Heliyon 6 (2020) e05499

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

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

Subrata Jana [1], Shuvam Mukherjee [1], Imran Ali, Bimalendu Ray, Sayani Ray [*]

Natural Products Laboratory, Department of Chemistry, The University of Burdwan, Golapbag, Burdwan, West Bengal 713 104, India

ARTICLE INFO

Keywords:
Food science
Borassus flabellifer fruit
Polysaccharide
Oligosaccharides
Characterisation
Antioxidant activity
Complexation
β-Lactoglobulin

ABSTRACT

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 rhamnopyranosyl units substituted with sizable side chains comprising of arabinofuranosyl, galactopyranosyl and esterified coumaric acid (CA) residues. Various series of oligosaccharides including (i) $Gal_{1,2,4\text{-}9}Ac_{5\text{-}29}$, (ii) $Ara_{2\text{-}3}Ac_{6\text{-}8}$, (iii) $Gal_3Ara_{1\text{-}3}Ac_{13\text{-}17}$, (iv) $Gal_{4\text{-}6}Ara_2Ac_{18\text{-}24}$, (v) $Gal_6Ara_1Ac_{22}$ and (vi) $Gal_1Ara_2CA_1Ac_7$ and $Gal_1Ara_3CA_1Ac_9$ 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 antioxidative 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 antioxidant 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;

* Corresponding author.
E-mail address: sray@chem.buruniv.ac.in (S. Ray).
[1] These authors equally contributed to this work.

https://doi.org/10.1016/j.heliyon.2020.e05499
Received 27 August 2020; Received in revised form 24 September 2020; Accepted 9 November 2020

Smestad Paulsen, 2002; Wijewardana et al., 2016), exploration on *B. flabellifer* fruit polysaccharide is meagre.

The goals of this study are to isolate a polysaccharide (**P**) from *B. flabellifer* fruit, and then determine its chemical profile and antioxidant activity. It was postulated that polysaccharide **P** will be gifted with antioxidant activity and be able to interact with β-lactoglobulin (β-lg). Provided right in that case this polymer can function as "triple agent" in the production of foodstuffs. Along with the its traditional role of as emulsifiers, thickeners, stabilizers, etc., this polysaccharide also serves as dietary antioxidant and as a protein stabilizer. Hence, the DPPH radical scavenging factor of this polymer was also evaluated. As well, the interaction between **P** and β-lg has been explored to display the impact of polymer concentration on complexation. Apropos, this animal protein is utilized in several functional foods (Kinsella and Whitehead, 1989; Liang et al., 2008). Overall, the uniqueness of this research leans with the unusual structural elements of *B. flabellifer* fruit polysaccharide and its possible multifarious functions.

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 (H_2O). 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 (H_2O, 100 mL), the solution filtered (G3 glass filter) 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 H_2O (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 WE (200 mL) was purified by precipitation with EtOH (800 mL) and the precipitate separated by centrifugation (10,000 × g, 0.5 h). This procedure i.e., dissolution in H_2O and ethanol precipitation repeated twice more. Thereafter, an aqueous solution of resultant precipitate was chilled (less than 4 °C) and subsequently mixed with chilled aqueous 10% CCl_3CO_2H solution for the removal of protein. Following centrifugation, the pH of supernatant was set to 6.0 by gradual addition of 2% NaOH solution. The resultant was then dialyzed (dialysis tubing, 12 kDa MWCO, Sigma Aldrich, Steinheim) extensively against water with unremitting stirring, reduced under vacuum and freeze-dried. The lyophilised product was purified once again by anion exchange chromatography. In brief, a 100 mL (0.05 M NaOAc, pH 5.5) solution of the lyophilized material was injected to a DEAE Sepharose Fast Flow ion-exchanger (2.6 cm × 30 cm). Thereafter, the column was eluted sequentially with NaOAc (0.05M, 0.5M, 2M) buffer (pH 5.5) at the flow rate of 60 mL h^{-1} in a step wise manner. The remaining attached polysaccharides were eluted from the anion exchanger using 0.2 N NaOH solution. Afterwards, the 0.5 M 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−H_2SO_4 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 gas 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., 2013).

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 N_2) 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 (2 N HCl). Finally, the Et_2O extracted phenolic acids were investigated by a RPHPLC 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 Bera et al. (2016).

2.8. Linkage analysis

Ahead of methylation, the galacturonic acid unit of fraction **P** was reduced to $-CH_2OH$ 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 ($NaBD_4$, 4 h, 33–40 °C) and then per-*O*-acetylated using perchloric acid catalysed acetylation with Ac_2O. 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 Shea, 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 ($\mathbf{P}_{OSE}$). Acetylation of a fraction of these oligosaccharides with acetic anhydride using N-methylimidazole as catalyst followed by partition with $CHCl_3$:H_2O (1:1::V:V) provided acetylated oligosaccharides (denoted as "**O**"). UV–visible spectroscopic, thin layer chromatographic and fluorescence study of these acetylated

oligosaccharides were carried out. TLC was made on kieselgel 60F plate (Merck) using methanol: dichloromethane (1:9::V:V) as mobile phase and detection was done utilizing ultraviolet light (λ_{max} = 366 nm). Fraction **O** was investigated as well with ESI-MS. Furthermore, aldoses liberated from $\mathbf{P}_{OSE}$ with CF_3CO_2H were converted into their alditol acetate derivatives and analysed.

2.10. Spectroscopic analysis

A FT-IR Spectrum RX 1 (PerkinElmer) was employed to acquire the Fourier Transform−Infrared spectrum in the frequency scale of 400–4000 cm^{-1}. For analysis, lyophilised polysaccharide (**P**) ground with KBr (1:20) was utilised for pellet assembling. Ultraviolet-visible and fluorescence spectra were acquired on Shimadzu UV-2450 and Hitachi F−7000 spectrophotometer, respectively. The ^1H NMR spectrum of fraction **P** was obtained via a Varian 400MR spectrometer at 25 °C. The hydroxylic protons of polysaccharide sample (10 mg) were replaced with deuterium using 99.9% D_2O (Sigma Aldrich) and this process repeated thrice to diminish the signal residual proton. This deuterium exchanged sample was dissolved in 500 μL D_2O 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.

2.11. 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.

2.12. Interaction of polysaccharide with β-lg

To study the polysaccharide-protein interaction uv-visible spectroscopically, absorption spectra of 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. UV–Vis spectra of **P** (0.0125, 0.025, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3 g L^{-1}) were also acquired using analogous conditions for omitting spectral inferences on the β-lg−**P** system.

Fluorometric analysis was made as per (Ghosh et al., 2013). Succinctly, inherent fluorescence of β-lg (0.75 g L^{-1}) lone and in presence of **P** (0.025, 0.05, 0.1, 0.2, 0.3, 0.5, 0.6 g L^{-1}) phosphate (0.1 M) buffer (pH 7.4) was recorded. To eradicate the interference of **P** on **P**−β-lg system, the emission spectra of **P** in the aforesaid span of concentration were also measured. Utilising the modified form of Stern–Volmer eq., we have calculated the association constant (K) of **P**−β-lg complex.

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) saccharides containing 7% (w/w) UA. Fraction WE comprised of arabinose, galactose, glucose, mannose, rhamnose and xylose residues in a 42:22:27:2:6:1 M proportion. Following purification through ethanol precipitation and exclusion of protein thru CCl_3CO_2H treatment, this fraction was further purified with a DEAE-Sepharose Fast Flow anion-exchanger. The first fraction, which consisted principally of glucose unit, contained 9% of the total material recovered from the exchanger with and therefore, is a glucan. The amount of starch in first fraction was 63% (w/w) as assessed as per McCleary 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*-trimethylsilyl 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.

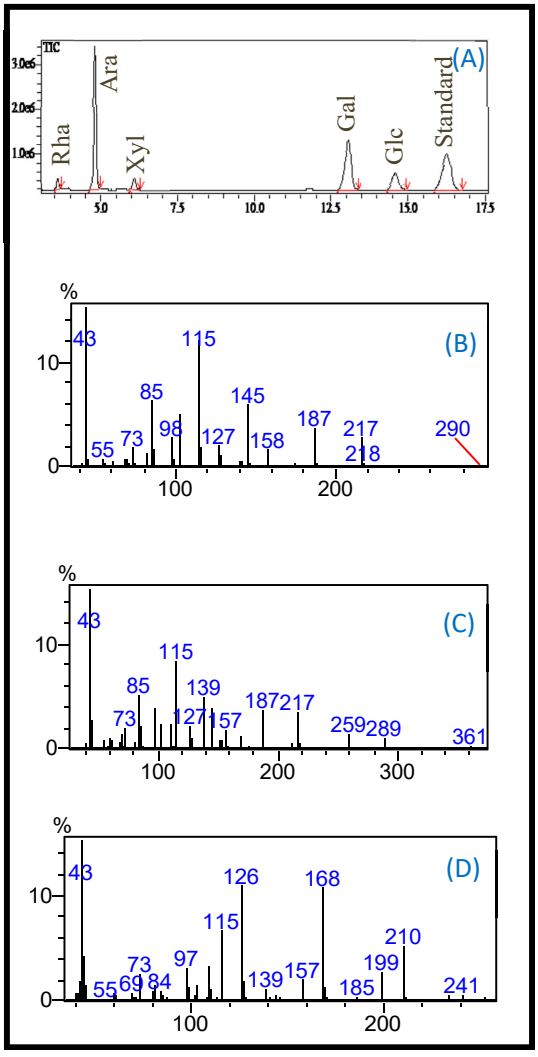

Figure 1. (A) Total ion chromatogram of alditiol acetates derived from *B. flabellifer* fruit polysaccharide (**P**) and the electron impact mass spectra of (B) penta-*O*-acetylarabinitol, (C) hexa-*O*-acetylgalactitol and (D) hexa-*O*-acetylmyoinositol, the internal standard. In the chromatogram (A) numerical values of the X-axis are in minutes.

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*f* and Gal*p* 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 Gal*p* units were present in pyranose ring, and, primarily, 1,3-, 1,6-, and 1,3,6-linked. Linkage pattern analysis of **PR**, the carboxyl ($-CO_2^-$) reduced derivative of **P**, demonstrated a rise in the 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylgalactitol content implying that Gal*p*A residue was 1,4-linked. The existence of variously linked Rha*p* 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.

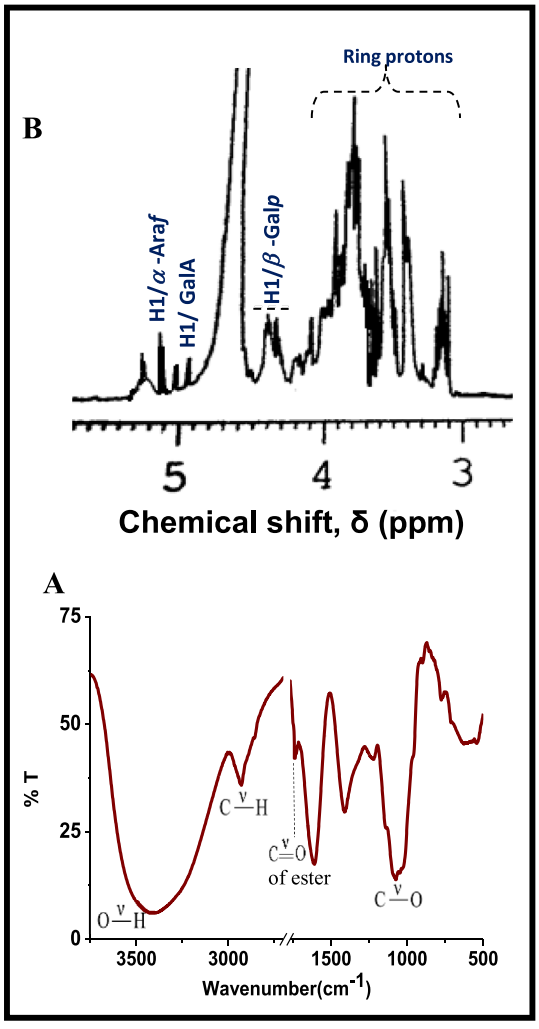

Figure 2. (A) Fourier-transform infrared (FTIR) and (B) ^{1}H-NMR (400 MHz, D_2O) spectra of *B. flabellifer* fruit polysaccharide (**P**).

3.5. NMR analysis

Assignment of ^{1}H 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 anomeric 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-Ara*f* units (Sims and Furneaux, 2003), while signals around δ 4.3–4.5 originate from H1 of the variously-linked β-Gal*p* units. The remaining signal in the H1 region around 4.95 was attributed to GalA 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) $Ara_{2\text{-}3}Ac_{6\text{-}8}$, (ii) $Gal_{1,2,4\text{-}9}Ac_{5\text{-}29}$, (iii) $Gal_3Ara_{1\text{-}3}Ac_{13\text{-}17}$, (iv) $Gal_{4\text{-}6}Ara_2Ac_{18\text{-}24}$, and (v) $Gal_6Ara_1Ac_{22}$. The initial series of oligomers ($Gal_{1,2,4\text{-}9}Ac_{5\text{-}29}$) implied the occurrence of a Gal comprising chunk, whereas the following series ($Ara_{2\text{-}3}Ac_{6\text{-}8}$) afforded indication for Ara encompassing block. Remarkably, the existence of $Gal_3Ara_{1\text{-}3}Ac_{13\text{-}17}$, (iv) $Gal_{4\text{-}6}Ara_2Ac_{18\text{-}24}$, and (v) $Gal_6Ara_1Ac_{22}$ offered testimony corroborating the co-existence of arabinose and galactose residues in single polymer. Apropos, these *O*-acetylated oligomers (**O**) displayed fluorescence emission (λ_{em}) around 370 nm, exhibited UV absorption (229 and 282 nm) and exposed fluorescent patch on TLC plate typical of phenolic acids (Fry, 1982). Collectively, pseudo- molecular ion at m/z 990 and 1206 may attributed to $Gal_1Ara_2CA_1Ac_7$ (CA, coumaric acid) and $Gal_1Ara_3CA_1Ac_9$. Finally, they afforded straight proof, for the first time, for the existence of oligomers containing galactose, arabinose and coumaric acid units. In particular, these *O*-acetylated oligomers afford a sign of the complicated finer structure of the side chain. As the apparent molecular weight of **P** is 21000 g mol^{-1} and because it comprised small amounts rhamnose comparing with the amount of arabinose and galactose residues, the petite rhamnogalacturonan I domain of this polysaccharide is definitely substituted with large side-chains. Overall, polymer **P** exhibited typical structural characteristics barely ever observed in any previous polysaccharides.

3.7. Polysaccharide P displayed dose dependent DPPH radical scavenging activity

Whilst several preceding reports revealed biological activities of polysaccharides, results of the present report too showed dose-dependent radical scavenging capacity of polysaccharide **P** (Figure 4) at 0.03–3.0 mg mL^{-1}. Highest scavenging capacity was attained at 1.5 mg mL^{-1}.

Table 1. Partially *O*-methylated alditol acetates obtained by glycosidic linkage analysis of *B. flabellifer* fruit polysaccharide (P) and its carboxyl reduced derivative (PR).

O-methyl alditol acetates[a]	Deduced linkages	RRT DB-225[b]	RRT SP-2340[c]	Mole percent[d] P	Mole percent[d] PR
2,3,5-Me_3-Ara	T-Ara_f	0.616	0.571	13	11
3,4-Me_2-Ara	1,2-Ara_f	0.899	0.86	1	1
2,3-Me_2-Ara	1,5-Ara_f	1.015	0.939	25	21
2-Me-Ara	1,3,5-Ara_f	1.207	1.532	15	13
2,3,4-Me_3-Rha	T-Rha_p	0.628	0.524	1	1
3,4-Me_2-Rha	1,2-Rha_p	0.932	0.857	2	2
2,4-Me_2-Rha	1,3-Rha_p	0.960	0.873	2	1
3-Me-Rha	1,2,4-Rha_p	1.176	1.318	1	2
2,3,4,6-Me_4-Gal	T-Gal_p	1.000	1.000	1	1
2,4,6-Me_3-Gal	1,3-Gal_p	1.239	1.369	11	12
2,3,4-Me_3-Gal	1,6-Gal_p	1.349	1.737	7	7
2,3,6-Me_3-Gal	1,4-Gal_p	1.263	1.518	0	8
2,4-Me_2-Gal	1,3,6-Gal_p	1.540	2.225	8	9
2,3,6-Me_3-Glc	1,4-Glc_p	1.279	1.553	10	9
2,3-Me_2-Glc	1,4,6-Glc_p	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.

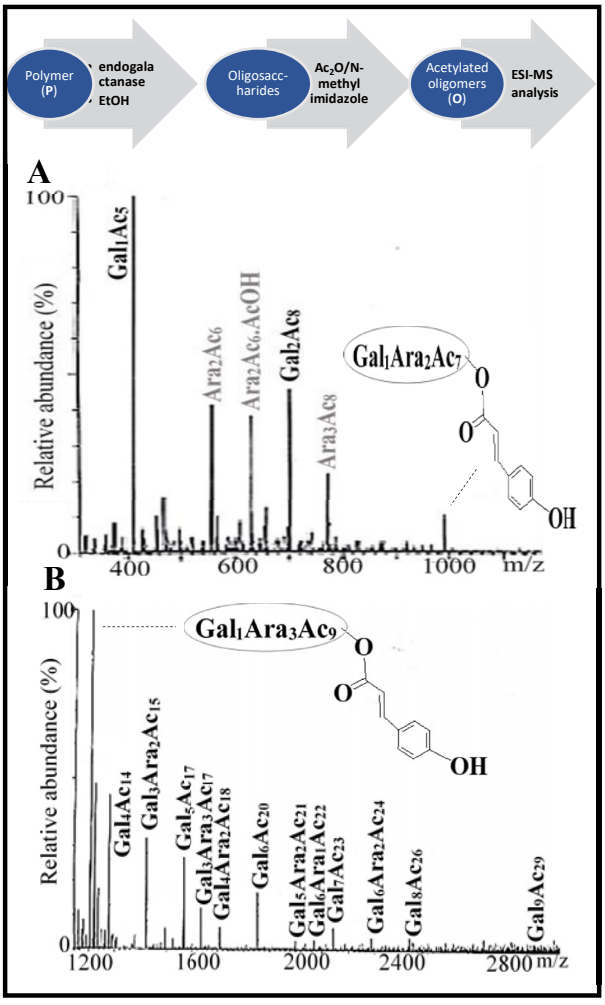

Figure 3. (A) Electrospray ionisation-mass spectral analyses of oligomers generated from *B. flabellifer* fruit polysaccharide (**P**) by enzyme digestion. (B): Expansion of m/z 1200–2900 region.

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•. 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

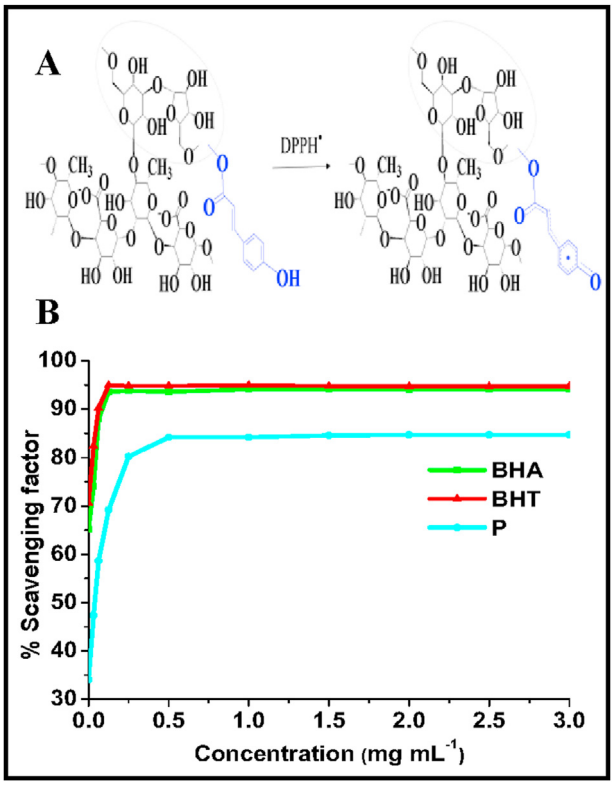

Figure 4. (A) Reaction of *B. flabellifer* fruit polysaccharide (**P**) with DPPH radical. This reaction scheme is not intended to be representative of the full sample composition. (B) In vitro scavenging activities of BHA, BHT in addition to *B. flabellifer* fruit polysaccharide (**P**) towards DPPH radicals.

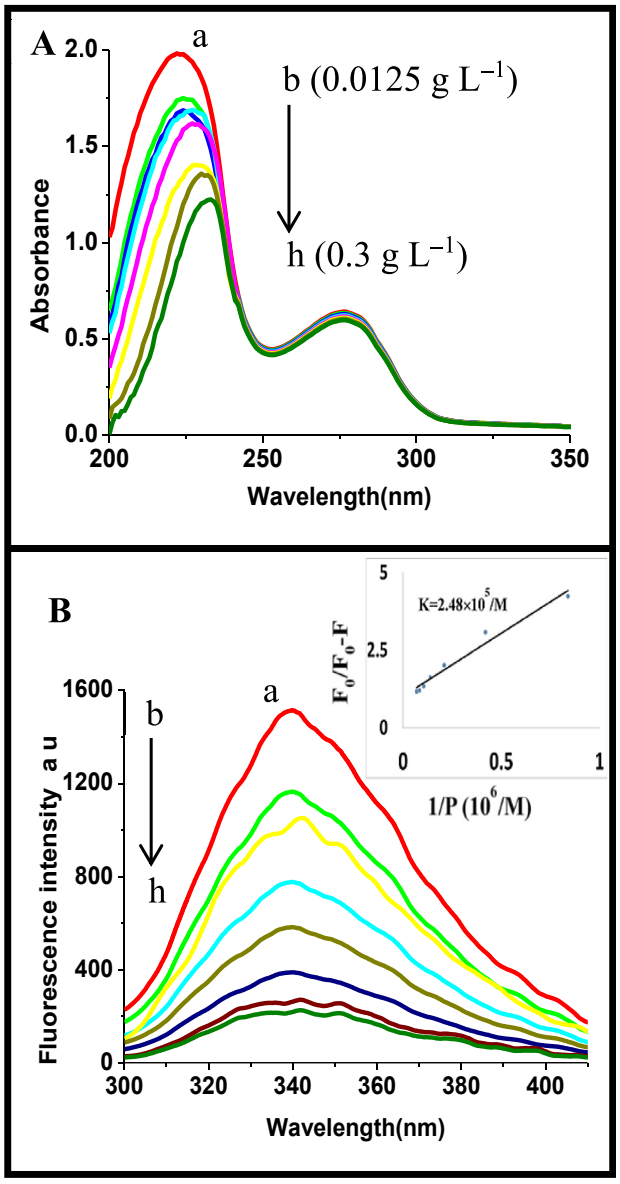

Figure 5. (A) Impact of **P** on UV absorption of β-lg. The red line (a) is the spectrum of native β-lg (0.75 g L^{-1}). The additional lines (b–h) stand for the spectra of β-lg in occurrence with 0.0125–0.3 g L^{-1} polysaccharide (**P**). (B) Fluorescence emission spectra of (a) β-lg (0.75 g L^{-1}) alone and (b–h) in occurrence with polymer **P** (0.025–0.6 g L^{-1}). λ_{ex} = 282 nm. Inset: The plot of F_0 ÷ (F_0–F) against 1/[P] (10^6 M^{-1}) including the association constant (K) for β-lg–**P** complex.

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 (λ_{max}) 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, pI 5.1, can be ascribed (Krebs 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 λ_{em} of 340 nm once incited at λ_{ex} 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. flabellifer* 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 seldomly 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.

Funding statement

This work was supported by University Grants Commission, New Delhi, India (Project No. F.30–425/2018 (BSR)). Fund granted to Sayani Ray.

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.

Acknowledgements

Subrata Jana and Shuvam Mukherjee thank CSIR, India for fellowship.

References

Agostini-Costa, T. da S., 2018. Bioactive compounds and health benefits of some palm species traditionally used in Africa and the Americas – a review. J. Ethnopharmacol. 224, 202–229.
Ahmed, A.E.R., Labavitch, J.M., 1977. A simplified method for accurate determination of cell wall uronide content. J. Food Biochem. 1 (4), 361–365.
Azadi, P., O'Neill, M.A., Bergmann, C., Darvill, A.G., Albersheim, P., 1995. The backbone of the pectic polysaccharide rhamnogalacturonan I is cleaved by an endohydrolase and an endolyase. Glycobiology 5 (8), 783–789.
Bera, K., Nosalova, G., Sivova, V., Ray, B., 2016. Structural Elements and Cough Suppressing Activity of Polysaccharides from *Zingiber officinale* Rhizome. Phytother Res. 30 (1), 105–111.
Blakeney, A.B., Stone, B.A., 1985. Methylation of carbohydrates with lithium methylsulphinyl carbanion. Carbohydr. Res. 140 (2), 319–324.
Blakeney, A.B., Harris, P.J., Henry, R.J., Stone, B.A., 1983. A simple and rapid preparation of alditol acetates for monosaccharide analysis. Carbohydr. Res. 113 (2), 291–299.
Can-Cauich, C.A., Sauri-Duch, E., Betancur-Ancona, D., Chel-Guerrero, L., González-Aguilar, G.A., Cuevas-Glory, L.F., Pérez-Pacheco, E., Moo-Huchin, V.M., 2017. Tropical fruit peel powders as functional ingredients: evaluation of their bioactive compounds and antioxidant activity. J. Funct. Foods 37, 501–506.
Capek, P., Matulová, M., Navarini, L., Suggi-Liverani, F., 2010. Structural features of an arabinogalactan-protein isolated from instant coffee powder of *Coffea arabica* beans. Carbohydr. Polym. 80 (1), 180–185.
Carpita, N.C., Shea, E.M., 1988. Linkage structure of carbohydrates by gas chromatography-mass spectrometry (GC-MS) of partially methylated alditol acetates. In: Bermann, C.J., McGinis, G.D. (Eds.), Analysis of Carbohydrates by GC and MS. CRC Press, Boca Raton, FL, pp. 157–212.
Chen, L., Huang, G., 2019. Antioxidant activities of phosphorylated pumpkin polysaccharide. Int. J. Biol. Macromol. 125, 256–261.
Dickinson, E., 2003. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. Food Hydrocolloids 17 (1), 25–39.
DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28 (3), 350–356.
Fry, S.C., 1982. Phenolic components of the primary cell wall. Feruloylated disaccharides of D-galactose and L-arabinose from spinach polysaccharide. Biochem. J. 203 (2), 493–504.
Fukumoto, L.R., Mazza, G., 2000. Assessing antioxidant and prooxidant activities of phenolic compounds. J. Agric. Food Chem. 48 (8), 3597–3604.
Ghosh, D., Ray, S., Ghosh, K., Micard, V., Chatterjee, U.R., Ghosal, P.K., Ray, B., 2013. Antioxidative carbohydrate polymer from *Enhydra fluctuans* and its interaction with bovine serum albumin. Biomacromolecules 14 (6), 1761–1768.
Ghosh, K., Ray, S., Ghosh, D., Ray, B., 2015. Chemical structure of the arabinogalactan protein from gum ghatti and its interaction with bovine serum albumin. Carbohydr. Polym. 117, 370–376.
Ghosh, T., Chattopadhyay, K., Marschall, M., Karmakar, P., Mandal, P., Ray, B., 2009. Focus on antivirally active sulfated polysaccharides: from structure–activity analysis to clinical evaluation. Glycobiology 19 (1), 2–15.
Guzey, D., McClements, D.J., 2007. Impact of electrostatic interactions on formation and stability of emulsions containing oil droplets coated by β-Lactoglobulin−Pectin complexes. J. Agric. Food Chem. 55 (2), 475–485.
Inngjerdingen, K.T., Coulibaly, A., Diallo, D., Michaelsen, T.E., Paulsen, B.S., 2006. A complement fixing polysaccharide from *Biophytum petersianum* Klotzsch, a medicinal plant from Mali, West Africa. Biomacromolecules 7 (1), 48–53.
Jones, O.G., Handschin, S., Adamcik, J., Harnau, L., Bolisetty, S., Mezzenga, R., 2011. Complexation of β-lactoglobulin fibrils and sulfated polysaccharides. Biomacromolecules 12 (8), 3056–3065.
Kacurakova, M., Capek, P., Sasinkova, V., Wellner, N., Ebringerova, A., 2000. FT-IR study of plant cell wall model compounds: pectic polyasaccharides and hemicelluloses. Carbohydr. Polym. 43 (2), 195–203.
Kim, J.B., Carpita, N.C., 1992. Changes in esterification of the uronic acid groups of cell wall polysaccharides during elongation of maize coleoptiles. Plant Physiol. 98 (2), 646–653.
Kinsella, J.E., Whitehead, D.M., 1989. Proteins in whey: chemical, physical, and functional properties. Adv. Food Nutr. Res. 33, 343–438.
Krebs, M.R.H., Bromley, E.H.C., Donald, A.M., 2005. The binding of thioflavin-T to amyloid fibrils: localisation and implications. J. Struct. Biol. 149 (1), 30–37.
Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E., Etherton, T.D., 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am. J. Med. 113 (9), 71–88.
Laurent, M.A., Boulenguer, P., 2003. Stabilization mechanism of acid dairy drinks (ADD) induced by pectin. Food Hydrocolloids 17 (4), 445–454.
Liang, L., Tajmir-Riahi, H.A., Subirade, M., 2008. Interaction of beta-lactoglobulin with resveratrol and its biological implications. Biomacromolecules 9 (1), 50–56.
Lindblad, M.S., Sjöberg, J., Albertsson, A.-C., Hartman, J., 2007. Hydrogels from polysaccharides for biomedical applications. In: Argyropoulos, D.S. (Ed.), Materials, Chemicals, and Energy from Forest Biomass, pp. 153–167.
Liu, Y., Huang, G., 2019. The antioxidant activities of carboxymethylated cushaw polysaccharide. Int. J. Biol. Macromol. 140, 1054–1063.
Mantovani, M.S., Bellini, M.F., Angeli, J.P., Oliveira, R.J., Silva, A.F., Ribeiro, L.R., 2008. β-Glucans in promoting health: prevention against mutation and cancer. Mutat. Res. 658 (3), 154–161.
Mazumdar, P., Pratama, H., Lau, S.-E., Teo, C.H., Harikrishna, J.A., 2019. Biology, phytochemical profile and prospects for snake fruit: an antioxidant-rich fruit of South East Asia. Trends Food Sci. Technol. 91, 147–158.
McCleary, B.V., Solah, V., Gibson, T.S., 1994. Quantitative measurement of total starch in cereal flours and products. J. Cereal. Sci. 20 (1), 51–58.
Mezzenga, R., Schurtenberger, P., Burbidge, A., Michel, M., 2005. Understanding foods as soft materials. Nat. Mater. 4 (10), 729–740.
Michels, K.B., Giovannucci, Edward, Joshipura, K.J., Rosner, B.A., Stampfer, M.J., Fuchs, C.S., Colditz, G.A., Speizer, F.E., Willett, W.C., 2000. Prospective study of fruit and vegetable consumption and incidence of colon and rectal cancers. J. Natl. Cancer Inst. 92 (21), 1740–1752.
Mirzadeh, M., Arianejad, M.R., Khedmat, L., 2019. Antioxidant, antiradical, and antimicrobial activities of polysaccharides obtained by microwave-assisted extraction method: a review. Carbohydr. Polym. 115421.
Nergard, C.S., Kiyohara, H., Reynolds, J.C., Thomas-Oates, J.E., Matsumoto, T., Yamada, H., Patel, T., Petersen, D., Michaelsen, T.E., Diallo, D., Paulsen, B.S., 2006. Structures and structure-activity relationships of three mitogenic and complement fixing pectic arabinogalactans from the malian antiulcer plants *Cochlospermum tinctorium* A. Rich and Vernonia kotschyana Sch. Bip. ex Walp. Biomacromolecules 7 (1), 71–79.
Park, J.M., Muhoberac, B.B., Dubin, P.L., Xia, J., 1992. Effects of protein charge heterogeneity in protein-polyelectrolyte complexation. Macromolecules 25 (1), 290–295.
Payet, B., Shum Cheong Sing, A., Smadja, J., 2006. Comparison of the concentrations of phenolic constituents in cane sugar manufacturing products with their antioxidant activities. J. Agric. Food Chem. 54 (19), 7270–7276.
Ray, S., Paynel, F., Morvan, C., Lerouge, P., Driouich, A., Ray, B., 2013. Characterization of mucilage polysaccharides, arabinogalactanproteins and cell-wall hemicellulosic polysaccharides isolated from flax seed meal: a wealth of structural moieties. Carbohydr. Polym. 93 (2), 651–660.
Renard, C.M.G.C., Crepeau, M.-J., Thibault, J.-F., 1999. Glucuronic acid directly linked to galacturonic acid in the rhamnogalacturonan backbone of beet pectins. Eur. J. Biochem. 266 (2), 566–574.
Samuelsen, A.B., Westereng, B., Yousif, O., Holtekjølen, A.K., Michaelsen, T.E., Knutsen, S.H., 2007. Structural features and complement-fixing activity of pectin from three *Brassica oleracea* varieties: white cabbage, kale, and red kale. Biomacromolecules 8 (2), 644–649.
Sawyer, L., Brownlow, S., Polikarpov, I., Wu, S.-Y., 1998. *β*-Lactoglobulin. Int. Dairy J. 8 (2), 65–72.
Schmitt, C., Sanchez, C., Desobry-Banon, S., Hardy, J., 1998. Structure and technofunctional properties of protein-polysaccharide complexes: a review. Crit. Rev. Food Sci. Nutr. 38 (8), 689–753.
Sims, I.M., Furneaux, R.H., 2003. Structure of the exudate gum from *Meryta sinclairii*. Carbohydr. Polym. 52 (4), 423–431.
Smestad Paulsen, B., 2002. Biologically active polysaccharides as possible lead compounds. Phytochemistry Rev. 1 (3), 379–387.
Westereng, B., Coenen, G.J., Michaelsen, T.E., Voragen, A.G.J., Samuelsen, A.B., Schols, H.A., Knutsen, S.H., 2009. Release and characterization of single side chains of white cabbage pectin and their complement-fixing activity. Mol. Nutr. Food Res. 53 (6), 780–789.
Wijewardana, R.M.N.A., Nawarathne, S.B., Wickramasinghe, I., Gunawardane, C.R., Wasala, W.M.C.B., Thilakarathne, B.M.K.S., 2016. Retention of physicochemical and antioxidant properties of dehydrated bael (*Aegle Marmelos*) and palmyra (*Borassus flabellifer*) fruit powders. Proc. Food Sci. 6, 170–175.
Wilson, D., Nash, P., Buttar, H., Griffiths, K., Singh, R., De Meester, F.D., Horiuchi, R., Takahashi, T., 2017. The role of food antioxidants, benefits of functional foods, and influence of feeding habits on the health of the older person: an overview. Antioxidants 6 (4), 81.
York, W.S., Darvill, A.G., McNeil, M., Stevenson, T.T., Albersheim, P., 1986. Isolation and characterization of plant cell walls and cell wall components. Methods Enzymol. 118, 3–40.