Extraction and characterization of a novel *Terminalia* pectin

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Abstract This study reports the investigation into pectin present in *Terminalia ferdinandiana*—a native Australian fruit utilised in dietary supplement industry. Citric acid extraction was carried out to extract pectin from two commercially available *T. ferdinandiana* products—frozen puree and freeze-dried puree powder. The yields of the extracted pectin were measured at various pHs (2.0, 3.0 and 4.0) and times (30, 60 and 120 min) at 75 °C. Pectin yield ranged between 4.8 and 21%. Freeze-dried powder had a higher pectin yield compared to puree. Extraction at pH 3 for 120 min resulted in the highest yield from both puree (15%) and powder (21%). *T. ferdinandiana* pectins were found to have low methoxyl content with degree of esterification of 35.07 and 34.74% for puree and powder, respectively. Fourier transform infrared spectroscopy confirmed that the extracts were pectin.

Keywords *Terminalia* · *Terminalia ferdinandiana* · Pectin · Degree of esterification · FT-IR

Introduction

Terminalia is the second largest genus of Combretaceae, and consists of ~200–250 species medium to large flowering trees, many of which have been used as traditional medicine for centuries around the world [1]. *Terminalia* plants have a wide distribution through the tropical and subtropical regions of Asia, Australia, Africa and the Americas. Australia is home to approximately 29 *Terminalia* species or subspecies and *Terminalia ferdinandiana* Exell, is one of such endemic Australian *Terminalia*. *T. ferdinandiana* has a significant history as a food source and traditional medicine, and has been used by the Australian aboriginal population for centuries. An increased awareness of potential health benefits of this fruit, has seen a rise in demand of this fruit as a functional ingredient in both national and international markets. In fact a number of food products, nutraceutical and cosmetic products containing *T. ferdinandiana* are currently available commercially.

The fruit of *T. ferdinandiana* is highly fibrous and may be a rich source of polysaccharides. Recently we reported the non-targeted metabolomic profiling of *T. ferdinandiana* fruit which identified 6 sugars namely—mannose; rhamnose; galacturonic acid; arabinose; glucose and fructose that are commonly associated with the polysaccharide pectin [2]. The possible presence of pectin in *T. ferdinandiana* tissue was further indicated by the observations that when dissolved in water (1:4 w/w), *T. ferdinandiana* powder formed a gel like consistency.

Pectin structure consists of galacturonic acid units covalently-linked by $\alpha(1\rightarrow4)$ glycosidic bonds [3]. Some galacturonic acid residues undergo esterification to form methyl esters. The percentage of esterified D-galacturonic acid residues per total number of D-galacturonic acid residues is termed as degree of esterification (DE). Pectins

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with a DE > 50% are known as high methoxyl (HM) pectins whereas those with <50% DE, are known as low methoxyl (LM) pectins [4]. Due to its gelling and stabilizing properties, pectin is widely employed as a functional ingredient in foods, such as gelling, stabilising and thickening agent in numerous products including jams, jellies, confectionery, fruit juice, yoghurt and milk blends [4]. Pectin based systems for drug delivery purposes has also been widely studied [5].

Gum polysaccharides have been reported in many *Terminalia* plants including—*T. catappa* [6], *T. sericea*, *T. superba* [7] and *T. belarica* [8]. Despite studies on *Terminalia* gums, to our knowledge this is the first study to identify, extract and subsequently report on the extraction and characterization of pectin polysaccharide from a *Terminalia* plant. Discovery of pectin in *T. ferdinandiana* could initiate important food and non-food applications for this versatile fruit. This study aims to extract pectin from two *T. ferdinandiana* products: frozen puree and freeze-dried powder using citric acid. In addition physico-chemical properties of the extracted pectins were determined.

Materials and methods

Plant material

T. ferdinandiana puree (processed in late August 2014 in Wadeye, Northern Territory, Australia) and freeze-dried powder were purchased from Traditional Homeland Enterprise (Victoria, Australia). Powder was milled to a fine consistency using a ball mill (MM301 cryomill, Retsch GmbH, Haan, Germany). All products were stored at −20 °C until further analysis.

Extraction of *T. ferdinandiana* pectin

For extraction, 5 g of puree or powder was mixed with 125 mL distilled water and acidified with different volumes of 0.1 N citric acid adjusted to a pH of 2.0, 3.0 and 4.0. The sample was stirred until a homogenous mixture in acidified water was obtained. The sample mixture was subjected to extraction for 30, 60 and 120 min in a shaking water bath at 75 °C. The parameters were selected based on preliminary analysis. The mixture was kept at room temperature overnight and precipitated pectin was recovered by centrifugation at 10,000 ×g for 10 min at 4 °C (4–16 K, Sigma Laborzentrifugen GmbH, Germany). The supernatant was vacuum filtered and combined with an equal volume of absolute ethanol and the pH was adjusted to 3.5, the pH of minimum pectin solubility. The mixture was stirred for 30 min, left to precipitate at 4 °C for 2 h and centrifuged (10,000 ×g for 20 min, at 4 °C). The pellet

was collected, washed with 70% (v/v) ethanol, re-centrifuged (10,000 ×g for 20 min at 4 °C) and dried at room temperature for 24 h. The dried pectin was milled to a fine powder using a ball mill (Supplementary Figure S1).

The percentage yield of the *T. ferdinandiana* pectin was determined by the following formula

$$\text{Pectin yield (\%)} = \frac{\text{Weight of dried pectin(g)}}{\text{Weight of original material(g)}} \times 100.$$

Characterization of the extracted pectin

Equivalent weight

Equivalent weight was determined as per the method reported by Ranganna [9]. Briefly, 0.5 g of the dried pectin sample was placed in a 250 mL conical flask and moistened with 5 mL absolute ethanol. To the above mixture, 0.1 g of sodium chloride was added to sharpen the end point followed by the addition of 100 mL distilled water. Six drops of phenolphthalein were added into the solution and was titrated against 0.1 N NaOH until the end point was reached, which was indicated by pale permanent pink colour. Equivalent weight was calculated by the following formula:

$$\text{Equivalent weight (g)} = \frac{\text{Weight of the sample(g)} \times 1000}{\text{Volume of NaOH(ml)} \times \text{Normality of NaOH(N)}}.$$

Methoxyl (MeO) content

The neutral solution was collected from the determination of equivalent weight and used to assess the MeO content by the saponification of pectin followed by titration of the liberated acid [9]. To the neutralized solution, 25 mL of 0.25 N NaOH was added to neutralize the polygalacturonic acid. The solution was shaken vigorously, and allowed to stand at room temperature for 30 min to de-esterify the pectin. To this mixture, 25 mL of 0.25 N HCl was added to neutralize NaOH and the sample was shaken until its pink colour disappeared. Six drops of phenolphthalein were added and the sample was further titrated with 0.1 N NaOH till the end point indicated by appearance of pale pinkish colour. MeO was calculated by the following formula:

$$\text{MeO (\%)} = \frac{\text{Volume of NaOH(ml)} \times \text{Normality of NaOH(N)} \times 3.1}{\text{Weight of sample(g)}}.$$

Fig. 1 Yield of pectin (%) extracted from *T. ferdinandiana* puree and powder (n = 3). Average yield were analysed with Student's *t* test. Different letters (i.e., *ABC*) across sample types denote significant differences between mean concentrations according to a Tukey–Kramer HSD. Pectin yield (%) = (weight of the extract × 100)/(weight of the original sample)

Anhydrouronic acid (AUA) content

Total AUA of the extracted pectin was calculated by the following formula.

$$\% \text{ of AUA} = \frac{176 \times 100}{Z}$$

where Molecular weight of AUA = 176 g and

Z =

$$\frac{\text{Weight of sample mg}}{\text{meq of Titration from Equivalent weight (ml)} + \text{meq of Titration from MeO (ml)}}$$

meq = millequivalents = N × mL.

Degree of esterification (DE)

DE of the extracted pectin was calculated using the following formula.

$$DE (\%) = \frac{176 \times \text{MeO} (\%)}{31 \times \text{AUA} (\%)} \times 100.$$

FT-IR spectroscopy of extracted pectin

FT-IR spectra of the pectin samples were obtained at room temperature with an Agilent Cary 630 FT-IR (Agilent Technologies, Santa Clara, United States) equipped with a monolithic design diamond attenuated total reflectance cell. The freeze-dried homogenized pectin samples were placed at the surface of the diamond crystal and were pressed with a system press tip flap. The samples were scanned at wavenumbers ranging from 4000 to 400 cm⁻¹ and corrected

against the background spectrum of air. The spectrum of each sample was obtained by taking the average of 32 scans.

Statistical analysis

All analyses were run in at least replicates of 3 (n = 3) and were expressed as mean ± standard deviation (SD). Statistical analysis was performed by using the XLSTAT-Pro software package version 7.0 (XLSTAT Addinsoft, Paris, France). Differences between means were analysed by the ANOVA test. Statistical significance were indicated by **p* < 0.05, ***p* < 0.01 and ****p* < 0.001.

Results and discussions

Pectin yield

Pectin yield (%) was significantly higher (****p* < 0.0001) from *T. ferdinandiana* powder than puree for all the extraction parameters (Fig. 1). The highest yield of 21% was obtained from *T. ferdinandiana* powder when extraction was performed at pH 3 for 120 min. These extraction parameters (of pH 3 and 120 min) also resulted in the highest yield from *T. ferdinandiana* puree at 14.96%. A similar trend of pectin yield was also observed in case of sugar beet pectins where the authors reported that pectin yield increased to a maximum value as pH decreased from 4.5 to 3.0 [10]. The effect of pH on pectin precipitation could be attributed to changes in the hydrophobic–hydrophilic balance of the pectin at different pHs.

Table 1 Characterization of *T. ferdinandiana* pectins

Sample	Equivalent weight (g)	MeO (%)	AUA (%)	DE %	Colour	Odour
Pectin from <i>T. ferdinandiana</i> puree ^a	334.92 ± 6.90	5.00 ± 0.19	80.96 ± 0.00	35.07 ± 1.33	Yellowish-brown	Neutral to slight acidic smell
Pectin from <i>T. ferdinandiana</i> powder ^a	329.81 ± 8.13	5.15 ± 0.16	81.78 ± 3.19	35.74 ± 0.79	Pale brown	Neutral to slight acidic smell

Average values with ± SD in pectin samples (n = 3) are shown

^aPectin extracted at pH 3 for 120 min from both puree and powder were used for chemical analysis

MeO Methoxyl content, AUA Anhydrouronic Acid and DE Degree of Esterification]

The higher yield observed for the powder may be due to the release of fibre during dehydration as suggested by Silva et al. [11]. These authors found that yield of pectin increased in the freeze-dried residues (70 and 50% DW) of passion fruit when compared to the fresh residue (41% DW).

Characterization of the extracted pectin

Characteristics of the *T. ferdinandiana* pectins are summarized in Table 1. Pectin from puree had an equivalent weight of 335 g, 5% MeO and 81% AUA content. Pectin from the powder also had similar characteristics with 330 g of equivalent weight, 5% MeO and 82% AUA content. Puree pectin had a yellowish brown colour whereas the powder pectin had a pale brown appearance. Both puree and powder pectin samples had neutral to slightly acidic odour. Results indicated that both *T. ferdinandiana* puree and powder pectins were LM pectins with DE < 50% (35% for puree pectin and 36% for powder pectin).

The physicochemical characters of the *T. ferdinandiana* pectin were lower than commercial citrus and apple pectin as commercial citrus pectin has an equivalent weight of 893 g, 9% MeO, 74% AUA with DE of 69% [12] and commercial apple pectin has an equivalent weight of 893 g, 7% MeO, 62% AUA and DE of 68% [13]. The MeO content and DE of the puree and powder pectins were close to the values reported in pectin from orange peels with MeO of 5%, DE of 32–33% [14], dragon fruit with MeO of 3–4%; DE of 31–47% [13] and pomelo fruit with MeO of 5% and DE of 41% [15]. The AUA contents were also close the pectin from orange peels (AUA 87–90%) and pomelo fruit (AUA 75%) however different than the dragon fruit (AUA 46%).

Although *T. ferdinandiana* pectins were found to be LM in nature, from our observations *T. ferdinandiana* puree and powder can form gel without adding any external Ca²⁺ source which is a necessity for LM pectins [16]. This can be explained by the fact that *T. ferdinandiana* has a high

Fig. 2 FT-IR spectra of pectin samples in the 4000–400 cm^{−1} region. (A) Pectin extracted from *T. ferdinandiana* puree, (B) pectin from *T. ferdinandiana* powder and (C) Apple pectin

level of calcium (273–287 mg/100 g DW) [17]. Presence of both calcium and pectin in the *T. ferdinandiana* tissue indicate that pectin from this source may exist in the ‘egg-box’ formation where the Ca²⁺ ions fit in the cavities formed by non-methoxylated galacturonic acid residues [18].

FT-IR spectral features of extracted pectin

FT-IR which is a simple and fast method with high sensitivity, was used to verify whether the extracted polysaccharide materials are indeed pectin by comparing the samples with a reference sample of a commercial apple pectin. The FT-IR spectra are presented in Fig. 2. Examination of the FT-IR spectra showed peaks visible at approximately 3400, 2939 cm^{−1} and between regions 990–1200 cm^{−1}, which are common to all polysaccharides and represent O–H stretching, C–H stretching of the –CH₂ groups and saccharides, respectively [19]. Observed peaks were typical of pectin molecules suggesting that the extracted materials are indeed pectin.

In pectin extracted from puree and powder of *T. ferdinandiana* as well as the reference sample of apple pectin, two major peaks were observed between the regions 3600 and 2500 cm^{−1}. The first peak was noted at 3325 cm^{−1} for pectin from the puree, while 3327 cm^{−1} for pectin from the powder and 3419 cm^{−1} for apple pectin. This corresponds to the absorption due to O–H stretching as a result of inter- and intra- molecular hydrogen bonding of the galacturonic acid backbone of the pectin molecule. The second peak within this region was observed around 2919 cm^{−1} for pectin derived from puree, 2920 cm^{−1} for powder and 2929 cm^{−1} for apple pectin. This corresponds to C–H absorption that includes CH, CH₂ and CH₃ stretching and bending vibrations.

The region between 1800 and 1500 cm^{-1} of the spectra corresponds to infrared absorption by the carboxylic acid ($-\text{COOH}$) and the carboxylic ester ($\text{O}=\text{C}-\text{O}$) groups of the pectin molecules and hence this region permits the evaluation of the degree of methylation of the pectin molecules. The first peak in this region at approximately 1730 cm^{-1} corresponds to $\text{C}=\text{O}$, ester carbonyl groups stretching while the second peak at around 1619 cm^{-1} corresponds to COO^- carboxylate ion stretching band [20]. As expected the spectra of the *T. ferdinandiana* pectin samples revealed two peaks in this region—first peak around 1735 cm^{-1} for both puree and powder and 1732 cm^{-1} for apple pectin. The second peak was observed at around 1619 cm^{-1} (for both puree and powder pectin) and 1608 cm^{-1} for apple pectin.

The region between 800 and 1200 are considered as the ‘fingerprint area’ which is unique to a carbohydrate [21]. Absorption patterns in this region cannot be assigned to any particular vibration because they correspond to complex interacting vibrating systems. As *T. ferdinandiana* pectin is a novel material, an explanation of these peaks by comparison with spectra derived from more established materials is problematic. The major peak observed in this region was around 1019 cm^{-1} for puree, 1019 cm^{-1} for powder and 1011 cm^{-1} for the apple pectin. Similarities of the FT-IR spectrum for the *T. ferdinandiana* pectins to the commercial apple pectin sample in the ‘fingerprint’ region suggest that the extract was predominantly pectin. Difference between the relative intensity of the bands between the *T. ferdinandiana* pectin and the apple pectin is due to the different DE values which depend on the non-esterified and methyl esterified carboxyl groups.

Functional groups that may present in the *T. ferdinandiana* pectin as indicated by FT-IR analysis are summarised in the supplementary Table S1.

To our knowledge this is the first study to report on the novel *Terminalia* pectin. Our findings indicate that the pectins obtained from *T. ferdinandiana* puree and powder can be classified as LM pectin and can be a potential source of a novel gelling pectin for food and non-food applications including—thickening agent, edible packaging films, delivery vehicle for drugs etc. Currently further research is in progress to further explore the structural, biological and functional properties of this unusual polysaccharide.

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