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Original Paper

Enzymatic-Assisted Extraction, Physicochemical Characteristics Evaluation and Structural Elucidation of Antioxidative Protein-Polysaccharides Gum from *Durio zibethinus* Murray Seeds

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Abstract*—*Durio zibethinus* Murray or durian is an exotic tropical fruit native to Southeast Asia. Durian seeds (DS) which are mainly composed of mucilage and fibers are generally discarded as agricultural waste post-consumption, yet have promising characteristics as innovative food ingredients with high economic value. Enzymatic-assisted extraction of protein-polysaccharide gum (E-PG) from DS improved the yield efficiency by 4.36-fold with 3.0% cellulase, and moisture content in the range of 4-6% (dry weight). Proximate composition for the total carbohydrate, dietary fiber, sugar and crude protein were found at 74.4, 24.0, 4.0 and 10.1 g/100 g of sample. E-PG recorded higher water-holding and oil-holding capacities, and solubility percentage as compared with non-enzymatic extract of protein-polysaccharide gum (N-PG) and DS flour. Physicochemical characteristics revealed E-PG was only 2.52-fold lower than xanthan gum (XG AD). Validation on E-PG$_{2.52\text{-fold}}$ revealed that the viscosity matched the quality of XG AD. Characterization with HPLC-RID, FT-IR and ^{1}N-NMR highlighted typical distribution of polysaccharides and protein, thus confirming the presence of protein/polysaccharides-based components in E-PG and N-PG. Antioxidant evaluation revealed that E-PG and E-PG$_{slurry}$ scavenged ABTS$^{\bullet+}$-radicals at an IC$_{50}$ of 5.759±0.719 and 6.083±0.131 mg/mL, and ORAC values of 57867±2873 and 70191±6291 µM TE/g, respectively, whereas ORAC activity for XG AD was not detected. The recovery of water-soluble protein-polysaccharides gum from non-traditional sources like DS via deployment of enzymatic-assisted extraction protocol has improved the extraction yield and enhanced antioxidative capacity of the gum, greater than commercial gums. Findings may significantly influence the food and/or nutraceutical industries on the utilization of value-added and innovative food ingredients in their product formulation.

Keywords*— *Enzymatic-assisted extraction, durian seeds, cellulase, protein-polysaccharides gum, antioxidant activity

I. INTRODUCTION

Durian (*Durio zibethinus* Murray) is a climacteric, popular seasonal fruit grown in Southeast Asia, including Malaysia. The edible portion of durian is only 30-35% and durian seeds (DS) (20-25%) [1-2], a by-product from durian industry, is usually discarded as agricultural waste [3-5]. Many reports have focused on the nutraceutical properties of the edible portion of durian [6-7] but transforming durian wastes such as the seeds to useful products is possible. DS contains protein [8-9] and approximately 74-75% (dry basis) carbohydrate [10-11]. Mucilage (gum) in DS has viscid texture and thickening ability with low gelatinization-temperature [12-13], and this characteristic could be exploited as new plant-based and value-added hydrocolloids for commercial food and nutraceutical applications; including but not limited to serve as a texture modifier, dietary fibre, stabilizer, emulsifier, thickener as well as encapsulating agent. Natural plant-based gums are generally preferred and regarded as safe for oral consumption due to their availability and safety (non-toxic) [1]. Scholars have reported on various plant-based gums [14-16], yet their application as food ingredients is limited as it requires high concentration in order to exhibit their function hence driving exploration for alternatives from other natural resources with low cost but high economic value.

DS contains about 1.8% of fatty acids which include palmitic and stearic acid [1, 17]. Oleic acid (18:1), linoleic (18:2) and γ-linolenic (18:3) constitute about 22% from the approximate 82% of the unsaturated fatty acids. DS also contains cyclopropene fatty acids (CFA), namely sterculic acid, dihydrosterculic acid and malvalic acid. Reported amino acids abundantly present in

DS include leucine, lysine, aspartic acid, glycine, alanine and glutamic acid [17].

Aqueous and chemical extractions have been used to yield protein-polysaccharides gum from DS [1, 16-18]. However, these methods require a large volume of solvents, high extraction temperature and lengthy period of extraction that may co-extract many non-active components, disrupt the structure of protein-polysaccharides of interest and affect the molecular weight of the polysaccharides. Enzyme-assisted extraction strategy harnesses the catalytic properties of enzymes to break down the plant cell wall and facilitate the release of intracellular polysaccharides from plant materials. Advantages of the technology include specificity, high extraction rates and efficiency, increased yield of targeted components [19], environmental-friendly and the capacity to enhance and preserve biological activities of the extracted plant polysaccharides, hence making the technology attractive to food and nutraceutical industries [20-21]. Enzymes such as pectinase, cellulase, papain, α-amylase and protease have been utilized in the extraction of plant polysaccharide. Song et al. [21] has employed α-amylase, cellulose, pectinase and protease to extract polysaccharides from lotus leaf. Gao et al. [22] used amylase to extract polysaccharides from the bulb of *Erythronium sibiricum*, and the yield (59.71 ± 2.72%) was much higher than the conventional hot aqueous extraction (37.25 ± 0.17%). Shang et al. [23] compared 4 different protocols to extract polysaccharides from *Trifolium repens* L, and concluded that enzymatic-assisted extraction process yielded polysaccharides with higher content of uronic acid and exhibited better antioxidant capacities against polysaccharides extracted using hot water, ultrasonic-assisted and ultrasonic-enzyme-assisted extraction protocols. A tailored approach by Shi et al. [24] to extract polysaccharides from *Dendrobium officinale* with a combination of cellulase and pectinase at a ratio of 2:1 demonstrated that optimized enzymatic-assisted extraction protocol able to standardize the yield with higher extraction efficiency rate. However, to our knowledge, there is limited information on enzymatic-assisted extraction and biological activities of water-soluble protein-polysaccharides gum from DS.

Industrial application of protein-polysaccharides gum from DS is vast. For instance, Kai et al. [1] replaced gelatine with DS gum in gummy candy formulation. Cornelia et al. [4] employed DS gum as emulsifier in mayonnaise and the outcome demonstrated exceptional capacity for water and oil absorption hence improved stability and quality of the mayonnaise. Others include DS gum as a thickening agent and alternative to wheat flour in the production of fermented rice, angkak [25]. Robust industrial applications warrant further exploration into DS-based protein-polysaccharides gum and its incorporation as value-added ingredients in functional food and nutraceutical formulations.

In this study, the objectives were to evaluate the efficacy of enzymatic-assisted extraction protocol to extract crude water-soluble protein-polysaccharides from DS, to examine the physicochemical characteristics of the mucilage fraction using a series of chemical processes against commercial gums largely used in food and nutraceutical industries, to characterize and elucidate structural composition of the protein-polysaccharides gum using chromatographic and spectroscopic procedures, and to determine antioxidant activities of water-soluble protein-polysaccharides from DS.

II. Materials And Methodology

A. Sample treatment and chemicals

Durian (*Durio zibethinus Murray*) was purchased from a local supplier in Raub, Pahang, Malaysia. The fruit were carefully selected; ripe, uniform in size and without defects. The fruit were de-husked, cut along the suture and the flesh was removed. The seeds were collected (fresh DS), rinsed with distilled water for 15-30 min, disinfected with 3% hydrogen peroxide (H_2O_2) for 5 min and washed with running water. Outer shells of the seeds were skinned-off and the washed seeds were sliced to a 0.5-1.0 mm thickness with a slicer. Then, the seeds were dried in an oven cabinet at 60°C until the moisture content was below 10%, verified using a moisture analyzer (Ohaus® MB27) as a rapid method. A grinder was used to pulverize the seeds into flour granules, sifted on 80-mesh size, seal-packed in a plastic bag and stored in the refrigerator (2-4ºC) until further use. All chemicals and reagents were analytical grade and purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) through a local distributor.

B. Enzymatic-assisted extraction of protein-polysaccharides gum (E-PG)

The extraction of protein-polysaccharides was carried out according to Cornelia et al. [4] with slight modification. Preliminary investigation was conducted and optimized to determine the rate of enzyme activity and optimum conditions (extraction time, extraction temperature, pH and solvent-to-seed ratio) needed during the extraction protocol. The E-PG was extracted enzymatically from DS flour using cellulase from *Aspergillus niger* (700 U/mg) at different percentages (0.2, 0.5, 1.0 and 3.0%), in a 50 mM sodium acetate buffer (w/s ratio 35.5:1 w/w) as the media at 37ºC for 24 h, in a 5 L solid-state bioreactor equipped with Rushton turbine-radial impellers. The pH of the solution was maintained at pH 4.5-5. Post-extraction, the water-seed slurry ($E\text{-}PG_{slurry}$) was subjected to enzyme inactivation in a waterbath (50-55°C) for 6 min, centrifuged at 1300 x g for 20 min at 20ºC and the supernatant was separated from the pellet. The supernatant containing water-soluble protein-polysaccharides was precipitated using 95% ethanol (3 times of the supernatant) for 30 min. The crude protein-polysaccharide was collected, washed with distilled water, dried in the cabinet dryer at 40ºC for 24 h and ground into powder. The E-PG flour was sifted through a sieve (120-mesh size), seal-packed and stored in a chiller (4-8°C) until further use. Moisture content was recorded. Control was prepared with the same protocol but without the addition of cellulase, and the yield was denoted as N-PG (non-enzymatic extract of protein-polysaccharide gum).

C. Proximate and mineral composition analysis

Official protocols adopted by AOAC International were adhered to quantitatively determine proximate composition of all samples [26-27]. Total dietary fiber was determined according to the enzymatic gravimetric method (AOAC 985.29); multi-enzyme digestion assay protocol using α-amylase, protease and amyloglucosidase at 60°C, ethanol precipitation and filtration [28]. Crude protein was evaluated using the Kjeldahl method (AOAC 2001.11 and ISO 1871:2009) with the total nitrogen being multiplied by a protein factor of 6.25. Total sugar employed AOAC 968.26 protocol [28]. Energy and total carbohydrate were examined according to Sullivan and Carpenter [26]. Total fat was determined according to Egan et al. [27]. Minerals were examined spectroscopically at their indicative wavelengths according to AOAC 999.11 and APHA 3120 [29].

D. Water-holding and oil-holding capacities

Water-holding capacity (WHC) for E-PG was determined according to Sciarini et al. [30] with minor modification. One g of E-PG powder was suspended in 10 mL of distilled water, vortexed for 2 min and then centrifuged (Multifuge X3 FR, Thermo Scientific) at 3000 x g for 30 min. The free water was decanted and the water absorbed by the samples was expressed as grams of water absorbed per 100 g of powder. Oil-holding capacity (OHC) was determined by dispersing 1 g of E-PG in 10 mL of refined sunflower oil and expressed as grams of oil absorbed per 100 g of powder. The same protocol was employed on N-PG and DS flour. Triplicate measurements were performed for each sample. The WHC and OHC were calculated based on (1) and (2):

$$WHC = (SSW - SW) / SW \quad (1)$$

$$OHC = (OSW - SW) / SW \quad (2)$$

where SSW, SW and OSW are the swollen sample weight, sample weight (initial) and oil-absorbed sample weight, respectively.

E. Solubility

The solubility was determined according to Dakia et al. [31] with minor modification. One g of E-PG was suspended in 100 mL of distilled water, and the solution mixture was agitated with mechanical stirring at ambient temperature (25 ± 1°C) and elevated temperature (>80°C) for 30 min. The solution was centrifuged at 6000 x g for 30 min to remove the insoluble material. The supernatant was transferred to clean disposable Petri dishes and oven dried at 105°C for 24 h until constant weight. The same protocol was conducted on DS flour, N-PG, XG AD and guar gum (GG) flour. Percentage of solubility was determined in triplicate using (3):

$$\text{Solubility (\%)} = (C_1 / C_2) \text{ X } 100 \quad (3)$$

where C_1 is the supernatant concentration (mg/mL) and C_2 is the initial solution concentration (mg/mL)

F. Viscosity, pH and total dissolved sugar measurements

Physicochemical characteristics of E-PG at a concentration of 5 mg/mL in distilled water were examined at ambient (25 ± 1°C) to elevated temperature (>80°C) against commercial gums, XG AD and GG, in terms of viscosity, pH and total dissolved sugar. Viscosity was measured using a digital viscometer (Atago VISCO^{TM}) at 250 rpm with A1 spindle and dissolved sugar via digital Brix refractometer (Atago PAL-1^{TM}). Concentration of E-PG required to exhibit similar or better physicochemical qualities than XG AD was determined and validated.

G. Monosaccharide composition by HPLC-RID

Chromatographic analysis of sugars was performed by high pressure liquid chromatography (HPLC) coupled with refractive index detector (RID) Chromaster 5450, and equipped with LC-20 AD pump and column thermostat. Operating conditions were conducted according to Hadjikinova et al. [32], adopting AOAC 980.13 and AOAC 2018.16. The chromatographic separation was carried out on Pb^{2+} Shodex Sugars SP0810 (300 mm x 8.0 mm i.d.). The column temperature and the flow rate were set at 80°C and 0.5 mL/min. The mobile phase (distilled water) was vacuum filtered through a 0.2 µm membrane filter. All samples were filtered through a 0.45 µm pore size filter and the injection volume was 20 µL.

H. Characterization via fourier transform-infrared (FT-IR) spectroscopy

The FT-IR is a useful analytical spectroscopy tool to examine the functional groups of a sample. The DS flour, N-PG and E-PG were characterized by FT-IR (Frontier, Perkin Elmer) by placing the sample in the Attenuated Total Reflection (ATR) chamber with infrared spectra in the wavenumber range of 4000-600 cm^{-1}. Test conditions were carried out at 29.7 ± 0.3°C and relative humidity of 55-58%.

I. Characterization via nuclear magnetic resonance (NMR) spectroscopy

The ^{1}H-NMR spectra were recorded in heavy water (deuterium oxide) solution at 400 MHz (70°C) with a Bruker DRX-400 spectrometer. Chemical shifts were expressed in parts per million (ppm) relative to the resonance of internal standard, 3-trimethylsilyl-1-propanesulfonic acid.

J. $ABTS^{\bullet+}$-radical scavenging activity

The $ABTS^{\bullet+}$ radical-scavenging assay is a spectrophotometric electron transfer-based assay. In principle, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$) is reduced by an antioxidant from a dark blue to colourless ABTS. E-PG and E-PG_{slurry} were tested for $ABTS^{\bullet+}$-radical scavenging activity. The untreated $ABTS^{\bullet+}$-radical solution was used as control blank. Data was reported as half-maximal inhibitory concentration (IC_{50}) by plotting the scavenging inhibition percentage over various concentrations on serial dilutions.

K. Oxygen radical absorbance capacity (ORAC)

The E-PG, E-PG_{slurry}, DS flour and XG AD were subjected to ORAC analysis. Samples (0.01 g/mL) were dissolved and

homogenized in phosphate buffer solution (PBS). Buffer (blank), Trolox (vitamin E analog as standard) and test samples were added separately in triplicate into each active well of a microplate. Then, the plate was placed in a microplate reader and incubated at 37°C. Fluorescein and a reactive oxygen species (ROS) generator, AAPH (2,2'-azobis(2-methylpropionamidine) dihydrochloride) solution were added using microplate injectors and reactions were initiated. Antioxidative capacity was represented as area under the fluorescence decay curve and data was expressed as Trolox equivalent (TE) per gram.

L. Statistical analysis

Tests were carried out in triplicates. All data were expressed as mean ± standard deviation (SD) of three measurements. Differences across multiple groups were tested by one-way analysis of variance (ANOVA), followed by post-hoc Tukey's test. The p values of <0.05 were considered significant. The ORAC values were calculated against linear regressions of the Trolox standard.

III. Results And Discussion

A. Yield of E-PG and N-PG from DS flour and fresh seeds

Findings as portrayed in Fig. 1 suggested that enzymatic-assisted extraction able to improve the extraction yield of protein-polysaccharides gum from DS by 1.17-fold using 0.2% cellulase, 1.36-fold with 0.5% cellulase, 3.45-fold and 4.36-fold for 1.0% and 3.0% cellulase, respectively. Similarly, data has demonstrated an increasing pattern of percentage yield of E-PG, directly proportional to the increased percentage of cellulase during the extraction. The colour of E-PG flour was light brown. Moisture content was reported to contribute towards microbial growth in food products hence must be kept below 10% [33]. Moisture content of E-PG flour was recorded in the range of 4-6%, unlike 13.42% by Mulyati et al. [34] and 17.9% as reported by Mat Amin et al. [2].

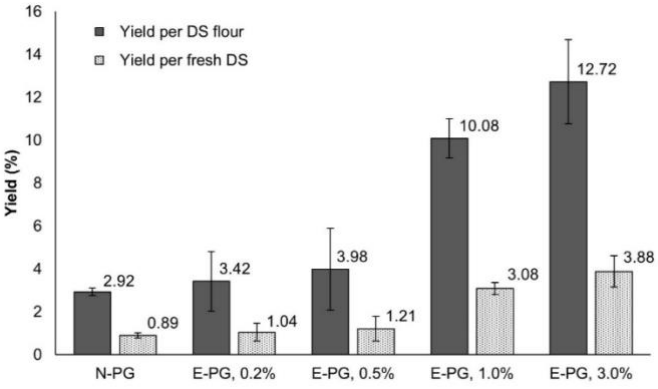

Fig. 1. Yield percentage of E-PG utilizing different percentages of cellulase during the enzymatic-assisted extraction process against N-PG as the control. Fresh DS refers to the seeds when they were initially collected and DS flour refers to the sample post-disinfection and pulverization protocols. Data expressed as mean ± SD (n = 3).

According to Wu et al. [35], extraction time and temperature play vital roles on the yield of crude gum. Operating conditions employed in the present research were subjected to the optimal conditions of cellulase for the catalysis. Amid and Mirhosseini [5] produced 56.4% yield of gum from aqueous extraction, however the protocol was conducted at high temperature (85°C) and alkaline pH (11.9) that may disrupt the quality and physicochemical attributes of the protein-polysaccharide gum. Mat Amin et al. [2] obtained 1.2% yield of air-dried and 0.5% of freeze-dried DS gum, chemically extracted and further purified via barium complexing. On similar note, Lertsiri and Kittivanichanon [36] obtained low yield of crude gum (0.05-0.21%) via chemical extraction protocol, thus the utilization of enzyme in the present work significantly improved the extraction efficiency and the yield of targeted mucilage from DS.

B. Functional and proximate analysis

Proximate compositions including total fat, total carbohydrate, total dietary fiber, total sugar and energy were found at <0.1, 74.4, 24.0, 4.0 g/100 g of sample and 290 kcal/100 g of sample, respectively. Crude protein content was found at 10.1 g/100 g of sample, higher than protein content of DS flour reported by Permatasari et al. [37] (6.26 - 7.49%) and Mulyati et al. [34] (7.73 - 9.08%). Total carbohydrate of 74.4 g/100 g was a good indicator of high polysaccharide content and the total protein may be due to the chain of amino acids. Total dietary fibre was higher than reported by Mat Amin and Arshad [11] with dehulled DS (7.7%) and fresh seeds by Srianta et al. [25]. In fact, study has reported higher total carbohydrate, total dietary fiber and total crude protein as compared with other seeds [38-39]. Vitamin D was detected at <2.0 µg/100 g of sample. Minerals of sodium, calcium, iron and potassium were 349.3, 57.4, 2.1 and 237.6 mg/100 g of sample, respectively.

C. Water-holding (WHC), oil-holding capacities (OHC) and solubility

Protein-polysaccharide backbone contributes to the viscosity or gelling ability of the dispersions. The E-PG has higher WHC at 249.33 g water/100 g flour, as compared with N-PG and DS (200.67 g water and 114.67 g water/100 g flour, respectively) (Fig. 2A). Scholars have reported that gum from aqueous extraction exhibited WHC from 139.5 to 274.0 g water/100 g flour [5], and chemical extraction at a broad range from 94.1 to 218.5 g water/100 g flour. According to Cornelia et al. [4], most amino acid constituents in DS assist water absorption. Arabic gum was reported to have WHC at 34.66 ± 0.5, GG at 200.66 ± 0.5 and XG at 1000.33 ± 0.5 [40].

OHC has demonstrated the same pattern but less absorption capacity as compared with water molecules. The OHC for E-PG, N-PG and DS was determined at 104.67 g water, 96.00 g water and 88.24 g water/100 g flour, respectively. The trace of fatty acid and hydrophobic amino acid present in the E-PG possibly had a role in its affinity towards oil absorption. Others have reported that OHC of DS gum from aqueous extraction at a range from 113.2 to 146.5 g oil/100 g flour [5].

Fig. 2B demonstrated that solubility increases with temperature, as expected. The E-PG and N-PG exhibited solubility of 58.37 ± 7.67 and 50.03 ± 8.69% at 25 ± 1°C, and 85.06 ± 8.71% and 81.76 ± 2.91% at elevated temperature (>80°C), respectively, yet still lower than commercial gums, XG

AD and GG, in the following rank order: XG AD>GG>E-PG>N-PG>DS flour. Amid and Mirhosseini [5] obtained 27.9% solubility from aqueous-extraction protocol. Findings suggested that enzymatic-assisted extraction protocol enhanced the solubility of the flour as compared with non-enzymatic protocol and corresponds to the degree of desirable solubility (>80%) advisable for the food and nutraceutical industries.

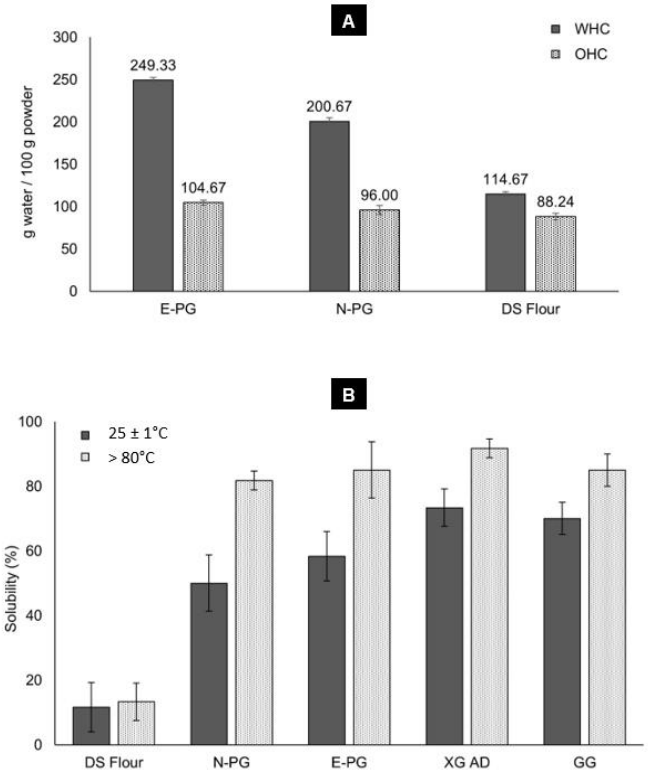

Fig. 2A. Water-holding (WHC) and oil-holding capacities (OHC) of E-PG, N-PG and DS flour. Results were expressed as g of water per 100 g of powder, and 2B. Percentage of solubility for DS flour, N-PG, E-PG, XG AD and GG at ambient (25 ± 1°C) and elevated temperature (>80°C). Data expressed as mean ± SD (n = 3).

D. Physicochemical characteristics

Viscosity of E-PG at 5 mg/mL as tabulated in **Error! Reference source not found.** was recorded in the range of 41.11±2.26 to 45.38±8.69 cP, with Brix value of 0.3 - 1.1% and pH in the range of 6.43 to 6.95.

Viscosity for E-PG was only 2.52-fold lower than XG AD (106.00±10.47 - 114.49±7.33 cP) but 7.08-fold lower than GG (108.20±2.83 - 321.47±1.08 cP). Total dissolved sugar of E-PG was comparable to XG AD. The GG is known to have larger particles thus exhibited higher viscosity in a water solution as compared with XG AD and E-PG. Physicochemical qualities of 2.52-fold E-PG were validated. E-$PG_{2.52\text{-fold}}$ has a viscosity in the range of 107.45±4.41 to 115.80±2.25 cP, closely within the range of XG AD. Brix was slightly higher (3.2 - 4.5%) but pH maintained (6.61 - 6.84). Data suggested that E-$PG_{2.52\text{-fold}}$ exhibited similar physicochemical qualities to XG AD.

TABLE I. PHYSICOCHEMICAL CHARACTERISTICS OF E-PG, XG AD, GG, AND E-PG_{SLURRY} IN DISTILLED WATER AT AMBIENT TEMPERATURE TO ELEVATED TEMPERATURE. PARAMETERS INCLUDE TOTAL DISSOLVED SUGAR AND VISCOSITY

Sample	Brix (%)[A]	Viscosity (cP)[B]	
		25±1°C	*>80°C*
E-PG	0.3 - 1.1	41.11 ± 2.26[b]	45.38 ± 8.69[c]
XG AD	0.4 - 1.1	106.00 ± 10.47[a]	114.49 ± 7.33[b]
GG	0.1 - 0.5	108.20 ± 2.83[a]	321.47 ± 1.08[a]
E-$PG_{2.52\text{-fold}}$	3.2 - 4.5	107.45 ± 4.41[a]	115.80 ± 2.25[b]

[A] Brix was reported from ambient to elevated temperature.

[B] Viscosity was expressed as mean ± SD (n = 3). Values for the measurement of viscosity with the same lowercase superscript letter within the same column are not significantly different (p > 0.05).

E. Characterization by HPLC-RID, FT-IR and NMR

The HPLC-RID chromatogram concluded that total sugar composition in DS flour was 9.83%, in which 5.33% was attributed to sucrose and the remaining 4.50% was dedicated to maltose with retention time at 14.33 and 17.33 min, respectively. The presence of fructose, galactose, glucose and lactose were insignificant (<0.5). Polysaccharides with a molecular weight of 3.765 x 10^5 Da by Xiao et al. [3] composed of rhamnose, galactose and galacturonic acid. Mat Amin et al. [2] reported the availability of glucose, D-galactose and rhamnose, with the absence of galactomannan.

Fig. 3A presented the comparison of functional groups spectra of DS flour and E-PG. The band around 3280.47 cm^{-1} indicated strong O-H stretching vibration. The stretching of the C-H bond was represented at 2930.24 cm^{-1}. The peak at 1637.84 cm^{-1} revealed the presence of C=O and N–H bond, showing the existence of protein. E-PG demonstrated higher intensity at this region as compared with DS flour.

Absorption wave with medium intensity at 1369.77 cm^{-1} indicated the presence of an alkane group with bonds between atoms C–H in the form of CH_3. The slightly different absorption peak for E-PG at 1541.84 cm^{-1} as compared with DS flour was dedicated to the presence of an alkene group with variable C=C bonds. High absorbency range between 1200–900 cm^{-1} was dedicated to the characteristic and absorption peak of polysaccharide, which was attributed to C-O-C and C-O-H link bands. This coupled with the peak at 1637.84 cm^{-1} justified the hydrocolloid properties in the sample. A study conducted by Amid et al. [17] reported that an aqueous mucilage extract of DS contain galactose (48.6-59.9%), glucose (37.1-45.1%), arabinose (0.58%-3.41%), xylose (0.3-3.21%) and 12 different essential amino acids. Absorption peaks between 800-700 cm^{-1} indicated that α- and β-configurations were available in the gum obtained from the enzymatic-assisted extraction process.

Distribution of functional groups between E-PG and N-PG were similar as shown in Fig. 3B, which indicates that the protein-polysaccharide content was not tampered during the enzymatic-assisted extraction process but improved efficiency as well as the yield. Findings from FT-IR suggested the presence of D(+)-glucose anhydrous and L(-)-glucose in abundance. The ^{1}H NMR spectroscopy analysis demonstrated that the signals were readily discernible and crowded in the region of δH 3.0-5.6 ppm, which is a typical distribution region of polysaccharides.

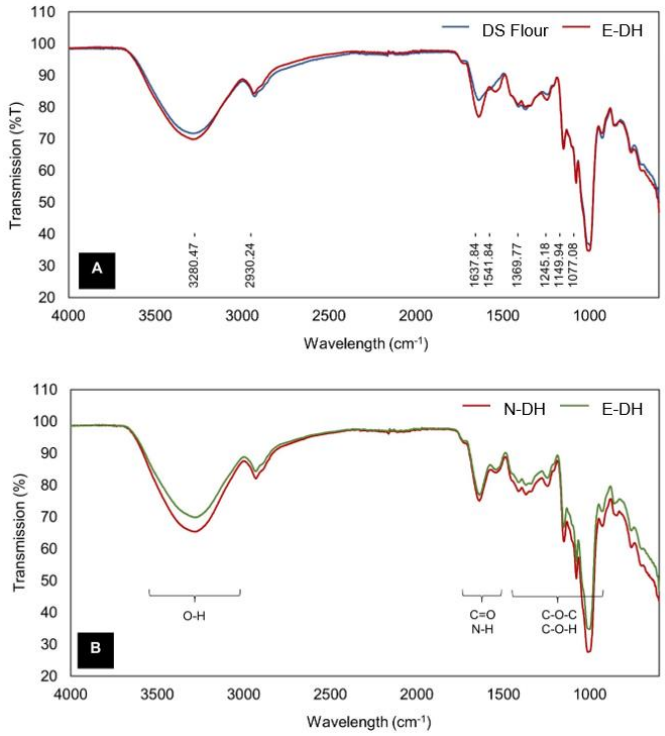

Fig. 3A. Fourier transform infrared spectrum of E-PG against DS flour, and **3B**. Spectrum of E-PG against N-PG at the wavelength 4000-600 cm^{-1}. Functional groups associated with hydrocolloid components were depicted.

F. Antioxidant evaluation of protein-polysaccharide gum

Antioxidant evaluation of E-PG, E-PG_{slurry}, XG AD and DS flour were tabulated in **Error! Reference source not found.**. It was evident that E-PG and E-PG_{slurry} scavenged $ABTS^{\bullet+}$-radicals at maximal inhibitory concentration (IC_{50}) of 5.759±0.719 mg/mL and 6.083±0.131 mg/mL, respectively. Ascorbic acid as the positive control exhibited an IC_{50} of 0.078±0.0006 mg/mL. Method of extraction via enzymatic hydrolysis probably accounts for the higher antioxidative capacities. Findings were in good agreement with Shang et al. [23] and Chen et al. [41], where antioxidant activity of enzymatic-assisted polysaccharide extracts from *Astragalus membranaceus* and *Trifolium repens* L. were higher than non-enzymatic extracts.

Peroxyl radicals are free radicals that are predominantly found during lipid oxidation under physiological conditions, both in foods and biological systems and ORAC assay monitors the inhibition of peroxyl radical–induced oxidation by measuring the capacity of antioxidant molecules to break the radical chains. Hence, ORAC values are biologically relevant as a reference indicator for antioxidant effectiveness. The ORAC analysis has shown that E-PG and E-PG_{slurry} obtained via enzymatic hydrolysis contained prominent antioxidants, with ORAC values of 57867±2873 and 70191±6291 µM TE/g, respectively against Trolox standard with correlation coefficient r^2 of 0.9941. The DS flour exhibited an ORAC value of 13800±686 µM TE/g which suggests that the enzymatic-bioprocess technology employed in the protocol is capable of extracting valuable antioxidant compounds from DS.

TABLE II. ANTIOXIDANT EVALUATION OF E-PG, E-PG_{SLURRY}, XG AD, AND DS FLOUR

Sample	Antioxidant assay[A]	
	IC_{50} for $ABTS^{\bullet+}$-radical scavenging activity (mg/mL)[B]	Oxygen radical absorbance capacity (ORAC) (µM TE/g)[C]
E-PG	5.759 ± 0.719[a]	57867 ± 2873[b]
E-PG_{slurry}	6.083 ± 0.131[a]	70191 ± 6291[a]
Ascorbic acid	0.078 ± 0.0006[b]	-
XG AD	-	ND
DS flour	-	13800 ± 686[c]

[A] ND is non-detectable. Values with the same lowercase superscript letter within the same column are not significantly different ($p > 0.05$).

[B] $ABTS^{\bullet+}$-radical scavenging activity was expressed as IC_{50}; concentration of test sample at which 50% of inhibition in its activity is achieved.

[C] ORAC was expressed as Trolox equivalent per gram of dried sample. Data are expressed as mean ± SD ($n = 3$).

The ORAC activity was not detected in the commercial gum/thickener, XG AD although Hu et al. [42] has reported antioxidant activities of a low-molecular-weight XG derived from biodegradation of the crude by the endophytic fungus. The results also concluded that E-PG and E-PG_{slurry} have much higher ORAC values as compared with reported acacia gums [43] and edible parts of durian [44]. The hydroxyl group that formed phenolic and flavonoid compounds in DS may have contributed to the antioxidative properties of E-PG and E-PG_{slurry}.

Error! Reference source not found. reviewed possible bioactives available in DS that are commonly associated with antioxidant capabilities. Classes of polyphenols have long been known as antioxidative compounds. They scavenge free radicals, reduce the level of oxidative stress, subsequently retard the harmful chains involving risks of cancer and coronary heart disease [1, 45]. Palmitic acid (16:0), stearic acid (18:0) and oleic acid (18:1) have also been reported to exhibit antioxidant properties. These bioactives might work synergistically and supported the characterization analysis via FT-IR and NMR, in which the E-PG contained valuable protein-polysaccharide hydrocolloids. Thus, DS which was normally underutilized has potential to be explored and exploited as a prospective value-added food ingredient with enhanced bioactivities due to the presence of valuable metabolites [45-50].

TABLE III. REVIEW OF POSSIBLE BIOACTIVES IN E-PG AND E-PG_{SLURRY} THAT MAY BE ACCOUNTABLE AS ANTIOXIDANT AGENT

Possible bioactives	Reference
Phytochemicals: Anthraquinones, flavonoids, saponins and terpenoids	[45]
Polyphenols: 367 ± 26.0 mg GAE/100 g dry seeds	[49]
Total phenolic content: 88.70 - 143.60 mg GAE/g Total flavonoid content: 165.80 - 193.50 mg QE/g	[50]
Fatty acids: Palmitic acid (16:0) (41.2%), stearic acid (18:0) (19.2%), oleic acid (18:1) (27.7%) and linoleic acid (18:2) (9.8%)	[36]

IV. CONCLUSION

Present work demonstrated that bioprocess technology employed was able to produce higher standardized yield of natural protein-polysaccharides gum (4.36-fold at 3.0% cellulase) from DS with enhanced organoleptic properties. Percentage of yield increased directly proportional to the percentage of cellulase. Composition analysis and

characterization of E-PG revealed the presence of hydrocolloid components. Physicochemical validation revealed that E-PG at 2.52-fold exhibited similar qualities to XG AD. Enzymatic-extract of DS containing water-soluble protein-polysaccharides exhibited antioxidant activity, both in its flour granules (E-PG) as well as its water-seed slurry (E-PG_{slurry}). Hence, tapping on the nutraceutical potential of DS in the present socio-economic scenario as a value-added food ingredient with the presence of hydrocolloids as alternative to commercial gums can benefit the food and nutraceutical industries.

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