WATER-SOLUBLE NONSTARCHY POLYSACCHARIDES OF COMPOSITE FLOURS. I. CHEMICAL NATURE OF POLYSACCHARIDES FROM YAM (*DIOSCOREA*) AND CASSAVA FLOURS[1]

S. SEFA-DEDEH and V. F. RASPER[2], Department of Food Science, University of Guelph, Guelph, Ontario, Canada

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ABSTRACT

Gas-liquid chromatographic analysis of derived hydrolysates of water-soluble nonstarchy polysaccharides (W.S.N.P.) extracted from flours milled from dried tubers of three species of yam (*Dioscorea rotundata* Poir., *D. alata* L., and *D. cayenensis* Lam.) and roots of cassava (*Manihot utilissima* Pohl., cv. Ankra) revealed marked differences in their composition. While D-glucose was the major component of material extracted from cassava flour, W.S.N.P. of *D. alata* L. and *D. cayenensis* Lam. origin were characterized by a high concentration of D-mannose, which accounted for almost 50% of their total carbohydrate content. Though pentoses were present in all tested samples, *D. rotundata* Poir. W.S.N.P. were the only ones in which 5-carbon ring sugars formed the major fraction; they represented 57% of the total carbohydrate content with arabinose being the major component. The uronic acid content in all extracted materials was relatively high, reaching over 24% in W.S.N.P. of *D. rotundata* Poir. flour. Viscometric measurements on unoxidized and oxidized solutions and uv absorption analysis indicated behavior similar to that characteristic of water-soluble wheat flour 'pentosans.' Data obtained by uv absorption analysis and thin-layer chromatography provided evidence for the involvement of a phenolic compound in the oxidative gelation of these solutions. The presence of ferulic acid and other phenolic acids in the tested materials was confirmed by thin-layer chromatography.

Results of an earlier study (1) indicated a pronounced effect of some nonstarchy components introduced into composite flours by wheat-flour substitutes on the baking performance of composite doughs. While in most cases it appeared preferable to substitute wheat flour with starch instead of flour (*e.g.*, cassava starch *vs.* cassava flour), some nonwheat flours exhibited a markedly less detrimental effect on the baking performance than pure starches of the same plant origin. This was observed with flours milled from tubers of some species of yam (*Dioscorea*), especially of West African 'white yam'—*Dioscorea rotundata* Poir. In spite of rather unfavorable bread-baking characteristics of starch present in its tubers, the flour not only had less deleterious effects on the

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[2]Respectively: Graduate Student and Associate Professor.

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performance of the dough than flours of other plant origin but, at replacement levels not exceeding 10%, a slight increase in loaf volume was noticed. It was speculated that components similar in functionality to water-soluble 'pentosans' of strong wheat flour might be responsible for this behavior. Therefore, a procedure identical with that used by several researchers for the isolation and purification of water-soluble pentosans from wheat flour was applied to flours milled from tubers of several West African species of yam (*Dioscorea*) and roots of one of the most common Ghanaian cultivars of cassava (*Manihot utilissima* Pohl.). The extracted and purified material was referred to as water-soluble nonstarchy polysaccharides (W.S.N.P.), though it was understood that the preparations contained measurable quantities of other noncarbohydrate components. In this part of the paper, the chemical nature of these polysaccharides and their viscosity characteristics in unoxidized and oxidized solutions were investigated and compared with those of water-soluble 'pentosans' of a typical bread flour.

MATERIALS AND METHODS

Preparation of Water-Soluble Nonstarchy Polysaccharides

Water-soluble nonstarchy polysaccharides (W.S.N.P.) were extracted from tubers of three of the most common West African species of *Dioscorea—D. rotundata* Poir. ("White Yam"), *D. alata* L. ("Water Yam"), and *D. cayenensis* Lam. ("Yellow Yam"). All tubers were grown in Ghana. Cassava roots of *M. utilissima* Pohl., cv. Ankra, were of the same geographical origin. After peeling, slicing, and sun drying, the slices were crushed and milled in a laboratory pulverizing mill (Weber Bros. and White Metal Works, Chicago, Ill.) to pass through screen No. 008. The extraction and purification of W.S.N.P. were carried out according to the procedure reported by Lin and Pomeranz (2) for the preparation of water-soluble pentosans from wheat flour. Water-soluble pentosans extracted from a straight-run Canadian hard red spring wheat flour were used for comparison. (Further in this paper, the term 'pentosans' will be used for this preparation of wheat-flour origin and will not mean pure pentosan fraction.)

Moisture, Fiber, and Protein Determinations

Moisture, fiber, and protein in flours used as starting material for the extraction of the tested polysaccharides were determined according to AOAC methods (3).

Furfural-Yielding Components

Furfural-yielding components in flours used were determined by volumetric bromine AACC Method 44-15 (4).

Gas-Liquid Chromatography of Sugars

Freeze-dried W.S.N.P. (10 mg) were hydrolyzed with 3.0 ml 2*N* H_2SO_4 at 100°C for 6 hr in sealed glass ampoules. The hydrolysate was neutralized by stirring with Amberlite IR-OB (OH^-) for 15 min. The sugars were transformed into alditol acetates (5) and injected into a column packed with 3% (w/w) ECNSS-M on Gas Chrom Q, 100–120 mesh, using ribitol pentaacetate as an

internal standard. Prior to derivation of sugars into alditol acetates, uronic acid components were removed by sodium carbonate to prevent any interference with sugar determination (6).

Uronic Acid Determination

Total uronic acids in tested extracted material were determined colorimetrically by Bitter and Muir's method based on the modification of the uronic-carbazole reaction (7). D-galacturonic acid monohydrate was used as standard.

Paper chromatography of uronic acids was performed according to the procedure described by Macek (8).

uv Spectroscopy of W.S.N.P.

Solutions (0.5% w/v) of freeze-dried W.S.N.P. in acetate buffer (pH 4.5) were measured with a Beckman DB-G Grating Spectrophotometer over the wavelength range of 240–360 nm. To study the changes in uv spectra upon oxidation of the tested material, 0.1 ml of 3% hydrogen peroxide was mixed with 10 ml of the solution at room temperature.

Thin-Layer Chromatography of Phenolic Compounds

The presence of phenolic compounds was determined by tlc after the tested material was saponified with 0.5*M* KOH using the procedure by Fausch *et al.* (9). The sample solutions were applied to plates precoated with silica gel G and calcium sulfate and were run in four different solvent systems: benzene:methanol:acetic acid (90:16:8), acetic acid:HCl:water (30:3:10), supernatant of toluene:acetic acid:water (4:1:5), benzene:dioxane:acetic acid (90:25:8). After each run, the spots were observed under uv light before and after exposure to ammonia vapors.

Protein Monitoring of DEAE-Cellulose Chromatography Fractions

W.S.N.P. were fractionated by DEAE-cellulose chromatography as described by Kuendig *et al.* (10). Protein monitoring of fractions collected in the eluates was done colorimetrically using the Folin-Ciocalteau reagent method (11). The same method was used for the determination of total protein in the extracted W.S.N.P.

Viscosity Measurements

Samples for viscosity measurements were prepared by using 10 ml of 1.0% (w/v) solutions of W.S.N.P. in acetone buffer (pH 4.5). Viscosity was measured at 20°C with an Ubbelohde kinematic viscometer. Intrinsic viscosity was determined by extrapolating the plot of reduced viscosities *vs.* concentration to zero concentration of the solution (12). The relative viscosity was determined by relating the flow times of 10 ml W.S.N.P. solutions (1.0%) to 10 ml acetate buffer. Oxidation of the W.S.N.P. solutions for viscosity measurement was done by mixing 10.0 ml with 0.1 ml of 3% hydrogen peroxide solution.

RESULTS AND DISCUSSION

Basic Chemistry of Tested W.S.N.P.

Considerably lower quantities of W.S.N.P. were obtained from tested yam

flours than from flour of *M. utilissima* origin. The yield of W.S.N.P. from the latter even exceeded that of water-soluble pentosans from HRS wheat flour (Table I). These differences in yields were recorded in spite of comparable quantities of furfural-yielding components in all tested materials as determined by the bromine volumetric method. This indicated that most of the furfural-yielding components in the nonwheat flours were present in water-insoluble form and did not get into the extract. High crude fiber content in these flours might be taken as evidence for the presence of hemicelluloses and similar compounds not extracted by the extraction procedure applied. Further tests revealed that the concentration of 5-carbon ring sugars in the extracted polysaccharides varied widely with their origin, so that no straightforward relation between the W.S.N.P. yield and the bromine method results could be expected.

Gas-liquid chromatograms of alditol acetates derived from sugars present in the hydrolyzed solutions of the tested nonstarchy polysaccharides are shown in Fig. 1. Quantitative evaluation of these chromatograms (Table II) revealed that all analyzed nonwheat materials were predominantly composed of hexose units, with the only exception of W.S.N.P. extracted from *D. rotundata* flour. While cassava W.S.N.P. were characterized by a high D-glucose content, mannose was determined as the major sugar in materials extracted from both *D. cayenensis* and *D. alata* flour. In both cases, it represented almost 50% of the total carbohydrate content of the lyophilized material. A considerable quantity of this sugar was also present in *D. rotundata* W.S.N.P. It may be noted that a small amount of this sugar (2.37%) was also found in wheat pentosans; the presence of mannose in this material has so far been reported in only one study (13). Pentoses in *D. alata* and *D. cayenensis* W.S.N.P. accounted for approximately one-third of the total sugar concentration. However, in *D. rotundata* W.S.N.P., they constituted the major sugar fraction accounting for 57% of the total carbohydrate content, compared with 77% for water-soluble pentosans of HRS wheat flour. Arabinose was the principal component (24.1%). Arabinose:xylose ratio was practically the same for all tested yam W.S.N.P.—in average 1:0.67, which differed markedly from that for wheat pentosans. The 1:0.91 ratio for the latter was in good agreement with that reported by Medcalf *et al.* (14).

TABLE I
Furfural-Yielding Components and Fiber Content of Flours Used in the Study Compared with the Yield of W.S.N.P. (All Data on Dry Solid Basis)

Origin of Flour	Furfural-Yielding Components %	Fiber %	Yield of W.S.N.P. %
C.H.R.S. wheat	3.34	0.20	0.35
Yam (*Dioscorea*)			
D. alata L.	3.66	1.37	0.18
D. rotundata Poir.	2.33	1.55	0.13
D. cayenensis Lam.	3.65	1.33	0.15
Cassava (*M. utilissima* Pohl. cv. Ankra)	3.54	2.83	0.50

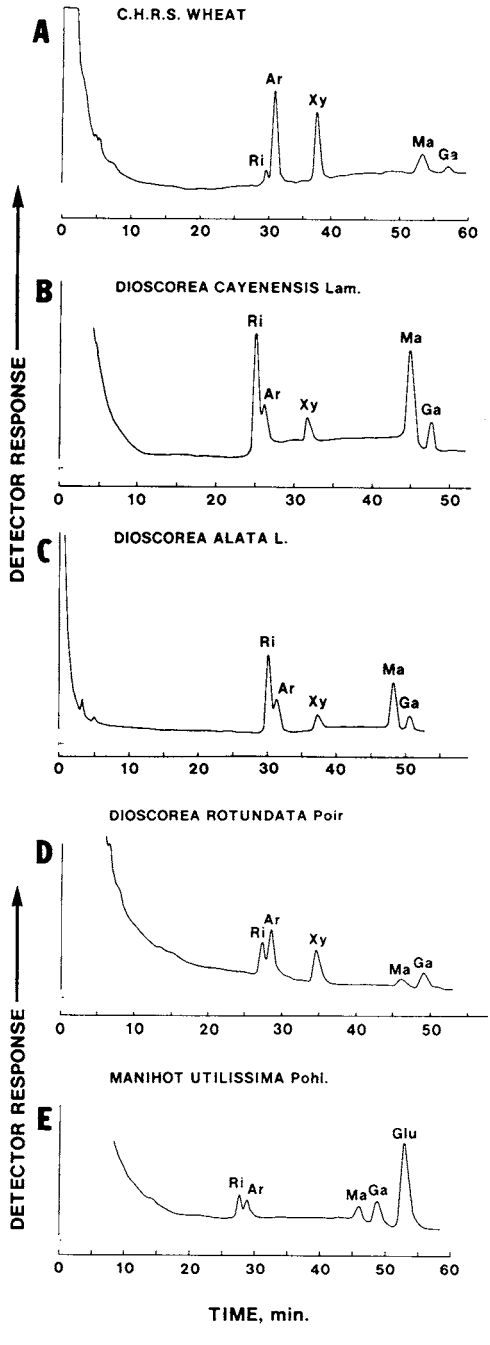

Fig. 1. Gas-liquid chromatograms of sugars in hydrolyzed water-soluble nonstarchy polysaccharides (Ri = ribose (internal standard); Ar = L-arabinose; Xy = D-xylose; Ma = D-mannose; Ga = D-galactose; and Glu = D-glucose).

TABLE II
Sugar Composition (Per Cent of Total) and Uronic Acid Content in Hydrolyzed Water-Soluble Nonstarchy Polysaccharides

Plant Origin of W.S.N.P.	L-Arabinose %	D-Xylose %	D-Galactose %	D-Glucose %	D-Mannose %	Ara/Xyl Ratio %	Total Carbohydrates %	Uronic Acid[a] %
C.H.R.S. wheat	25.65	23.45	12.45	0	2.37	0.91	63.92	4.25
D. rotundata Poir.	24.11	15.66	13.74	0	15.50	0.65	69.01	24.30
D. alata L.	13.50	9.00	15.41	0	33.45	0.67	71.36	17.75
D. cayenensis Lam.	13.03	8.75	14.58	0	31.14	0.67	67.50	10.20
M. utilissima Pohl.	5.36	...	14.89	54.34	6.26	...	80.85	12.25

[a]Determined by a modified uronic acid carbazole reaction (Bitter and Muir, 1962).

TABLE III
Protein Content of Flours Used in the Study and of Extracted W.S.N.P.

Plant Origin	Protein in Flour[a] %	Protein in W.S.N.P.[b] %	Protein in W.S.N.P. as % of the Total Protein in Flour
C.H.R.S. wheat	13.80	20.4	0.51
Yam (*Dioscorea*)			
D. alata L.	2.98	4.06	0.24
D. rotundata Poir.	2.28	3.88	0.22
D. cayenensis Lam.	2.78	3.90	0.21
Cassava (*M. utilissima* Pohl., cv. Ankra)	1.50	1.14	0.50

[a]Factor 5.7 for wheat flour, 6.25 for tuber flours; data on 14% m.b.
[b]Lowry method (11).

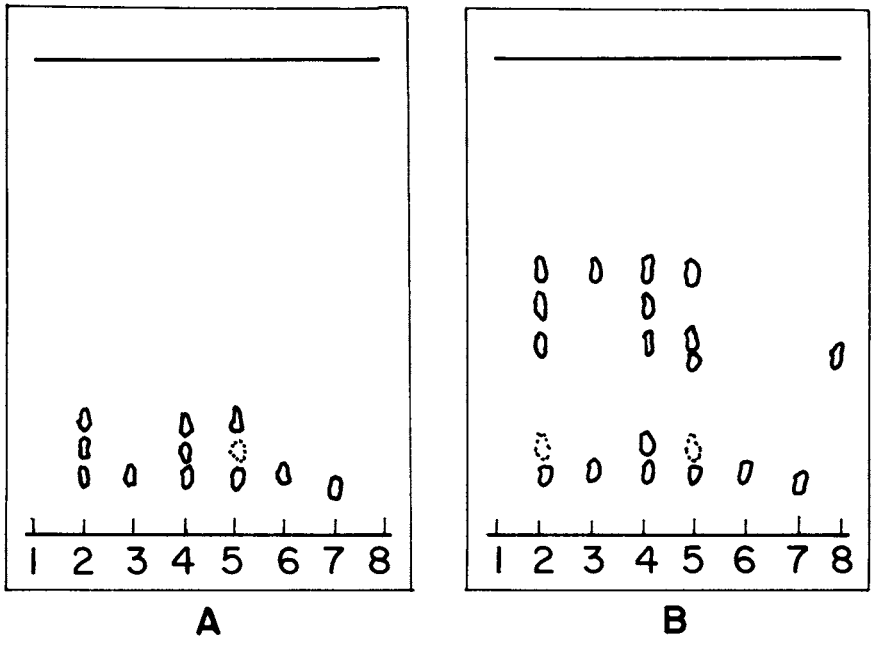

Fig. 2. Paper chromatograms of uronic acids in hydrolyzed W.S.N.P. A) Ethyl acetate:pyridine:acetic acid:water (5:5:1:3); sprayed with periodic acid-$KMnO_4$ reagent. B) Pyridine:ethyl acetate:acetic acid:water (5:5:1:3); sprayed with bromophenol blue-boric acid reagent. Spots on the starting line: 1) wheat water-soluble pentosan, 2) *D. alata* L., 3) *D. rotundata* Poir., 4) *D. cayenensis* Lam., 5) *M. utilissima* Pohl., 6) D-glucuronic acid, 7) D-galacturonic acid, and 8) D-glucose.

All reported glc data were obtained after the removal of uronic acids from the samples prior to formation of alditol acetates to avoid any possible interference of these acids with sugar determination (15–17). The procedure was found essential for the glc analysis of all nonwheat W.S.N.P. in which the concentration of uronic acids, as determined colorimetrically by carbazole reaction, was significantly higher than in material extracted from wheat flour (Table II). An attempt was made to identify these acids by paper chromatography. Though the colorimetric method gave positive reaction with all tested samples, including that of wheat-flour origin, paper chromatography of hydrolyzed samples did not reveal the presence of any uronic acid in wheat W.S.N.P. Most likely, the very low quantities present in this material were destroyed under the conditions of

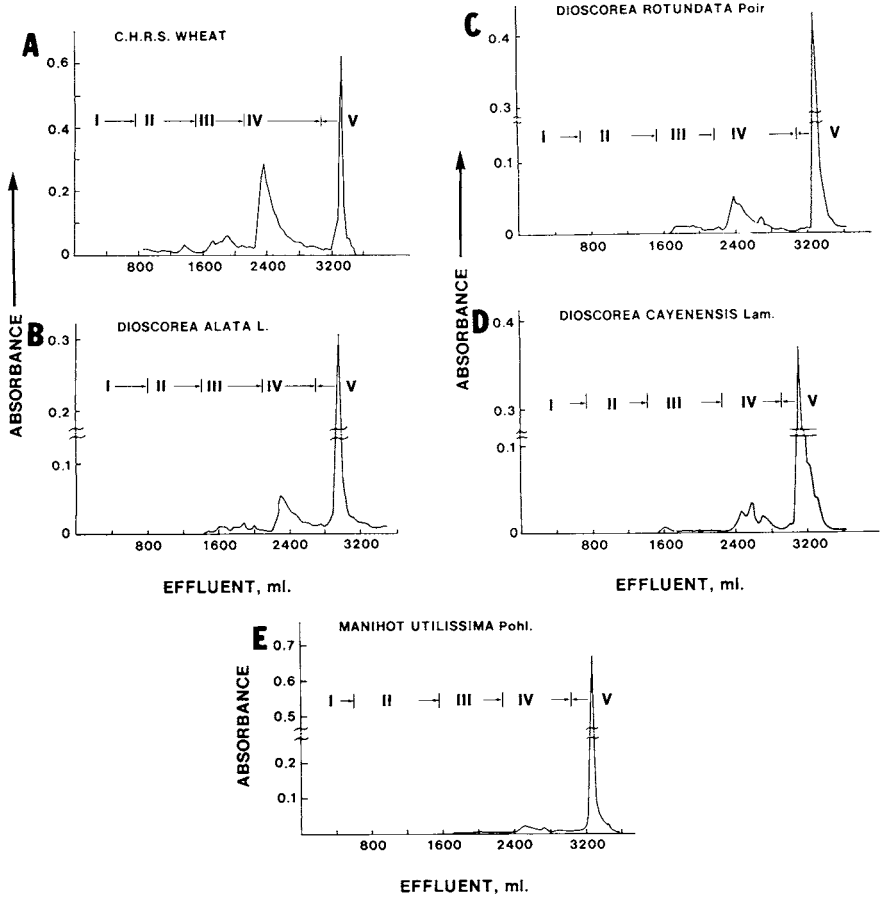

Fig. 3. Distribution of protein in DEAE-cellulose chromatography fractions of water-soluble nonstarchy polysaccharides. Fraction I) distilled water, Fraction II) 0.0025*M* sodium borate, Fraction III) 0.025*M* sodium borate, Fraction IV) 0.125*M* sodium borate, and Fraction V) 0.5*M* sodium borate.

hydrolysis applied. Though glucuronic linkages are more stable to acid hydrolysis than most glycosidic linkages, once liberated the free uronic acids can be easily destroyed before the hydrolysis is completed (18). In all nonwheat W.S.N.P., D-glucuronic acid was detected; other spots which appeared on the chromatograms have not been identified (Fig. 2).

TABLE IV
Intrinsic and Relative Viscosities of Tested W.S.N.P.

Plant Origin of W.S.N.P.	Intrinsic Viscosity	Relative Viscosity of 1% Solution		% Increase in Rel. Visc. due to Oxidation
		Before oxidation	After oxidation	
C.H.R.S. wheat flour	0.80	2.80	2.94	5.0
Yam (*Dioscorea*)				
D. alata L.	1.30	8.97	9.83	9.6
D. cayenensis Lam.	1.45	3.32	3.47	4.6
D. rotundata Poir.	0.30	1.90	1.94	2.1
Cassava (*M. utilissima* Pohl cv. Ankra)	0.02	1.16	1.17	0.9

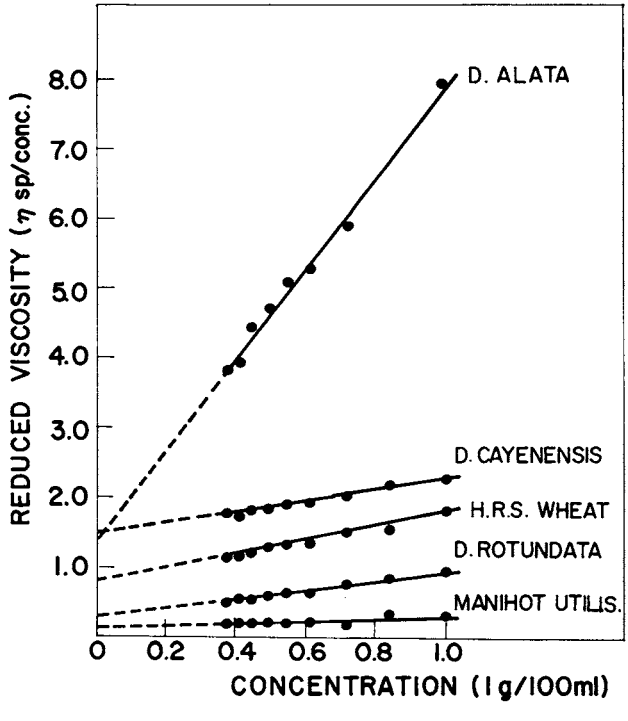

Fig. 4. Reduced viscosities of W.S.N.P. solutions in acetate buffer (pH 4.5).

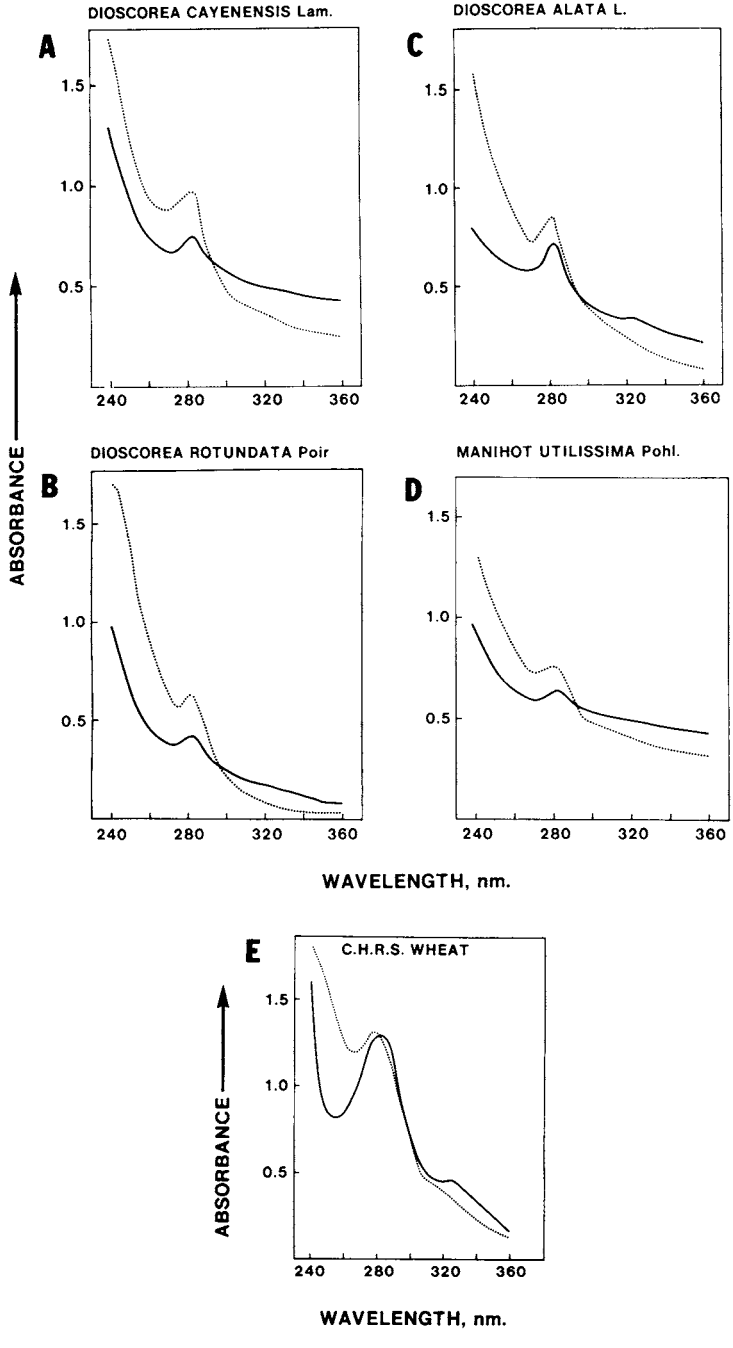

Fig. 5. uv spectra of tested W.S.N.P. before and after oxidation with H_2O_2. Before oxidation _____, after oxidation - - - - - - - -.

Protein content of W.S.N.P. extracted from all nonwheat flours was considerably less than that of wheat pentosans (Table III). This correlated closely with a considerably lower protein content in the original flours. Monitoring of fractions obtained by DEAE-cellulose chromatography showed a considerably lower concentration of protein in lower fractions of nonwheat W.S.N.P.; this was most noticeable with W.S.N.P. extracted from cassava flour (Fig. 3).

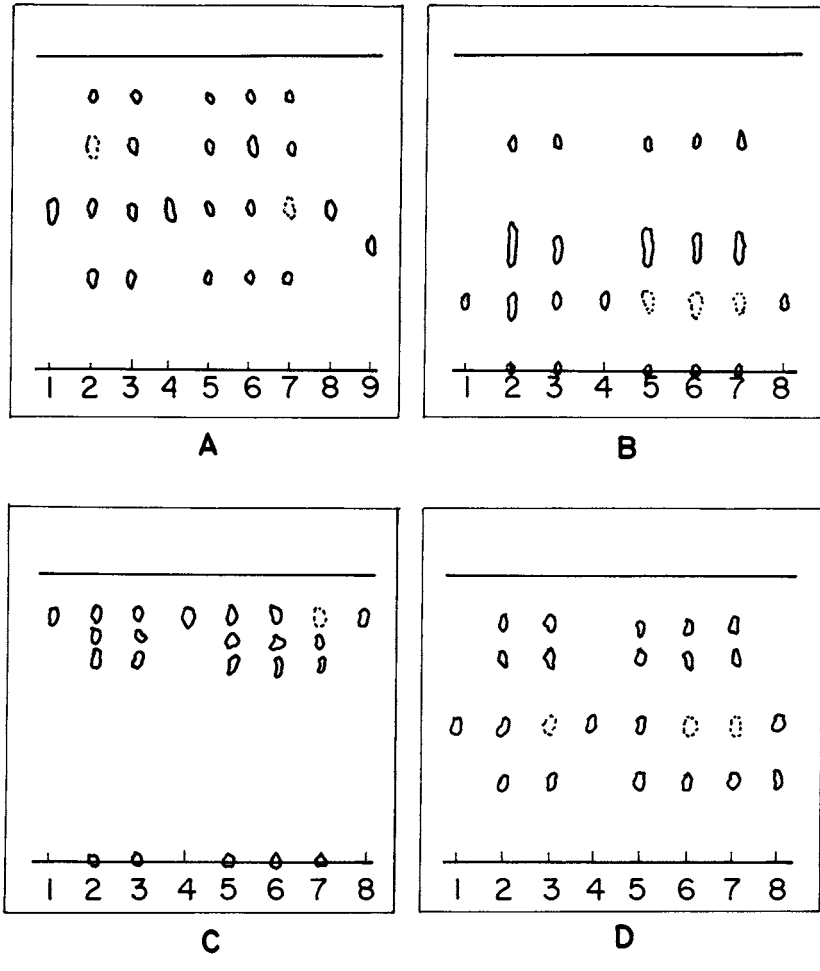

Fig. 6. Thin-layer chromatograms of phenolic acids present in tested W.S.N.P. Solvent systems: A) Benzene:methanol:acetic acid (90:16:8), B) Toluene:acetic acid:water (4:1:5) supernatant, C) Acetic acid:HCl:water (30:3:10), and D) Benzene:dioxane:acetic acid (90:25:8). Spots on starting line: 1) ferulic acid, 2) C.H.R.S. wheat W.S.N.P., 3) *D. alata* L. W.S.N.P., 4) ferulic acid, 5) *D. rotundata* Poir. W.S.N.P., 6) *D. cayenensis* Lam. W.S.N.P., 7) *M. utilissima* Pohl. W.S.N.P., 8) ferulic acid, and 9) caffeic acid.

Viscosity Measurements and Oxidative Gelation

All solutions for viscometric measurements were prepared by dissolving the lyophilized material in acetate buffer (pH 4.5). This buffer was used instead of commonly used dilute sodium hydroxide because of difficulties encountered in dissolving yam W.S.N.P. in the latter.

Though the values of intrinsic viscosity for all tested materials were found in a rather narrow range (Fig. 4), *D. alata* W.S.N.P. yielded the most viscous solutions, the relative viscosity of which increased markedly with increasing concentration. At 1% concentration, the reduced viscosity of *D. alata* W.S.N.P. was four to eight times higher than viscosities of other materials at the same concentration.

Viscosity measurements were also performed on solutions after they had been oxidized by 3% H_2O_2 to find out whether an effect similar to the oxidative gelation of wheat pentosans might be expected. In all cases the viscosity was affected by oxidation; however, with the solution of cassava W.S.N.P., the increase was hardly noticeable. The highest increase (9.6%) was recorded with *D. alata* W.S.N.P. (Table IV). It may be noted that the change in viscosity of the wheat pentosans solution did not reach the magnitude reported by some other workers (19–23). This may be due to the dependence of the gelation mechanism on many factors, including the reaction of the solvent (24). All reported data are, therefore, to be considered of relative value only.

Although the mechanism of oxidative gelation of these types of polysaccharides has not been fully elucidated, studies on aqueous extracts from wheat flour indicated that some polyphenol or polyphenol derivatives were involved in this reaction (25). The polyphenolic component was found responsible for the 320-nm maximum in the uv absorption spectrum of the unoxidized solutions; the disappearance of this maximum upon oxidation was taken as evidence for the involvement of this component in the reaction mechanism (24,26). To find out whether the gelation of tested nonwheat W.S.N.P. followed the same pattern, uv spectra of their solutions before and after oxidation were compared (Fig. 5). A distinct maximum at 320 nm was recorded with material extracted from wheat flour and *D. alata* flour only; with both samples, the maximum disappeared after oxidation. With other materials, the maximum was hardly detectable at concentrations used. Since difficulties were encountered in preparing solutions of higher concentration due to a rather limited solubility of the material, thin-layer chromatography of saponified samples was used to prove the presence of any phenolic compounds in all other tested materials. By use of four different solvent systems, four spots reacting as phenolic acids or their derivatives were detected (Fig. 6). Ferulic acid was positively identified in all samples, though its concentration in yam and cassava W.S.N.P. was visibly lower than in wheat-flour pentosans. Caffeic acid, which was earlier found in tubers of yam (27, 28), did not appear on any of the tlc chromatograms of W.S.N.P. derived from these tubers. Phenolic acids responsible for spots other than ferulic acid were not identified.

Like uv spectrum of wheat-flour pentosans, all spectra of yam W.S.N.P. showed an increase in protein absorbance (280 nm) after oxidation. This phenomenon, reported for wheat pentosans to take place not only in *in vitro* experiments but also during mixing of dough from flour treated with oxidizing agents, was explained by possible aggregation of glycoproteins associated with

the soluble polysaccharides (23). Provided this explanation was equally valid for nonwheat material tested in this study, it might be suggested that most of the studied W.S.N.P. possessed a higher aggregation capacity than wheat pentosans, judging from a more pronounced increase in the 280-nm maximum. It would, however, seem too speculative to draw any conclusions on the basis of available data. It was also noticed that the shift of this maximum toward lower wavelengths, characteristic for oxidized solutions of wheat-flour pentosans, did not appear with any of the tested W.S.N.P. No unambiguous explanation of this shift has been given. It was suggested that it could be attributed to a bathochromic shift caused by hydrogen bonding in the presence of a solute acting as a hydrogen donor (29). Changes in protein structure caused by transferring the chromophoric groups from a hydrophobic environment in the protein matrix to an aqueous environment of lower refractive index were also considered as a possible explanation. A more detailed study with pure materials must be conducted to find out whether the absence of any distinct shift of the protein peak in uv spectra of tested W.S.N.P. was due to a different structure of the protein moiety in the glycoprotein fraction of these polysaccharides or simply due to its markedly lower concentration.

CONCLUSIONS

Presented data gave evidence for the differences in the qualitative and quantitative distribution of sugars in the W.S.N.P. of several flours considered as possible substitutes for wheat flour in composite blends. A considerable concentration of pentoses was found in W.S.N.P. extracted from the flour of *D. rotundata* Poir. Material extracted from this flour was also rich in uronic acids or uronic acid derivatives. This chemical nature may offer a lead to the explanation of a noticeably less adverse effect of the above flour on the baking performance of wheat/yam flour mixture than that exhibited by flours from other tested species. In Part II of this paper, an attempt will be made to relate the chemical nature of these polysaccharides and their oxidative gelation characteristics to their effect on the viscoelastic character of composite doughs.

Acknowledgments

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