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SHORT COMMUNICATION

COCOA POD HUSK PECTIN

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Abstract—Dry pectin has been prepared from fresh cocoa pod husk by mild acid extraction with yields of 8–11% of the dry ripe pod husk and 25–29% of the same tissue of immature fruits. Its carbohydrate composition is similar to that of apple pectin, containing mainly the sugars associated with the pectic substances. Partial acid hydrolysis of the crude material yields about 60% galacturonic acid.

INTRODUCTION

PECTIC substances have been shown[1] to form a considerable proportion of the water-, and ammonium oxalate-soluble polysaccharide fractions of cocoa pod husk. Dittmar[2] analysed several South American varieties of cocoa and showed that the sun-dried pod husk contained 5·3–7·1% pectin. The present paper reports the pectin content of fresh ripe pod husk as well as husk of 2- to 3-month-old fruits (cherelles).

Of the various methods commonly used for pectin extraction[3,4] the use of mild acid was chosen to facilitate extraction of maximum amounts of high-methoxyl pectin and to preserve them as intact as possible for the study of their physico-chemical properties.

RESULTS

Yield and Solubility of Crude Pectin from Cherelle and Ripe Pod Husk

Cherelle husk was found to contain a higher proportion of pectin than ripe pod husk. Four different preparations from cherelle husk gave 25–29% crude pectin on a dry weight basis whilst the yield from ripe pod husk in four different experiments was 8–11%. Extraction with 0·05 N HCl gave similar results. The mean dry weight of the husk of five randomly picked ripe pods was 53·75 g (s. 13·10) compared to the value of 5·69 g (s. 45) recorded for the husk of four cherelles.

Most acetic acid preparations yielded viscous solutions when the dry powder was suspended in ethanol, followed by addition of water with vigorous stirring, and warming. Solutions up to 0·3% could be prepared in this way. However, preparations made by extraction with 0·1 N or 0·05 N HCl failed to dissolve and showed no capacity to swell. After exhaustive dialysis against distilled water the pH of solutions or suspensions was 3·4–3·7 for the HCl preparations, and between 4·3 and 4·5 for the acetic acid preparations. Extraction with lower HCl concentrations was not attempted. Extraction with 0·2 N acetic acid was used for most of the work.

[1] W. R. BLAKEMORE, E. T. DEWAR and R. A. HODGE, *J. Sci. Food Agric.* **17**, 558 (1966).
[2] H. F. K. DITTMAR, *Gordian* 58–1387, **48** (1958).
[3] R. M. MCCREADY and E. A. MCCOMB, *Analyt. Chem.* **24**, 1986 (1952).
[4] D. R. NANJI and A. G. NORMAN, *Biochem. J.* **22**, 596 (1928).

Ash

Two determinations each (using 0·3–1·2 g samples) of different ripe pod and cherelle husk preparations were made. The ash content of cherelle husk preparations was 7·9–8·8% and for the ripe pod husk preprations, 8·9–9·8%. Further analysis revealed that Ca and K were the major elements present. Exhaustive dialysis against water reduced the ash content to 0·05% in both cases. However, dialysis was found to be unsuitable as a step in the preparation of the dry pectin, as the extracts, when dialysed against 0·05 N acetic acid or 0·005 N HCl (where relevant), required about 75–85% ethanol concentration to precipitate the pectin. Such preparations were insoluble in water although they showed ability to imbibe water to form gels. These findings support the widely held view that flocculation of pectinic acids by ethanol requires the presence of small amounts of salts.[5]

Carbohydrate Composition

A comparative study was made of the carbohydrate composition of the crude preparations and of apple pectin (purified[6] from exhaustively dialysed B.D.H. 250 grade apple pectin). Paper chromatographic analysis of acid hydrolysates showed that the apple and cocoa pectins were identical with respect to their qualitative sugar composition, viz. galacturonic acid, galactose, arabinose, rhamnose, xylose and glucose. The differences were essentially of a quantitative nature. Thus glucose was absent from several preparations of cocoa pectin of both ripe and cherelle pod-husk origin, whereas it formed a relatively significant proportion of the apple pectin. The galactose and arabinose contents also varied in the apple and cocoa pectins. Their proportions relative to galacturonic acid (= 100) in apple pectin were: galactose, 6·7; glucose, 22·5; arabinose, 16·7; and xylose 13·9, with trace amounts of rhamnose (see cocoa pectin, Table 1).

In the apple pectin hydrolysate as well as some cocoa pectin hydrolysates, trace amounts of an unidentified substance (probably a pentose) with R_G 1·34 and 1·82 in solvent-systems 1 and 2 respectively were found. Acid hydrolysates of the cocoa pectin obtained by extraction with HCl had little or no rhamnose.

Table 1 gives the yields and the general composition of two preparations from ripe and cherelle pod husk. The results show that even without correction for its losses during hydrolysis galacturonic acid forms by far the largest proportion of the sugars liberated by acid hydrolysis. Generally cherelle preparations were more viscous than those from ripe pod husk.

TABLE 1. THE YIELD AND GENERAL COMPOSITION OF POWDERED COCOA PECTIN FROM RIPE AND CHERELLE POD HUSK

Source	Initial dry wt. of husk (g)	Yield of pectin (as g alcohol-insoluble ppt)	Residual husk fibre (g)	Ash (% powder)	Protein N (% powder)	Galacturonic acid (%)	Galactose (%)	Rhamnose (%)	Arabinose	Xylose	η_i
Ripe pod husk	24·70	2·38	11·28	8·90	1·10	62·10	4·65	2·90	1·70	1·20	10·10 (c.O.14)
Cherelle husk	20·60	6·29	7·30	7·90	1·80	62·00	8·30	3·30	2·00	1·20	4·00 (c.O.05)

Analysis for sugar components was conducted on acid hydrolysates of dialysed solution or suspension of acetic acid-extracted powders. The value of each monosaccharide is corrected for losses during hydrolysis (see Experimental) and is expressed as percentage of dry powder hydrolysed.

[5] Z. I. KERTESZ, *The Pectic Substances*, 628 pp., Interscience, New York (1951).
[6] Z. I. KERTESZ, in *Methods in Enzymology* (edited by S. P. COLOWICK and N. O. KAPLAN), Vol. 3, p. 27, Academic Press, New York (1957).

DISCUSSION

Both quantitative and qualitative differences occur between the present results and previous ones,[1,7] which can be explained in terms of the isolation and analytical procedures employed. For example, Blakemore *et al.*,[1] studying the general polysaccharide content of the pod husk, precipitated the polysaccharides with four volumes of ethanol, and found the galactose content of the acid hydrolysate to be only slightly less than the galacturonic acid (21% galactose, 27% galacturonic acid). Substantial amounts of mannose and glucose were also present. The latter is not considered to be a component of the pectic group of substances but occurs mainly as a contaminant.[8] Whistler *et al.*[7] could not detect galacturonic acid in their work on cocoa husk polysaccharides. This may be attributed to the fact that they neutralized the acid hydrolysates with $BaCO_3$ and Ag_2O. In the present work, the high proportion of galacturonic acid, the absence of mannose and the very small amounts in which glucose occurs show that partial purification has been achieved by the use of low alcohol concentrations in the precipitation step (see ref. 5).

The yield of dry pectin from pod husk as reported in the present work cannot be directly compared with the values reported by Dittmar,[2] as different cocoa selections are involved. Moreover, the latter worked on sun-dried husk, and the extent to which the pectic substances are degraded during sun-drying is not known.

It is generally believed that the importance of pectic substances in plants is the role they play during the development of the cell-wall.[8–10] It is not surprising, therefore, that in immature cocoa fruit the pectic substances form a higher proportion of the husk than in the ripe pod husk. A similar situation exists in apples.[5] It must be mentioned, however, that the ripe pods have about 9 times more dry husk material per pod than do the cherelles of the size used in the investigations. Thus the total yield of pectin from the husk of one ripe pod is estimated (from the data in Table 1) to be 3 times more than can be obtained from the husk of one immature fruit. In the course of maturation, pectic substances are converted from their more complex insoluble form (protopectin) to the simpler soluble forms by the action of pectic enzymes. The finding that cherelle preparations were more viscous than those from ripe pod husk probably reflects the more polymerized form of the pectin from the former source.

Extraction with 0·05 N or 0·1 N HCl at 70–80° appears to be too drastic as judged by loss of solubility and the ability to imbibe water. As acid hydrolysates of such preparations showed little or no rhamnose, it may be concluded that the loss of these properties resulted from partial degradation of pectin.

EXPERIMENTAL

Preparation of powdered pectin. Husk of ripe pods or cherelles was chopped, mixed thoroughly and weighed amounts placed in boiling 0·2 N HOAc (pH 2·8) (1:3, w/v). Boiling was continued for 20 min with frequent stirring and the solution removed by filtration through previously washed cheese-cloth. The residual solids were mildly homogenized and extraction was repeated until extracts were no longer viscous. The combined extract was filtered through a double thickness of cheese-cloth and mixed thoroughly. EtOH was added to 55–60% concentration. After 10–15 hr the white gelatinous precipitate was removed by centrifugation at 5000 *g* for 10 min, suspended in 95% EtOH, filtered through a sintered-glass crucible and washed with EtOH–Et_2O (1:1, v/v) followed by Et_2O. The product was dried for 24 hr under partial vacuum over silica gel at 40°, then at 60° to constant weight, and ground to pass a 60-mesh sieve. Extraction with 0·05 N and 0·1 N HCl was carried out in the same manner, except that heating was maintained at 80°.

[7] R. L. Whistler, E. Messak and R. A. Plunkett, *J. Am. Chem. Soc.* **78**, 2851 (1956).
[8] D. A. Rees and N. J. Wight, *Biochem. J.* **115**, 431 (1969).
[9] D. H. Northcote and A. J. Barett, *Biochem. J.* **94**, 617 (1965).
[10] R. R. Selvendran and B. P. M. Perera, *Chem. & Ind.* 577 (1971).

Preliminary experiments showed that acid hydrolysates of the dialysed supernatant solutions remaining after the 55–60% EtOH precipitation contained no uronic acid as measured by the carbazole reaction.[3,11]

Acid hydrolysis. Dialysed solutions were made 0·1 N with HCl and heated under reflux for 14 hr at 100° in the presence of small amounts of Zeokarb 225(H^+). The mixture was filtered through glass fibre 'paper' and undegraded material plus glass fibre 'paper' was further digested with 0·2 N HCl for 1·5 hr and filtered. The filtrate plus washings was combined with the above hydrolysate. The solution was evaporated to dryness under reduced pressure and excess HCl removed by addition of water and repeated evaporation. Aliquots of the resulting syrup were analysed colorimetrically for galacturonic acid.[3,11] For the determination of the neutral sugar components the syrup was neutralized by shaking with Amberlite IR (OH) and Zeokarb 225(H^+) prior to evaporation. Hydrolysate from the first step was found to contain the neutral sugars together with about 70% of the total polygalacturonic acid content, about 80% of the latter being in the form of higher galacturonic acid oligomers with R_G 0·08 and 0·12 in solvent systems 5 and 4 respectively. The 0·2 N HCl fraction contained mainly galacturonic acid with trace amounts of arabinose.

Paper chromatography and determination of sugars. Descending paper chromatography of hydrolysates before or after neutralization was on Whatman No. 1 paper using the following solvent systems (v/v): (1) and (2) EtOAc–pyridine–H_2O (10:4:3) and (8:2:1) respectively. (3) MeCOEt–HOAc–H_2O (9:1:1). (4) EtOAc–HOAc–HCO_2H–H_2O (18:3:1:4). (5) EtOAc–pyridine–HOAc–H_2O (5:5:1:3). Spots were located with $AgNO_3$–alc. NaOH, aniline hydrogen phthalate and *p*-anisidine-HCl. Sugars were eluted and determined with the phenol-sulphuric acid reagent in the manner described by Dubois *et al.*[12]

Recovery of sugars. In duplicate experiments in which, (1) 125 mg galacturonic acid, and (2) a mixture containing 125 mg galacturonic acid, 140 mg rhamnose, 140 mg galactose and 120 mg arabinose were treated with, respectively, (1) 0·2 N HCl for 1·5 hr and (2) 0·1 N HCl for 14 hr in the presence of Zeokarb 225 (H^+) and analysed as above, 80·5% and 75·5% of the galacturonic acid was recovered for treatments (1) and (2) respectively. Under treatment (1) 70·3% rhamnose was recovered; but heavy losses of galactose and arabinose occurred, the recoveries being 51·7% and 42·2% respectively.

Corrections for losses were made for the individual sugars in Table 1 according to the figures given above. In the case of galacturonic acid, values were corrected by a factor of 1·40. The same correction was made for xylose as for arabinose.

Inherent viscosity. Was determined[13] with a Master U-tube viscometer at 25°.

Ash and protein nitrogen determination. Ash content of samples heated at 520° for 2 hr was determined. Protein nitrogen determination was by the Kjeldhal method.

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[11] E. A. DAVIDSON, in *Methods in Enzymology* (edited by E. F. NEUFIELD and V. GINSBURG), Vol. 8, p. 52, Academic Press, New York (1966).
[12] M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS and M. SMITH, *Analyt. Chem.* **28**, 350 (1956).
[13] R. R. MYERS and R. J. SMITH, in *Methods in Carbohydrate Chemistry* (edited by R. L. WHISTLER), Vol. 4, p. 124, Academic Press, New York (1964).

Key Word Index—Theobroma cocao; Sterculiaceae; cocoa pod; pectin.