Research article

Isolation, structural features, in vitro antioxidant activity and assessment of complexation ability with β-lactoglobulin of a polysaccharide from *Borassus flabellifer* fruit

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**A R T I C L E   I N F O**

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**A B S T R A C T**

This research was intended to investigate the structural feature, antioxidative activity and interaction with β-lactoglobulin (β-lg) of a polysaccharide (P) isolated from *Borassus flabellifer* fruit thru aqueous extraction, protein elimination and chromatographic techniques. Polysaccharide P (molecular weight: 21,000 g mol⁻¹) was constituted of arabinose, galactose, glucose, and rhamnose in a 50:24:20:6 M ratio alongside 9% (w/w) galacturonic acid. It encompassed a petite backbone entailing galacturonopyranosyl and rhamnopranosyl units substituted with sizable side chains comprising of arabinofuranosyl, galactopyranosyl and esterified coumaric acid (CA) residues. Various series of oligosaccharides including (i) Gal1,2,4,9Ac5-29, (ii) Ara2-3Ac6-8, (iii) Gal3Ara1-3Ac13-17, (iv) Gal4-6Ara2Ac18-24, (v) GalAra1Ac22 and (vi) GalAra2Ca1Ac7 and GalAra3Ca1Ac9 epitomizing polysaccharide structure were generated and characterised. Fraction P exhibited dose-dependent antioxidant activity and possessed a strong β-lactoglobulin binding capability. Accordingly, *B. flabellifer* fruit offers an antioxidant polysaccharide having novel structure that can associate with β-lg and, hence, useful in formulating novel food possessing adjustable composition.

**1. Introduction**

Polysaccharides/proteins alongside their function as building blocks also influence taste, flavour as well as nourishment in the physical world (Jones et al., 2011). Complex formation reaction engaging these polymers is beneficial not only for their participation in diverse bioprocess, but also because they encounter several industrial usages. For example, food-processing industry utilized complexation reaction in beverages, microencapsulation, etc (Dickinson, 2003; Guzey and McClements, 2007; Laurent and Boulenguer, 2003; Schmitt et al., 1998). Low cost, accessibility, sustainability, etability and consumer preference for natural products rendered biopolymers suitable raw material for formulating functional food (Mezzenga et al., 2005). Consequently, production of unique polysaccharide capable of forming water soluble complex with protein should be tempting as it enhances their probable uses.

Antioxidants, whether natural or synthetic in origin, are additives that conserve food and counteract oxidative deterioration during storage and processing (Wilson et al., 2017). However, the possible carcinogenic properties of synthetic antioxidant molecules make them undesirable in food industry. Of late, enormous attempts are being made to isolate molecules possessing antioxidant activity from natural resources. Epidemiological body of knowledge suggests that food flourished in vegetables along with fruits, as a result of the existence of varieties of bioactives, are beneficial for human health (Kris-Etherton et al., 2002; Michels et al., 2000). Albeit information on bioactive molecules from vegetables/fruits of tropical zones are lacking, current reports show that these commodities are filled with bioactives as well (Can-Cauich et al., 2017; Mazumdar et al., 2019). *Borassus flabellifer* (Palmyra), also known as palmyra palm, is well-known for its distinctive odour, tastes and culinary uses. For instance, local people enjoyed the soft orange fruit-pulp raw; otherwise cooked with milk or else baked after blending with wheat flour, ripe banana and grated coconut. Also, extracts of this fruit possess several beneficial effects including antioxidative activity (Agostini-Costa, 2018; Lindblad et al., 2007). Thus far, secondary metabolites have only been chemically characterized from this fruit (Agostini-Costa, 2018; Lindblad et al., 2007). Even though polysaccharides demonstrate various bioactivities (Ghosh et al., 2009, 2013; Ghosh et al., 2015; Lindblad et al., 2007; Mantovani et al., 2008; Nergard et al., 2006;...

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2. Materials and methods

2.1. Plant resources

Ripened fruits of *Borassus flabellifer* (1.8–3.1 kg) from ‘Garden of Medicinal Plants’, The University of Burdwan (www.buruniv.ac.in) were thoroughly washed (H₂O). Subsequently, the orange coloured fruit pulp was taken apart from the peel and stone using a scalpel. An aliquot of 9 fruits was randomly selected which had a total weight of 21.6 kg; a yield of 9.1 kg of pulp was obtained. In the first of a series of experiments, a portion (500 g) of the pulp was then diluted with 3 L of chilled ethanol. After centrifugation (10 000 g, 20 min), the collected precipitate was solubilized (H₂O, 100 mL), the solution filtered (G3 glass frit) and then lyophilized to yield the alcohol insoluble residue (AIR, 30 g). This process of preparation of the alcohol insoluble residue was repeated (∗5).

2.2. Extraction and purification

The resulting AIR (100 g) was stirred with H₂O (2 L) at 100 °C for 2 h. Following filtration (fritted glass, G-2), the resulting residue was subsequently extracted twice more under identical conditions. Following mixing, the volume of the combined extracts was reduced (Eyela N-1100 Rotary Evaporator), and the concentrated solution lyophilised (ScanVac Cool Safe 55-F freeze drier) to produce the water extract (WE; 11.2 g) containing, inter alia, polysaccharides. Afterwards, an aqueous solution of NaOAc buffer eluted material was dialyzed, concentrated and, at last, lyophilized to yield a purified polysaccharide named as P (0.305 g).

2.3. Analytical methods

The general experimental conditions were as per (Ray et al., 2013).

2.4. High performance size exclusion chromatography (HPSEC)

The molecular weight of polysaccharide (P) was evaluated using a HPSEC system as said by Ghosh et al. (2015).

2.5. Saccharide analysis

The PhOH–H₂SO₄ assay (DuBois et al., 1956) was employed to estimate the quantity of saccharide colorimetrically by assessing the absorbance at λ_max = 490 nm using galactose as reference standard, whereas quantification of uronic acid was performed by 3-phenylphenol colour reagent (Ahmed and Labavitch, 1977) utilising galacturonic acid as standard. GC in addition to GC-MS investigation of alditol acetates generated from oligo- and polysaccharides analysed according to Blakeney et al. (1983) provides neutral saccharide composition. The saccharide makeup was determined as well by chromatographic analysis of the per-O-methyl-silylated derivative of the methyl glycosides (York et al., 1986). The working condition used for GC and GC–MS analyses were as presented beforehand (Ghosh et al., 2019).

2.6. Phenolic analysis

Assessment of the phenolic content was done using the Folin–Ciocalteu reagent as reported (Payet et al., 2006). For determining the composition of the ester-linked phenolic acids, the polymer (P, 50 mg) was hydrolysed with 1 mL NaOH (0.1 N, under N₂) solution (24 h, 32–37 °C). A solution of 2,3,5-trimethoxy-(E)-cinnamic acid, employed as internal standard, was inserted into the hydrolysate prior to adjustment of the pH to 2 (N HCl). Finally, the EtOH extracted phenolic acids were investigated by a RP-HPLC system as described earlier (Ghosh et al., 2013).

2.7. Starch assessment

The estimation of starch was executed according to McCleary et al. (1994) as communicated by Bern et al. (2016).

2.8. Linkage analysis

Ahead of methylation, the galacturonic acid unit of fraction P was reduced to -CH₂OH as per (Kim and Carpita, 1992). Afterward the parental polysaccharide fraction (P) along with its carboxyl reduced derivative (PR) were per-O-methylated independently according to Blakeney and Stone (1985). Next, the glycosidic linkages of both per-O-methylated polysaccharides were hydrolysed (2.5 M TFA, 120 °C, 75 min) and the released methylated saccharides were reduced (NaBD₄, 4 h, 33–40 °C) and then per-O-acetylated using perchloric acid catalysed acetylation with Ac₂O. The partially methylated alditol acetates (PMAAs) were subsequently investigated with GC plus GC–MS on columns of DB-225 (J&W Scientific, Folsom, CA, USA) and 3% SP-2340 on Supelcoport 100–120 mesh. The temperature programme for these columns were as stated beforehand (Ray et al., 2013) and (Carpita and Shen, 1988), respectively.

2.9. Enzymatic hydrolysis

The polysaccharide (32 mg) solution (2 mL) in 100 mM sodium acetate (NaOAc) buffer (pH 5.5) was digested (33–40 °C) using a purified enzyme (1.5 mL) as said beforehand (Ghosh et al., 2013). Afterwards, the resultant mixture was boiled (97 °C, 0.5 h), cooled and precipitated with ice-cold EtOH (10 mL). Succeeding centrifugation, the ethanol soluble oligomeric fraction was lyophilised (P₉₀). Acetylation of a fraction of these oligosaccharides with acetic anhydride using N-methylimidazole as catalyst followed by partition with CHCl₃/H₂O (1:1:v:v) provided acetylated oligosaccharides (denoted as “O”). UV-visible spectroscopic, thin layer chromatographic and fluorescence study of these acetylated...
3. Results and discussion

3.1. Extraction, purification and chemical constitution

The fruit pulp (AIR), gotten in 6% yield from the orange coloured pulp of ripened B. flabellifer fruit, possessed 51% (w/w) saccharides out of which around 5% was uronic acid (UA). Aqueous extraction of AIR produces a fraction (named as WE, yield 11.2%) comprised 57% (w/w) of which around 5% was uronic acid (UA). Aqueous extraction of AIR was investigated as well with ESI-MS. Furthermore, aldoses liberated from P_9 with CF_3CO_2H were converted into their alditol acetate derivatives and analysed.

3.2. Interaction of polysaccharide with β-lg

To study the polysaccharide-protein interaction uv-visible spectrophotometrically, absorption spectra of a solution (0.75 g L\(^{-1}\)) of β-lg alone and in the presence of 0.0125–0.3 g L\(^{-1}\) polysaccharide (P) in a phosphate (0.1 M) buffer (pH 7.4), was recorded at 25 °C. The hydroxyl protons of polysaccharide sample (10 mg) were replaced with deuterium using 99.9% D\(_2\)O (Sigma Aldrich) and this process repeated thrice to diminish the signal residual proton. This deuterium exchanged sample was dissolved in 500 μL D\(_2\)O for subsequent proton NMR measurement. The electrospray ionization mass spectrum of per-O-acetylated oligomers (O) was acquired by means of a QTOF 60 Micro YA 263 mass spectrometer.

3.3. DPPH radical scavenging assay

The quantification of in vitro antioxidative activity of polysaccharide (P) utilizing DPPH radicals was accomplished following Ghosh et al. (2013). Concisely, a portion of the polysaccharide (P) solution in the concentration range 0.03–3.0 mg mL\(^{-1}\) and positive controls (BHA and BHT) solution each was mixed individually with fresh DPPH radical. Previous to recording the absorbance at 515 nm the reaction mixture was wobbled and stored in absence of light for a period of 1 h at 30 °C.

Figure 1. (A) Total ion chromatogram of alditol acetates derived from B. flabellifer fruit polysaccharide (P) and the electron impact mass spectra of (B) pent-O-acetyl arabinitol, (C) hexa-O-acetylgalactitol and (D) hexa-O-ace-tylxyloinositol, the internal standard. In the chromatogram (A) numerical values of the X-axis are in minutes.

with and therefore, is a glucan. The amount of starch in first fraction was 63% (w/w) as assessed as per McIerley et al. (1994). Then again, the key fraction (P), eluted with 0.5 M NaOAc buffer, comprised of 47% of the sugars eluted from the anion-exchanger. It contained of 74% (w/w) saccharides incorporating 9% (w/w) UA. GC-MS study of alditol per-O-acetates (Figure 1), resulting through hydrolysis with acid, reduction and acetylation of P indicated the existence of arabinose, galactose, glucose, and rhamnose in a 50:24:20:6 M ratio. GC study of the O-tri-methylsilyl methyl glycoside derivatives of P by usual procedure as well as TLC examination of CF_3CO_2H released saccharides confirmed the existence of galacturonic acid. Fraction P, the key polysaccharide, was analysed further. This polymer showed UV absorption bands (228 nm and 281 nm) and displayed fluorescence emissions at 373 nm (λ\(_{em}\)) idiosyncratic of phenolic acid. The phenol content of fraction P was 53 GAE g \(^{-1}\). As per RPHPLC analysis of the released phenolic acids, the chief constituent was (E)-p-coumaric acid (1.7 μg mL\(^{-1}\)). Other constituents include ferulic acid (0.1 μg mL\(^{-1}\)), and trace amounts of 8,5'-benzofuran-diferulic acid, 8,5'-diferulic acid and 8-O'-4'-diferulic acid.

Figure 1.
3.2. Molecular weight

Fraction P was further characterized using HPSEC-MALLS and only one population was detected on the basis of light scattering, differential refractometer, and UV detections. The molecular weight was determined to be 21,000 g mol⁻¹.

3.3. Fourier-transforms infrared (FTIR) analysis

The FT-IR spectrum of P exhibited peaks between 1000 and 1150 cm⁻¹ characteristic for polysaccharide skeletons (Kacurakova et al., 2000). The band ~1734 cm⁻¹ corresponding to the C=O stretching vibration of esters was also detected (Figure 2A). A weak band ~2931 cm⁻¹ resulting from C-H stretching vibrations as well as the one between 3000 and 3600 cm⁻¹ (str) related to O-H stretching vibration were present as well.

3.4. Glycosidic linkage pattern analysis

GC-MS investigation of PMMAs derived from P fraction demonstrated the presence of Ara and Galp residues in nonreducing ends indicative of a polysaccharide having branch structure (Table 1). The residual Ara residues were 1,2-, 1,5-, and 1,3,5-linked and are present in furanoid form. On the other hand, the Galp units were present in pyranose ring, and, primarily, 1,3-, 1,6-, and 1,3,6-linked. Linkage pattern analysis of PR, the carboxyl (-CO₂⁻) reduced derivative of P, demonstrated a rise in the 1,4,5-tri-O-acetyl-2,3,6-tri-O-methylgalactitol content implying that GalpA residue was 1,4-linked. The existence of variously linked Rhap units, a constituent of RG I was observed as well in the methylation product of PR. The relatively low Rha content in addition to the preponderance of Ara in addition to Gal units suggest that the petite RG I backbone of the investigated polymer comprised of large side-chain encompassing arabinose along with galactose units.

3.5. NMR analysis

Assignment of ¹H NMR signals of P were done by taking into account the saccharide composition and glycosidic linkage pattern of the polymer, and literature data (Azadi, O'Neill, Bergmann, Darvill and Albersheim, 1995; Capek et al., 2010; Ghosh et al., 2013; Samuelsen et al., 2007; Sims and Furneaux, 2003). As expected, the spectrum (Figure 2B) was complicated, comprising several anumeric signals (δ 4.3–5.3) and ring protons (δ 3.3–4.3). The signals in the δ 5.0–5.3 region are typical of H1 of differently linked α-Araf units (Sims and Furneaux, 2003), while signals around δ 4.3–4.5 originate from H1 of the variously-linked β-Galp units. The remaining signal in the H1 region around 4.95 was attributed to Gal residues (Renard et al., 1999). Since P was mainly made up of arabinose (50%) and galactose (24%) residues, their signals can unmistakably be identified. Signals typical of ring protons are distinctly visible too between 3.1 and 4.3 ppm.

3.6. Enzyme produced oligosaccharides comprised esterified coumaric acid (CA)

Oligosaccharides generated by a purified enzyme preparation as per Ghosh et al. (2013) were isolated, acetylated and then analysed. These oligosaccharides designated as “O” contained Gal and Ara residues as neutral saccharide. Their electrospray ionization (ESI) mass spectrum demonstrated the occurrence of various fragments (Figure 3). In keeping with the molecular masses as well as saccharide composition, sodium adducts at various m/z values were assigned largely to five series of oligosaccharides: (i) Ara₂₃Ac₆₈, (ii) Gal₁₂₄₄₅₂⁰, (iii) Gal₃Ara₁₃₁₇, (iv) Gal₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆₆ąc

Figure 2. (A) Fourier-transform infrared (FTIR) and (B) ¹H-NMR (400 MHz, D₂O) spectra of B. flabellifer fruit polysaccharide (P).
Notably, three different batches of polysaccharide (P) exhibited the activity reliably. At 1.5 mg mL\(^{-1}\), fraction P scavenged 84.7% of the DPPH radical. This value for P paralleled to 89.9% and 89.3% of BHA and BHT activity, respectively. Incidentally, several naturally occurring polysaccharides (Ghosh et al., 2013; Ghosh et al., 2015; Mirzadeh et al., 2019) and their derivatives (Chen and Huang, 2019; Liu and Huang, 2019) possess antioxidant activity. Palmyra encompasses multitude of phytochemicals.

| O-methyl alditol acetatesa | Deduced linkages | RRT DB-225b | RRT SP-2340c | Mole percentd |
|---------------------------|-----------------|-------------|--------------|--------------|
| 2,3,5-Me\(_3\)-Ara | T-Ara\(_2\) | 0.616 | 0.571 | 13 | PR |
| 3,4-Me\(_2\)-Ara | 1,2-Ara\(_2\) | 0.899 | 0.86 | 1 | 1 |
| 2,3-Me\(_2\)-Ara | 1,5-Ara\(_2\) | 1.015 | 0.939 | 25 | 21 |
| 2-Me-Ara | 1,3,5-Ara\(_3\) | 1.207 | 1.532 | 15 | 13 |
| 2,3,4-Me\(_3\)-Rha | T-Rha\(_3\) | 0.628 | 0.524 | 1 | 1 |
| 3,4-Me\(_2\)-Rha | 1,2-Rha\(_2\) | 0.932 | 0.857 | 2 | 2 |
| 2,4-Me\(_2\)-Rha | 1,3-Rha\(_2\) | 0.960 | 0.873 | 2 | 1 |
| 3-Me-Rha | 1,2,4-Rha\(_3\) | 1.176 | 1.318 | 1 | 2 |
| 2,3,4,6-Me\(_4\)-Gal | T-Gal\(_4\) | 1.000 | 1.000 | 1 | 1 |
| 2,4,6-Me\(_3\)-Gal | 1,3-Gal\(_3\) | 1.239 | 1.369 | 11 | 12 |
| 2,3,4,6-Me\(_4\)-Gal | 1,4-Gal\(_4\) | 1.349 | 1.737 | 7 | 7 |
| 2,3,6-Me\(_3\)-Gal | 1,4-Gal\(_4\) | 1.263 | 1.518 | 0 | 8 |
| 2,4-Me\(_2\)-Gal | 1,3,6-Gal\(_3\) | 1.540 | 2.225 | 8 | 9 |
| 2,3,6-Me\(_2\)-Glc | 1,4-Glc\(_4\) | 1.279 | 1.553 | 10 | 9 |
| 2,3-Me\(_2\)-Glc | 1,4,6-Glc\(_4\) | 1.475 | 2.151 | 3 | 3 |

\(^a\) 2,3,5-Me\(_3\)-Ara denotes 1,4-di-O-acetyl-2,3,5-tri-O-methylarabinitol, etc. RRT: Relative retention time.
\(^b\) RRT values as obtained from DB-225 column.
\(^c\) RRT values as obtained from SP-2340 column.
\(^d\) Mole percent of the identified peaks.

Figure 3. (A) Electrospray ionisation-mass spectral analyses of oligomers generated from B. flabellifer fruit polysaccharide (P) by enzyme digestion. (B): Expansion of m/z 1200–2900 region.

Figure 4. (A) Reaction of B. flabellifer fruit polysaccharide (P) with DPPH radical. This reaction scheme is not intended to be representative of the full sample composition. (B) In vitro scavenging activities of BHA, BHT in addition to B. flabellifer fruit polysaccharide (P) towards DPPH radicals.
comprising of coumaric acid (CA), a compound known to have antioxidant property (Fukumoto and Mazza, 2000). Notably, the purified P too contained CA although in small quantity. Reports linking up chemical profile of polysaccharides with its bioactivity underlined the significance of chains attached to the hairy domain for potency (Inngjerdingen et al., 2006; Nergard et al., 2006; Samuelsen et al., 2007). Particularly, sizable neutral side chains holding vast extents of (1,3), (1,6)- and (1,3,6)-linked galactose units are important (Westereng et al., 2009). In a separate investigation, Ghosh and workers, stressed the influence of esterified phenolic acids on antioxidant potency (Ghosh et al., 2013). In view of the existence of important neutral side chains in polysaccharide P, this domain can as well be the active site. Likewise, coumaric acid can as well reduce free radicals by self-oxidation (Figure 4).

3.8. Water-soluble complex formation between this polysaccharide and β-lactoglobulin

The UV-visible absorption spectrum of β-lg displays peaks at values ~222 nm and 277 nm. By incessant incorporation of polysaccharide P solution into β-lg, the intensity of the 222 nm peak diminished (Figure 5A) and concurrently the maximum absorption wavelength (λmax) headed for longer wavelength (+9 nm) at pH 7.4. Spectral alterations like these can be accredited to association between P and β-lg. This type of complexation that happened at value greater than the isoelectric point of β-lg, pl 5.1, can be ascribed (Krebts et al., 2005; Sawyer et al., 1998) to the electrostatic interaction amongst +vely charged domain of β-lg and −vely charged acidic polysaccharide. The consequences of charge heterogeneousness during protein-polyelectrolyte complex formation has been testified previously (Park et al., 1992).

In normal form, β-lg exhibited fluorescence at λex of 340 nm once incited at λem of 277 nm. Remarkably, the intrinsic fluorescence emission of β-lg (Figure 5B) was extinguished at pH 7.4 by raising the concentration of polymer (P). A binding of polymer molecule to β-lg induces changes in its conformation and consequently its fluorescence. This outcome is compatible with the information acquired from UV measurement. In accordance with the modified form of Stern-Volmer eq, the association constant (K) of the P–β-lg combination was found to be 2.48 × 10^5 M^-1.

4. Conclusions

Results presented herein emphasize some unique features of B. flabelifera fruit polysaccharide (P) linking its structure and properties. In conjunction with the existence of two oligosaccharides linked to coumaric acid via ester bond; this polysaccharide (21000 g mol^-1) comprised a small backbone substituted with sizeable side chains seldom found in erstwhile naturally occurring polysaccharides. Nineteen oligomers representing polysaccharide fine-structure were created and later characterized. The DPPH radical scavenging factor of this polymer that exhibits dose dependency, is comparable to BHA and BHT, the synthetic antioxidants used as reference molecules. The decidedly branched side chains coupled with phenolic acid residues be the active region. Lastly, of water-soluble complex formation with β-lg improves the ability of this polysaccharide in devising innovative functional food having adaptable morphology.

Declarations

Author contribution statement

Subrata Jana, Shuvam Mukherjee: Performed the experiments; Analyzed and interpreted the data.
Imran Ali: Performed the experiments.
Bimalendu Ray: Conceived and designed the experiments; Analyzed and interpreted the data.
Sayani Ray: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.
Declaration of interests statement
The authors declare no conflict of interest.

Additional information
No additional information is available for this paper.

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