C and the ratio of water to raw material was 20 g/mL. Under these conditions, the yield was 20% ± 1.76.

3.3. Chemical composition

The chemical composition was determined in GSP. Total sugar (72.63% ± 1.78) was the most abundant element in GSP, followed by reducing sugar (10.36% ± 0.18). GSP has small amount of soluble protein (0.2% ± 0.01) and total phenol (0.0332 ± 0.24 g GAEs/ 100g).

The UV spectrum of polysaccharide sample was shown in Fig. 2. The GSP sample was clearly emerged a stronger absorption peak at 200–220 nm. This showed that the sample might contain unsaturated carbonyl, carboxyl, etc. There was no absorption at 260 and 280 nm, indicating that the polysaccharides contained trace of protein or polypeptide (Lei, 2010).

Results from phenol–sulfuric acid assay showed that GSP contained significant amount of carbohydrate (72.63% ± 1.78). GSP total sugar content was higher than the one from tea (59.48%) (Wang et al., 2013). However, it is lower than crude polysaccharide from finger citron (81.32%) and from Hyriopsis cumingii (76.42%) (Qiao et al., 2009; Wu et al., 2013).

The monosaccharide composition of GSP was analyzed by HPLC (Fig. 3). Compared with the monosaccharide standards, GSP was mainly composed of glucose (71.72%),
galactose (9.87%), mannose (6.37%) and xylose (4.78%). According to the results of HPLC we come to conclusion that glucose is the component of main-chain structure of GSP, and galactose, mannose and xylose may be in the position of branched structure of GSP.

In order to further characterize GSP and to identify its structure, FT-IR analysis was performed. The infrared spectrum of this polysaccharide is given in Fig. 4A. Two characteristic absorptions of polysaccharides, a strong absorption band of about 3100–3700 cm\(^{-1}\) for O–H stretching vibrations and a small absorption peak of about 2800–3000 cm\(^{-1}\) for C–H stretching vibrations, were observed. The band towards 1720 indicated the trace of uronic acids (Chen et al., 2008). Absorptions at 1142 and 1099 cm\(^{-1}\) are both assigned to the coupling of C-O, C-C, and O-H bond stretching, bending, and asymmetric stretching of the C-O-C glycosidic bridge (Aguirre et al., 2009). Absorbance at 1014 cm\(^{-1}\) is assigned to the vibration of C-O-H deformation, and absorbance at 957 cm\(^{-1}\) is assigned to C-H bending (Sebastian et al., 2009). These results indicated that GSP possesses typical absorption peak of polysaccharides.

The \(^{13}\)C NMR spectrum of GSP (Fig. 4B) showed four signals. The anomeric carbon signals of various sugars were tentatively assigned by comparison with the data reported in the literature (Serrero et al., 2010). Signal at 103.4 is assigned to C-1 in polysaccharides that are relatively well ordered. Additionally signals were observed at 82.53 ppm, 71.92 and 70.2 ppm. The spectrum showed signal at 104.2 which may be assigned to glucose. The carbon resonances in GSP in the range 82.53–70.2 ppm were due to the carbons C2–C6 of various sugar moieties.

3.4. Antioxidant activity

3.4.1. DPPH free radical scavenging activity
The model of scavenging stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of natural compounds (Bersuder et al., 1998). In the DPPH test, the antioxidants were able to reduce the stable DPPH radical to the yellow-coloured diphenylpricrylhydrazine. The effect of antioxidants on DPPH radical scavenging was conceived to be due to their hydrogen-donating ability. At concentrations of 0.1–2 mg/mL, the scavenging abilities of GSP on DPPH radicals were in the range of 10.17–86.9% (Fig. 5A). The IC\textsubscript{50} values of GSP and BHA were 740 µg/mL and 63 µg/mL, respectively. The results indicated that GSP had a noticeable effect on scavenging DPPH free radicals, especially at high concentrations. However, the radical-scavenging activity of GSP was lower than that of BHA used in this study. In comparison, the DPPH IC\textsubscript{50} value of the GSP was higher than the IC\textsubscript{50} value presented in hot water extracted polysaccharide (HWP) from fruiting bodies of wild \textit{S. commune} (0.6 mg/mL) (Klaus et al., 2011), but it was similar than the polysaccharide extracted from \textit{Inonotus obliquus sclerotia} (Du et al., 2013). The GSP expressed significantly higher scavenging capacity than did those obtained from different substrates based on selected agricultural wastes composed of asparagus straw, maize straw, cottonseed hull, bean straw, cotton straw, corncob, soybean cake and gypsum (IC\textsubscript{50} values were between 2.19 - 3.50 mg/mL) (Wang et al., 2013). The possible mechanism by which GSP acts as an antioxidant may be attributed to their electron donation power to the free radicals, thereby terminating the radical chain reaction (Lai et al., 2010).

3.4.2. Antioxidant activity by the conjugated diene method

The conjugated diene method is widely used for monitoring lipid oxidation \textit{in vitro}. In fact, the oxidation of linoleic was measured as an increase in 234 nm absorbance due to conjugated diene formation (Lingnert et al., 1979). As shown in Fig. 5B, GSP exhibited a strong inhibitory effect on lipid peroxidation and the inhibitory effect was concentration
dependent. The inhibition ratios of GSP ranged from 10 to 81.62% when the concentrations varied from 0.1 mg/mL to 2 mg/mL, which was lower than BHA. The IC_{50} values of GSP and BHA were 480 µg/mL and 25 µg/mL, respectively. The inhibition percentage of GSP reached 65.45% at 1 mg/mL, which was higher than the polysaccharides of 10 L. edodes strains (14.56% to 58.27% at 1.5 mg/mL) (Lo et al., 2011). Our data suggest that GSP has a significant effect on inhibiting lipid peroxidation.

3.4.3. Activity of reducing power

The reducing power serves as a significant potential antioxidant index. The presence of reductant in the reaction can be monitored by the formation of Perl’s Prussian blue at 700 nm (Yildirim et al., 2001). As can be seen in Fig. 5C, the reducing capacity of GSP ascended with increasing concentration, which indicated that GSP was electron donors and could react with free radicals to convert them into more stable products (Ma et al., 2012). The higher the absorbance values were, the stronger reducing power was. However, the reducing power of GSP was much weaker compared with that of BHA. The RP_{0.5AU} values of BHA and GSP were 0.727 mg/mL and 3.125 mg/mL, respectively. The absorbance value of GSP is higher than the absorbance values present in polysaccharides extracted from Lilium davidii var. unicolor Salisb and Ganoderma (Zhao et al., 2013; Kan et al., 2015). Shimada et al. (1992) suggested that reductone-associated and hydroxide groups of polysaccharides can act as electron donors and can react with free radicals to convert them to more stable products and thereby terminate radical chain reactions.

3.5. Antimicrobial activity

The antimicrobial activity of GSP was evaluated against a panel of 7 bacteria and 2 fungal strains using agar diffusion method. The inhibitory effect of GSP on the growth of test
microorganism is presented in Table 3. The sensitivity to GSP was found to differ among the test microorganisms. From the results it was observed that E. coli (Gram-negative) was found to be very resistant and M. luteus (Gram-positive) was found to be more sensitive among the organisms applied in the experiment. As the concentration increasing, the antimicrobial abilities of GSP improved significantly. GSP exhibited antimicrobial activity against M. luteus, E. faecalis, S. aureus and B. turengensis with inhibition zones of 11.5, 10.5, 10 and 8.5 mm, respectively (Table 3). Though the exact mode of action of the polysaccharide on bacteria was still not clear, it was proposed that the polysaccharide disrupted the cell wall and cytoplasmic membrane, leading to the dissolution of the protein and leakage of essential molecules, resulting in cell death. Moreover, DNA might be decomposed into small pieces after the polysaccharide entered the cell. Therefore, there might be multiple possible targets of the polysaccharide against bacteria, including cell wall, cytoplasmic membrane and DNA, which might result in bacteria being unable to develop resistance (He et al., 2010). Thus, the microbial inhibitory effect of GSP was more effective on Gram-positive than on Gram-negative bacteria. This is in agreement with results obtained in others studies (Du et al., 2011). This result can be due to the differences in the cell envelope composition between Gram-positive and Gram-negative bacteria, which affect permeability and susceptibility of these microorganisms to different compounds (Sikkema et al., 1995). Water used as negative control had no inhibitory effects on the seven bacteria tested. However, ciprofloxacin used as positive control showed antibacterial activity more important than GSP.

The GSP presented similar antibacterial capacity to the one isolated from Cyclocarya paliurus (Batal.) Iljinskaja. Previous reports showed that polysaccharides from L. japonicum possess significant broad-spectrum anti-microorganism activity (Li et al., 2006). Significant antibacterial activity was also shown for polysaccharide isolated from the broth of Streptomyces virginia H03 (He et al., 2010).
3.6. Application of GSP as a natural preservative in chilled minced beef meat

Results of the present study have shown that GSP exhibited exciting antioxidant and antimicrobial activities; it was applied as a natural preservative in beef patties during refrigerated storage. Its effects on lipid oxidation, meat colour stability, microbial growth and sensory evaluation were then investigated.

3.6.1. Measurement of lipid oxidation

Lipid oxidation was analyzed in minced beef patties subjected to chilled storage using the TBARS distillation method (Fig. 6A). The TBARS method has been widely used to determine the degree of lipid oxidation. TBARS is produced through second stage auto-oxidation during which peroxides are oxidized to aldehydes and ketones (e.g., MDA). Significant changes in TBARS occurred over the 9-day sampling period. The respective treatments influenced TBARS values and also impacted the change over time, as evidenced by interactions between treatments and time. As expected, TBARS values increased significantly in the negative control patties at the onset of lipid oxidative reactions. Among the patties, the samples without antioxidants had the highest TBARS values by the end of storage (slope: 0.89). The results show that the TBARS values of all beef treated with 2% and 4% GSP increased from an initial 0.452 and 0.4 mg MDA per Kg patties to 2.04 and 1.3 mg MDA per Kg patties, respectively (slope: 0.514-0.3). Nonetheless, the TBARS value of the PC group with BHA (0.1%) was much lower than those of the control counterparts (slope: 0.121).

To our best knowledge and literary survey, there is no report available describing the effect of polysaccharides from garlic straw as a minced meat beef preservative. Generally, a higher intake of antioxidant compounds results in a deposition of these molecules in muscle with a consequent improvement of the overall muscle antioxidant capacity and stability to
oxidative deterioration (Descalzo and Sancho, 2008). The protective effect of the diets
containing GSP against lipid peroxidation found in the present study might be explained
considering the presence of antioxidant compounds in this by-product. The results of the
present study show that adding GSP protects beef patties against lipid oxidation. Lipid
peroxidation reducing effects of certain polysaccharides are described in the literature
(Albertini et al., 2000). The mechanism of these lipid protecting effects seems to be the
chelation of transition metal ions. Therefore, GSP may serve as possible functional foods in
diets to help the human body reduce oxidative damage.

3.6.2. Effects of GSP on total viable count of beef patties meat

The microbiological changes of the minced beef patties during chilled storage at 4°C are
shown in Table 3. Results show that the microbial population increased with time during the
storage period and reached the highest values at the end of chill period. Among the
experimental groups, the NC group showed the most rapid increase in the number of
microorganisms, followed by samples treated with GSP and PC. According to the legislation
(Regulation EC, 2005) the limit established for bacterial counts is $10^6$ CFU/g, but the spoilage
can be detected, mainly due to odour, in most foods with more than 6 log CFU/g (Dainty and
Mackey, 1992). Therefore, the shelf-life of samples from control group would be 3 days,
while for samples from GSP and BHA groups this shelf-life could be extended over 3 and 6
days of storage, respectively. However, on day 9 of storage there were no significant
differences in log values of total viable count among NC and GSP ($p > 0.05$) (Table 4). The
observed inactivity of polysaccharide against microorganisms can be explicated by the
moderate antimicrobial activity of the polysaccharide. Similarly, no inhibitory effect of
chitosan on microbial growth in meat samples has been documented by other authors (Park et
al., 2010).
3.6.3. Colour deterioration during refrigerated storage of beef patties

The changes in L*, a*, b* (lightness, redness, and yellowness) were analyzed during storage. In fact, a* value is the most important colour parameter in evaluating meat oxidation as a decrease in redness makes the meat product unacceptable to consumers (Renerre, 2000). The lightness (L*) significantly decreased during the storage period in all treatments and the parameter b* did not show significant modification in this stage (P ≥ 0.05) (data not shown). All types of beef patties suffered a considerable decrease in redness (a*) under chilled storage conditions, illustrating dark discoloration. In our study, the negative control sample had relatively lower a* values (P ≤ 0.05) compared with the other antioxidant treatments examined. The patties with BHA had a higher value (P ≤ 0.05) than the other samples at 9 days (Fig. 6B). Overall treatment means indicated a significant (P ≤ 0.05) difference in redness among patties. The addition of GSP (2% and 4%, w/w) had a significantly negative effect on the colour of the beef patties (day 9). The a* values showed that the GSP extracts had better colour than the negative control (P ≤ 0.05) (Fig. 6B). Such result suggested that GSP can be used in minced beef such as garlic (*Allium sativum* L.) aerial parts, lemon grass (*Cymbopogon citratus*) leaves, licorice (*Glycyrrhiza glabra*) root and pomegranate (*P. granatum* L.) peel extract (Tayel and El-Tras, 2012).

We infer that the antioxidant compounds in the polysaccharide retarded metmyoglobin formation when incorporated at the level of 2% (w/w). The bright red colour of fresh meat cuts is caused by the presence of oxymyoglobin, an oxygenated myoglobin (Leward, 1991). These meat products are exposed to high levels of oxygen during chilled storage, in which oxymyoglobin is transformed to brown-colored metmyoglobin. This discoloration is mainly defined by the loss of redness, which is related to the accumulation of metmyoglobin. Primary lipid oxidation products such as hydroperoxides and other free radicals are known to oxidize the ferrous ion (Fe$^{2+}$) from oxymyoglobin into the ferric form (Fe$^{3+}$) present in metmyoglobin.
Recent studies have highlighted that secondary lipid oxidation products (e.g., unsaturated aldehydes) can accelerate the formation of metmyoglobin in meat products (Faustman et al., 2010).

3.6.4. Sensory characteristics evaluation

Preserved meat quality assessment by sensory evaluation is largely based on personal judgment and subjective qualitative evaluation; the results cannot be absolute but reflect the influences of consumer preferences. The results of the sensory evaluation are presented in Table 5. In meats non-treated with GSP, after 9 days, due to oxidative changes the panelists mostly disliked the colour and odour. As for the beef meat treated with 2 and 4% GSP, changes in colour and odour was recorded by panelists, but meats containing GSP were significantly different from control sample (P < 0.05) and was more acceptable (P < 0.05). No unusual or uncharacteristic flavors such as might be attributed to the garlic were detected by the panelists. There were no differences between the GSP treatment levels. Sensory evaluation for beef flavor revealed that the BHA treatments tended to score significantly higher than the control and GSP treatments. Meat in which oxidation reactions have occurred is brown in colour; the flavor is rancid and stale and such meat would likely be rejected by the consumer (Greene and Price, 1975). Changes in meat colour are due to oxidation of red oxymyoglobin to metmyoglobin (MMG), which gives rise to an unattractive brown colour (Velasco and Williams, 2011).

Conclusion

This study has revealed that garlic straw is a rich source of many important nutrients. It has relatively high levels of carbohydrate and some minerals. Hot water technique was used for the extraction of polysaccharide from garlic straw (GSP) with a relatively high yield of 20 ± 1.76% under the optimal extraction condition (Temperature of 90°C, extraction time of 2h
and solvent to raw material of 20 mL/g). Mannose, galactose, glucose and xylose were detected in GSP. Moreover, GSP showed a relatively important DPPH scavenging activity, high reducing power and inhibited the peroxidation of linoleic acid. Besides, this study showed that the incorporation of GSP in minced beef patties could effectively reduce lipid oxidation, improve sensory attributes and extend its shelf-life during refrigerated storage. In conclusion, GSP could be used in many biotechnological fields as natural preservative ingredient of food.

Acknowledgement

This work was funded by “Ministry of Higher Education, Scientific Research and Technology-Tunisia”.

References


**Figure captions**

Fig. 1: Effect of different (A) extraction temperatures, (B) extraction times and (C) ratios of water to raw material on extraction yield of garlic straw polysaccharide. Means ± standard deviations values of three replicates.

Fig. 2: UV–vis absorption spectra of garlic straw polysaccharide.

Fig. 3: High Performance Liquid Chromatography of garlic straw polysaccharide.

Fig. 4: Structural characterization of garlic straw polysaccharide: (A): $^{13}$C NMR spectra of garlic straw polysaccharide; (B): FT-IR spectroscopy of garlic straw polysaccharide.

Fig. 5: Antioxidant activity of garlic straw polysaccharide compared to a synthetic antioxidant BHA by (A) free radical DPPH scavenging activity, (B) conjugated diene method and (C) reducing power assay. BHA (■) was used as positive control and (○) GSP. Means ± standard deviations values of three replicates.

Fig. 6: Application of garlic straw polysaccharide on beef patties: (A): TBARS values (MDA mg/Kg meat) of beef patties treated with 2% and 4% of polysaccharide during refrigerated storage; (B): Changes in instrumental colour ($a^*$ value, redness) of beef patties treated with 2% and 4% of polysaccharide during refrigerated storage. NC: Negative control (non-treated group), GSP: 2% and 4% of garlic straw polysaccharide, PC: Positive control (BHA-treated group). Means ± standard deviations values of three replicates.

Fig. 7: Appearance of meat after 9 days of storage at 4°C for the control sample (A) and the sample treated with GSP (B).
Table

**Table 1:** Chemical composition of garlic straw (g/100g dry matter)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Content(^a) (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>13.18 ± 0.45</td>
</tr>
<tr>
<td>Protein</td>
<td>4.38 ± 0.21</td>
</tr>
<tr>
<td>lipid</td>
<td>2.66 ± 0.13</td>
</tr>
<tr>
<td>Dietary fiber (DF)</td>
<td>24.10 ± 1.70</td>
</tr>
<tr>
<td>Insoluble DF</td>
<td>20.50 ± 1.30</td>
</tr>
<tr>
<td>Soluble DF</td>
<td>3.60 ± 0.40</td>
</tr>
<tr>
<td>Ethanol extractive</td>
<td>1.20 ± 0.06</td>
</tr>
<tr>
<td>Lignin</td>
<td>6.32 ± 0.36</td>
</tr>
<tr>
<td>Ash</td>
<td>10.08 ± 0.30</td>
</tr>
</tbody>
</table>

\(^a\) Expressed on a dry basis with the exception of moisture data. Data are means ± standard deviations values of three replicates.
Table 2: Mineral composition of garlic straw (mg/100 g dry matter)

<table>
<thead>
<tr>
<th>Minerals elements</th>
<th>Content a (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(^{2+})</td>
<td>292.30 ± 15.26</td>
</tr>
<tr>
<td>K(^+)</td>
<td>206.76 ± 10.30</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>198.35 ± 7.60</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>53.26 ± 0.150</td>
</tr>
<tr>
<td>Fe(^{2+})</td>
<td>4.60 ± 0.021</td>
</tr>
<tr>
<td>Mn(^{2+})</td>
<td>0.474 ± 0.013</td>
</tr>
<tr>
<td>Zn(^{2+})</td>
<td>0.44 ± 0.0016</td>
</tr>
<tr>
<td>Cu(^{2+})</td>
<td>&lt;0.088 ± 0.001</td>
</tr>
</tbody>
</table>

a Expressed on a dry basis with the exception of moisture data. Data are means ± standard deviations values of three replicates.
**Table 3:** Diameters (mm) of inhibition zones determined after 24 h incubation at 37°C for bacteria and 72 h incubation at 30°C for fungi.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Garlic straw polysaccharide (mg/mL)</th>
<th>Control</th>
<th>Ciprofloxacin</th>
<th>Amphotericin B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. turengensis</td>
<td>ND</td>
<td>7.5 ± 0.5</td>
<td>ND</td>
<td>23.5 ± 0.5</td>
</tr>
<tr>
<td>S. aureus</td>
<td>7 ± 0.5</td>
<td>10 ± 0.5</td>
<td>ND</td>
<td>26 ± 0.5</td>
</tr>
<tr>
<td>M. luteus</td>
<td>9.5 ± 0.5</td>
<td>11.5 ± 0.5</td>
<td>ND</td>
<td>32 ± 0.5</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>ND</td>
<td>7 ± 0.5</td>
<td>ND</td>
<td>27 ± 0.5</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>6.50 ± 0.5</td>
<td>10.5 ± 0.5</td>
<td>ND</td>
<td>19 ± 0.5</td>
</tr>
<tr>
<td>E. coli</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>6.75 ± 0.5</td>
<td>8.5 ± 0.5</td>
<td>ND</td>
<td>19 ± 0.5</td>
</tr>
<tr>
<td>A. niger</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>R. oryzae</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
</tr>
</tbody>
</table>

Note. ND: not detected; Amphotericin B and Ciprofloxacin were used as positive control.

Values represent averages ± standard deviations for triplicate experiments.
Table 4: Microbial changes in beef patties treated with 2% and 4% (w/w) of garlic straw polysaccharide (GSP) during storage at 4°C. Unit: log CFU/g.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage time (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>NC</td>
<td>4.35 ± 0.04&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% GSP</td>
<td>4.55 ± 0.24&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>4% GSP</td>
<td>4.77 ± 0.20&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>PC</td>
<td>4.30 ± 0.02&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means not sharing the same letters (a–c) within a column are significantly different (p < 0.05).

Means not sharing the same letters (A–E) within a row are significantly different (p < 0.05).

Table 5: Influence of minced meat preservation treated with 2% and 4% (w/w) of garlic straw polysaccharide (GSP) and BHA on the sensory attributes after storage for 9 days at 4°C.

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>Treatment with GSP (%)</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2%</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Odour</td>
<td>1.51 ± 0.17</td>
<td>2.86 ± 0.27</td>
<td>3.57 ± 0.50</td>
</tr>
<tr>
<td>Colour</td>
<td>1.58 ± 0.19</td>
<td>2.35 ± 0.37</td>
<td>3.34 ± 0.43</td>
</tr>
<tr>
<td>Texture</td>
<td>1.48 ± 0.38</td>
<td>2.65 ± 0.58</td>
<td>3.49 ± 0.41</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>1.84 ± 0.27</td>
<td>2.55 ± 0.22</td>
<td>3.61 ± 0.51</td>
</tr>
</tbody>
</table>

Means followed by the same letter within a row are non-significantly different (P < 0.05).

NC: Negative control (non-treated group). GSP: garlic straw polysaccharide; PC: Positive control (BHA-treated group).
Fig. 1:
Fig. 2:
Fig. 3:
Fig. 5:
Fig. 6:
Fig. 7: Appearance of meat after 9 days of storage at 4°C for the control sample (A) and the sample treated with 2% GSP (B).