Characterisation of Polysaccharides of Copra Meal

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(Manuscript received 16 July 1982)

To investigate the polysaccharide composition of copra meal, defatted meal was treated successively by hot water extraction, delignification with sodium chlorite and protein elimination with protease. The deproteinised holocellulose obtained was treated by extraction with 16\% NaOH to give an alkaline extract and an alkali insoluble fraction. Acidification of the alkaline extract with glacial acetic acid gave a product termed polysaccharide A and subsequent ethanol precipitation of the acidified liquor gave a product termed polysaccharide B. The hot water extract (HWE) and polysaccharide B were further fractionated by gel permeation chromatography. Sugar composition analyses of the extraction products examined by gas-liquid chromatography indicate that mannan is the major polysaccharide in copra meal and accounts for about 61\% of the total polysaccharides in the deproteinised holocellulose. Other polysaccharides present in copra meal are, in descending order, cellulose, arabinoxylagalactan, galactomannan, arabinomannagalactan and galactoglucomannan.

1. Introduction

Copra, the dried kernel of nuts of the coconut palm (Cocos nucifera), is an important vegetable oil source, especially in tropical countries. World production of copra reaches 5.1 million tonnes annually. The extraction of coconut oil yields 33-42\% residue, called copra meal or copra cake, with a world production of copra meal of 1.7-2.1 million tonnes annually. Copra meal typically contains 43-45\% carbohydrates, 19-20\% protein, 10-11\% oil and 12\% crude fibre. Due mainly to unhygienic conditions of production, its utilisation at present is limited to livestock feeding but more efficient methods of utilisation as food, feed or industrial materials could make an important contribution to global food and feed supplies. Balasubramaniam reported that galactomannan formed 61\% of the total polysaccharides in the coconut kernel followed by mannan (26\%) and cellulose (13\%). Therefore, in this laboratory, an attempt was made to utilise copra meal as a galactomannan source for industrial use. However, it was found that the main polysaccharide present was mannan rather than galactomannan. This paper reports an investigation of fractionation and characterisation of mannan and other polysaccharides in copra meal.

2. Experimental

2.1. Preparation of deproteinised holocellulose

Dried copra meal was obtained from an oil mill in Bangkok in 1981. It was pulverised using a high speed grinder (Kyoritsuriko Co. Ltd) for 10 min and extracted twice with ether-ethanol (1:1) at room temperature to remove lipids. The air-dried defatted copra meal was sieved through a 32 mesh screen. The defatted powder (100 g) was extracted twice with 2 litres of distilled water over boiling bath for 2 h, and hot water-soluble material was then separated by centrifuging. The residue, after washing with distilled water and acetone, was dried (68 g). The water extract was filtered, concentrated under reduced pressure to about 700 ml, and then was precipitated in twice its volume of ethanol. The precipitate was centrifuged, washed successively with 95\% ethanol and acetone,
and dried in a vacuum desiccator to give a light yellowish powder. This hot water extract (HWE) yielded about 5 g (5%).

The hot water-insoluble matter was delignified by the treatment with acidified sodium chlorite solution\(^4\) to yield a holocellulose 46 g (68%). The product was then treated with a fungal protease (Tokyokasei) in phosphate buffer solution, pH 8, at 37\(^\circ\)C for 24 h to remove coexisting protein. The treated residue was filtered, washed thoroughly with water and acetone, and dried to give a porous and white powder, 34 g (74%).

2.2. Extraction and isolation of polysaccharides from deproteinised holocellulose

The deproteinised holocellulose (15 g) was extracted thrice with 16\% NaOH (600 ml) at room temperature under nitrogen atmosphere with continuous stirring for 24 h. The combined alkaline extract was acidified to pH 5 with glacial acetic acid and let stand overnight at 4\(^\circ\)C, then the precipitate was separated by centrifuging (15 400 \(\times\) g, 10 min) washed several times with 50\% ethanol and acetone, and dried to yield a white powder, named polysaccharide A, 8.25 g (55\%).

The supernatant from the acid precipitation was concentrated under reduced pressure and allowed to stand overnight at 4\(^\circ\)C to precipitate sodium acetate which was later removed by filtration. To the filtrate, twice its volume of 99\% ethanol was added and a white precipitate thus formed, collected by centrifuging, washed with 95\% ethanol and acetone, and dried in vacuo. The yield of the product, termed polysaccharide B, was 1.2 g (8\%).

The residue from the alkali extraction was washed thoroughly with water until free of alkali then with acetone and dried. The yield was 3 g (20\%).

2.3. Gel permeation chromatography

For gel permeation chromatography, the hot water extract (50 mg) was dissolved in distilled water (2 ml), and the polysaccharide B (50 mg) was dissolved in 1N NaOH (2 ml) and acidified to pH 5–6 with glacial acetic acid. The solution was chromatographed on a column (2.9 \(\times\) 60 cm) packed with TSK-gel, Toyopearl HW-55s (Toyo Soda Co.) and eluted with distilled water, 30 or 60 ml h\(^{-1}\). Eluate was detected with a refractive index detector (JAI, model RI-2) and also an aliquot (5 ml) of the eluate was taken and detected for carbohydrates by the phenol–sulphuric acid method.\(^5\) The molecular weight was estimated by gel permeation chromatography using dextran T70, T40 and T10 (Pharmacia) as a standard.

2.4. Sugar analysis by gas–liquid chromatography (g.l.c.)

Hydrolysis of the polysaccharide B and HWE (10 mg or less) was done with 2 ml of 1N sulphuric acid in a sealed tube at 100\(^\circ\)C for 16 or 20 h, if the mixture remained turbid as in the case of mannose rich sample. The defatted copra meal, polysaccharide A and residue were first dissolved in cold 72\% sulphuric acid and then diluted with water to 3\% acid concentration and refluxed for 3 h as described by Whistler and Gaillard.\(^6\) After hydrolysis, the acid solution was neutralised with barium carbonate, filtered and treated with Amberlite IR 120 (H\(^{+}\)) resin. The hydrolysate was concentrated to dryness and trimethylsilylated to determine their sugar components by gas chromatography.

G.l.c. was made with a Shimadzu model 4BMPF equipped with a 3.0 mm i.d. \(\times\) 200 cm glass column of 15\% EGS (polyethylene glycol succinate) on 80–100 mesh Chromosorb W (AW). The temperature rise was programmed at a rate of 2\(^\circ\)C min\(^{-1}\) from 100 to 190\(^\circ\)C and the nitrogen flow rate was 50 ml min\(^{-1}\). The amount of each sugar was calculated from the integrated peak area using methyl \(\alpha\)-D-mannopyranoside as an internal standard.

2.5. Total carbohydrate and lignin content

Total carbohydrate in copra meal and each fraction was determined by the phenol–sulphuric acid method\(^8\) using mannose as a standard. Lignin content was determined by the sulphuric acid method.\(^7\)
3. Results and discussion

3.1. Extraction of the polysaccharides from the defatted copra meal

The defatted copra meal contained 48% carbohydrate and 5% lignin. Its sugar composition determined by g.l.c. was 72% mannose, 25% glucose, 3% galactose and traces of xylose and arabinose. Delignification and the subsequent treatment of the holocellulose with fungal protease to remove coexisting protein resulted in significant improvements in extraction and purification as well as sugar analysis of the polysaccharides. By the procedures described, 100 g of the defatted copra meal yielded about 34 g of a white deproteinised holocellulose which contain about 90% carbohydrate. The yield, total carbohydrate content and sugar composition of each polysaccharide fraction and residue are shown in Table 1.

Table 1. Products of extraction of defatted copra meal and deproteinised holocellulose

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Yield (%)</th>
<th>Total carbohydrate content (%)</th>
<th>Monosaccharide composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot water extract</td>
<td>5</td>
<td>31</td>
<td>tr.  tr.  60  17  23</td>
</tr>
<tr>
<td>Polysaccharide A</td>
<td>55</td>
<td>98</td>
<td>---  ---  98  2  ---</td>
</tr>
<tr>
<td>Polysaccharide B</td>
<td>8</td>
<td>74</td>
<td>8  5  69  14  4</td>
</tr>
<tr>
<td>Residue</td>
<td>20</td>
<td>80</td>
<td>---  ---  42  ---  58</td>
</tr>
</tbody>
</table>

* Yield of HWE is based on defatted copra meal while of polysaccharide A, B and residue is based on deproteinised holocellulose.

* Determined by the phenol–sulphuric acid method.

* Determined by gas chromatography: ara = arabinose; xyl = xylose; man = mannose; gal = galactose; glu = glucose.

3.2. Polysaccharide A

The results in Table 1 clearly show that polysaccharide A is the main part of the holocellulose of the copra meal and can be assigned as almost pure mannan according to Aspinall's definition of a mannan as 'a polysaccharide containing 95% or more of mannose residues'. However, no further investigation of this fraction was made since it was insoluble even in the alkaline solution in which initially it had been dissolved. This mannan, when added to the mannan from the residue, gave a total which accounted for about 61% of the total polysaccharides in the deproteinised holocellulose. Although copra meal is a by-product of dried coconut kernel, it is unlikely that the oil extraction process would substantially alter the composition of the polysaccharides in the coconut kernel. Therefore, similar polysaccharides should be found in both mature coconut kernel and copra meal. In several reports on the polysaccharides of coconut kernel and copra meal, galactomannan and galactoglucomannan have been considered to be the main polysaccharides in mature coconut kernel and copra meal respectively. In contrast, the present results show that mannan, rather than galactomannan or galactoglucomannan, is the major polysaccharide in both copra meal and mature coconut kernel. The presence of mannan in coconut kernel has been reported earlier and its structure has been indicated as mannose units which are joined through $\beta$-(1–4) linkage and the mannan is highly branched.

3.3. Alcohol-precipitated products: polysaccharide B and HWE

3.3.1. Polysaccharide B

The polysaccharide B was soluble in dilute alkali, thereby, it was further fractionated by use of gel permeation chromatography. The gel chromatogram (Figure 1) indicates that the polysaccharide B is a mixture of at least five fractions. Further purification of each fraction was done by repeated
gel chromatography. Fractions B₁ and B₂ were minor ones and their molecular weights were estimated to be above $1 \times 10^5$. They had a similar sugar composition of xylose, arabinose and galactose in the ratio of 1:4:5 with trace amounts of mannose and glucose. Fraction B₃ was shown to be a mannan of molecular weight about $4 \approx 5 \times 10^3$. Fractions B₄ and B₅ were oligosaccharides of molecular weight about $1 \approx 2 \times 10^3$ and not well separated from each other by the use of a HW-55s gel column, so further fractionation was made by a HW-40s column. As shown in Figure 2, B₅ gave six peaks and each was collected, concentrated to dryness and analysed for its sugar composition. The sugar components, their molecular ratio and weight percentage of the fractions of the polysaccharide B are summarised in Table 2. It shows that the polysaccharide B is composed of three types of polysaccharides (i.e. arabinoxylogalactan, mannan and galactomannan). Fractions B₄ and B₅(Δ) did not clearly separate and might be mixtures of oligosaccharides which produced degradatively by the alkali extraction and/or probably by further degradation of larger molecule fractions of the polysaccharide B when the latter was dissolved in the alkaline solution in the presence of air prior to the gel chromatography. The fractions from B₄ to B₅(Δ) were short chain arabinoxylogalactan and probably degraded from the long chain arabinoxylogalactan fractions B₁ and B₂. This was presumed because gel permeation chromatography of the polysaccharide B occasionally gave two large peaks of B₁ and B₂ but with tiny peaks of B₄ and B₅. However, this phenomenon was difficult to reproduce and further purification of B₁ and B₂ yielded only peaks B₄ and B₅.
3.3.2. Hot water extract

The hot water extract (HWE) was obtained as a minor fraction and it contained about 31% carbohydrates along with a considerable amount of unidentified material, presumably protein. The gel chromatogram of HWE gave four carbohydrate fractions (Figure 3). Fractions 1 and 2 were larger polymers having molecular weights about $1 \times 10^5$, while fractions 3 and 4 were smaller ones, about $2 \times 10^3$ and $1 \times 10^3$, respectively. The sugar composition of each fraction is shown in Table 3. It shows that the HWE is a mixture of, probably, arabinomannogalactan and galactoglucomannan but they are present only as a trace.

![Figure 3. Gel chromatogram of the hot water extract (HWE) on a Toyopearl HW-55s column (2.9 × 45 cm). The elution condition was the same as in Figure 1, except that the flow rate was 60 ml h⁻¹.](image)
3.4. The residue of the alkali extraction

The residue of the alkali extraction contained mannose (42\%) and glucose (58\%) (Table 1). From the sugar composition, this fraction can be interpreted as glucomannan or a mixture of mannan and cellulose or a combination of both alternatives. Glucomannans from lily bulbs (Lilium candidum, L. henryii, and L. umbellatum) had been extracted with cold water\(^1\) and that from Iris seeds (Iris ochroleuca and I. sibirica) with 10\% NaOH\(^14\). However, in spite of employing the high concentration of the alkali, no glucomannan could be detected in other fractions. Neither Rao et al.\(^{10}\) nor Mukherjee and Rao\(^9\) reported the presence of glucomannan in coconut kernel. Balasubramaniam\(^3\) extracted coconut kernel with 4\% and 17\% NaOH and obtained a non-extractable fraction which yielded glucose and mannose in almost equal amounts and concluded that the fraction was a mixture of mannan and cellulose. However, from our observation and these reports, it is reasonable to assume that the residue is a mixture of mannan and cellulose. To confirm the presence of cellulose, removal of cellulose by enzymic hydrolysis was tried by treating the residue (1 g) with 10 mg of Aspergillus niger cellulase (Nagase Biochemicals Ltd) in acetate buffer (pH 4.5) at 37°C for 48 h, however, unlike Balasubramaniam's experience,\(^3\) the treatment did not alter the sugar composition of the residue significantly. This was probably due to the fact that cellulose is denatured when treated severely with alkali.\(^15\) The mannan in this fraction accounts for 6.72\% (20\% x 0.8 x 0.42) of the total polysaccharides in the deproteinised holocellulose and, when added to the mannan of the polysaccharide A, the total mannan accounts for about 61\% of the total polysaccharides in the deproteinised holocellulose as described earlier.

4. Conclusion

The above results have demonstrated that the major polysaccharide in copra meal is mannan, accounting for about 61\% in the deproteinised holocellulose, followed by substantial amounts of cellulose, minor amounts of arabinoxylogalactan, galactomannan and traces of arabinomannogalactan and galactoglucomannan. Utilisation of mannan from copra meal as human food and for industrial use is being investigated in this laboratory.

References