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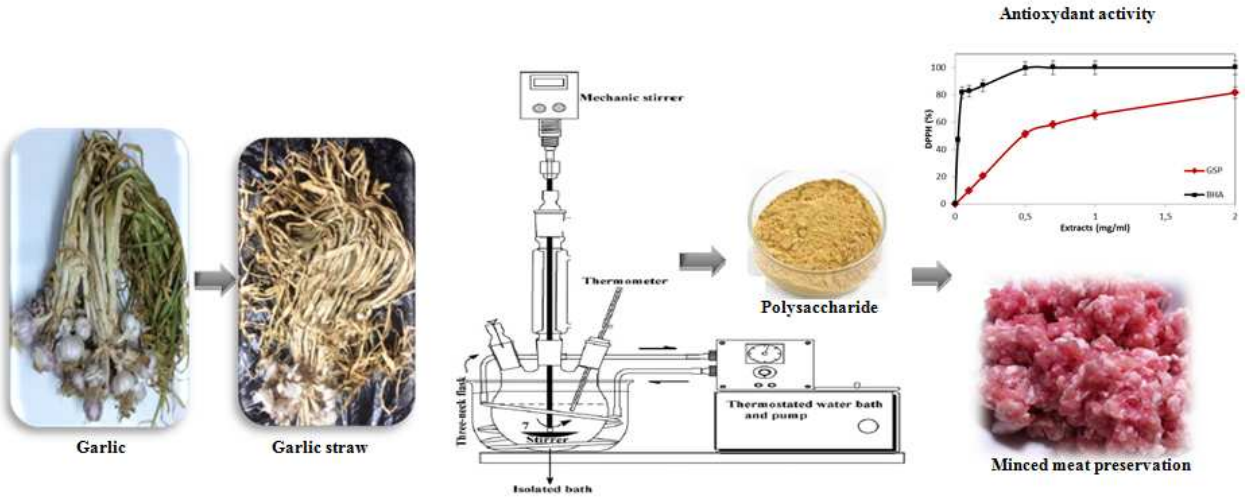
Garlic
Garlic straw
Mechanic stirrer
Thermometer
Three-neck flask
Stirrer
Thermostated water bath and pump
Isolated bath
Polysaccharide
Antioxydant activity
DPPH (%)
Extracts (mg/ml)
GSP
BHA
Minced meat preservation

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

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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 ($p \leq 0.05$) 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 ZEEnit700 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_2SO_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^{-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 ^{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:

$$\text{AOA (\%)} = \left[\frac{\Delta A_{234} \text{ of control} - \Delta A_{234} \text{ of sample}}{\Delta A_{234} \text{ of control}}\right] * 100$$

The blank comprises DI water and the control, which consisted of DI water and reagent solution without the solvent extract. IC_{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 $FeCl_3$ (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 ($RP_{0.5AU}$) 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 turengensis, 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 10^6 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.

245 *2.7.2. Evaluation of lipid oxidation*

246 Lipid oxidation was evaluated using the thiobarbituric acid-reactive substance (TBARS)
247 as previously performed (Witte et al., 1970). Four samples taken at the 1st, 3rd, 6th and 9th
248 day of the chilled storage were used. Briefly, 20 g from each prepared formula were
249 homogenized with 50 mL reagent solution containing 20% TCA in2 M phosphoric acid, at
250 4°C. The mixture was then adjusted to 100 mL distilled water, shaken and filtered using
251 Büchner funnel through Whatman paper. 5 mL of the filtrate was then transferred into a glass
252 test tube and an equal volume of 2-thiobarbituric acid (5 mM), freshly prepared, was added at
253 4°C. The test tube was then well shaken and incubated at room temperature for 17 h. The
254 absorbance of the mixture was measured at 530 nm (A530) and the results were expressed, as
255 mg malondialdehyde (MDA)/Kg meat as follows: TBA (mg (MDA)/Kg meat) = A530 × 5.2.
256
257 *2.7.3. Microbial analysis*
258 Microbial count was carried out at days 0, 1st, 3rd, 6th and 9th of storage at 4°C
259 according to the following procedure. 225 mL sterilized peptone solution (25.5 g/L) was
260 added to the 25 g minced meat, with and without GSP, then homogenized. Decimal dilutions
261 up to 10^{-8} prepared from the initial concentration (100 mg/mL) and aliquots of the appropriate
262 dilutions were placed on PCA media. Total viable count (TVC) were determined using plate
263 count agar (PCA) after incubation at 30°C for 72 h. Microbiological count was expressed as
264 the $\log_{10}$ of colony-forming units per gram of patty (log CFU/g of minced beef patties).
265
266 *2.7.4. Colour changes in minced beef patties*
267 Colour changes in the patties during storage were monitored with a tristimulus
268 colorimeter (model DP-400 with chroma meter model CR-400, Konica Minolta Sensing, Inc.,
269 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 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%) (Adeyi, 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

345 decreased with increasing the extraction time. The extraction yield of GSP reached a maximal
346 value of (15% ± 0.75) at 120 min, and no longer changed as the extraction time prolonged, as
347 seen in Fig. 1B. Ratios of water to raw material were set at 10, 20, 30 and 40 mL/g in order to
348 investigate the effect of different extracting ratio of water to raw material on the yield of GSP
349 (Fig. 1C). The extraction yield of GSP firstly increased with the ratio of water to materials
350 and then decreased and the highest value was obtained with the ratio 20 mL/g. This is
351 probably due to the increase in the driving force for the mass transfer of polysaccharides
352 (Bendahou et al., 2007). The optimum extraction conditions were as follows: extraction time
353 was 120 min, extraction temperature at 90°C and the ratio of water to raw material was 20
354 g/mL. Under these conditions, the yield was 20% ± 1.76.
355
356 3.3. Chemical composition
357 The chemical composition was determined in GSP. Total sugar (72.63% ± 1.78) was the
358 most abundant element in GSP, followed by reducing sugar (10.36% ± 0.18). GSP has small
359 amount of soluble protein (0.2% ± 0.01) and total phenol (0.0332 ± 0.24 g GAEs/ 100g).
360 The UV spectrum of polysaccharide sample was shown in Fig. 2. The GSP sample was
361 clearly emerged a stronger absorption peak at 200–220 nm. This showed that the sample
362 might contain unsaturated carbonyl, carboxyl, etc. There was no absorption at 260 and 280
363 nm, indicating that the polysaccharides contained trace of protein or polypeptide (Lei, 2010).
364 Results from phenol–sulfuric acid assay showed that GSP contained significant amount
365 of carbohydrate (72.63% ± 1.78). GSP total sugar content was higher than the one from tea
366 (59.48%) (Wang et al., 2013). However, it is lower than crude polysaccharide from finger
367 citron (81.32%) and from *Hyriopsis cumingii* (76.42%) (Qiao et al., 2009; Wu et al., 2013).
368 The monosaccharide composition of GSP was analyzed by HPLC (Fig. 3). Compared
369 with the monosaccharide standards, GSP was mainly composed of glucose (71.72%),

370 galactose (9.87%), mannose (6.37%) and xylose (4.78%). According to the results of HPLC
371 we come to conclusion that glucose is the component of main-chain structure of GSP, and
372 galactose, mannose and xylose may be in the position of branched structure of GSP.

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

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

391

392 3.4. Antioxidant activity

393 *3.4.1. DPPH free radical scavenging activity*

394 The model of scavenging stable DPPH radical is a widely used method to evaluate the
395 free radical scavenging ability of natural compounds (Bersuder et al., 1998). In the DPPH test,
396 the antioxidants were able to reduce the stable DPPH radical to the yellow-coloured
397 diphenylpricrylhydrazine. The effect of antioxidants on DPPH radical scavenging was
398 conceived to be due to their hydrogen-donating ability. At concentrations of 0.1–2 mg/mL,
399 the scavenging abilities of GSP on DPPH radicals were in the range of 10.17–86.9% (Fig.
400 5A). The IC_{50} values of GSP and BHA were 740 μg/mL and 63 μg/mL, respectively.The
401 results indicated that GSP had a noticeable effect on scavenging DPPH free radicals,
402 especially at high concentrations. However, the radical-scavenging activity of GSP was lower
403 than that of BHA used in this study. In comparison, the DPPH IC_{50} value of the GSP was
404 higher than the IC_{50} value presented in hot water extracted polysaccharide (HWP) from
405 fruiting bodies of wild *S. commune* (0.6 mg/mL) (Klaus et al., 2011), but it was similar than
406 the polysaccharide extracted from *Inonotus obliquus* s*clerotia* (Du et al., 2013). The GSP
407 expressed significantly higher scavenging capacity than did those obtained from different
408 substrates based on selected agricultural wastes composed of asparagus straw, maize straw,
409 cottonseed hull, bean straw, cotton straw, corncob, soybean cake and gypsum (IC_{50} values
410 were between 2.19 - 3.50 mg/mL) (Wang et al., 2013). The possible mechanism by which
411 GSP acts as an antioxidant may be attributed to their electron donation power to the free
412 radicals, there by terminating the radical chain reaction (Lai et al., 2010).
413
414 *3.4.2. Antioxidant activity by the conjugated diene method*
415 The conjugated diene method is widely used for monitoring lipid oxidation *in vitro*. In
416 fact, the oxidation of linoleic was measured as an increase in 234 nm absorbance due to
417 conjugated diene formation (Lingnert et al., 1979). As shown in Fig. 5B, GSP exhibited a
418 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

444 microorganism is presented in Table 3. The sensitivity to GSP was found to differ among the
445 test microorganisms. From the results it was observed that *E. coli* (Gram-negative) was found
446 to be very resistant and *M. luteus* (Gram-positive) was found to be more sensitive among the
447 organisms applied in the experiment. As the concentration increasing, the antimicrobial
448 abilities of GSP improved significantly. GSP exhibited antimicrobial activity against *M.*
449 *luteus, E. faecalis, S. aureus* and *B. turengensis* with inhibition zones of 11.5, 10.5, 10 and 8.5
450 mm, respectively (Table 3). Though the exact mode of action of the polysaccharide on
451 bacteria was still not clear, it was proposed that the polysaccharide disrupted the cell wall and
452 cytoplasmic membrane, leading to the dissolution of the protein and leakage of essential
453 molecules, resulting in cell death. Moreover, DNA might be decomposed into small pieces
454 after the polysaccharide entered the cell. Therefore, there might be multiple possible targets of
455 the polysaccharide against bacteria, including cell wall, cytoplasmic membrane and DNA,
456 which might result in bacteria being unable to develop resistance (He et al., 2010). Thus, the
457 microbial inhibitory effect of GSP was more effective on Gram-positive than on Gram-
458 negative bacteria. This is in agreement with results obtained in others studies (Du et al.,
459 2011). This result can be due to the differences in the cell envelope composition between
460 Gram-positive and Gram-negative bacteria, which affect permeability and susceptibility of
461 these microorganisms to different compounds (Sikkema et al., 1995). Water used as negative
462 control had no inhibitory effects on the seven bacteria tested. However, ciprofloxacin used as
463 positive control showed antibacterial activity more important than GSP.

464 The GSP presented similar antibacterial capacity to the one isolated from *Cyclocarya*
465 *paliurus* (Batal.) *Iljinskaja*. Previous reports showed that polysaccharides from L. *japonicum*
466 possess significant broad-spectrum anti-microorganism activity (Li et al., 2006). Significant
467 antibacterial activity was also shown for polysaccharide isolated from the broth of
468 *Streptomyces virginia* H03 (He et al., 2010).

469 3.6. Application of GSP as a natural preservative in chilled minced beef meat

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

474

475 *3.6.1. Measurement of lipid oxidation*

476 Lipid oxidation was analyzed in minced beef patties subjected to chilled storage using
477 the TBARS distillation method (Fig. 6A). The TBARS method has been widely used to
478 determine the degree of lipid oxidation. TBARS is produced through second stage auto-
479 oxidation during which peroxides are oxidized to aldehydes and ketones (e.g., MDA).
480 Significant changes in TBARS occurred over the 9-day sampling period. The respective
481 treatments influenced TBARS values and also impacted the change over time, as evidenced
482 by interactions between treatments and time. As expected, TBARS values increased
483 significantly in the negative control patties at the onset of lipid oxidative reactions. Among
484 the patties, the samples without antioxidants had the highest TBARS values by the end of
485 storage (slope: 0.89). The results show that the TBARS values of all beef treated with 2% and
486 4% GSP increased from an initial 0.452 and 0.4 mg MDA per Kg patties to 2.04 and 1.3 mg
487 MDA per Kg patties, respectively (slope: 0.514-0.3). Nonetheless, the TBARS value of the
488 PC group with BHA (0.1%) was much lower than those of the control counterparts (slope:
489 0.121).

490 To our best knowledge and literary survey, there is no report available describing the
491 effect of polysaccharides from garlic straw as a minced meat beef preservative. Generally, a
492 higher intake of antioxidant compounds results in a deposition of these molecules in muscle
493 with a consequent improvement of the overall muscle antioxidant capacity and stability to

494 oxidative deterioration (Descalzo and Sancho, 2008). The protective effect of the diets
495 containing GSP against lipid peroxidation found in the present study might be explained
496 considering the presence of antioxidant compounds in this by-product. The results of the
497 present study show that adding GSP protects beef patties against lipid oxidation. Lipid
498 peroxidation reducing effects of certain polysaccharides are described in the literature
499 (Albertini et al., 2000). The mechanism of these lipid protecting effects seems to be the
500 chelation of transition metal ions. Therefore, GSP may serve as possible functional foods in
501 diets to help the human body reduce oxidative damage.
502
503 *3.6.2. Effects of GSP on total viable count of beef patties meat*
504 The microbiological changes of the minced beef patties during chilled storage at 4°C are
505 shown in Table 3. Results show that the microbial population increased with time during the
506 storage period and reached the highest values at the end of chill period. Among the
507 experimental groups, the NC group showed the most rapid increase in the number of
508 microorganisms, followed by samples treated with GSP and PC. According to the legislation
509 (Regulation EC, 2005) the limit established for bacterial counts is 10^6 CFU/g, but the spoilage
510 can be detected, mainly due to odour, in most foods with more than 6 log CFU/g (Dainty and
511 Mackey, 1992). Therefore, the shelf-life of samples from control group would be 3 days,
512 while for samples from GSP and BHA groups this shelf-life could be extended over 3 and 6
513 days of storage, respectively. However, on day 9 of storage there were no significant
514 differences in log values of total viable count among NC and GSP ($p > 0.05$) (Table 4). The
515 observed inactivity of polysaccharide against microorganisms can be explicated by the
516 moderate antimicrobial activity of the polysaccharide. Similarly, no inhibitory effect of
517 chitosan on microbial growth in meat samples has been documented by other authors (Park et
518 al., 2010).

519 *3.6.3. Colour deterioration during refrigerated storage of beef patties*

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

536 We infer that the antioxidant compounds in the polysaccharide retarded metmyoglobin
537 formation when incorporated at the level of 2% (w/w). The bright red colour of fresh meat
538 cuts is caused by the presence of oxymyoglobin, an oxygenated myoglobin (Leward, 1991).
539 These meat products are exposed to high levels of oxygen during chilled storage, in which
540 oxymyoglobin is transformed to brown-colored metmyoglobin. This discoloration is mainly
541 defined by the loss of redness, which is related to the accumulation of metmyoglobin. Primary
542 lipid oxidation products such as hydroperoxides and other free radicals are known to oxidize
543 the ferrous ion (Fe^{2+}) from oxymyoglobin into the ferric form (Fe^{3+}) present in metmyoglobin.

544 Recent studies have highlighted that secondary lipid oxidation products (e.g., unsaturated
545 aldehydes) can accelerate the formation of metmyoglobin in meat products (Faustman et al.,
546 2010).

547 *3.6.4. Sensory characteristics evaluation*

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

563

564 **Conclusion**

565 This study has revealed that garlic straw is a rich source of many important nutrients. It
566 has relatively high levels of carbohydrate and some minerals. Hot water technique was used
567 for the extraction of polysaccharide from garlic straw (GSP) with a relatively high yield of 20
568 ± 1.76% under the optimal extraction condition (Temperature of 90°C, extraction time of 2h

569 and solvent to raw material of 20 mL/g). Mannose, galactose, glucose and xylose were
570 detected in GSP. Moreover, GSP showed a relatively important DPPH scavenging activity,
571 high reducing power and inhibited the peroxidation of linoleic acid. Besides, this study
572 showed that the incorporation of GSP in minced beef patties could effectively reduce lipid
573 oxidation, improve sensory attributes and extend its shelf-life during refrigerated storage. In
574 conclusion, GSP could be used in many biotechnological fields as natural preservative
575 ingredient of food.
576

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580

581 **References**

582 Adeyi, O. (2010). Proximate composition of some agricultural wastes in Nigeria and
583 their potential use in activated carbon production. *Journal of Applied Sciences and*
584 *Environmental Management,* 14, 55-58.

585 AFNOR (1986). Association Franc¸ aise de Normalisation. Céréales et produits
586 tcéréaliers. Détermination de la teneur en matières grasses totales. N.F.V. 03-713. 39–155.

587 Aguirre, M. J., Isaacs, M., Matsuhiro, B., Mendoza, L., & Zúñiga, E. A. (2009).
588 Characterization of a neutral polysaccharide with antioxidant capacity from red wine.
589 *Carbohydrate Research*, 344, 1095-1101.

590 Albertini, R., Passi, A., Abuja, P. M., & De Luca, G. (2000). The effect of
591 glycosaminoglycans and proteoglycans on lipid peroxidation. *International Journal of*
592 *Molecular Medicine,* 6, 129-136.

593 Alvo, P., Savoice, P., Tremblay, D., Emond, J. P. & Turcotte, G. (1996). A system
594 approach for evaluation of ethanol production based on forages. *Bioresource Technology,* 56:
595 61–68.
596 AOAC (1997). Official methods of Analysis. Assoc. of Analytical Chemists,
597 Washington, D.C. USA.
598 AOAC (1999). Methods of Analysis of Association of official Analytical chemists (16th
599 ed). Washington, D.C.1: 600-792.
600 Banerjee, S. K., & Maulik, S. K. (2002). Effect of garlic on cardiovascular disorders: a
601 review. *Nutrition Journal*, 1, 4.
602 Bendahou, A., Dufresne, A., Kaddami, H., & Habibi, Y. (2007). Isolation and structural
603 characterization of hemicelluloses from palm of *Phoenix dactylifera* L., *Carbohydrate*
604 *Polymers*, 68, 601–608.
605 Bersuder, P., Hole, M., & Smith, G. (1998). Antioxidants from a heated histidine-
606 glucose model system. I. Investigation of the antioxidant role of histidine and isolation of
607 antioxidants by high performance liquid chromatography. *Journal of the American Oil*
608 *Chemists' Society*, 75, 181-187.
609 Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram
610 quantities of protein utilizing the principal of dye binding. *Analytical Biochemistry*, 72, 248-
611 254.
612 Brewer, M.S. (2011). Natural Antioxidants: Sources, Compounds, Mechanisms of
613 Action, and Potential Applications. *Comprehensive Reviews in Food Science and Food*
614 *Safety*, 10, 221–247.
615 Buenaventura, M. L., Dong, F. M., & Rasco, B. A. (1986). The total dietay fiber content
616 of wheat, corn barley, sorghum, and distillers' dried grains with soluble. *Cereal Chemistry*,
617 64, 135-136.

618 Chen, Y., Xie, M.Y., Nie, S.P., Li, C., & Wang, Y.X. (2008). Purification, composition
619 analysis and antioxidant activity of a polysaccharide from the fruiting bodies of *Ganoderma*
620 *atrum*. *Food Chemistry,* 107, 231–241.
621 Dainty, R. H., & Mackey, B. M. (1992). The relationship between the phenotypic
622 properties of bacteria from chill-stored meat and spoilage processes. The Journal of Applied
623 Bacteriology, 73, 103-114.
624 Davidson, P.M., in: M. P. L. R. Doyle, T. J. Beuchat, Montville (Eds.), (2001) Food
625 Micro-biology, Fundamentals and Frontiers, second ed., *American Society for Microbiology*,
626 *Washington, DC*, 593–627.
627 Descalzo, A.M., & Sancho, A.M. (2008). A review of natural antioxidants and their
628 effects on oxidative tatus, odor and quality of fresh beef produced in Argentina. *Meat Science*,
629 79, 423–436.
630 Devatkal, S. K., & Naveena, B. M. (2010). Effect of salt, kinnow and pomegranate fruit
631 by-product powders on color and oxidative stability of raw ground goat meat during
632 refrigerated storage. *Meat Science*, 85(2), 306-311.
633 Du, W. X., Olsen, C. W., Avena-Butillos, R. J., Friedman, M., & McHugh, T. H.
634 (2011). Physical and antibacterial properties of edible films formulated with apple skin
635 polyphenols. *Food Science*, 76, 149–155.
636 Du, X. J., Mu, H. M., Zhou, S., Zhang, Y., & Zhu. X. L. (2013). Chemical analysis and
637 antioxidant activity of polysaccharides extracted from *Inonotus obliquus sclerotia*.
638 *International Journal of Biological Macromolecules*, 62, 691–696.
639 Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956).
640 Colorimetric method for determination of sugars and related substances. *Analytical*
641 *Chemistry*, 28, 350–356.

642 Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2010).
643 Dietary fibre and fibre-rich by-products of food processing: characterisation, technological
644 functionality and commercial applications: a review. *Food Chemistry*, 124, 411–421.
645 Estévez, M., & Cava, R. (2006). Effectiveness of rosemary essential oil as an inhibitor
646 of lipid and protein oxidation: contradictory effects in different types of frankfurters. *Meat*
647 *Science*, 72, 348–355.
648 Faustman, C., Sun, Q., Mancini, R., & Suman, S. P. (2010). Myoglobin and lipid
649 oxidation interactions: mechanistic bases and control. *Meat Science*, 86, 86-94.
650 Galanakis, C.M., Tornberg, E., & Gekas, V. (2010). Recovery and preservation of
651 phenols from olive waste in ethanolic extracts. *Journal of Chemical Technology and*
652 *Biotechnology*, 85, 1148-1155.
653 Greene, B. E., & Price, L. G. (1975). Oxidation induced colour and flavour changes in
654 meat. Journal of Agricultural and Food Chemistry, 23, 164–167.
655 Guo, X., Zou, X., Sun. M. (2010). Optimization of extraction process by response
656 surface methodology and preliminary characterization of polysaccharides from *Phellinus*
657 *igniarius. Carbohydrate Polymers*, 80, 344–349.
658 He, F., Yang, Y., Yang, G., & Yu, L.J. (2010). Studies on antibacterial activity and
659 antibacterial mechanism of a novel polysaccharide from *Streptomyces Virginia H03*. *Food*
660 *Control*, 21, 1257–1262.
661 Kallel, F., Driss, D., Chaari, F., Belghith, L., Bouaziz, F., Ghorbel, R., & Chaabouni,
662 S.E. (2014). Garlic (*Allium sativum* L.) husk waste as a potential source of phenolic
663 compounds: Influence of extracting solvents on its antimicrobial and antioxidant properties.
664 *Industrial Crops and Products*, 62, 34–41.

Kan, Y., Chen, T., Wu, Y., Wu, J., & Wu, J. (2015) Antioxidant activity of polysaccharide extracted from *Ganoderma lucidum* using response surface methodology. *International Journal of Biological Macromolecules,* 72, 151-157.

Kiassos, E., Mylonaki, S., Makris, D. P., & Kefalas, P. (2009). Implementation of response surface methodology to optimise extraction of onion (*Allium cepa*) solid waste phenolics. *Innovative Food Science and Emerging Technologies*, 10, 246–252.

Kinsella, J. E., Frankel, E., German, B., & Kanner, J. (1993). Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technology*, 47, 85-89.

Klaus, A., Kozarski, M., Niksic, M., Jakovljevic, D., Todorovic, N., & Van Griensven, L.J.L.D. (2011) Antioxidative activities and chemical characterization of polysaccharides extracted from the basidiomycete *Schizophyllum commune*. *LWT - Food Science and Technology*, 44, 2005-2011.

Lai, F. R., Wen, Q. B., Li, L., Wu, H., & Li, X. F. (2010). Antioxidant activities of water soluble polysaccharide extracted from mung bean (*Vigna radiata* L.) hull with ultrasonic assisted treatment. *Carbohydrate Polymers*, 81, 323–329.

Lei, S. (2010). Separation, Purification and Structure Characterization of a Polysaccharide from Root of *Cudrania tricuspidata*. *Asian Journal of Experimental Biological Sciences,* 1, 311-314.

Lennette, E. H., Balows, A., & Hausler, W. J. (1980). Manual of Clinical Microbiology. American Society for Microbiology, Washington, D.C, USA.

Li, X.L., Zhou, A.G., & Han, Y. (2006). Anti-oxidation and anti-microorganism activities of purification polysaccharide from *Lygodium japonicum* in *vitro. Carbohydrate Polymers*, 66, 34–42.

Lingnert, H., Vallentin, K., & Eriksson, C. E. (1979). Measurement of antioxidative effect in model system. *Journal of Food Processing and Preservation*, 3, 87-103.

690 Liu, Z. D., Wei, G. H., Guo, Y. C., & Kennedy, J. F. (2006). Image study of pectin
691 extraction from orange skin assisted by microwave. *Carbohydrate Polymers*, 64, 548–552.
692 Lo, T.C-T., Chang, C.A., Chiu, K-H., Tsay, P-K., & Jen, J-F. (2011). Correlation
693 evaluation of antioxidant properties on the monosaccharide components and glycosyl linkages
694 of polysaccharide with different measuring methods. *Carbohydrate Polymers*, 86, 320–327.
695 Mielnik, M. B., Abby, K., & Skrede, G. (2003). Synthetic antioxidants control lipid
696 oxidation in mechanically deboned turkey meat. *Meat Science*, 65, 1147-55.
697 Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for the determination of
698 reducing sugars. *Analytical Chemistry*, 31, 426-428.
699 Nwinuka, N. M., Ibeh, G. O., & Ekeke, G. I., (2005). Proximate composition and levels
700 of some toxicants in four commonly consumed spices. *Journal of Applied Sciences and*
701 *Environmental Manage*, 9, 150–155.
702 Park, S., Marsh, K.S., & Dawson, P. (2010). Application of chitosan-incorporated
703 LDPE film to sliced fresh red meats for shelf life extension. *Meat Science,* 85, 493–499.
704 Prosky, L., Asp, N. G., Schweizer, T. F., De Vries, J. W., & Furda, I. (1988).
705 Determination of insoluble, soluble and total dietary fiber in foods and food products:
706 Interlaboratory study. *Journal of the Association of Official Analytical Chemists*, 71, 1017-
707 1988.
708 Pujol, D., Liu, C., Gominho, J., Olivella, M. À., Fiol, N., Villaescus, I., Pereira, H.
709 (2013). The chemical composition of exhausted coffee waste. *Industrial Crops and Products*,
710 50, 423–429
711 Puupponen-Pimiä, R. Nohynek, L. Meier, C. Kähkönen, M. Heinonen, M. Hopia, A. &
712 Oksman-Caldentey, K. M. (2001). Antimicrobial properties of phenolic compounds from
713 berries. *Journal of Applied Microbiology,* 90, 494–507.

714 Qiao, D., Ke, C., Hu, B., Luo, J., Ye, H., Sun, Y., Yan, X., & Zeng, X. (2009).
715 Antioxidant activities of polysaccharides from Hyriopsis cumingii. *Carbohydrate Polymers.*
716 78, 199–204.

717 Renerre, M. (2000). Review: biochemical basis of fresh meat color. In Proceedings of
718 the 45th international congress of meat science and technology. Yokohama, Japan, 344-352.

719 Romano, R. T., & Zhang, R. (2011). Anaerobic digestion of onion residuals using a
720 mesophilic Anaerobic Phased Solids Digester. *Biomass and Bioenergy*, 35, 4174-4179.

721 Rusendi, D., & Sheppard, J. D. (1996). Hydrolysis of potato processing waste for the
722 production of poly-β-hydroxybutyrate. *Bioresource Technology*, 54, 191-196.

723 Sallam, K. I., Ishioroshi, M., & Samejima, K. (2004). Antioxidant and antimicrobial
724 effects of garlic in chicken sausage. *Lebenson Wiss Technology*, 37, 849–855..

725 Sebastian, S., Sundaraganesan, N., & Manoharan, S. (2009). Molecular structure,
726 spectroscopic studies and first-order molecular hyperpolarizabilities of ferulic acid by density
727 functional study. *Spectrochimita Acta Part A*, 74, 312-323.

728 Sebranek, J. G., Sewalt, V. J. H., Robbins, K. L., & Houser, T. A. (2005). Comparison
729 of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork
730 sausage. *Meat Science*, 69(2), 289-296.

731 Serrero, A., Trombotto, S., Cassagnau, P., Bayon, Y., Gravagna, P., Montanari, S., &
732 David, L. (2010). Polysaccharide gels based on chitosan and modified starch:Structural
733 characterization and linear viscoelastic behavior. *Biomacromolecules*, 11, 1534–1543.

734 Shimada, K., Fijikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidative
735 properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of*
736 *Agricultural and Food Chemistry*, 40, 945-948.

737 Sikkema, J., De Bont, J. A. M., & Poolman, B. (1995). Mechanisms of membrane
738 toxicity of hydrocarbons. *FEMS Microbiology Reviews*, 59, 201–222.

739 Singh, N. & Rajini, P. S. (2004). Free radical scavenging activity of an aqueous extract
740 of potato peel. *Food Chemistry*, 85, 611–616.
741 Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with
742 phosphomolybdic phosphotungstic acid reagents. *American Journal of Enology and*
743 *Viticulture.* 16, 144-158.
744 TAPPI method T 222 om-83, (1999). Acid-insoluble lignin in wood and pulp. In: Test
745 Methods 1998–1999. TAPPI Press, Atlanta, USA.
746 TAPPI UM 250, (1991). Acid-soluble lignin in wood and pulp in: 1991 TAPPI Useful
747 Methods, Tappi, Atlanta, GA, USA.
748 Tayel, A. A., & El-Tras, W. F. (2012). Plant extracts as potent biopreservatives for
749 *Salmonella typhimurium* control and quality enhancement in ground beef. *Journal of Food*
750 *Safety*, 32, 115–121.
751 Tombs, M., & Harding, S.E. (1998). An introduction to polysaccharide technology.
752 Taylor and Francis, London.
753 Trommer, H., & Reinhard H. H. (2005). The examination of polysaccharides as
754 potential antioxidative compounds for topical administration using a lipid model system.
755 *International Journal of Pharmaceutics*, 298, 153–163.
756 Vanden Berghe, D. A., & Vlietinck A. J. (1991). In Dey, P. M., & Harborne, J. B. (Ed.),
757 *Methods in plant biochemistry*, 6, 47-67.
758 Vandercook, C. E., Hasegawa, S., Maier, V. P., 1979. Quality and nutritive value of
759 dates as influenced by their chemical composition. *Date Growers Institute*. 54, 3–11.
760 Velasco, V., & Williams, P. (2011). Improving meat quality through natural
761 antioxidants. Chilean Journal of Agricultural Research, 71, 313–322.

762 Venkatesan, J., Qian, Z. J., Ryu, B. M., Ashok Kumar, N., & Kim, S. K. (2011).
763 Preparation and characterization of carbon nanotube-grafted-chitosan-Natural hydroxyapatite
764 composite for bone tissue engineering. *Carbohydrate Polymer*, 83, 569–577.

765 Vinogradov, E. V., Brade, L., Brade, H., & Holst, O. (2003). Structural and serological
766 characterisation of the O-antigenic polysaccharide of the lipopolysaccharide from
767 *Acinetobacter baumannii* strain 24. *Carbohydrate Research*, 338, 2751–2756.

768 Wang, J.T., Wang, Q., & Han, J.R. (2013) Yield, polysaccharides content and
769 antioxidant properties of the mushroom *Agaricus subrufescens* produced on different
770 substrates based on selected agricultural wastes. *Scientia Horticulturae*, 157, 84–89.

771 Wang, Y., Xian, J., Xi, X., & Wei, X., 2013. Multi-fingerprint and quality control
772 analysis of tea polysaccharides. *Carbohydrate Polymers*. 92, 583– 590.

773 Witte, V. C., Krause, G. F., & Baily, M. E., (1970). A new extraction method for
774 determiniy 2- thiobarbiturie acid values of pork and beef during storage. *Journal of Food*
775 *Science*, 35, 582-585.

776 Wu, Z., Li, H., Tu, D., Yang, Y., & Zhan, Y. (2013). Extraction optimization,
777 preliminary characterization, and in vitro antioxidant activities of crude polysaccharides from
778 finger citron. *Industrial Crops and Products.* 44, 145–151.

779 Yao, Y., Wei, J. Z., Wang, J., Zhang, J. P., Liu, J. H., & Feng, W. H. (2005). Extraction
780 of *Mytilus coruscus* polysaccharides and study on their immunoactivities. *Academic Journal*
781 *of Second Military Medicine University*, 26, 896–899.

782 Yildirim, A., Mavi, A., & Kara, A. A. (2001). Determination of antioxidant and
783 antimicrobial activities of *Rumex crispus* L. extracts. *Journal of Agricultural and Food*
784 *Chemistry*, 49, 4083-4089.

785 Zhao, B., Zhang, J., Guo, X., & Wang. J. (2013). Microwave-assisted extraction,
786 chemical characterization of polysaccharides from *Lilium davidii var. unicolor Salisb* and its
787 antioxidant activities evaluation. *Food Hydrocolloids,* 31, 346-356.
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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)

Parameter	Content [a] (g/100g)
Moisture	13.18 ± 0.45
Protein	4.38 ± 0.21
lipid	2.66 ± 0.13
Dietary fiber (DF)	24.10 ± 1.70
Insoluble DF	20.50 ± 1.30
Soluble DF	3.60 ± 0.40
Ethanol extractive	1.20 ± 0.06
Lignin	6.32 ± 0.36
Ash	10.08 ± 0.30

[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)

Minerals elements	Content [a] (mg/100g)
Ca^{2+}	292.30 ± 15.26
K^{+}	206.76 ± 10.30
Na^{+}	198.35 ± 7.60
Mg^{2+}	53.26 ± 0.150
Fe^{2+}	4.60 ± 0.021
Mn^{2+}	0.474 ± 0.013
Zn^{2+}	0.44 ± 0.0016
Cu^{2+}	<0.088 ± 0.001

[a] Expressed on a dry basis with the exception of moisture data. Data are means ± standard deviations values of three replicates.

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877 **Table 3:** Diameters (mm) of inhibition zones determined after 24 h incubation at 37°C for
878 bacteria and 72 h incubation at 30°C for fungi.

Strains	Garlic straw polysaccharide (mg/mL)		Control	Ciprofloxacin	Amphotericin B
	20	40			
B. turengensis	ND	7.5 ± 0.5	ND	23.5 ± 0.5	-
S. aureus	7 ± 0.5	10 ± 0.5	ND	26 ± 0.5	-
M. luteus	9.5 ± 0.5	11.5 ± 0.5	ND	32 ± 0.5	-
P. aeruginosa	ND	7 ± 0.5	ND	27 ± 0.5	-
E. faecalis	6.50 ± 0.5	10.5 ± 0.5	ND	19 ± 0.5	-
E.coli	ND	ND	ND	24 ± 1	-
K. pneumoniae	6.75 ± 0.5	8.5 ± 0.5	ND	19 ± 0.5	-
A.niger	ND	ND	ND	-	13 ± 0.5
R. oryzae	ND	ND	ND	-	22 ± 0.5

879 Note. ND: not detected; Amphotericin B and Ciprofloxacin were used as positive control.
880 Values represent averages ± standard deviations for triplicate experiments.

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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.

	TVC				
Treatments	Storage time (day)				
	0	1	3	6	9
NC	4.35 ± 0.04^{aA}	5.11 ± 0.09^{aB}	6.06 ± 0.20^{aC}	6.76 ± 0.20^{aD}	7.89 ± 0.30^{aE}
2% GSP	4.55 ± 0.24^{aA}	5.25 ± 0.18^{bB}	5.94 ± 0.09^{bB}	6.30 ± 0.80^{aC}	7.71 ± 0.08^{aD}
4% GSP	4.77 ± 0.20^{aA}	4.69 ± 0.13^{bB}	5.90 ± 0.08^{bB}	6.21 ± 0.82^{aC}	7.54 ± 0.17^{aD}
PC	4.30 ± 0.02^{aA}	4.43 ± 0.08^{cA}	5.76 ± 0.15^{cB}	5.81 ± 0.13^{bB}	6.15 ± 0.16^{bB}

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 ($p < 0.05$). NC: Negative control (non-treated group). GSP: garlic straw polysaccharide; PC: Positive control (BHA-treated group). TVC: total viable count.

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.

	NC	Treatment with GSP (%, w/w)		PC
		2	4	
Odour	1.51 ± 0.17^{a}	2.86 ± 0.27^{b}	2.36 ± 0.36^{b}	3.57 ± 0.50^{c}
Colour	1.58 ± 0.19^{a}	2.35 ± 0.37^{b}	2.30 ± 0.21^{b}	3.34 ± 0.43^{c}
Texture	1.48 ± 0.38^{a}	2.65 ± 0.58^{b}	2.32 ± 0.32^{b}	3.49 ± 0.41^{c}
Overall acceptability	1.84 ± 0.27^{a}	2.55 ± 0.22^{b}	2.47 ± 0.21^{b}	3.61 ± 0.51^{c}

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).

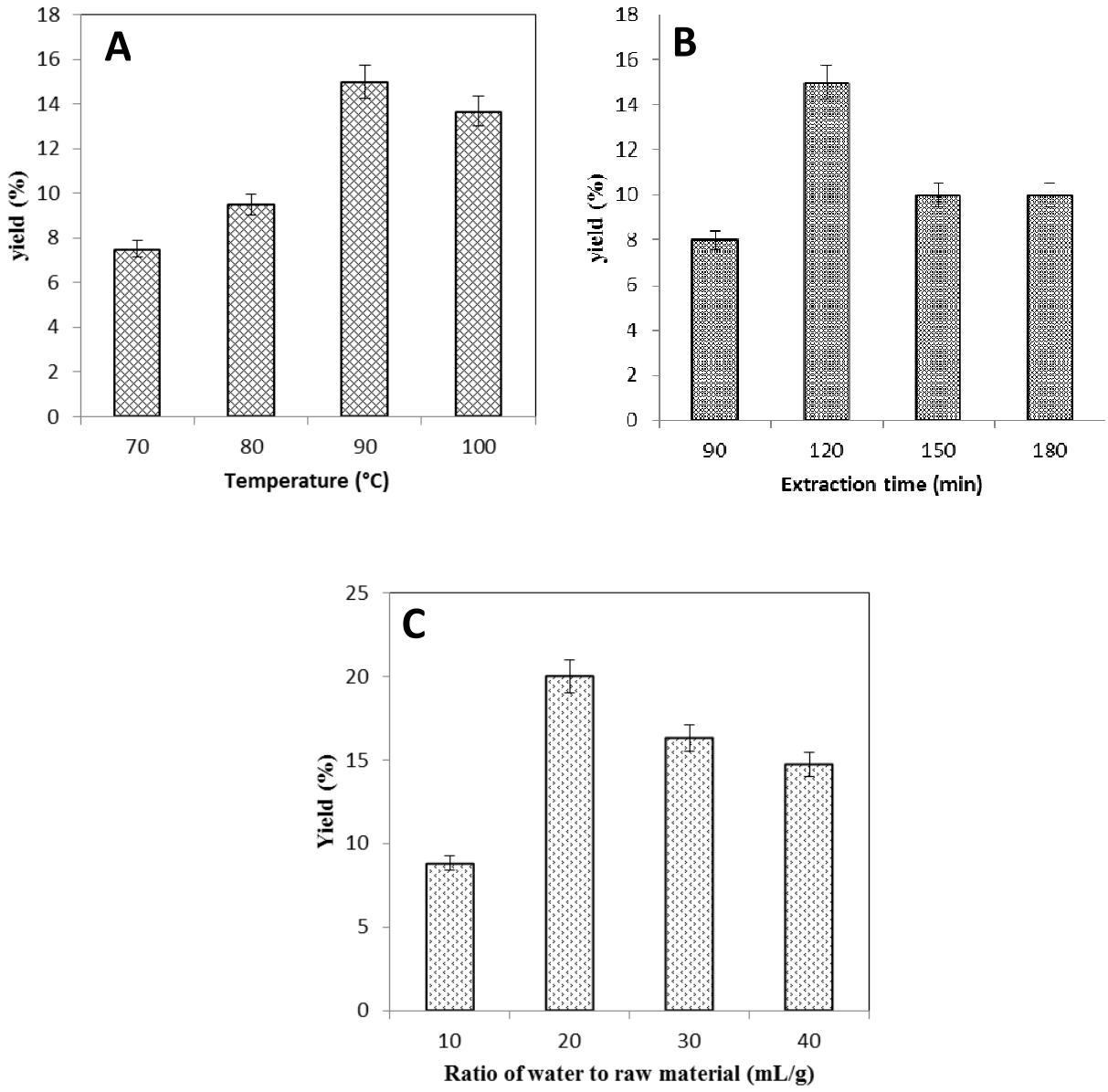
A
yield (%)
Temperature (°C)
70
80
90
100
B
yield (%)
Extraction time (min)
90
120
150
180
C
Yield (%)
Ratio of water to raw material (mL/g)
10
20
30
40

Fig. 1:

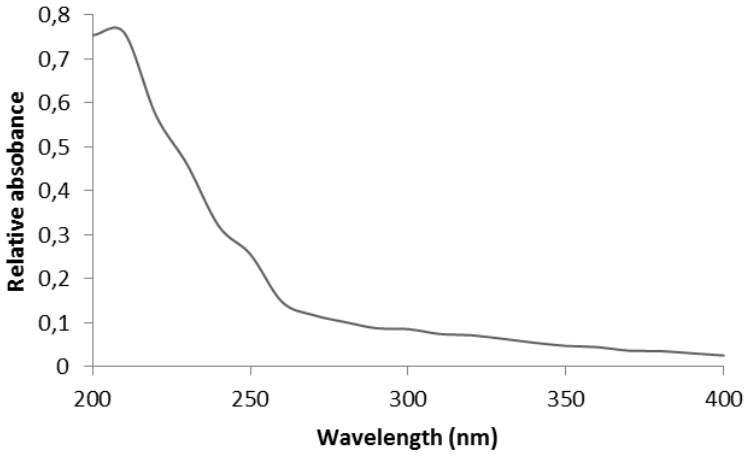
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
Relative absobance
200
250
300
350
400
Wavelength (nm)

Fig. 2:

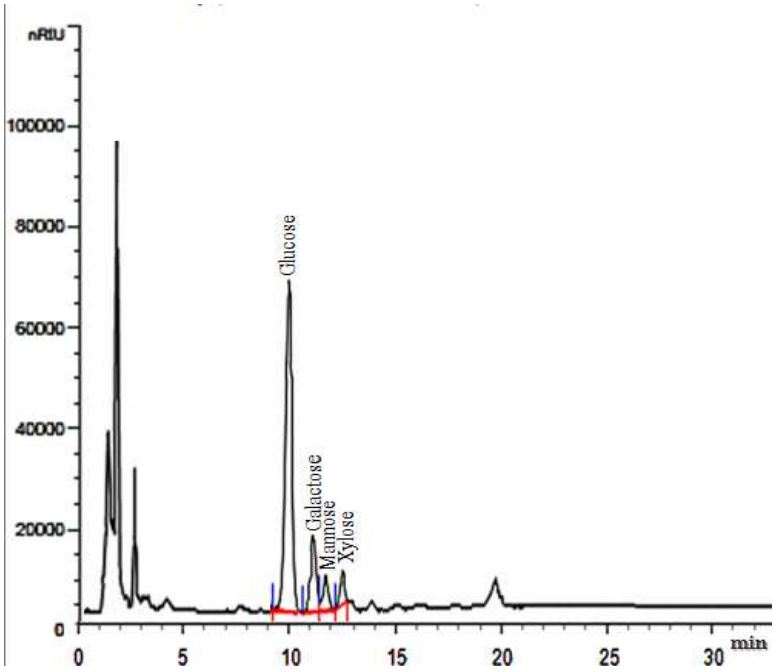
nRIU
100000
80000
60000
40000
20000
0
Glucose
Galactose
Mannose
Xylose
0
5
10
15
20
25
30
min

Fig. 3:

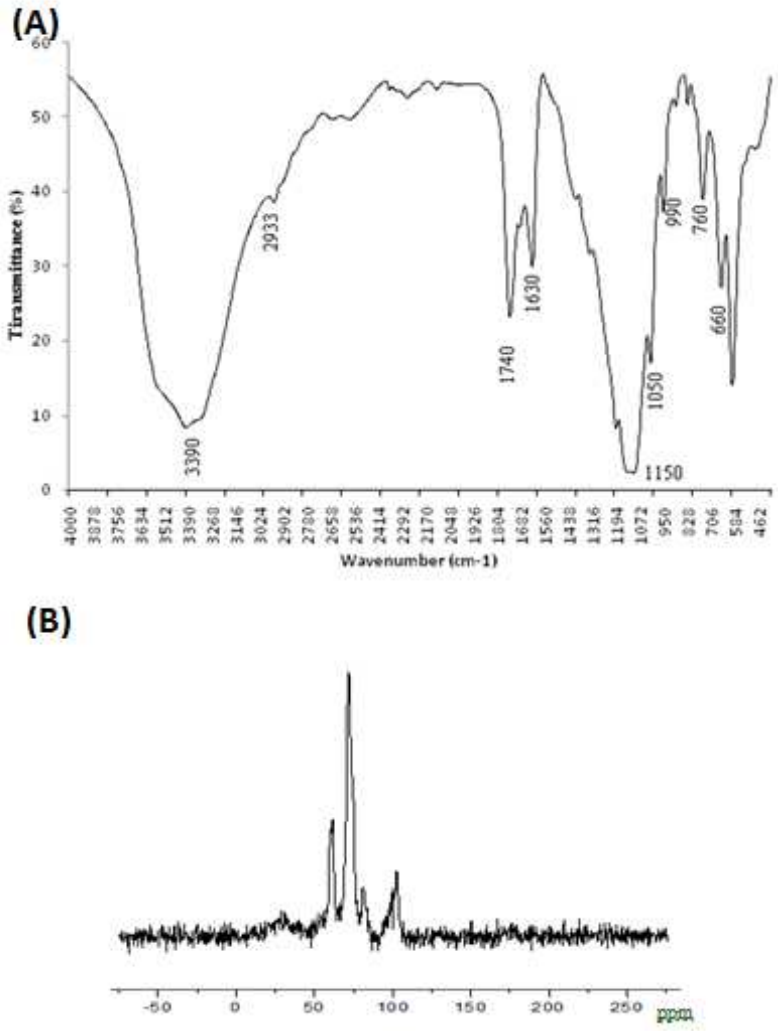
(A)
Transmittance (%)
Wavenumber (cm-1)
3390
2933
1740
1630
1150
1050
990
760
660
(B)
ppm

Fig. 4:

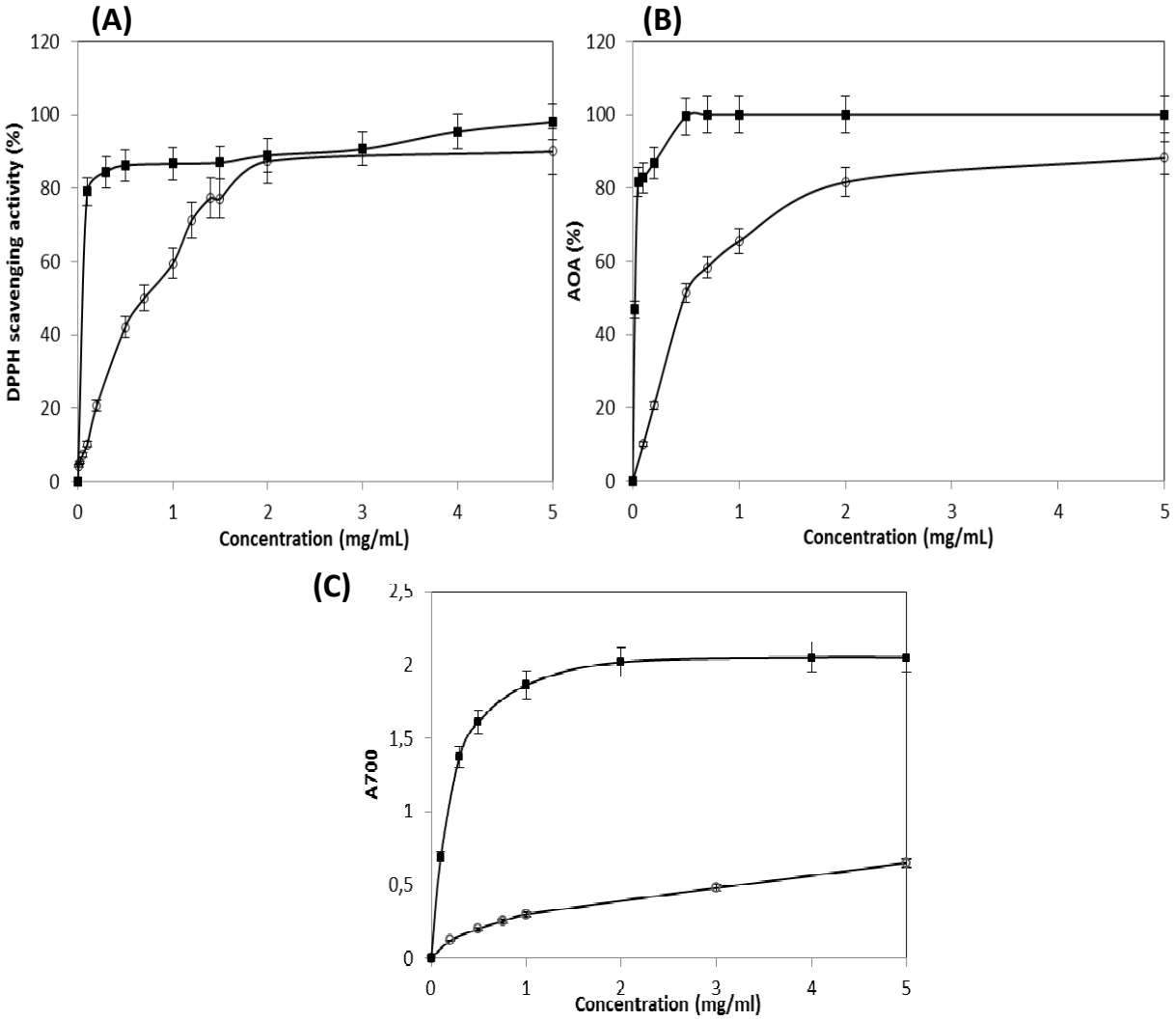
(A)
DPPH scavenging activity (%)
Concentration (mg/mL)
(B)
AOA (%)
Concentration (mg/mL)
(C)
A700
Concentration (mg/ml)

Fig. 5:

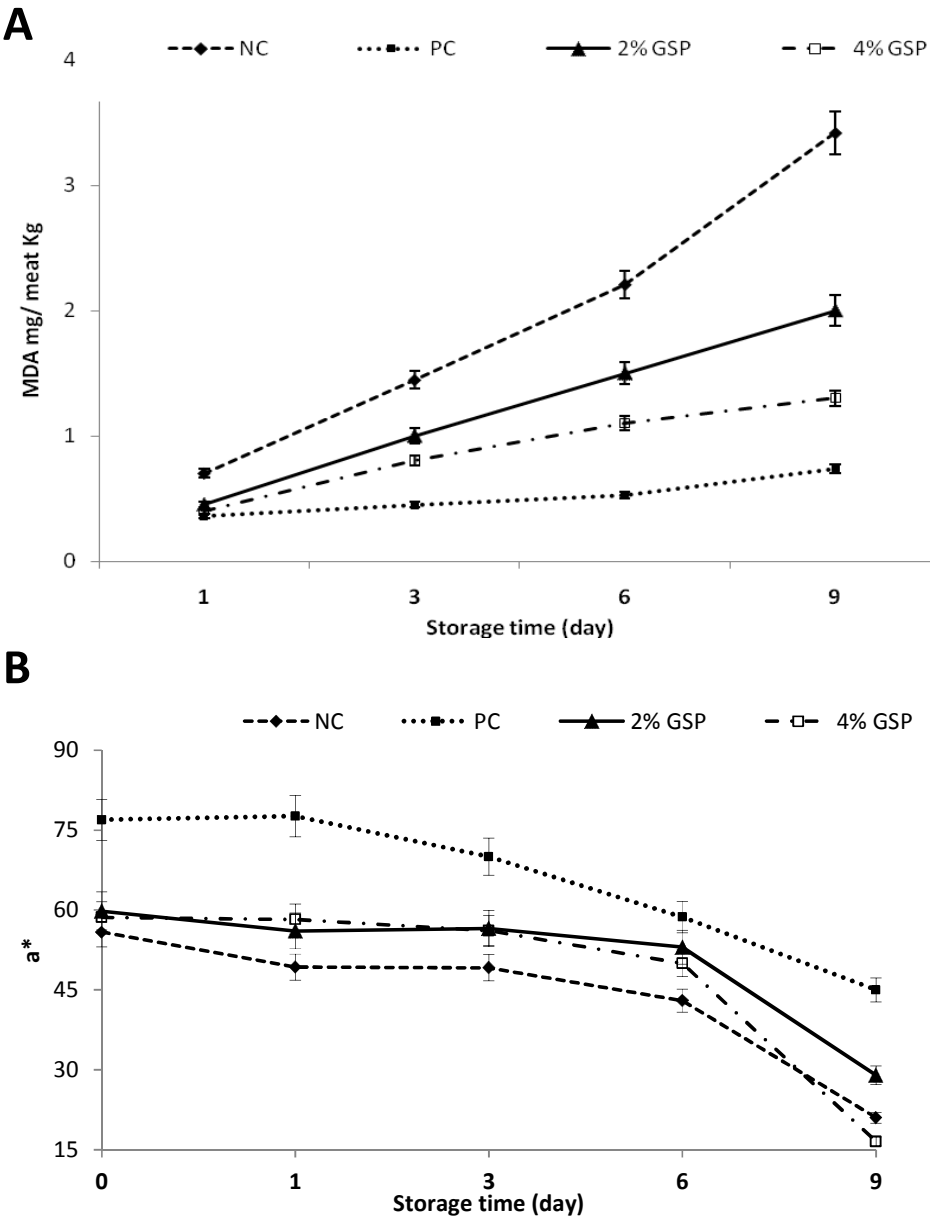
A
NC
PC
2% GSP
4% GSP
MDA mg/ meat Kg
0
1
2
3
4
1
3
6
9
Storage time (day)
B
NC
PC
2% GSP
4% GSP
a*
15
30
45
60
75
90
0
1
3
6
9
Storage time (day)

Fig. 6:

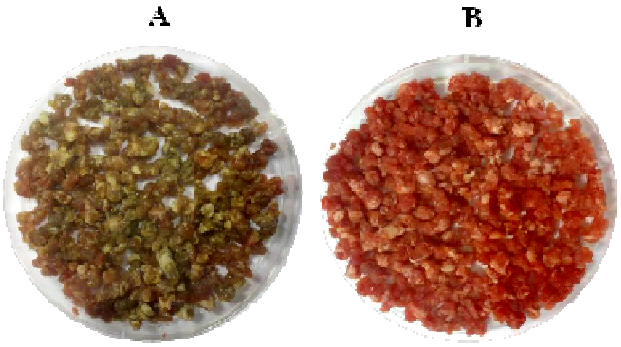

Fig. 7: Appearance of meat after 9 days of storage at 4°C for the control sample (A) and the sample treated with 2% GSP (B).