ISOLATION AND CHARACTERIZATION OF TAMARIND SEED (TAMARINDUS INDICA L.) POLYSACCHARIDE

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The alcohol-insoluble fraction from the water extract of tamarind seed meal, constituting 60 to 65 per cent of the husked kernel, has been described as a rich source of pectin (1–3). Although it forms a firm jelly in the presence of appropriate amounts of sugar and acid (1, 4) and has been suggested for commercial use as a substitute for pectin (5), it has been shown in preliminary communications by the authors (4, 6–8) and later by others (2, 3, 9–11) that it differs fundamentally from fruit pectins. Pectins are characterized by the presence of methyl ester groups and galacturonic acid units (12) with varying amounts of arabinose and galactose, presumably derived from associated araban and galactan, loosely attached to the molecule (13–15). On decomposition pectins also yield pectic acid, a product of definite chemical composition (16, 17), which is obtained either directly (18) or, better, through the insoluble calcium salt (19, 20) after hydrolysis by mild alkali followed by neutralization with acid. This paper records the results of investigations on the nature of the polysaccharide fraction of tamarind seed which show how in all these respects it differs from pectins, a conclusion necessitating revision of the views held hitherto to account for many of the phenomena of pectin-sugar-acid jelly formation (21, 22).

EXPERIMENTAL

The results of analysis of decorticated tamarind seed meal are given in Table I. The testas, which formed 28.5 to 33 per cent of the whole seed, were removed from the kernel after parching with sand, light crushing, and winnowing. All determinations were carried out according to methods of the Association of Official Agricultural Chemists (23); the values for the polysaccharide were obtained by precipitation of the aqueous extract with 50 per cent alcohol according to the method of Ghose and Krishna (1).

The alcohol precipitate yielded 8.57, 8.71, and 8.82 per cent respectively of crude proteins, expressed in terms of the weight of the original seed meal; these accounted for nearly half the total proteins of the seed meal and therefore also for the surplus over 100 per cent in Table I.

In view of the close protein-polysaccharide association (Table I), a fractionation study of the chief types of proteins in the seed meal appeared worth while. This was effected as follows:

20 gm. of the seed meal (Sample I) were extracted by refluxing with 80 per cent ethanol. The residue from the extract after removal of alcohol gave, on treatment with acidulated water or dilute alkali, a product with 0.22 gm. of total protein having certain of the characteristic properties of prolamins, which yielded on hydrolysis glutamic acid, proline, and ammonia.

The residual seed meal after extraction with alcohol was taken up in the cold in 2 liters of distilled water with a mechanical stirrer. A considerable part (8.1 gm.) of the polysaccharide and all of the albumins of the seed meal, amounting to 1.79 gm., were thus brought into solution; the latter could also be extracted with 5 per cent sodium chloride or 0.5 per cent sodium hydroxide and was completely precipitated on full saturation with ammonium sulfate.

TABLE I

Analysis of Tamarind Seed Meal

Determinations	Sample I	Sample II	Sample III
	per cent	*per cent*	*per cent*
Moisture	12.64	10.80	11.30
Ash, water-insoluble	3.73	4.37	3.87
" water-soluble	5.36	6.18	5.13
" total	8.09	10.55	9.00
" acid-insoluble	1.47	2.10	1.29
Polysaccharide (alcohol ppt., ash-free)	58.47	62.88	63.30
Crude proteins	15.69	15.28	15.35
Ether extractives	8.19	6.50	6.81
Crude fiber	4.93	1.46	2.60
Sugars	None	None	None
Tannins	"	"	0.83
Total	108.01	107.47	109.19

Ash present as Na, K, Ca, Mg, P, and Si.

The hot water-insoluble portion of the seed meal contained 1.13 gm. of glutelins insoluble in water, salt solution, and dilute alcohol, but soluble in dilute alkali.

Thus the proteins of tamarind seed meal consist of 1.1 per cent prolamin, 8.5 per cent albumin, and 5.65 per cent glutelin. It should, however, be stated here that these data were obtained by the application of conventional methods, no study having been given to the development of specific methods suitable for the extraction of the proteins of the tamarind seed.

Extraction of Gel-Forming Constituent—A convenient quantity of the seed meal (about 200 gm.) was made into a thin paste with the required quantity

of cold water and poured slowly into about 5 liters of boiling water, the heating being continued for about 20 minutes. The mixture was then filtered and the solution, after treatment with sulfur dioxide to decolorize it, was concentrated to a small bulk *in vacuo* and the gel-forming constituent precipitated by addition of an equal volume of alcohol. The product, when dried, has the following per cent composition: moisture 2.90, ash 3.98, albuminoids 14.83, crude fiber 0.96, ether extractives 1.79; the rest constituted the polysaccharide fraction.

Preparation of Pure Polysaccharide—For the purification from its associated proteins of the fraction obtained as above, several methods were tried with varying degrees of success. The proteins were not removed, or even appreciably diminished on repeated solution in water and reprecipitation with alcohol or by fractional precipitation with alcohol of different strengths. Mild acid hydrolysis resulted in simultaneous degradation, to varying extents, of both the protein and polysaccharide constituents. The latter were also precipitated together from solution by treatment with protein precipitants such as phosphotungstic and tannic acids or by full saturation with ammonium and sodium sulfates. Prolonged digestion with proteolytic enzymes like pepsin and papain removed only 30 to 35 per cent of the nitrogenous fraction. Allowing the extract to stand overnight or using centrifugation for coagulation of the proteins was also only partially successful.

The most effective method of removal of proteins was found to be by considerable dilution of the aqueous extract. Thus, when the seed meal extract was diluted with increasing amounts of water and let stand overnight, increasing amounts of albuminoids settled; the supernatant liquid could be concentrated *in vacuo* after centrifuging and the gel-forming constituent precipitated with alcohol. Table II gives a typical set of results.

Dilution of the extract beyond 1:6 did not yield a purer product and, in various trials with different lots of seed meal, the purest product, obtained as above, yielded protein varying from 1.8 to 1.9 per cent. The jellies obtained with this preparation, in the presence of appropriate amounts of sugar and acid, had the maximum gel strength.[1]

Differences between Tamarind Seed Polysaccharide and Fruit Pectins—In spite of its undoubted property of forming sugar-acid jellies, the polysaccharide of tamarind seed differs fundamentally from fruit pectins, as may be seen from Table III; the purified pectins used for this study were prepared according to Savur and Sreenivasan (24).

In addition to the foregoing, the polysaccharide, unlike pectins, forms copper, calcium, and barium salts which separate as flocculent precipitates

[1] Savur, G. R., and Sreenivasan, A., unpublished work.

in alkaline medium. It also forms a highly viscous, gelatinous product when treated with small amounts of borax, resembling in this respect gum tragacanth. Pectic enzymes, such as pectin esterase and pectinase, which hydrolyze pectins to various cleavage products such as arabinose, galactose, and pectic or galacturonic acid (30–33), do not hydrolyze tamarind seed polysaccharide. On the other hand, taka-diastase, although without action on fruit pectins, hydrolyzes the polysaccharide with the liberation of a

TABLE II

Variations in Protein Content with Dilution

1% seed meal extract, times diluted.....	0	2	3	4	5	6
Proteins in alcohol ppt., %.....	10.24	8.66	3.15	2.49	2.15	1.85

TABLE III

Comparison of Tamarind Seed Polysaccharide with Fruit Pectins

Determinations*		Apple pectin	Lemon pectin	Wood-apple pectin	Orange pectin	Tamarind seed polysaccharide
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
Fresh basis	Moisture	11.6	9.3	4.1	8.7	4.0
	Ash	2.22	1.05	2.98	2.38	3.05
	Ether extractives	0.83	1.87	0.90	2.13	1.60
	Alcohol ppt. (ash-free)	91.76	94.14	96.37	95.32	97.50
	Albuminoids	0.66	1.16	2.76	1.87	1.85
	Crude fiber	0	0	0	0	0
Dry basis	Reducing sugars	12.50	20.72	22.40	26.82	0
	Calcium pectate (19)	80.35	91.72	91.96	89.60	0
	Pectic acid (16–18)	70.82	84.94	81.47	80.16	0
	Galacturonic acid (25)	24.20	26.49	22.48	23.21	0
	Mucic acid (26)	24.70	28.81	28.08	29.22	18.84
	Uronic acid (27)	41.80	62.20	58.70	62.90	3.44
	Pentosans (28)	12.14	15.47	18.28	16.97	27.54
	Methyl ester groups (29)	7.10	9.38	8.76	9.07	0

* *Cf.* (23). The figures below in parentheses refer to bibliographic references.

maximum of 86 per cent of reducing sugars. The purified product does not reduce Fehling's solution except when hydrolyzed with acids, whereas fruit pectins, even when highly purified, readily reduce Fehling's solution, evidently on account of the associated occurrence of free reducing sugars.

It would appear therefore that classification of tamarind seed polysaccharide as a pectin would rest solely on its property of forming sugar-acid jellies (2, 3); such a classification, based purely on physical behavior and not on chemical constitution, would be haphazard and confusing (34),

especially since the Committee on Pectin Nomenclature of the American Chemical Society has clearly defined the group designation for pectins (35).

The polysaccharide cannot be classed as a mucilage either (10), for recent work has shown that substances commonly described as gums and mucilages are in fact polyuronides and that their acidic properties are due to the presence of uronic acid; hence the name, "acidic polyuronides" sometimes given to them ((34) p. 122). In an earlier communication (6), the authors had referred to the polysaccharide as a gluco-galacto-xylan and there is little doubt that it is a "polyose" or "hexopentosan" (34), a nomenclature which is based on hydrolytic and oxidation studies referred to below.

Products of Hydrolysis—As already stated, tamarind seed polyose does not reduce Fehling's solution unless hydrolyzed by dilute acids. Reducing

TABLE IV

Acid Hydrolysis of Tamarind Seed Polyose and of Fruit Pectins

Time	Sugars, expressed as glucose						
	Tamarind seed polyose		Apple pectin	Wood-apple pectin	Lemon pectin	Orange pectin	Glucose and xylose
	Sulfuric acid, per cent						
	2	4	4	4	4	4	5
min.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0	0	0	12.5	14.4	20.2	11.8	29.9
60	12.3	17.3	16.6	16.2	23.8	14.1	28.4
120	22.5	28.4	18.8	19.2	26.1	16.8	27.7
180	34.9	41.3	22.4	21.5	27.2	19.9	25.2
240	35.5	37.4	25.9	24.4	29.1	22.9	24.4
300	34.9	37.1	25.9	26.8	30.1	27.7	20.7
480	25.6	32.7	37.4	32.7	37.4	31.0	17.1

sugars formed on hydrolysis by boiling, under a reflux, a 1 per cent suspension for different periods of time with 2 and 4 per cent sulfuric acid are given in Table IV. The hydrolysates were partially neutralized with baryta and, towards the end, with barium carbonate, filtered, and the sugars in the filtrate determined by Bertrand's method (36). For comparison, the results of hydrolysis of fruit pectins with 4 per cent acid are included.

It is evident that hydrolysis with acids does not result in quantitative formation of reducing sugars and that prolonged hydrolysis results in partial destruction of sugars, an observation reported earlier (37) and confirmed by the results of hydrolysis, for varying periods, of a mixture of equal parts of glucose and xylose with 5 per cent sulfuric acid (included in the last column of Table IV). In a number of trials, the maximum amount of re-

ducing sugars found in hydrolysates of tamarind seed polyose was about 41 per cent at the end of 6 hours boiling with 3 or 4 per cent sulfuric acid.

Using 1 per cent extract of tamarind seed meal and hydrolyzing with 0.1 gm. of taka-diastase at 37.5° for 24 and 48 hours gave 74.7 and 86.7 per cent, respectively, of reducing sugars.

Identification of Sugars—The only sugars that could be identified in the acid or enzymic hydrolysate, obtained as above, were glucose, galactose, and xylose. The hydrolysate was neutralized, filtered, and concentrated *in vacuo*. An aliquot of this concentrate was heated on a water bath and treated with phenylhydrazine and glacial acetic acid. The mixture was allowed to react for 45 minutes and then cooled. The osazones of the hexoses that crystallized were removed in batches and recrystallized. Glucosazone (m.p. 204°) and galactosazone (m.p. 155.5°) were the only products identified, although the latter was, to a certain extent, contaminated with xylosazone.

Another aliquot of the concentrated hydrolysate was fermented with yeast to remove the hexoses and at the end of 24 hours the solution was treated as above for the osazone reaction. Only xylosazone (m.p. 154°) could be obtained in this way. The presence of xylose was confirmed by Bertrand's reaction (38) with the help of bromine and cadmium carbonate, when characteristic boat-shaped crystals, identified under the microscope, of the double salt cadmium bromide-cadmium xylonate were obtained. The absence of arabinose was conclusively proved by the fact that no characteristic diphenylosazone could be obtained (39). It is possible, although not probable in the light of the evidence presented here, that there may be other components of the polyose which have escaped detection.

The data for reducing sugars in the acid hydrolysate were obviously unsuited for the quantitative characterization of the polyose, in view of the destruction, to varying degrees, of the sugars under these conditions (Table IV). Calculations based on specific rotation of acid or enzymic hydrolysates were also ruled out, as it frequently happens that small experimental errors are enormously magnified and that the final results, even with mixtures of pure sugars, can be regarded as only roughly approximate (36). The actual quantities of galactose and glucose in the polyose were therefore ascertained from a study of the yield of mucic and saccharic acids on oxidation with nitric acid, while that of xylose was deduced from the yield of furfural on distillation with dilute hydrochloric acid. The procedures are detailed below.

Products of Oxidation—5 gm. of the seed polyose were treated with 300 cc. of 25 per cent nitric acid (sp. gr. 1.15) and the mixture was heated on a water bath for about 3 hours, when its volume was reduced to 50 to 75 cc. On dilution, mucic acid separated out ((36) p. 691). This was allowed to

stand for 2 hours and then filtered. The precipitate was dissolved in dilute sodium carbonate solution, filtered, and the mucic acid reprecipitated by addition of a slight excess of hydrochloric acid. It was then filtered, dried, and weighed. The product had a melting point of 225–227°, corresponding to mucic acid. It was quantitatively determined according to Tollens (26).

The filtrate obtained after removal of mucic acid was neutralized by the addition of potassium carbonate, treated with a few drops of acetic acid, and evaporated to a syrupy consistency. After addition of a few more drops of acetic acid, it was then allowed to cool, when potassium acid saccharate separated out. This was filtered and recrystallized from a small amount of water. The acid salt was neutralized with ammonia and the excess ammonia removed by boiling. On addition of silver nitrate, a white

TABLE V

Products of Oxidation

Substance	Mucic acid	Saccharic acid
	per cent	*per cent*
Apple pectin	24.70	0
Wood-apple pectin	28.08	0
Orange pectin	29.22	0
Lemon "	28.81	0
Tamarind seed polyose	18.84	64.59

precipitate was obtained. This was separated, dried, and carefully ignited. The residue was cooled and weighed. The equivalent weight of the acid was calculated from the formula

$$\text{Equivalent weight} = \frac{\text{Weight of Ag salt}}{\text{Weight of Ag}} - 1$$

A value of 206.56, corresponding to that of saccharic acid (210), was obtained.

The yields of mucic and saccharic acids, obtained as above, are given in Table V in comparison with the values for mucic acid of purified fruit pectins (24); the latter did not yield saccharic acid.

Probable Molecular Proportion of Sugars in Polyose—The purified polyose on distillation with 12 per cent hydrochloric acid yielded furfuraldehyde, which as phloroglucide amounted to 30.0 per cent (26). The xylose equivalent of this was calculated from Krober tables ((36) p. 1276). From this and from the yields of mucic and saccharic acids (Table V) obtained on oxidation with nitric acid ((36) p. 691) the amounts of xylose, galactose, and glucose were calculated to be 28.42, 16.19, and 55.36 per cent respectively;

these values correspond approximately to a molecular ratio of 2:1:3 of xylose-galactose-glucose. On the basis of the results recorded here the polyose may therefore be classed as a xylo-galacto-glucosan. There is no evidence on record of a similar polyose with these sugars as components ((34) p. 70). The constitution of the polyose remains to be worked out, but, considering the fibrous nature of the product and its gel-forming property, it may be presumed to have a chain structure rather than a ring structure (40).

SUMMARY

1. An aqueous extract of tamarind seed meal gives, on precipitation with 50 per cent alcohol, 60 to 65 per cent of a polysaccharide which forms jellies with sugar and acid as in the case of fruit pectins.

2. The polysaccharide obtained as above is associated with 14 to 15 per cent of crude proteins, accounting for nearly half of the total seed proteins: the jelling property is not, however, related to the protein fraction.

3. The polysaccharide could be freed from most of the proteins by excessive dilution (1:100 or above), followed by standing overnight to coagulate the latter.

4. The purified polysaccharide is free from methyl ester groups and galacturonic acid units, does not give the Carre and Haynes reaction for pectins, and differs also in other respects from fruit pectins.

5. The only sugars formed on acid or enzymic hydrolysis of the polysaccharide are xylose, galactose, and glucose. From a quantitative study of the products of oxidation and hydrolysis, it is concluded that the approximate molecular proportion in which these sugars are present in the polysaccharide is 2:1:3 respectively.

6. The polysaccharide is designated as a "polyose" or "hexo-pentosan" which may be specifically termed gluco-galacto-xylan.

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