Note

Simple Method for Refining Arabinan Polysaccharides by Alcohol Extraction of the Prune, *Prunus domestica* L.

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1-Arabinose is a useful sugar in the food industry. We demonstrate here simple methods for refining arabinan polysaccharides by alcohol extraction from prune, *Prunus domestica* L., as a source of 1-arabinose. Alcohol-soluble polysaccharides were purified from a solution of prune extracted by 80% ethanol. After fractionating the polysaccharides by ion-exchange chromatography, arabinans were identified as mainly constituted by (1→5)-linked arabinofuranosyl units.

Key words: arabinan; 1-arabinose; alcohol-soluble polysaccharide; prune

Arabinans appear in the primary cell walls of different families of plants in seeds, fruits, and roots, usually as pectic polysaccharide side-chains or as free polymers unattached to pectic domains.¹⁻⁷ The arabinosyl-linkage composition of arabinans consists of a backbone of (1→5)-α-L-arabinofuranosyl units, variably branched at O-2 and/or O-3 by single arabinosyl residues or short side chains.⁶⁻⁸ The degree of branching has been variously reported, depending on the source of the arabinan. Terminal arabinosyl residues mostly occur as furanoses, but the pyranose form has also been identified in an arabinan from a cotyledon.⁹⁻¹¹ A β-L-arabinofuranosyl terminal unit has also been detected in an arabinan from olive pectic polysaccharides,³⁻¹⁰ although most of the arabinofuranosyl terminal units have an α-anomeric configuration.

Prunes, which are dried plums, are a popular dried fruit enjoyed by people worldwide. They are also well known as a healthy food. The consumption of prunes or a prune extract involves the ingestion of all components present, including polysaccharides. Studies on the composition of the cell walls of prunes, including *Prunus domestica* L., have been reported.¹¹⁻¹⁵ Prune cell wall polysaccharides are composed mainly of pectic polysaccharides and cellulose. Chilling-injured plum (*P. salicina*) had the highest yield of uronic acids in the water and dilute carbonate fractions, with a change from water- to dilute alkali-soluble pectins when compared with normally ripened plum.¹⁵ The composition of the cell walls of the flesh and skin from five different plums revealed a varying galactose and arabinose ratio between the varieties.¹³⁻¹⁵

Prunes, *P. domestica* L. cv. d’Agen, imported from United States as the material for a commercial prune extract (concentrated prune juice), were supplied by MIKI Corporation (Hyogo, Japan). The moisture level of the prunes was adjusted to 21%. Arabinans are soluble in 70% alcohol;¹⁷ alcohol-soluble polysaccharides (AlSPs) were therefore extracted with 80% ethanol from concentrated prune juice as a soluble extract. Briefly, the commercial prune extract (150 g) was diluted with 10 volumes of distilled water and stirred at room temperature. AlSPs were extracted from the diluted prune solution by ethanol (an 80% final concentration) for 30 min at room temperature. The resulting extract was passed through a 26GP100 glass filter (Shibata Scientific Technology, Saitama, Japan) with Celite 545 (Wako Pure Chemical Industries, Osaka, Japan). Additional 80% ethanol was added to the residue after filtration. This procedure was conducted three times. The combined extract was evaporated *in vacuo* to remove ethanol and then the concentrated extract was dialyzed against distilled water with a size 36 dialysis membrane (Wako). AlSP was finally obtained through lyophilization. The isolated AlSP was obtained in an overall yield of 0.827% and consisted of 15.2% of proteins and 84.8% of sugars (70.4% of neutral sugars and 14.4% of uronic acids). To characterize AlSP, we attempted to isolate it by stepwise ion-exchange chromatography in a DEAE-cellulose column (Supplemental Fig. 1; see the Biosci. Biotechnol. Biochem. Web site). This chromatography afforded two main fractions (Table 1): neutral fraction N₁ eluted with distilled water (55.1% yield calculated by measuring the dry weight after lyophilization) and acidic fraction A₁ eluted with 0.3 M NaHCO₃ (21.3% yield).

The fractions obtained by ion-exchange chromatography were subjected to a neutral monosaccharide analysis after acid hydrolysis. Briefly, the glycosyl residues (neutral sugars) of the polysaccharide were analyzed by gas-liquid chromatography (GLC) as alditol acetates after acid hydrolysis.¹⁸ GLC was carried out with GC-18A apparatus (Shimadzu, Kyoto, Japan)

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Abbreviations: AlSP, alcohol-soluble polysaccharide; GLC, gas-liquid chromatography
equipped with a flame ionization detector. A capillary column (25 m × 0.22 mm i.d., 0.25 μm Hicap CBP-10, Shimadzu) was used and operated at 220 °C with a nitrogen gas flow rate of 0.8 mL/min with a 1/60 split ratio. Peak areas were measured with a Chromatocorder 21 integrator (System Instruments, Tokyo, Japan). The composition of neutral sugars was calculated from GLC peak areas. Since arabinose was the major component (94.9%) of fraction N1 (Table 2), the polysaccharide contained in this fraction would have been the arabinoxylan polysaccharide. In contrast, acidic fractions A1 and A2 would have been composed of heteropolysaccharides. L-Arabinose is widely used as a functional sweetener. The industrial production of arabinose, however, is difficult, because the structure of arabinoxylan, which is the main source of L-arabinose, is complex and a combination of enzymes is required to digest it. Our findings therefore suggest that the arabinoxylan polysaccharides in prunes would be a reasonable source of L-arabinose.

Major retained fraction N1 in the prune samples was subjected to gel filtration chromatography in a Toyopearl HW-55S column (Tosoh Corp., Tokyo, Japan) to determine the molecular weight distribution by using technical grade dextrans (Supplemental Fig. 2; see the Biosci. Biotechnol. Biochem. Web site). Fraction N1 gave an almost symmetrical peak (an 82–114 mL elution volume) through the column, and the Mw of arabinan polysaccharides was calculated by a linear regression analysis to be 5,240 ± 260 Da (n = 6). Pectic polysaccharides have been extracted from sugar beet pulp to yield fractions including arabinans. It was found that the average degree of polymerization of arabinian side chains must be higher than 45, indicating that Mw of the arabinans was more than 6,000 Da. It has also been previously reported that the degrees of polymerization of arabinan, galactan, and arabinogalactan side chains might vary from 1 up to 50.7,8

The structure of arabinan, which was the major polysaccharide in fraction N1, was determined by a methylation analysis. The polysaccharide of fraction N1 was methylated by the Hakomori method, as modified by Harris. Each product was confirmed to show no absorption for the hydroxyl group in its IR spectrum. The methylated polysaccharide was hydrolyzed with 90% formic acid at 100 °C for 2 h, and then with 0.25 M sulfuric acid at 100 °C for 16 h. The partially methylated sugars thus obtained were converted to their alditol acetates, and the partially methylated alditol acetates were analyzed by GLC and gas chromatography-mass spectrometry (GC-MS; GC Mate II GCMS system, Jeol Ltd., Tokyo, Japan) at 160 °C to 220 °C in the column, increasing at 2 °C min⁻¹ and using He as the carrier gas with flow of 50 mL min⁻¹. Table 3 shows that the polysaccharide was a small branched arabinan. This polymer was composed of terminal (T)-Araf, (1→5)-Araf, (1→3)-Araf, (1→3,5)-Araf, and (1→2,5)-Araf in relative proportions of approximately 3:10:2:3:1 (Table 3). This structure shows that the backbone was relatively longer than the side chain. This structure characterized this polysaccharide as an AlSP. The percentage of methylated derivatives in Table 3 shows that non-reducing terminal sugars (1,4-di-O-acetyl-2,3,5-tri-O-methyl-pentitol) did not correspond to the percentage of mono-methylated derivatives of arabinose (1,3,4,5-tetra-O-acetyl-2-O methyl-pentitol and 1,2,4,5-tetra-O-acetyl-3-O methyl-pentitol). This difference was approximately 50%. The chemical structure proposed will therefore need to be further analyzed in future.

The alcohol-insoluble fraction extracted from the cell wall polysaccharides of prunes has recently been analyzed. Pectic arabinian chains are composed of 1,5-α-linked L-arabinofuranosyl residues and can be branched at O-2 and/or O-3 by single arabinosyl residues or short side chains.8 On the basis of this data, the structure of the polysaccharide of fraction N1 can thus be attributed to that of pectic arabinans. In addition, the arabinan-rich pectic polysaccharide from the cell walls of the almond seed, P. dulcis, has been reported to be composed of T-Araf, (1→5)-Araf, (1→3,5)-Araf, and (1→2,3,5)-Araf in relative proportions of approximately

Table 1. Yield and Composition of Fractions N1, A1, and A2 Determined from DEAE-Cellulose Chromatography

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Yield, %</th>
<th>Neutral sugars</th>
<th>Uronic acids</th>
<th>Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>55.1</td>
<td>80.1</td>
<td>6.6</td>
<td>13.3</td>
</tr>
<tr>
<td>A1</td>
<td>21.3</td>
<td>36.5</td>
<td>12.4</td>
<td>51.1</td>
</tr>
<tr>
<td>A2</td>
<td>6.5</td>
<td>n.d.</td>
<td>n.d.</td>
<td>47.4</td>
</tr>
</tbody>
</table>

n.d., not determined

Table 2. Molar Ratio of Neutral Sugars in Extracts

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Rha</th>
<th>Ara</th>
<th>Xyl</th>
<th>Man</th>
<th>Gal</th>
<th>Glc</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>5.8</td>
<td>16.1</td>
<td>7.0</td>
<td>10.8</td>
<td>6.5</td>
<td>53.9</td>
</tr>
<tr>
<td>A1</td>
<td>5.8</td>
<td>16.1</td>
<td>7.0</td>
<td>10.8</td>
<td>6.5</td>
<td>53.9</td>
</tr>
<tr>
<td>A2</td>
<td>5.8</td>
<td>16.1</td>
<td>7.0</td>
<td>10.8</td>
<td>6.5</td>
<td>53.9</td>
</tr>
</tbody>
</table>

Rha, rhamnose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose

Table 3. GC-MS Results of Methylation Analysis of Extracts

<table>
<thead>
<tr>
<th>Methylation product</th>
<th>Type of linkage</th>
<th>Molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4-Di-O-acetyl-2,3,5-Tri-O-methyl-pentitol</td>
<td>1→Araf</td>
<td>12.9</td>
</tr>
<tr>
<td>1,3,4-Tri-O-acetyl-2,3-Di-O-methyl-pentitol</td>
<td>→3→Araf</td>
<td>10.1</td>
</tr>
<tr>
<td>1,4,5-Tetra-O-acetyl-2-O-methyl-pentitol</td>
<td>→3→Araf</td>
<td>16.6</td>
</tr>
<tr>
<td>1,2,4,5-Tetra-O-acetyl-1,3-O-methyl-pentitol</td>
<td>→2,5→Araf</td>
<td>4.8</td>
</tr>
<tr>
<td>1,3,5-Tri-O-acetyl-2,4,6-Tri-O-methyl-hexitol</td>
<td>→3→D-Glcj</td>
<td>5.4</td>
</tr>
<tr>
<td>1,4,5-Tri-O-acetyl-2,3,6-Tri-O-methyl-hexitol</td>
<td>→4→D-Galp</td>
<td>1.1</td>
</tr>
<tr>
<td>1,4,5-Tri-O-acetyl-2,3,6,5-Tri-O-methyl-hexitol</td>
<td>→4→D-Galp</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Molar ratio was calculated from the results of reduced samples.
The structural analysis of an arabinan isolated from the alkaline extract of the endosperm of seeds of Nata Karanja, **Caesalpinia bonduc**, which is an important medicinal plant widely distributed throughout coastal regions in tropical and subtropical areas, has also recently been reported. The polymer of the water-soluble arabinan isolated from *C. bonduc* was composed of T-Araf, (1→5)-Araf, (1→2,5)-Araf, and (1→2,3,5)-Araf in relative proportions of approximately 3:2:1:1. These figures are similar to each other, but are totally different from our results, suggesting that the characterized AlSP in prunes would be attributed to its unique structure.

We suggest the simple and easy refinement method for arabinans, mainly constituted by (1→5)-linked arabinofuranosyl units, by alcohol extraction from prune, *P. domestica* L., and purification by ion-exchange chromatography in a DEAE-cellulose (HCO$_3$ form) column eluted with water. The $M_w$ of arabinan polysaccharides was around 5,000 Da. This method will contribute to efficiently providing a source of L-arabinose.

**Acknowledgments**

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**References**