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Preliminary Studies on the Mucilages Extracted from Okra Fruits, Taro Tubers, Jew's Mellow Leaves and Fenugreek Seeds

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ABSTRACT

Okra fruits, Taro tubers, Jew's mellow leaves and Fenugreek seeds are commonly used in Egypt to prepare popular diets with desired slimy consistency.

The mucilages were extracted and preliminary studies conducted to characterise them physically. The pH values of a 1% solution of the mucilages varied from 6·9–7·5 for Okra and Taro, 7·1–7·8 for Jew's mellow, and 5·9–6 for Fenugreek, depending upon extraction conditions. The highest viscosity was observed in Okra solutions, followed by Fenugreek, Jew's mellow and Taro mucilages. Okra and Jew's mellow mucilages are acidic polysaccharides which contain higher amounts of ash than the Taro and Fenugreek mucilages which are neutral polysaccharides. All mucilages are associated with protein. Gel chromatography indicated strong interaction of protein with the polysaccharide. The acid hydrolysis of the mucilages followed by paper chromatography revealed that all mucilages contain methyl pentose, glucose, galactose, and fructose, in different proportions. Taro and Fenugreek mucilages are free of rhamnose. All mucilages are devoid of arabinose and mannose except Fenugreek which contained these two sugars.

INTRODUCTION

Mucilages are polysaccharides, widespread in a number of plants, which form viscous colloidal dispersions in water from which they can often be precipitated with the usual protein precipitants (Jones & Smith, 1945). In Egypt, Okra fruits (*Hibiscus esculentus* L.), Taro tubers (*Colocasia antiquorum*) and Jew's mellow leaves (*Corchorous olitorius*) are commonly used to prepare local dishes with a desired slimy consistency. The ground Fenugreek seeds (*Trigonella foenum graecum* L.) are used to brew a viscous hot drink.

Previous investigations on the composition and properties of Okra mucilage have been reviewed (BeMiller, 1973) and different compositions of the mucilage are given. Whistler & Concard (1954*a*, *b*) found Okra mucilage to be an acidic polysaccharide consisting of galactose, rhamnose and galacturonic acid. Amin (1956) also found these three carbohydrates, as well as arabinose, while Kelkar *et al.* (1962), on hydrolysis of the mucilage, found glucose and glucosamine and quantitatively determined six amino acids. Taro and Fenugreek mucilages were reported to be neutral mucilages (Jones & Smith, 1945). They reported that the Taro mucilage contained D-glucose but little is known about the detailed sugar composition. Fenugreek mucilage has been identified as a mannogalactan which contains D-galactose, and D-mannose (Daoud, 1932). The structure of Fenugreek galactomannan is based upon a chain of six $1 \rightarrow 4$ linked D-mannopyranose units, and these are attached to D-galactopyranose residues (Smith & Montgomery, 1959). There are no available data concerning the chemical composition of Jew's mellow leaves mucilage. Thus this study was undertaken to investigate the chemical composition of the mucilages prepared from Okra fruits, Taro tubers, Jew's mellow leaves and Fenugreek seeds, in an attempt to assess their functional properties and technological uses.

MATERIALS AND METHODS

Materials

Okra fruits (*Hibiscus esculentus* L.), Taro tubers (*Colocasia antiquorum*), Jew's mellow leaves (*Corchorous olitorius* and Fenugreek seeds (*Trigonella foenum graecum* L.), were purchased from a local market at Alexandria, Egypt.

Preparation of samples
All the samples were cleaned, washed and air-dried. Okra fruits and Taro tubers were trimmed and sliced. The leaves of Jew's mellow were separated. Fenugreek seeds were ground in a laboratory Wiley mill to pass through a 40 mesh sieve.

Methods

Extraction of mucilage
All the prepared samples were homogenised (Waring blender) with four times their weight of water except for Fenugreek where the ratio was 6:1. The viscous homogenate was heated at 70 °C for 15 min to inactivate enzymes; then cooled and squeezed through a cheese cotton cloth. The crude mucilage was precipitated, from the resulting viscous solution, with three volumes of acetone, and washed using ethanol followed by acetone. The coloured crude preparation was dried overnight at 35 °C on trays, followed by milling through a 60 mesh screen.

Purification of mucilages
The method used was that described by Woolfe *et al.* (1977), which is an adaptation of the general method of gum isolation from foods (Glicksman, 1969). The crude mucilage (1 %) was homogenised (Unipan homogeniser) with cold dilute trichloroacetic acid solution, (5 %). The solution was centrifuged (3500 rpm for 20 min), neutralised with sodium hydroxide by dropwise addition, and then dialysed for 30 h against distilled water at 4 °C. The dialysis water was changed every 6 h. The mucilage was precipitated with ethanol (three volumes), washed successively with ethanol, acetone and diethyl ether, allowed to dry at room temperature on stainless steel trays, and finally ground.

Proximate analysis of mucilages
Crude and purified mucilages were analysed for moisture, ash and total nitrogen (TN) by the methods of the Association of Official Analytical Chemists (1975). The micro-Kjeldahl method was used for the TN estimation and the crude protein was calculated by multiplying total nitrogen by the factor 6·25. True protein was determined by the method of Lowry *et al.* (1951) after the samples had been dissolved in 0·1 M sodium hydroxide. Crude fibre was determined by the neutral detergent method of VanSoest and Wine (1967). The mucilage content of crude and purified

preparations was gravimetrically determined as described by Hassan and Boctor (1962). The ethanol-soluble carbohydrates, obtained by exhaustive extraction with hot 70% ethanol, as described by Lineback and Ke (1975), were determined by the phenol sulphuric acid method of Dubois *et al.* (1956) and arbitrarily expressed as glucose equivalent.

Measurement of physical properties

Bulk density (g/5 ml) of the mucilage preparations (40 mesh) was determined by the accurate weighing of 5 ml. Specific gravity (g/ml) of 0·3% aqueous solutions of the mucilage was determined using the pycnometer method as described by the AOAC (1975).

pH measurements were carried out using 1% aqueous solutions of the mucilages. Refractive index readings of mucilage solutions having the same density (0·9970) were estimated using a Carl Zeiss–Jena Abbé refractometer at 30 °C. The specific rotations ($[\alpha]_D^{20}$) of different mucilage solutions (0·3% in 1 M sodium hydroxide) were measured by a Carl Zeiss–Jena polarimeter at 30 °C, and the readings corrected for temperature difference.

Viscosity measurements of the 1·0% mucilage solutions were carried out at 30 °C using the rotary viscometer type RN211-VEB MLW. The viscosity was calculated and expressed in centipoise units.

The solubility of the mucilages in distilled water was determined as follows: 10 g of the mucilage were soaked in 100 ml distilled water at 30 °C, and homogenised for 5 min using a Waring blender at high speed. The suspension was centrifuged at 3500 *G* for 30 min. An aliquot was taken from the supernatant to determine the dry weight (at 105 °C for 12 h) as criterion of solubility. The solubility of mucilage was expressed as mg per ml distilled water.

Infra-red spectra

A potassium bromide disc of the purified and dried (over phosphorus pentoxide in an evacuated desiccator) mucilage was prepared and the infra-red spectrum was recorded on a Shimadzu IR 400 spectrophotometer between 4000 and 650 cm^{-1}.

Fractionation of mucilages by gel chromatography

The purified mucilages were fractionated by gel chromatography on a column (1·6 × 30 cm) of Sephadex G-100 (40–120 U) run at a flow rate of

18 ml/h at room temperature (25–28 °C) using 0·1 M citric acid as eluent. A Unipan fraction collector was used to collect 3·5-ml fractions which were analysed for protein as described by Lowry *et al.* (1951) and for carbohydrates using the phenol–sulphuric acid method of Dubois *et al.* (1956). The molecular weights of the fractionated polysaccharides and proteins were calculated as described by Fischer (1969).

Acid hydrolysis of mucilages

Three degrees of hydrolysis were used (Pearson, 1970). The first (a) was 0·5 M H_2SO_4 for 1 h at 100 °C, which cleaves 6-deoxyhexoses; the second (b) was 1 M H_2SO_4 for 4 h at 100 °C which cleaves hexose and pentose glycosidic bonds; and the third (c) was 2 M H_2SO_4 for 4 h at 100 °C to hydrolyse uronic acid linkages. Purified mucilage (100 mg) was weighed into a stoppered tube and 5 ml of the appropriate acid added. Nitrogen gas was bubbled through the suspension, and the tubes were stoppered and placed in a boiling water bath for the appropriate time. The hydrolysates were cooled, neutralised with saturated barium hydroxide solution and filtered through Whatman No. 1 filter paper. The filtrate was evaporated to dryness in a rotary evaporator (Gallenkamp) and finally dissolved in 3 ml distilled water.

Quantification of sugars and uronic acids in mucilage hydrolysates

The mucilage hydrolysates were analysed for total soluble carbohydrates by the phenol–sulphuric acid method of Dubois *et al.* (1956). The hydrolysates were analysed quantitatively by paper chromatography using the method of Lineback and Ke (1975). Standard sugar and uronic acid mixtures were applied beside the hydrolysates and the spots were detected on the dried papers with the aniline–diphenylamine spray reagent (Dawson *et al.*, 1969). The corresponding sugar spots were eluted with water and quantified by the phenol–sulphuric acid method of Dubois *et al.* (1956). The separated and identified uronic acids were determined by the carbazole method (Dietz & Rouse, 1953). The sugar and uronic acid contents of different mucilages are expressed in mole ratios of the anhydroforms.

RESULTS AND DISCUSSION

The process of the crude mucilage extraction yielded 20, 25, 44 and 95 g/kg of Okra fruits, Taro tubers, Jew's mellow leaves and Fenugreek seeds,

respectively. The purification of these crude preparations afforded friable solids in 64, 81, 59 and 32% yield for Okra, Taro, Jew's mellow and Fenugreek, respectively.

The results of the proximate analysis of the different crude and purified mucilages on dry weight basis are shown in Table 1. These results are useful in giving a hint of the relative proportions of the different components, although preparations may vary from one to another and the results of the chemical analysis of the mucilages must not be considered as absolute. As shown in Table 1, of the crude preparations Taro contained the highest percentage of mucilage followed by Okra and Fenugreek, while Jew's mellow preparation contained the lowest percentage. After purification the percentage of mucilage increased, being approximately doubled in Okra, Jew's mellow and Fenugreek preparations. The most interesting feature of the results is the presence of crude protein in the different mucilage preparations, both crude and purified. This was expected for the crude mucilages considering the method of extraction and precipitation. The percentage of true protein was found to be comparatively low in crude preparations and decreased to about half its percentage in Okra, Jew's mellow and Fenugreek after purification.

The decrease in true protein of purified Taro mucilage was less and amounted to 16%. This may be explained on the basis that the proteins are associated with the polysaccharides in such a manner that they are not precipitable with trichloroacetic acid during the purification. McGarvie & Parolis (1979) reported that the mucilages of *Opuntia ficus indica* contain 0·34–1·56% total nitrogen. Crude Fenugreek mucilage contained the highest percentage of crude fibre, followed by Jew's mellow, Okra and Taro mucilages. After purification the crude fibre content decreased to about 60·3, 60·3, 43·3 and 55·3% of its original content in Okra, Taro, Jew's mellow and Fenugreek, respectively. The high contents of crude fibre of some mucilages, either crude or purified, indicates the possible association of crude fibre constituents with other components which are present in the preparations (Aspinall, 1959). Grant *et al.* (1969) reported that mustard mucilage contains cellulose in a crystalline condition.

Alcohol-soluble carbohydrates were the highest in crude Okra mucilage followed by Taro, Jew's mellow and Fenugreek mucilages. On the other hand, the purified mucilages were found to be free of the alcohol-soluble carbohydrates. The ash content of the crude and purified preparations obtained from Okra and Jew's mellow is higher than that

TABLE 1
Proximate Analysis of Different Preparations of Crude and Purified Mucilages[a,b]

	Okra		*Taro*		*Jew's mellow*		*Fenugreek*	
	Crude	*Purified*	*Crude*	*Purified*	*Crude*	*Purified*	*Crude*	*Purified*
Moisture	11·6	14·7	12·1	8·2	8·7	14·2	13·6	11·3
Mucilage	42·2	83·9	79·6	90·2	22·8	49·6	41·9	81·1
Total nitrogen	3·19	0·94	1·24	0·85	5·87	2·52	6·69	2·91
Crude protein (N × 6·25)	19·9	5·87	7·72	5·32	36·68	15·8	41·8	18·18
True protein	6·38	3·97	5·34	4·56	13·8	7·9	16·3	8·76
Crude fibre	21·0	8·31	6·19	2·46	29·7	16·8	51·2	22·9
Ethanol-soluble carbohydrates	3·28	0·0	2·16	0·0	1·8	0·0	1·3	0·0
Ash	7·0	6·0	3·7	1·8	11·1	8·6	2·3	1·1

[a] All data presented as percentage of dry weight except moisture content.
[b] Mean of three determinations.

prepared from Taro and Fenugreek. These data may indicate that Okra and Jew's mellow mucilages are acidic in their nature and consequently may combine better with metallic ions than those mucilages which are neutral.

Physical properties of mucilage preparations

Table 2 shows some physical properties of the mucilage preparations. Taro and Fenugreek mucilages had the highest bulk density, followed by Jew's mellow and Okra mucilages. Specific gravity was approximately the same in all mucilages. Refractive index measurements indicate that all mucilage solutions with the same density have the same refractive index.

TABLE 2
Some Physical Characteristics of Different Mucilage Preparations[a]

	Mucilage of:			
	Okra	*Taro*	*Jew's mellow*	*Fenugreek*
Colour	Cream	Light brown	Olive green	Cream
Bulk density (g/5 ml)	1·159	3·679	2·325	3·543
Specific gravity	0·996 5	0·996 4	0·997 1	0·997 0
pH	6·9–7·5	6·9–7·5	7·1–7·8	7·9–6·0
Solubility (mg/ml)	62·5	65·4	38·3	47·6
Refractive index	1·336 2	1·336 5	1·336 5	1·336 8
Specific rotation $[\alpha]_D^{20}$	+22·2	+27·4	+57·6	+31·6
Viscosity (cP)	19·2	2·96	9·86	17·8

[a] Purified mucilages

The results indicate that all mucilage preparations are soluble in water. The solubility of mucilages and gums in water was previously reported by Hirst & Jones (1958) and that of Fenugreek mucilage in water by Shankaracharya & Natarajan (1972).

The viscosity of a 1% solution of the mucilage was highest in Okra mucilage followed by Fenugreek, Jew's mellow and Taro. The viscosity of Taro mucilage was very low compared with that of Okra mucilage.

Infra-red analysis

As indicated by Glicksman (1969), the amount of information that can be obtained from the infra-red spectrum of polysaccharides is rather limited. The usual bands for hydroxyl (3700–3100 cm^{-1}), ester carbonyl

(1730–1720 cm^{-1}) protein (carbonyl stretch), amide deformation (1700–1550 cm^{-1}) and phosphate (1150–1000 cm^{-1}) can be distinguished in the spectra of purified mucilages.

Gel chromatography of purified mucilages

Gel chromatography of purified mucilages was used as an analytical guide to protein and carbohydrate molecular weight distribution (Woolfe *et al.*, 1977). The results are shown in Fig. 1. The elution patterns indicate that, in all tested mucilages, most of the polysaccharides are totally excluded from the gel and eluted at the void volume. This means that these polysaccharides have average molecular weights greater than 100 000 (Fischer, 1969) (Table 3). The results indicate that Taro mucilage contains a minor peak, which represents 18·8% of the total polysaccharides, with molecular weight about 84 000. Although, the purification process of different mucilages depends on the treatment of the crude preparation with trichloroacetic acid, proteins are found in all the purified preparations. Most of the proteins in Jew's mellow and Fenugreek mucilages are eluted in the excluded fraction associated to the polysaccharide peak. This means that these proteins have molecular weights greater than 150 000 (Fischer, 1969). Further experiments are needed to explore the nature of the polysaccharides of the purified mucilages using other types of gel.

Carbohydrate analysis of purified mucilages

The three degrees of hydrolysis of the four mucilages gave the same neutral sugars for each hydrolysis as previously reported by Woolfe *et al.* (1969).

The results are presented in Table 4. The carbohydrate composition of the Okra mucilage agrees with previous findings of other workers (Whistler & Concard, 1954*a*, *b*) of rhamnose, galactose and galacturonic acid; moreover, glucose, fructose, xylose and methyl pentose were detected. The concentration of glucuronic acid was found to be about four times that of galacturonic acid. The Okra mucilage was free of arabinose, a result which agrees with that of Woolfe *et al.* (1977). These results differed from those of Amin (1956), who found that the proportions of arabinose, galactose, rhamnose, and galacturonic acid present in Okra mucilage were 3:80:10:6. Jew's mellow mucilage contained the same neutral sugars except xylose. It appears to have high proportions of uronic acids. The mole ratio of glucuronic acid was two

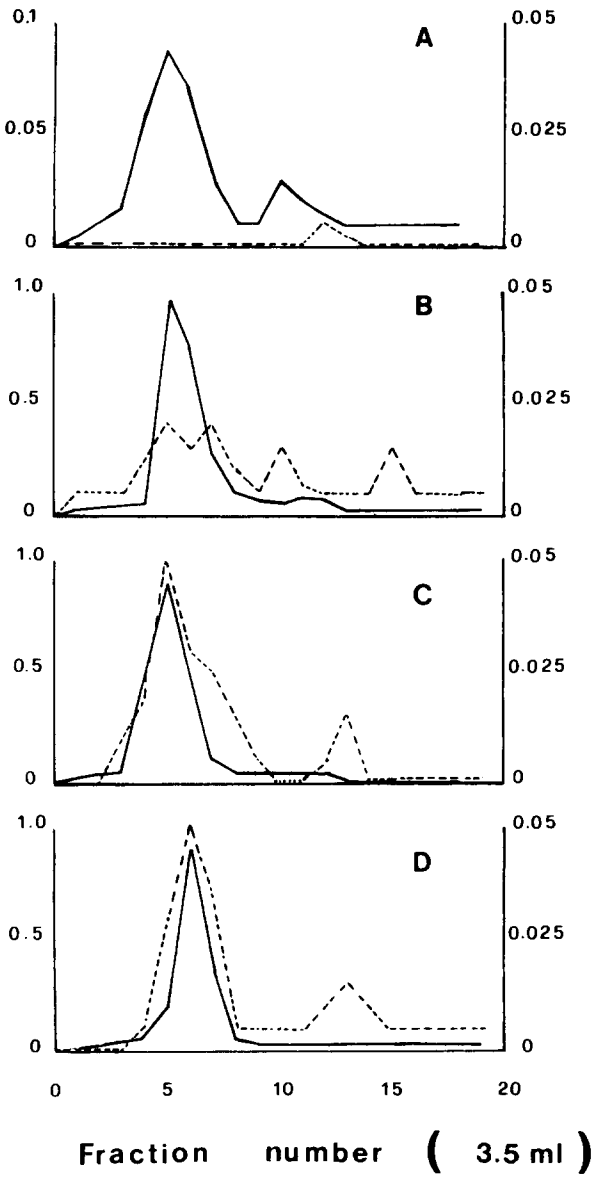

Fig. 1. Gel chromatography of purified mucilages on Sephadex G-100. ——, Absorbance at 480 nm; – – –, absorbance at 740 nm. A, Taro; B, Okra; C, Jew's mellow; D, Fenugreek.

TABLE 3
Molecular Weight of Polysaccharides and Proteins Fractionated from Different Mucilages on Sephadex G-100

Mucilage	*Molecular weight*	
	Polysaccharides	*Proteins*
Taro	> 100 000	98 900
	84 000	
Okra	> 100 000	> 150 000
		133 000
		65 000
Jew's mellow	> 100 000	> 150 000
		137 000
		78 000
Fenugreek	> 100 000	> 150 000
		137 000

TABLE 4
Quantitative Carbohydrate Analysis of Purified Mucilage Preparations

Carbohydrate	*Okra*		*Taro*		*Jew's mellow*		*Fenugreek*	
	% of dry weight	*Mole ratio*	*% of dry weight*	*Mole ratio*	*% of dry weight*	*Mole ratio*	*% of dry weight*	*Mole ratio*
Neutral sugars								
Arabinose	0·0	0·0	0·0	0·0	0·0	0·0	25·1	2·9
Xylose	4·35	0·5	1·88	0·2	0·0	0·0	0·0	0·0
Methyl pentose	7·2	0·9	4·35	0·5	7·8	2·8	15·4	2·0
Glucose	6·75	0·6	23·7	2·1	8·25	2·1	3·3	0·3
Galactose	11·1	1·0	11·6	1·0	3·9	1·0	10·7	1·0
Mannose	0·0	0·0	0·0	0·0	0·0	0·0	22·1	2·0
Fructose	8·25	0·8	13·4	1·2	6·75	1·8	6·3	0·6
Rhamnose	15·8	1·6	0·0	0·0	10·2	2·9	0·0	0·0
Uronic acids								
Galacturonic	4·17	0·4	0·0	0·0	16·6	3·9	0·0	0·0
Glucuronic	16·7	1·4	0·0	0·0	35·8	8·5	0·0	0·0

times that of galacturonic. Both Okra and Jew's mellow mucilages, whilst having different compositions, could be classed as glucuronorhamnans. Aspinall (1969) classed Okra mucilage in the galacturonorhamnose group. The results revealed that the Okra and Jew's mellow mucilages are acidic polysaccharides which contain different proportions of uronic acids. This gives the clue to why these two mucilages contain higher amounts of ash when compared to Taro and Fenugreek.

Taro and Fenugreek mucilages were found devoid of uronic acids, so they may be classed as neutral polysaccharides. The absence of uronic acids in Taro and Fenugreek mucilages agrees with previously mentioned work by Jones & Smith (1945), who reported Taro and Fenugreek to be neutral mucilages. The presence of mannose and galactose in the Fenugreek mucilage is in agreement with Daoud (1932) and Smith & Montgomery (1959) who classed Fenugreek as a mannogalactan.

From the above data it may be concluded that the prepared mucilages exhibited pronounced physical properties. Work is now in progress to evaluate the functional and technological properties of these mucilages.

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