Studies on the Mucilages Extracted from Okra Fruits *(Hibiscus esculentus* L.*)* and Baobab Leaves *(Adansonia digitata* L.*)*

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Okra fruits and baobab leaves are just two examples of foods used to give a mucilaginous quality to West African food dishes. The mucilages were extracted from both foods and purified. Preliminary studies have been conducted to characterise the mucilages chemically, as well as study their viscous behaviour in relation to their use in West African dishes. Both mucilages are acidic polysaccharides with associated protein and minerals. Neither the quantity of protein nor minerals were significantly reduced during purification. The protein was not separated from the polysaccharide by either gel chromatography or disc electrophoresis. Hydrolysis of okra mucilage revealed that the polysaccharide was composed of galacturonic acid, galactose, rhamnose and glucose (1.3:1.0:0.1:0.1). Baobab mucilage on hydrolysis was found to contain mainly galacturonic and glucuronic acids with minor quantities of galactose, rhamnose, glucose and arabinose (11.7:11.3:1.0:0.6:0.4:0.1). The mucilages form viscous solutions at low concentrations (5–10 g/litre). They attain maximum viscosity in the neutral pH range. However, the mucilage solutions are not stable to heat and lose much of their viscosity when heated.

1. Introduction

Mucilages and gums are water soluble polysaccharides found in a widespread number of plants,[1] and also in some microorganisms. In many areas of West Africa, mucilaginous foods are commonly used to impart a desired slimy consistency to local soups and stews. Two foods are prominently used, and have been chosen for this investigation; these are the fruits of the okra plant (*Hibiscus esculentus* Linn.) and the leaves of the baobab tree (*Adansonia digitata* Linn.)

Okra, originally an Indian plant, is now grown in many other areas of the world including the Middle East, Africa and the Southern States of the USA. There appears to be a large number of different varieties which are used because of their differing content of mucilage, the mucilaginous varieties being popular in West African and Creole cooking. A typical Ghanaian okra soup would contain approximately 0.2–0.3 % mucilage by weight. In West Africa, the plant is widely cultivated because the leaves can also be consumed and the stem is used for fibre and rope. The fruits are used both in the fresh and dried forms, the latter form being common in the Sahel.

The baobab tree occurs in many parts of the tropics, mainly as a component of secondary forest. Its occurrence in savannah areas usually indicates human introduction. The consumption of baobab leaves and fruits is restricted to the Sahel. Only the leaves are mucilaginous and are consumed fresh or dried. Soups prepared from the leaves are particularly popular as a weaning food.

Previous investigations into the composition and properties of okra mucilage have been reviewed by BeMiller.[2] Various workers have given different compositions of the mucilage.[3–6] Whistler and Conrad[3,4] found okra mucilage to be an acidic polysaccharide consisting of galactose, rhamnose and galacturonic acid. Amin[5] also found these three carbohydrates, and arabinose in addition.

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Kelkar et al.[6] on hydrolysis of the mucilage found glucose and glucosamine and quantitatively determined six amino acids. Okra mucilage has been found to have potential uses as an extender for serum albumin and as an additive to dried egg white.[2] There does not appear to have been any investigation into the composition or uses of mucilage extracted from baobab leaves.

This study was undertaken primarily to investigate the use of the two mucilages in local foods and their possible wider uses as food additives. Viscosity measurements were made under conditions of varying concentration, pH and temperature. Chemical analysis was also carried out to characterise the mucilages.

2. Experimental

2.1. Extraction of the mucilages

Okra fruits were purchased from a local market; a variety of okra used by the Ewe tribe and noted for its sliminess was chosen. The seeds do not contain any mucilage and were removed prior to extraction. The okra was sliced, homogenised with five times its weight of water, centrifuged at 4000 g for 15 min and the clear, viscous solution decanted. The solution was heated at 70°C for 5 min to inactivate enzymes, and recentrifuged. The mucilage was precipitated with three volumes of ethanol and washed with more ethanol followed by acetone. The cream coloured solid was dried under vacuum (less than 1 Torr at 25°C for 12 h) and gave a yield of 16 g mucilage/kg okra.

Baobab leaves were obtained from a tree on the University campus. The leaves were blanched for 3 min at 100°C, before following the above extraction procedure; no further heat treatment was given. The yield of crude baobab mucilage was 18 g mucilage/kg leaves.

2.2. Purification of the mucilages

An adaption of the general methods of isolation of gums from food was used.[7] The crude mucilages (1 %) were homogenised (Potter homogeniser) with cold dilute trichloroacetic acid solution (5 %). The solution was centrifuged (3500 g for 20 min), neutralised with sodium hydroxide by dropwise addition, and then dialysed for 30 h against distilled water. The mucilage was reprecipitated with ethanol (three volumes), and washed successively with ethanol, acetone and diethyl ether.

Final purification for analytical purposes was effected by gel chromatography on a column (2.5 × 65 cm) of Sephadex G-100 run at 10 ml/h at 4°C in 0.1 M citric acid. Fractions were analysed for protein spectrophotometrically at 280 nm, and for carbohydrate by using the cysteine assay on a microscale.[9] The high molecular weight peak was bulked for colorimetric sugar analyses.

2.3. Disc electrophoresis

The mucilages were analysed by disc electrophoresis using a concentrating buffer at pH 6.9, and a running buffer at pH 9.5.[9] The gels were stained for protein using Coomassie Brilliant Blue, and for carbohydrate using periodate/Schiff's reagent.

2.4. Proximate analysis of the mucilages

Moisture and ash were determined by AOAC methods.[10] Total nitrogen was determined by the semi-micro Kjeldahl method.[10] Potassium, calcium and magnesium content were measured by atomic absorption spectroscopy (Techtron Model AA 100) on the digested mucilages. Total phosphorus was determined by the vanado-molybdate colorimetric method.[11]

2.5. Analysis of the sugars in the mucilages

2.5.1. Acid hydrolysis

Three degrees of hydrolysis were used.[12] The first which cleaves 6-deoxy hexoses was with 0.5 M-H_2SO_4 for 1 h at 100°C. The second, which cleaves hexose and pentose glycosidic bonds, was the 1 M-H_2SO_4 for 4 h at 100°C. The third was with 2 M-H_2SO_4 for 4 h at 100°C to break uronic acid linkages.

Purified mucilage (50 mg) was weighed into a stoppered tube and 5 ml of the appropriate acid added. Nitrogen gas was bubbled through the suspension, the stopper replaced and the tubes placed in a boiling water bath for the appropriate time. The hydrolysates were cooled, neutralised with barium carbonate, filtered and concentrated under vacuum.

2.5.2. Thin-layer chromatography

The hydrolysates prepared above were analysed qualitatively by t.l.c. Reducing sugars were run on silica gel G plates (0.25 mm) buffered with 0.02 M sodium acetate using the solvent system acetone-water–chloroform–methanol (75:5:10:10).[13] Standard sugar mixtures were applied beside the hydrolysates and the spots developed using an aniline/diphenylamine or anisaldehyde/sulphuric acid spray.

Uronic acids were analysed qualitatively on 20 cm square cellulose plates (0.25 mm) in the solvent system iso-propanol–pyridine–acetic acid–water (8:8:1:4) and developed with aniline/diphenyl-amine.[14]

Quantitative analysis of the uronic acids in baobab mucilage was also performed by scraping off bands (1 cm) from an undeveloped plate, eluting with distilled water, and assaying for uronic acids as in section 2.5.3.

2.5.3. Colorimetric sugar analysis

The bulked mucilage solution obtained by gel chromatography was analysed for hexoses and 6-deoxy hexoses by the cysteine-sulphuric acid reaction.[15] Total uronic acid was determined by the carbazole reaction.[16]

2.5.4. Quantitative neutral sugar analysis

Mucilages were acid hydrolysed with 2 M trifluroacetic acid for 6 h at 100°C, and analysed for neutral sugars by automatic carbohydrate analysis using borate ion-exchange chromatography/orcinol-sulphuric acid assay system on a Jeol-6AH carbohydrate analyser.

2.5.5. Incubation of mucilage with pectin esterase

The mucilage solutions were tested for the presence of pectin by titration with sodium hydroxide (0.05 M) after incubation with tomato pectinesterase (0.2 mg, E.C. 3.1.1.11, Sigma) at 30°C for 1 h at pH 7.5.

2.6. Infrared spectra of the mucilages

A potassium bromide disc of each of the dried purified mucilages was prepared, and the infrared spectra recorded (Perkin-Elmer 720) between 4000 and 650 cm^{-1}.

2.7. Viscosity measurements on the mucilages

2.7.1. Measurement of viscosity

Relative viscosities (η mucilage/η water) of the mucilage solutions were measured using a Canon-Fenske capillary viscometer (Technico, Size 300), held in a temperature controlled water bath. Flow times were measured in triplicate and the average taken.

2.7.2. The effect of concentration on viscosity

A 1% solution of each mucilage was prepared using a Potter homogeniser (higher concentrations being difficult to disperse), and the exact concentration determined by removing an aliquot and drying in a vacuum oven. The stock solution was then diluted and the relative viscosities measured. The pH was checked to make certain it was constant in all the solutions.

2.7.3. The effect of pH on viscosity

A stock solution of each mucilage was prepared and divided in half. The pH was varied by the separate addition of very small volumes of either concentrated sodium hydroxide or hydrochloric acid to each half of the stock solution. The use of concentrated reagents minimised any dilution effects. The relative viscosity with respect to change of pH was determined.

Changes in relative viscosity in the presence of added calcium ions were also measured. Solutions of each mucilage were prepared to give similar initial viscosities. 50 μl aliquots of 1 M calcium chloride, were added, stepwise to 50 ml of each mucilage solution. After each addition, the relative viscosity was measured.

2.7.4. The effect of heating on viscosity

The two mucilage solutions were heated in steps of 10°C up from room temperature to 90°C, and then cooled back to room temperature again in steps of 10°C and the relative viscosity measured at each 10°C step.

The effect of boiling the solution for differing lengths of time was also measured, using fresh stock solution for each boiling time.

3. Results and discussion

3.1. Introduction

The physical properties of the two foods have been examined by study of their extracted mucilages. Results of chemical analysis have been used to explain this behaviour. It is recognised, however, that although the mucilage is probably the most important contributor to these physical properties, it might not be the only one. Interactions between mucilage and other components of the plant material might be taking place, and after extraction are unable to do so.

A change was noticed on precipitation of the mucilage. Whereas the homogenates of okra and baobab were clear after centrifugation, solutions of either crude or purified mucilages were always cloudy and opaque at neutral or acid pHs. Precipitation of the mucilage causes the coprecipitation of some other material, that gives a visible colloidal suspension on rehomogenisation.

3.2. Chemical analysis

The results of the chemical analysis of the mucilages must not be considered as absolute. Extracts will vary from one to another, but the results are useful in giving a hint of relative proportions of the different components.

The contents of moisture, protein and minerals are given in Table 1, for the crude and purified mucilages. The most interesting feature of the results is the high protein and mineral content. This was expected for the crude mucilage considering the method of extraction. The change of protein content during purification is small, despite precipitation with t.c.a. This could indicate a fairly

Table 1. Protein, ash and mineral content of crude and purified okra and baobab mucilages (percentage of wet wt)

	Okra		Baobab	
	Crude	Purified	Crude	Purified
Moisture content	9.35	9.37	8.00	7.80
Protein (N × 6.25)	9.42	7.31	6.58	6.32
Ash	5.95	4.81	9.88	8.80
Magnesium	0.51	0.41	0.33	1.13
Calcium	2.02	2.83	5.13	4.82
Potassium	0.68	0.11	1.11	0.09
Phosphorus	0.18	0.14	0.21	0.07

strong interaction of protein with the polysaccharide. The mineral content also is not decreased by dialysis in the purification process. The ash content can be accounted for by calcium, magnesium, potassium (calculated as oxides) and phosphate ions. Purification alters the distribution of the ions, the divalent ion concentration increasing. The retention of these ions could indicate binding by the acid groups of the polysaccharide.
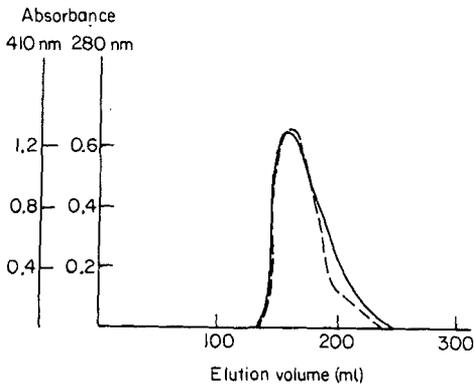

Figure 1. Elution profile of purified okra mucilage on Sephadex G-100 for —— protein (absorbance at 280 nm), and – – – – carbohydrate (absorbance at 410 nm after cysteine assay).
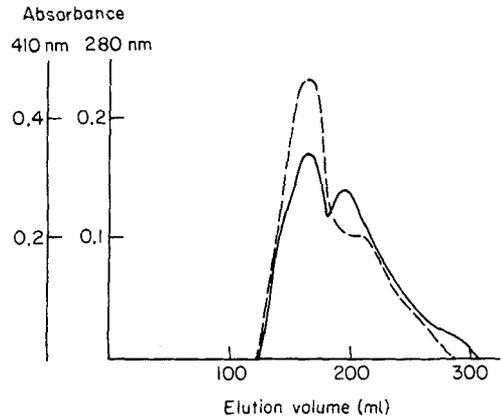
Figure 2. Elution profile of purified baobab mucilage on Sephadex G-100 for —— protein (absorbance at 280 nm), and – – – carbohydrate (absorbance at 410 nm after cysteine assay).

Gel chromatography of the purified mucilages was used as an analytical guide to protein and carbohydrate molecular weight distribution. The results are shown in Figure 1 and 2. The curves indicate that okra mucilage has a relatively narrow range of molecular weight distribution for both protein and carbohydrate. Most of the protein and carbohydrate is eluted in the excluded fraction which means that they have a molecular weight greater than 100 000. There is a minor peak (mol. wt. about 50 000) on the tail of the main peak. Baobab mucilage is slightly less homogeneous, but has a major high molecular peak (greater than 100 000). The second peak is more prominent (mol. wt. about 40 000) and there is also a minor peak (mol. wt. about 20 000).[17] The protein and carbohydrate curves follow one another closely. This could be coincidental, or could indicate strong interaction between the protein and carbohydrate.

After bulking the high molecular weight peaks, total protein was measured by absorption at 280 nm, using bovine serum albumin as a standard. These values (Table 2) if compared to the total nitrogen in Table 1, show reasonable agreement for the okra mucilage but the baobab gives a much higher value. Examination of the absorption spectra (Figure 3) reveals that baobab has a large shoulder in the 280 nm region. This absorption is removed by raising the pH to 10.2, indicating a u.v. active contaminant. The protein content estimation at this pH is in better agreement with the Kjeldahl determination (Table 1). Both mucilages were also treated with 1 % hydrogen peroxide for 4 h at 30°C, a usual method of detecting polyphenols present in mucilage. However, no change was noted in the absorption spectra.

Table 2. Protein content of purified mucilages by spectro-photometric analysis

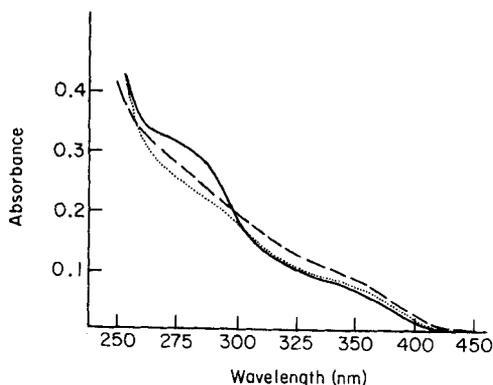| Protein (% of wet wt)	Okra	Baobab
At 280 nm	9.2	30.2
At 294 nm, pH 10.2	—	7.0

Figure 3. Absorption spectra of purified okra and baobab mucilages. 0.83 g/litre okra mucilage – – – pH 4.9; 0.30 g/litre baobab mucilage —— pH 5.4; . . . . pH 10.2.

3.3. Electrophoresis of the two mucilages

Both mucilages gave two fast moving bands (okra—major 61 mm, minor 57 mm; baobab—major 59 mm, minor 56 mm) indicating a highly negatively charged species. Both bands absorbed the protein and carbohydrate stains. This could again indicate a fairly strong protein–carbohydrate interaction in the mucilage.

3.4. Carbohydrate analysis

3.4.1. Qualitative carbohydrate analysis

The three degrees of hydrolysis of the two mucilages gave the same neutral sugars for each hydrolysis. The neutral sugars identified were rhamnose, galactose and glucose in okra mucilage, and rhamnose and galactose in baobab mucilage. The uronic acids present in the hydrolysis with 2 M-H_2SO_4 were also identified. Okra mucilage contained only galacturonic acid, whereas baobab mucilage was a mixture of galacturonic and glucuronic acids.

3.4.2. Quantitative carbohydrate analysis

The bulked mucilage solutions were analysed colorimetrically for both neutral sugars and uronic acids. Since the carbazole method did not distinguish between galacturonic and glucuronic acids, the two uronic acids were measured after separation by t.l.c.

Neutral sugars were analysed quantitatively by an automatic carbohydrate analyser, and these results along with those of the colorimetric analyses are summarised in Table 3. Although an

Table 3. Quantitative carbohydrate analyses of the purified mucilages

g sugar/100 g mucilage	Okra			Baobab		
	Colorimetric	Mole ratio	Automatic	Colorimetric	Mole ratio	Automatic
Neutral sugars						
Rhamnose	—	0.12	3.6	—	0.60	1.8
Galactose	—	1.00	33.0	—	1.00	3.2
Glucose	—	0.10	3.3	—	0.44	1.4
Arabinose	—	0.0	0.0	—	0.15	0.4
Total	40.0	—	39.9	9.3	—	6.8
Uronic acids						
Galacturonic acid	45.8	1.30	—	40.2	11.7	—
Glucuronic acid	0.0	0.0	—	39.1	11.3	—
Total	45.8	—	—	79.3	—	—

attempt was made to use the colorimetric analysis to distinguish between rhamnose and galactose, the high uronic acid content interfered with this method and prevented quantitative results.

The carbohydrate content of the okra mucilage agrees with previous workers findings[3,5] of galactose, galacturonic acid and rhamnose. No arabinose was detected and quantitatively the results differed from those of Amin,[5] who found the sugars present in the ratio galactose, arabinose, rhamnose and galacturonic acid (80:3:10:6). It is very possible that glucose is present as a contaminant rather than a component of the mucilage.

Baobab mucilage appears to have an interesting composition. It has a very high proportion of uronic acids and a very small quantity of neutral sugars. The uronic acids are composed of equal quantities of galacturonic and glucuronic acids. This could therefore be a mixture of two polysaccharides each separately containing galacturonic and glucuronic acids, or it could be a polysaccharide composed of a mixture of the two acids.

Both mucilages, whilst having different compositions, could be classed in the galacturonorhamnan group of polysaccharides.[18] This group are acidic polysaccharides, and include the Sterculia and Khaya gums. Some Sterculia gums have also been found to contain mixtures of galacturonic and glucuronic acids.

3.4.3. Incubation of mucilages with pectin esterase

The mucilage solutions gave no increase in acidity after incubation with pectin esterase. This indicates the absence of pectin in the mucilages and also pectin-like units.

3.5. Infrared analysis

The amount of information that can be obtained from the i.r. spectrum of polysaccharides is rather limited. The spectra (Figure 4) are reported principally as a fingerprint for any future identification, since the absorption bands for such complex compounds are usually broad and diffuse.[7] Apart from the usual bands for hydroxyl (915–955 cm $^{-1}$) and ester carbonyl (1730 cm $^{-1}$) groups, protein (carbonyl stretch 1660–1680 cm $^{-1}$), amide deformation (1625–1650 cm $^{-1}$) and phosphate (1260–1280 cm $^{-1}$) can be distinguished.
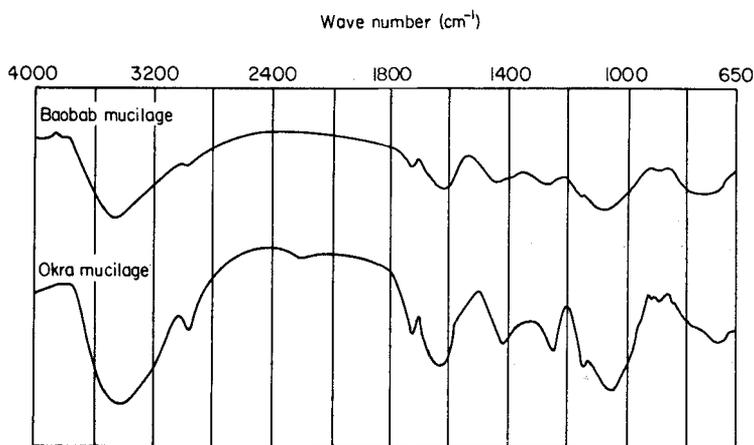

Figure 4. Infrared spectra of purified okra and baobab mucilages.

3.5. Viscosity behaviour of the mucilages

3.5.1. Introduction

Changes in viscosity reflect gross changes in conformation and interaction of the mucilages. Since it has been shown that they are complex mixtures of polysaccharide, protein and mineral matter, changes observed in the behaviour of the mucilage solutions might be a result of different opposing or combining effects.

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3.5.2. Effect of concentration on viscosity

Both mucilages show rapid increases in viscosity with concentration (Figure 5). Baobab mucilage appears to be twice as viscous at the same concentration as okra mucilage. The curves resemble those for many plant gums, and would suggest gelation at higher concentrations. Dispersion of the mucilages is quite difficult at concentrations exceeding 10 g/litre and severe homogenisation is required.
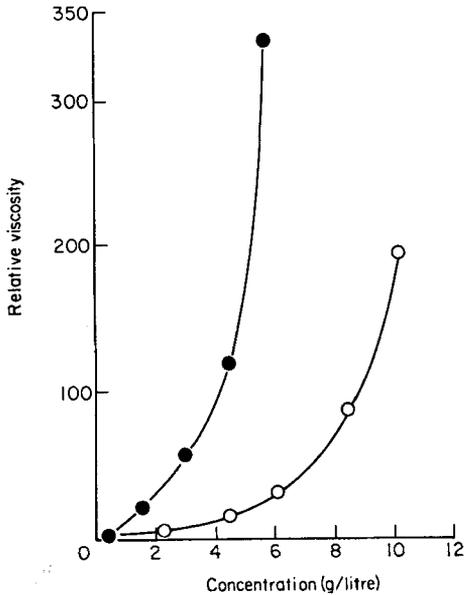
Figure 5. Effect of concentration on the relative viscosities of purified okra (pH 6.0) ○, and purified baobab (pH 6.2) ● mucilage solutions at 25°C.

3.5.2. The effect of pH on viscosity

The viscosity of both mucilages is at a maximum in the neutral pH range (Figure 6). Okra mucilage has a slightly broader maximum (pH 6–9) than baobab. This behaviour is similar to other acidic polysaccharides, such as gum arabic.[19] The curves are interesting because they explain certain practices that are used when cooking okra. Those individuals who prefer to reduce the sliminess of okra will often add lemon or lime juice to stews. Where the cooked vegetable is consumed, lemon juice or vinegar is sprinkled over it. Certain people, mainly from the Ewe tribe in West Africa, increase the sliminess of stews prepared from okra by adding a local stone to the stew. This stone is composed mainly of sodium carbonate and bicarbonate, and hence increases the pH of the stew towards neutrality.

Addition of calcium ions results in a decrease in viscosity of both mucilages (Figure 7). This behaviour is again similar to gum arabic,[19] and is a result of the interaction of the divalent ion with the acid groups of the polysaccharide causing a change in molecular shape and hence viscosity.

3.5.4. The effect of heat on viscosity

Heating both mucilage solutions to 90°C causes similar decrease in viscosity (Figure 8) of each. On cooling the solution back to room temperature, there is an irreversible loss of viscosity of both mucilages.

Boiling the mucilages also causes a marked decrease in viscosity (Figure 9). This decrease occurs mainly in the first few minutes of boiling. After this initial decrease, the viscosity, of baobab mucilage in particular, stabilises.

It was noticed when conducting the heating experiments that a precipitate was formed which did not redissolve on cooling. This was most probably denatured protein. It therefore appears likely that a
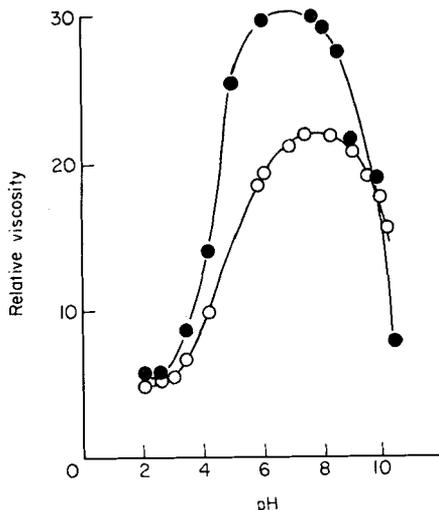
Figure 6. Effect of pH on the relative viscosities of purified okra (5 g/litre) ○, and purified baobab (2 g/litre) ● mucilage solutions at 25°C.
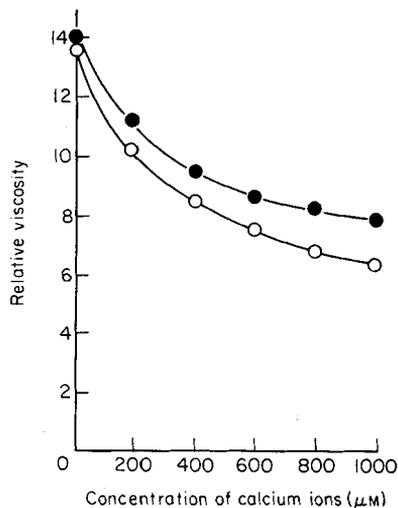

Figure 7. Effect of addition of calcium ions to the relative viscosities of purified okra (5 g/litre, pH 6.0) ○, and purified baobab (2 g/litre, pH 6.2) ● mucilage solutions at 25°C.
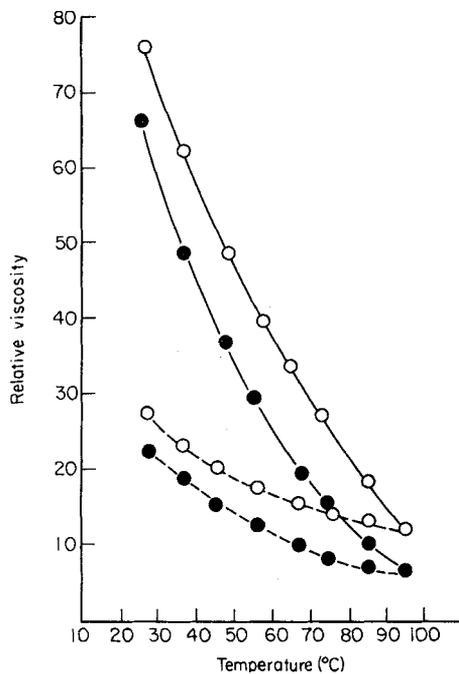

Figure 8. Effect of heating (solid line) and cooling (broken line) on the relative viscosities of okra (8 g/litre) ○, and baobab (4 g/litre) ● mucilage solutions.
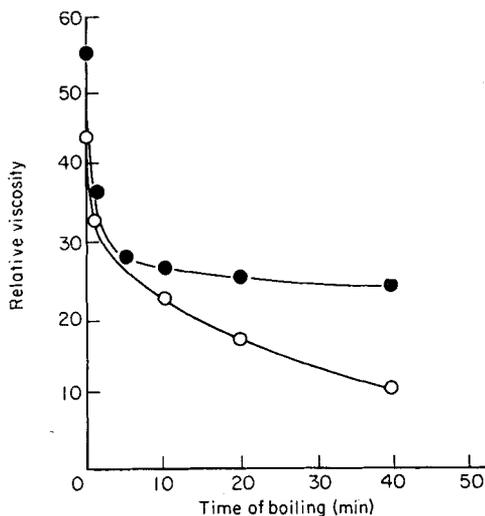

Figure 9. Effect of boiling on the relative viscosities of crude okra (7 g/litre) ○, and baobab (3 g/litre) ● mucilage solutions at 25°C.

significant component of the viscosity is provided by a protein–carbohydrate interaction. This would also explain the boiling curves, where the initial loss of protein occurs in the first few minutes of boiling. Depolymerisation of the polysaccharides would also be occurring above 90°C and would account for loss of viscosity. The results have implications when considering the behaviour of actual okra fruit and baobab leaves. When these are incorporated into foods other protein carbohydrate interactions might be taking place, which enhance their viscous behaviour.

4. General conclusions

Mucilaginous or slimy ingredients are commonly incorporated into soups or stews in West Africa, but are not encountered in many other diets of the world. The characterisation and properties, as well as nutritional effects of these foods is an area which has received little attention.

The mucilages extracted from okra fruits and baobab leaves are both complex macromolecules composed of polysaccharide, protein and minerals. Plant gums normally have protein and minerals associated with the polysaccharide, but these particular mucilages have non carbohydrate components in unusually high concentrations.

The sugar composition for okra mucilage resembles that of many pectic substances. Baobab mucilage, on the other hand, is very unusual in consisting mainly of an equal mixture of galacturonic and glucuronic acids. It remains to be seen whether this is a mixed polymer and not separate compounds.

Considering the differences in carbohydrate composition of the two mucilages, it is surprising that they behave so similarly in terms of viscosity. The results confirm their role as effective thickening agents, and baobab mucilage in particular appears to have most potential for this function. However, their viscosity does decrease markedly with high temperature and with boiling although this does not obviously prevent their use in cooked food. The variation of viscosity with pH is interesting and explains some of the culinary practices associated with okra. It should be pointed out that the capillary viscometer is limited in its ability to examine the rheological properties of the mucilages. Okra mucilage solution, for example, behaves like egg white at higher concentrations forming threads and stabilising foams, hence its use as a dried egg white substitute and extender has already been exploited.[2] Therefore studies using other rheological instruments and further chemical analyses are in progress in an attempt to try and evaluate the potential of these two mucilages.

Acknowledgements

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