sample, using relative peak heights from a GLC curve to follow changes in oil composition with maturity.

The ratio of octanal to decanal in grapefruit oils has been suggested as a quality index. Kesterson et al. (1971) reported ratios of 1:1.1 to 1:1.4 for white grapefruit oil and ratios of 1.2:1 to 1.3:1 for red grapefruit oil. A later study (Braddock and Kesterson, 1976) indicated that the ratio was greater than 1 in a white grapefruit oil sample. The ratio we found was about 1.2:1.

Several of the identified compounds were part of mixtures and/or present in quantities too small to be accurately quantitated. Nootkatone eluted as small, broad peak with a very long retention time (95 min) and could not be integrated accurately. Only the peak for one mixture (myrcene and sabinene) was sufficiently large and sufficiently well resolved from surrounding peaks to make integration possible. The response factor for the sabinene–myrcene peak was determined from that of myrcene only in the synthetic mixture.

In other cases, adjustments had to be made for accurate quantitation. Sufficient quantities of chromatographically pure β-elemene were not available to allow us to determine its response factor; so its corrected weight percent is based on the response of β-copaene. The response factor for citral (containing 51% geranial) was used as that for geranial, and the corrected weight percentage of geranial is based on the percentage of geranial present in the citral sample.

Thus, we quantitated 19 major components of a typical Florida white grapefruit oil by a procedure involving GLC without preliminary separation steps that uses response factors and corrects for the presence of nonvolatiles. Only few comparisons could be made, but our results are similar to previously reported values. We quantitated ten components not previously quantitated, including octyl and neryl acetates. These esters were reported by Moshonas (1971) to be two of the major carbonyl flavor components present in grapefruit oil.

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Isolation and Characterization of Arabinogalactan from Black Gram (*Phaseolus mungo*)

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An arabinogalactan type of polysaccharide has been isolated from black gram by extracting the meal with aqueous 10% (w/v) trichloroacetic acid and precipitation with acetone. Reprecipitation and dialysis gave an ultracentrifugally homogeneous preparation with a high molecular weight (ca. 144 000) as determined by gel filtration through Bio-Gel P-200. Aqueous dispersions possessed high viscosity around pH 5–7 which decreased with increase in temperature.

Black gram (*Phaseolus mungo*) has been the traditional choice among the common grain legumes as an essential ingredient of some of the most popular and typical Indian breakfast foods which possess a characteristic soft, spongy texture and are made out of leavened mixtures of the legume and cereals (usually rice). A noteworthy feature of batters containing this legume is their high viscosity ascribed to the presence in it of a mucilagenous principle that is also held to be responsible for the gas-holding and dough-raising qualities. Other legumes lack this principle and hence are considered unsuitable for such food preparations.

Kadkol et al. (1961) attempted to isolate the mucilagenous principle from black gram by extracting with an acetate buffer, deproteinizing by repeated treatment with Sevag's solvent, and finally precipitating with acetone. The resultant crude preparation still contained 20% protein and was designated as a mucopolysaccharide. In our preliminary studies on the texture principles of this legume (Susheelamma and Rao, 1974), it was demonstrated that the highly surface-active proteins in the grain function producing the spongy texture and an arabinogalactan type polysaccharide closely associated with the proteins (and generally coprecipitated with them during isolation) protect this spongy framework against thermal disruption

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at high (culinary) temperatures. As the simple techniques for separating the contaminating proteins from the polysaccharide, including adsorption on calcium phosphate gel, were unsuccessful in reducing it to below 10%, recourse was taken to extracting the polysaccharide with 10% trichloroacetic acid (TCA) to completely eliminate the protein. The polysaccharide preparation was subsequently obtained by solvent precipitation. Such a preparation after purification by reprecipitation was homogeneous and functionally satisfactory and has been used for physical and chemical characterization and related studies.

EXPERIMENTAL SECTION

Isolation of the Polysaccharide. Twenty-five grams of black gram flour (100 mesh) prepared as described earlier (Susheelamma and Rao, 1974) was dispersed in 10% w/v trichloroacetic acid (TCA), stirred for 4 h, and centrifuged at 10000*g* for 15 min. The supernatant was precipitated with acetone at 75% (v/v) concentration. After removal of acetone the precipitate was dispersed in 10% TCA, reprecipitated as described above, and thoroughly washed with acetone. It was dispersed in water, dialyzed, and lyophilized. All of these operations (extraction, centrifugation, precipitation, dialysis, etc.) were carried out in the cold at 5–7 °C. Similar preparations were also obtained from 40, 60, and 80 mesh flours.

The polysaccharide was dispersed in 0.01 M Tris–HCl buffer (pH 7.8) and centrifuged at 8000*g* for 10 min. The residue was dispersed in 0.01 N NaOH. Gel filtration of these two fractions was carried out on Sephadex G-100 column. Ultracentrifugation of the TCA–polysaccharide was carried out (0.9% solution) in a Spinco Model E analytical ultracentrifuge.

Molecular weight was estimated according to Anderson et al. (1965) on a Bio-Gel P-200 column and washed and equilibrated with 0.2 M NaCl. Standard dextrans T-10, T-20, T-40, and T-70 were used for calibration (Granath and Kvist, 1967).

Composition. Acid hydrolysate (20 mg/mL of 1 N HCl at 95 °C and 4 h) was flash evaporated after neutralization, taken in 80% ethanol, and subjected to paper chromatography on Whatman No. 1 filter paper in (1) 1-butanol–acetic acid–water (4:1:1), (2) 1-butanol–ethanol–water (4:1:1), and (3) 1-propanol–ethyl acetate–water (7:1:2) solvent systems. The zone of sugar was located with the help of guide strips containing authentic monosaccharide samples and sprayed with benzidine–trichloroacetic acid (Bacon and Edelman, 1951) and aniline phosphate (Bryson and Mitchell, 1951) reagents. The ratio of sugars was determined after eluting the corresponding zones from the paper chromatograms (run in solvent system 1) with 0.2 M acetic acid and assayed with orcinol–H_2SO_4 reagent (Winzler, 1955).

HCl hydrolysate was passed through an Amberlite IR-4B column, eluted with water to remove neutral sugars, and then with ethanolic ammonia to obtain the uronic acids. For further identification and confirmations, the column eluates were pooled, concentrated, and subjected to paper electrophoresis (Block et al., 1955) along with authentic standards in 0.2 M borate buffer, pH 9.0, at 330 V for 5 h and sprayed with benzidine trichloroacetic acid (Bacon and Edelman, 1951). The phenyl osazones were prepared according to Oser (1965).

Optical rotations were determined in a Hilger Model MK III polarimeter.

Determination of Viscosity. Viscosity of aqueous dispersions of the polysaccharide was determined in an Ostwald viscometer and also in a Brook-field viscometer with LVT model spindles. The viscosity of guar gum (Dealca P/225) and gelatinized soluble starch dispersions was also determined under similar conditions for comparison.

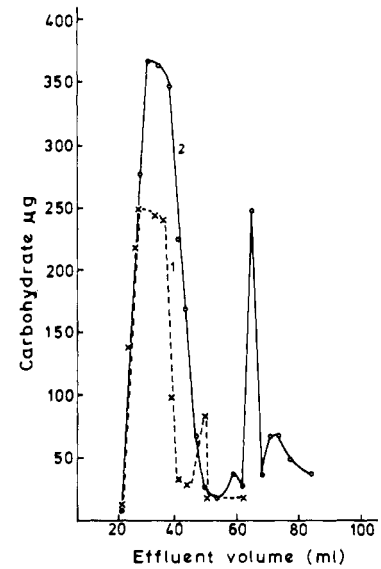

Figure 1. Gel filtration of the TCA–polysaccharide through Sephadex G-100: Column dimensions, 1.9 × 25.5 cm; temperature, 20 °C, flow rate, 20 mL/h; (1) 1.92 mg of buffer soluble fraction in 4 mL of 0.01 M Tris–HCl buffer (pH 7.8) and eluted with the same; (2) 2.8 mg of alkali dispersible fraction in 2 mL of 0.01 N NaOH and eluted with the same. The recoveries were 95 and 96%, respectively.

RESULTS AND DISCUSSION

Preliminary experiments with black gram flour indicated that the polysaccharide obtained by extraction with water (hot or cold), 40 or 70% ethanol or acetone or extraction with alkali, and precipitation with acetone or alcohol gave preparations containing varying amounts of protein. However, extraction of the flour with TCA and precipitation with acetone gave a polysaccharide (yield 4.5%) which was nondialyzable and free from starch. It was biuret negative but contained Kjeldahl nitrogen over a range of 0.6–1%. Reprecipitated preparations (Kjeldahl nitrogen 0.55–0.6%) were used for further characterization. The yield of polysaccharide from 40, 60, and 80 mesh flours was 10, 27, and 85% of that obtained from 100 mesh flour.

Chakraborty (1975) has reported that as many as eight fractions could be obtained from black gram flour after extraction with water and sodium hydroxide and precipitation with alcohol. All the fractions contained different amounts of arabinose and galactose with varying amounts of glucose, but the protein content of these fractions has not been indicated.

About 35% of the TCA polysaccharide was soluble in 0.01 M Tris–HCl buffer (pH 7.8) and 55% was dispersible in 0.01 N NaOH. The elution pattern of these during gel filtration is shown in Figure 1. The buffer dispersion had a minor peak of 5–6%, while the alkali dispersion had a minor peak of 15–16%. The major peaks did not show significant differences in monosaccharide composition. The entire fraction has been used for further characterization.

Upon ultracentrifugation the TCA polysaccharide (Figure 2a) gave a peak with sedimentation coefficient of 9.94. The experiment was repeated in presence of the surface active protein from black gram (from which it does not separate out easily during extraction) to see whether any interaction occurs between them. But neither the sedimentation coefficient nor the pattern (Figure 2b,c)

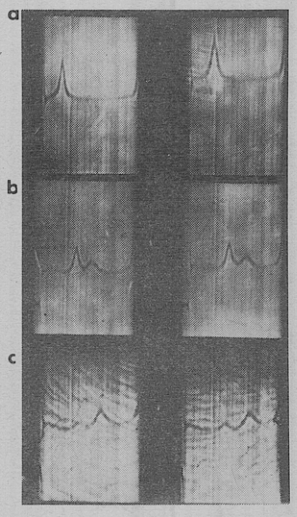

Figure 2. Sedimentation pattern of the TCA polysaccharide from black gram. Sedimentation pattern as obtained with the Spinco Model E analytical ultracentrifuge at 59700 rpm: (a) (left to right) TCA–polysaccharide at t (time after attaining maximum speed) = 30 and 40 min; (b) (left to right) TCA–polysaccharide along with the surface-active proteins from black gram (obtained after DEAE–cellulose column chromatography of the 40–75% $(NH_4)_2SO_4$ precipitate from the saline extract of the meal) at t = 30 and 45 min; (c) (left to right) surface-active protein alone at t = 35 and 40 min.

indicated the possibilites of any firm molecular interactions between isolated protein and polysaccharide although they were coprecipitated during the initial stages of isolation. $S_{20,w}$ values were 11.08 for protein and 9.63 and 12.08 for the polysaccharide and protein, respectively, when they were centrifuged together. Molecular weight was found to be around 144 000 as determined by gel filtration (by extrapolation of the calibration curve), higher than larch arabinogalactan with a molecular weight around 100 000.

Composition. Paper chromatography of the acid hydrolysate of the polysaccharide indicated a composition of arabinose–galactose–galacturonic acid–rhamnose as 30:20:3:5, somewhat similar to that reported by Kadkol et al. (1961) as 18:13:2:3. Thus arabinose and galactose were the major constituents in the ratio of 3:2. These were identified and confirmed by cochromatography and galacturonic acid by electrophoresis. Rhamnose was identified only by paper chromatography and paper electrophoresis.

The specific rotations for arabinose and galactose (in water at 25 °C) were quite close to those of authentic samples (arabinose standard −105° and arabinose test specimen −102°, galactose standard −82° and galactose test specimen −80°). The melting points and mixed melting points of phenyl oxazones were also in good agreement with those obtained from authentic sugars [arabinose standard 166 °C, test sample (chromatographically separated) 164 °C and mixed melting point 165 °C. Galactose standard 184 °C, test sample (chromatographically separated), 185 °C and mixed melting point 184 °C].

During partial hydrolysis of the TCA polysaccharide with dilute HCl, arabinose was the first sugar released and galactose was released afterwards. Only those two sugars were detected up to 50% hydrolysis of the polysaccharide. Rhamnose and galacturonic acid were detected only after complete hydrolysis with strong acid, suggesting that arabinose and galactose may be present in the peripheral regions of the molecule.

Properties. The variation of viscosity with concentration of aqueous dispersions of the polysaccharide as compared to guar gum and gelatinized soluble starch is

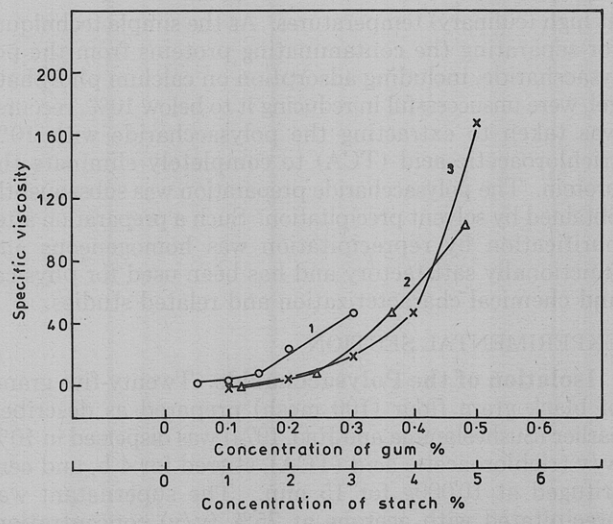

Figure 3. Comparison of the viscosities of polysaccharides as determined by the Brook field viscometer: (1) gelatinized soluble starch; (2) TCA–polysaccharide from black gram; and (3) guar gum.

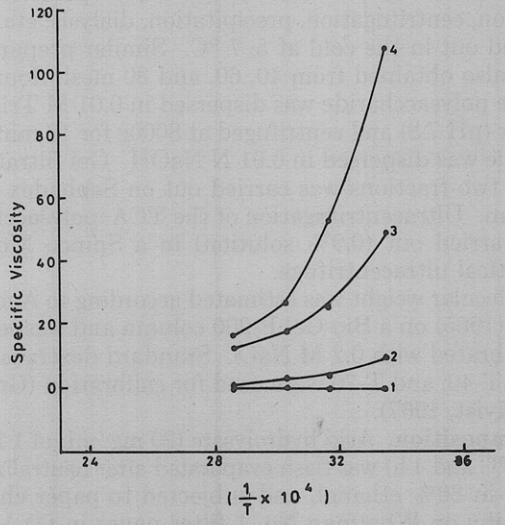

Figure 4. Influence of temperature on the viscosity of aqueous dispersions of TCA–polysaccharide from black gram: (1) 0.12%; (2) 0.24%; (3) 0.36%; (4) 0.48%.

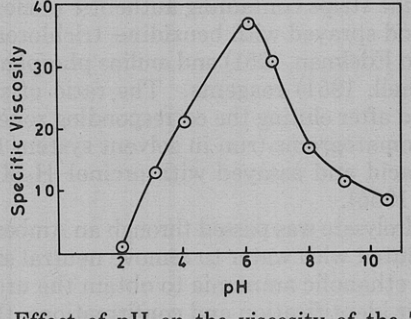

Figure 5. Effect of pH on the viscosity of the TCA–polysaccharide from black gram.

shown in Figure 3. A 0.36% solution had a viscosity of 43 cps, while that of 30% solution of larch gum had 40 cps (Glicksman, 1969).

The specific viscosity decreased considerably with increase in temperature at concentrations above 0.2% (Figure 4). Heat treatment of aqueous dispersion at 95 °C (30 min) and cooling to room temperature also caused decrease in specific viscosity, unlike the larch gum dispersions where the viscosity decreased with increase in temperature, but the original values were regained upon

cooling. The specific viscosity was maximum at pH 5-7 (Figure 5) and showed a decrease in acidic as well as basic pH ranges in contrast to that of larch gum which at 5 to 20% concentrations did not exhibit any significant changes over a broad pH range (Nazarath et al., 1961).

Recently the structure of cell-wall polysaccharides from sycamore (dicotyledon) species has been studied by Peter Albersheim and co-workers (1975) and a tentative model (which seems to be partly supported by electron microscopic studies) has been put forth wherein xyloglucan, arabinogalactan, and rhamnogalacturonan type of polysaccharides are embedded into the cellulosic network of primary cell walls. Our observation that very fine grinding of black gram seeds is essential for complete extraction of the polysaccharide seems to imply that this polysaccharide may also be a similar cell-wall constituent.

Arabinogalactan type of polysaccharides occur as part of pectic substances from several sources (McCready and Gee, 1960; Aspinall and Molloy; 1968, Aspinall et al, 1969; Aspinall and Cotterell, 1970). The structure of those from wheat (Pomeranz, 1968; D'Appolonia et al., 1970; Wall, 1971; Fincher and Stone, 1974a, 1974b; Neukom and Markwalder, 1975; Patil et al., 1975) and soybean (Morita, 1965; Aspinall et al., 1967a,b,c; White, 1942) have been partially characterized. Those reported to occur in green gram and buckwheat (Desikachar and Subba Rao, 1977) are not fully characterized. Arabinogalactan from black gram has a high molecule weight and is also highly viscogenic and may have a branched chain structure somewhat similar to that of guar gum (Whistler, 1954, 1975, Glicksman, 1969). Further characterization of this functionally important polysaccharide may prove valuable from both the structural and food application points of view.

Guar and other hydrocolloid gums have found wide use in several food preparations. The manner is which this highly visogenic gum from black gram exerts its effect along with the protein in the texture of leavened foods will be discussed elsewhere.

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