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Composition and Properties of Mucilaginous Polysaccharide from Native and Fermented Black Gram Flour

G. Changala Reddy, N. S. Susheelamma & R. N. Tharanathan*

Department of Food Chemistry, Biochemistry Section, Central Food Technological Research Institute, Mysore 570013, India

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ABSTRACT

A mucilaginous polysaccharide was isolated from black gram under different processing conditions. The trichloroacetic acid (TCA) extracted and acetone precipitated polysaccharides contained L*-rhamnose,* L*-arabinose,* D*-xylose and* D*-galactose along with* D*-galacturonic acid (12–13%). The polysaccharide preparations showed considerable changes in galactose and rhamnose contents during processing. The α-galactosidase activity as well as the viscosity increased by 25–30% after fermentation. Rheological constants such as yield stress and consistency index showed considerable changes but flow behaviour index did not show much change. The zero shear intrinsic viscosity or consistency index appeared to be related to the galactose content of the polysaccharide.*

INTRODUCTION

Black gram (*Phaseolus mungo*) dhal, both in dry and wet-ground forms, finds use in several sweet and savoury preparations and to a lesser extent even the whole seeds are used after dehusking as and when necessary. Among them the '*idli*', an autofermented and steamed pudding made from finely ground black gram flour and rice semolina (1:2 ratio), is relished as a breakfast food for its soft and spongy texture. The factors responsible for this are the surface active proteins imparting a porous texture and a highly viscous polysaccharide, which stabilizes the porous

*To whom correspondence should be addressed.

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texture against thermal disruption during steaming (Susheelamma & Rao, 1974, 1979). The polysaccharide is an acidic arabinogalactan (Susheelamma & Rao, 1978; Ramadas Bhat *et al.*, 1987). Nothing is known so far about the physico-chemical and structural changes induced in the polysaccharide by various processing (native or fermented) or pre-processing (dry or wet grinding) conditions of black gram. An attempt is made in the present investigation to understand them.

MATERIALS AND METHODS

Black gram flour

Black gram, whole seeds and dhal (dehusked and split seeds), were purchased from the local market, cleaned and stored at 5–7°C in sealed containers until use. Dhal was powdered to pass through 60 mesh (BSS). For wet grinding black gram dhal was washed with water, soaked for 3–4 h at room temperature (22–27°C) and wet-ground in an edge runner for 15–20 min to get a fine paste (dhal:water = 1:4). Whole seeds were soaked in water for 7–8 h at room temperature, rubbed with hands under running water to remove the husk and then wet-ground to a fine paste.

All the reagents used were of analytical grade. *p*-Nitrophenyl, α- and β-D-galactopyranosides were obtained from Sigma Chemicals (USA).

Determination of water uptake

Black gram seeds or dhal were individually weighed, soaked in water for different intervals of time and the gain in weight was recorded. About 50 mg of flour, in triplicate, was mixed with 5 ml of water, centrifuged after 3, 5, 10 and 15 min standing to determine the gain in weight.

Isolation of the polysaccharide

Aqueous pastes (20%) were prepared (dry or wet-ground) in three lots and two of them were allowed to autoferment at 25°C for 16 h with (F^+) and without (F^-) added NaCl (4% on flour basis). Batters were extracted to get the polysaccharides, as described earlier (Susheelamma & Rao, 1974).

Composition

Analyses were carried out for total sugar (Dubois *et al.*, 1956), reducing sugar (Nelson, 1944), pentoses (Ashwell, 1966), uronic acid (Knutson & Jeanes, 1968) and protein (Lowry *et al.*, 1951) contents. Polysaccharides were hydrolysed with 0·5 N H_2SO_4 at 95°C for 4 h and their alditol acetates were analysed by GLC (Savardekar *et al.*, 1965).

α-Galactosidase activity

This was carried out according to Suzuki *et al.* (1970) with slight modification. Native and fermented batters with and without added NaCl (N^+, N^- and F^+, F^-, respectively) were extracted with 0·05 M sodium acetate buffer, pH 5·3, containing 0·01 M EDTA, for 2 h at 5–7°C and centrifuged at 5000 g for 15 min. Suitably diluted aliquots of supernatants were incubated with *p*-nitrophenyl, α- and β-D-galactopyranosides (2 μmol in 1·0 ml) at 30°C for 30 min. The reaction was stopped by the addition of 2 ml of 2% Na_2CO_3 and the released *p*-nitrophenol was determined by measuring the OD at 405 nm. One unit of activity was expressed as the amount of extract that releases 1 μmol of *p*-nitrophenol at 30°C min^{-1} and the specific activity expressed as units mg^{-1} of protein.

Rheological constants

Aqueous dispersions of the polysaccharide (0·5–1·5%) were prepared and their flow curves at shear rates of 6–3000 s^{-1} were determined in a Haake rotovisco PK 100 Viscometer with M-500 sensor and PK V 1° cone. Rheological constants were calculated using the Casson equation.

Viscosity determination

Aqueous dispersions of the polysaccharide (0·1–0·6%) were prepared and their viscosities were determined in a multi-bulb capillary viscometer (Swenson, 1963) at 25 ± 0·5°C. $[\eta]$, $[\eta]_0$ and slopes ($\log \eta$ Sp_0 versus $\log C[\eta]_0$) were calculated. The viscosity — average molecular weight (M_v) was calculated according to the coefficient determined by Owens *et al.* (1946).

Entanglement density

The ratio of reduced concentration over slope was calculated to get the average number of entanglements per molecule, $\bar{n}_e$ (Launay *et al.*, 1986).

RESULTS AND DISCUSSION

The mucilaginous polysaccharide is released only after fine grinding (dry or wet) of black gram. The rate of hydration will be different for these and whether this influences the nature of the polysaccharide is not known. Hence water uptake was studied as a function of time.

Water uptake

Black gram dhal or whole seeds take up water according to the weight or size of the grains (Fig. 1). The dhal reaches a saturation point at about 2 h after soaking, while the whole seeds show a continuously increasing trend even after 24 h. This may be due to further water imbibition by the endosperm with the germ and hypocotyledon intact, while these are removed during the preparation of dhal. It is seen that dry-ground flour takes up water in a relatively shorter period (about 75–85% water in 5–12 min). The bigger grains take up less water when compared with small or medium-sized grains (dhal or seeds). As obtained in the bulk the black gram sample is a mixture of grains of different sizes and maturities.

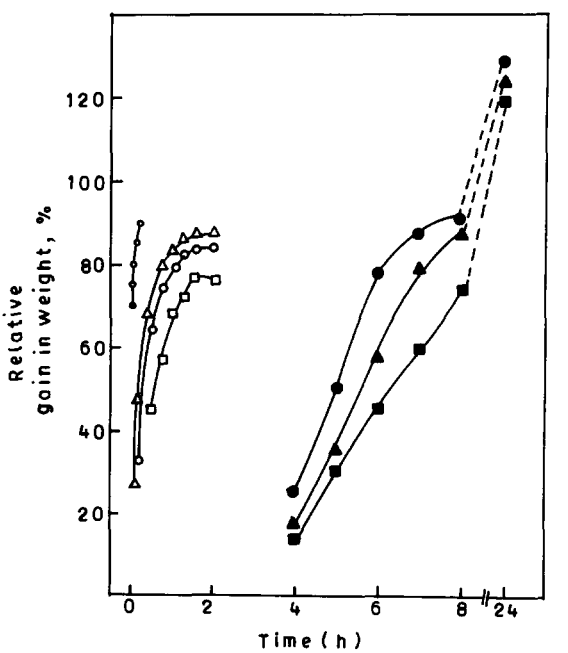

Fig. 1. Changes in water up-take by black gram flour, dhal or whole seeds with time. △/▲, small, ○/●, medium and □/■, big size (by eye selection) dhal or whole seeds, respectively; —○—○—○—○—, black gram flour.

Isolation of the polysaccharide

Data shown in Table 1 indicate that TCA extracts show 120–130% higher values for η_r over that of water extracts. This may be due to lower protein and higher carbohydrate content of the former. Both of them showed an increase in η_r(20–30%) after fermentation. F^+(TCA) samples had 8–12% lower values of η_r as compared to F^- samples, probably due to the presence of acid and NaCl, which restricts the swelling of the polysaccharide and thus lowers its viscosity. But in the water extracts, F^+ showed a marginal (10–12%) increase in η_r over that of the F^- sample, perhaps due to the presence of proteinaceous material which may influence the viscosity. The extracts after precipitation with acetone, dialysis and redispersion in the same volume of water showed significant increase in viscosity. The η_r values were about 75% greater than those of N^- in TCA-extracted samples and only 20% more in the case of water-extracted samples; F^+ samples showed η_r values nearly double that of F^- in all the samples. From the water extracts, only N^- and F^+ were significantly different and hence only these two were used in further analyses. TCA extracts from wet-ground flours had slightly higher values

TABLE 1
Relative Viscosities of Water and TCA-Extracted Polysaccharides from Native and Fermented Black Gram Flour (Values Represent Averages of Three Independent Experiments)

Sample	*Processing condition*[a]	*Extract*	*Acetone ppt.*	*Relative increase*
Dry-ground dhal	N^-	5·6	8·3	1·5
	F^-	7·6	14·8	1·9
	F^+	6·6	26·3	4·0
	N^{-b}	2·3	4·4	1·8
	F^{-b}	2·5	5·5	2·2
	F^{+b}	2·8	11·6	4·1
Wet-ground dhal	N^-	5·5	8·5	1·6
	F^-	7·6	15·0	2·0
	F^+	7·0	28·5	4·0
Wet-ground gram	N^-	5·6	8·6	1·6
	F^-	7·6	15·4	2·0
	F^+	7·0	28·6	4·0

[a]N^-, F^- and F^+ represent native, fermented without and with added NaCl batters from black gram flour.
[b]Water-extracted polysaccharides.

when compared with those from dry-ground flours. The increase in viscosity may be associated with both difference in polysaccharide fractions and their concentrations.

Composition

The yields of TCA polysaccharides were comparable from dry or wet-ground dhal or seeds, and the increase in yield was about 25% after fermentation (Table 2). Slightly higher yields (33–35%) obtained with the water-extracted polysaccharides may be due to the presence of proteins (50%) in them. Water-extracted polysaccharides had only 50% total sugar while TCA-extracted polysaccharides had 80–90%. The reducing sugar content showed considerable increase after fermentation. Pentoses showed about 15–20% decrease, while uronic acid content, identified as galacturonic acid, showed about 12–15% decrease after fermentation. Only the water-extracted polysaccharide showed about 45% increase in uronic acid content after fermentation, the reasons for this are not known at present.

TABLE 2
Yield (%) and Carbohydrate Composition (%) of Polysaccharides from Native and Fermented Black Gram Flour

Sample	*Processing condition*[a]	*Yield*	*Total sugar (TS)*	*Reducing sugar (RS)*	*Pentoses*	*Uronic acid*
Dry-ground dhal	N^-	3·3	83	0·8	58	12·8
	F^-	4·2	87	1·6	48	10·3
	F^+	4·4	96	1·6	49	11·5
	N^{-b}	4·4	48	ND[c]	ND	5·4
	F^{+b}	6·5	50	ND	ND	7·8
Wet-ground dhal	N^-	4·0	82	0·7	51·3	13·6
	F^-	4·2	80	1·8	47·5	9·2
	F^+	4·8	80	1·5	47·5	12·0
Wet-ground gram	N^-	3·6	94	0·5	55·0	13·2
	F^-	3·9	96	ND	52·5	11·0
	F^+	4·4	96	1·4	52·5	13·2

[a]Abbreviation as in Table 1.
[b]Water-extracted polysaccharides.
[c]ND — Not determined.

Relative distribution of neutral sugars

It is seen in Table 3 that xylose content was significant only in the dry-ground dhal TCA-extracted polysaccharide and showed a considerable decrease in the F^+ sample. In the wet-ground samples xylose content was rather low and F^+ showed negligible change, but F^- showed 50 and 200% increase over N^- from gram and dhal, respectively. The arabinose content was around 70% and after fermentation showed marginal changes in the TCA polysaccharide but about 17% increase in the water-extracted polysaccharide (dry-ground dhal). Variation of arabinose in the polysaccharides from dhal (wet-ground) was negligible but those from gram showed about 10% increase and decrease in F^- and F^+, respectively.

The galactose content showed more than 35–40% decrease in the water — extracted polysaccharide, and only marginal (10–12%) decrease in the TCA polysaccharide after fermentation. The content of galactose was lower in the polysaccharides from dry-ground flour, but nearly twice in those of wet-ground dhal or whole seeds. However, F^- samples showed higher Ara/Gal ratios compared to F^+ samples from both dry or wet-ground flours. Rhamnose showed marginal changes in the wet-

TABLE 3
Relative Proportion (%) of Neutral Sugars (as their Alditol Acetates) of Polysaccharides from Native and Fermented Black Gram Flour

Sample	*Processing condition*[a]	*Rha*	*Ara*	*Xyl*	*Gal*	*Ara/Gal*	*Rha:Gal A*
Dry-ground dhal	N^-	3·0	73·6	2·3	8·3	8·9	1:4·3
	F^-	3·3	76·5	2·3	7·6	10·1	1:3·1
	F^+	3·8	75·1	1·3	9·4	8·0	1:3·0
	N^{-b}	0·9	60·6	0·4	32·8	1·9	1:6·3
	F^{+b}	2·0	70·3	—	19·9	3·5	1:3·9
Wet-ground dhal	N^-	1·5	70·3	0·3	14·9	4·7	1:9·5
	F^-	1·2	71·5	0·9	11·3	6·3	1:8·0
	F^+	1·5	69·1	0·4	17·8	3·9	1:8·0
Wet-ground gram	N^-	1·2	71·9	0·6	13·4	5·4	1:11·0
	F^-	1·4	80·5	0·9	7·1	11·3	1:8·0
	F^+	1·6	65·1	0·5	20·1	3·2	1:8·0

[a]Abbreviations as in Table 1.
[b]Water-extracted polysaccharides.

ground samples but an increase of 25–30% in others after fermentation, probably indicating some depolymerization, as rhamnose is known to be involved in branching points for side chains (Dey & Brinson, 1984). The Rha/Gal A ratio showed about 30–50% decrease after fermentation.

Galactosidase activity

From Table 4 it is obvious that the α-galactosidase activity of N^+ was 33% more than that of N^- sample. The activity increased only marginally (11%) in F^- but significantly (50–70%) in F^+ samples. The α-galactosidase activity of black gram flour has been shown to increase after 16 h of fermentation (Reddy & Salunkhe, 1980). In our studies even β-galactosidase activity increased significantly after fermentation, as the values were 14 and 28 units ml^{-1} of extract and specific activities were 4·5 and 6·0 mg^{-1} protein, respectively for N^- and F^+ extracts. This increase in activity accounts for 35–40% decrease in galactose content of F^+ from water-extracted polysaccharides (see Table 3). However, TCA-extracted polysaccharide shows a considerable increase (marginal in the dry-ground flour and significant in the wet-ground dhal or seeds, see Table 3), probably due to the release of this and other galactose-containing polysaccharides from the cell wall (Ramadas Bhat & Tharanathan, 1986) during fermentation with NaCl. Similar observations have been made on the water, oxalate and NaCl-extracted pectins from the pulp of grapes (Saulnier & Thibault, 1987). The presence of NaCl adds to the ionic strength (Jarvis, 1982) and may also partially sub-

TABLE 4
Galactosidase Activities of Native and Fermented Black Gram Flour

Sample	*Processing condition*[a]	*α-Galactosidase activity (U/ml)*	*Specific activity (U/mg)*
Dry-ground dhal	N^-	27	3·9
	N^+	35	4·3
	F^-	30	4·3
	F^+	46	5·0
Wet-ground dhal	N^-	20	6·0
	N^+	30	10·2
	F^-	25	8·0
	F^+	35	12·0

[a] N^-, N^+ and F^-, F^+ represent extracts without and with added NaCl from native and fermented flour, respectively.

stitute the calcium ions and enhance a further release of pectin-type polysaccharides. It is likely that similar types of polysaccharide(s) might be co-extracted along with the mucilaginous polysaccharide of black gram, although α- and β-galactosidase would have exerted their depolymerizing action on the polysaccharides during fermentation. The Rha/Gal A ratio also supports this, as it shows a decrease in F^+ over N^- samples (see Table 3). A similar decreasing trend in Rha/Gal A was observed in the water and NaCl-extracted pectins from the pulp of grapes (Saulnier & Thibault, 1987).

Rheological constants

The flow curves (given in Fig. 2(a) for only F^+ from wet-ground dhal) indicated that all the polysaccharide preparations exhibited pseudoplasticity. The corresponding data giving variation of viscosity with shear rate is shown in Fig. 2(b) and plots of root of shear stress versus root of

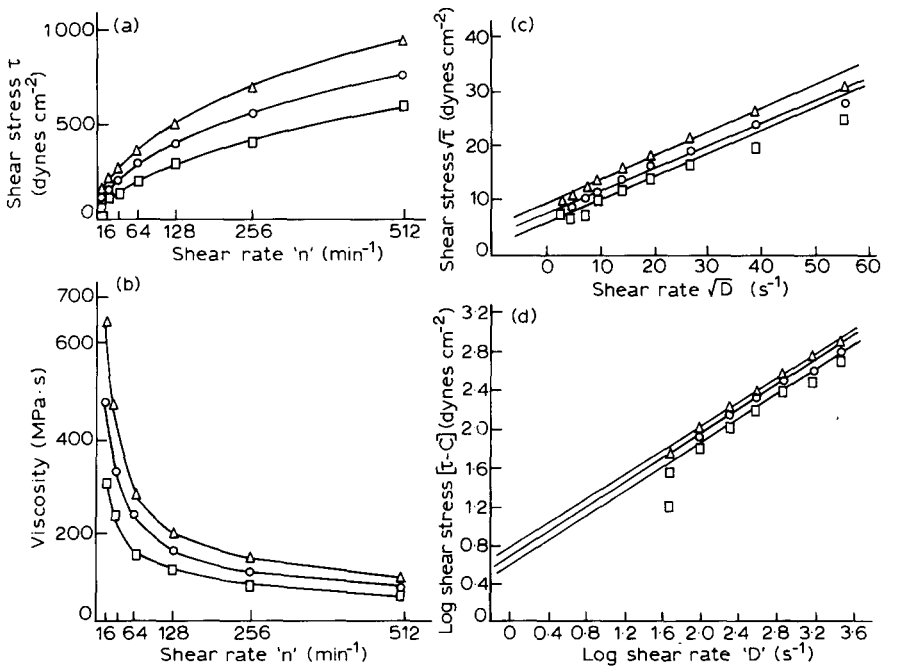

Fig. 2. Variation of rheological constants with concentration for F^+ polysaccharide from black gram (wet-ground) dhal. (a) Shear stress versus shear rate; (b) viscosity versus shear rate; (c) square root of shear stress versus root of shear rate; (d) log of shear stress − yield stress versus log of shear rate. □, ○, and △ represent 0·65, 1·0 and 1·3% concentration of the polysaccharide, respectively.

shear rate are shown in Fig. 2(c). A point of deflection was noticed in some samples around a shear rate of 10^2 s^{-1}, but most of the samples exhibited linearity between shear rates of 10^2–10^3 s^{-1}. Hence, the line passing through this region was extrapolated to zero shear rate to get the yield stress. The yield values were higher than could be found for mucilaginous polysaccharides. Probably the device (cone and plate geometry) used to determine this might have exerted some influence and the true yield values may not be that high (Launay *et al.*, 1986). The apparent yield stress values ranged from 4–25 dynes cm^{-2} for those from dry-ground flour and 25–80 or 120 dynes cm^{-2} for those from wet-ground dhal or seeds, respectively. The values decreased by about 50% in F^- samples, but in F^+ samples, the values were comparable to those for N^- samples. Double log plots of corrected shear rates, i.e. shear stress $-$yield stress $(\tau - C)$ versus shear rate are shown in Fig. 2(d). Here also linearity was observed around shear rates of 10^2–10^3 s^{-1}, and lines passing through these were extrapolated to zero shear rate to get the log of consistency index, '*K*'. The values of '*K*' showed a trend similar to that of yield stress and ranged from 2–8 dynes cm^{-2}. The flow behaviour index '*n*' (slopes from the double log plots) also showed only marginal changes (ranging from 0·65–0·45), but all of them showed a decrease in '*n*' value with increase in concentration. These data indicate that the inherent nature of the polysaccharide may not be affected under these conditions, but other co-extracted polysaccharides might exert their influence on the consistency index and yield stress values.

Viscosity and solution properties

The $[\eta]$ and $[\eta]_0$ values were 5·0 and 7·5 dl g^{-1} for N^- when determined at 0·1–0·25% level. As the concentration of the polysaccharide in most of the food preparations varies between 0·3 and 0·8%, $[\eta]$ values were also determined at the higher concentration ranges. The variation of specific viscosity with concentration is shown in Fig. 3 for N^-, F^- and F^+ polysaccharides from wet-ground dhal. The $[\eta]$ values for polysaccharides from dry and wet-ground flour were 30 and 36 dl g^{-1} for N^-, 12·5 and 25 dl g^{-1} for F^+ and 25 and 29 dl g^{-1} for F^-, respectively. The corresponding Huggin's coefficients were 2·47 and 2·68 for N^-, 2·00 and 2·30 for F^+ and 2·14 and 2·36 for F^- polysaccharides from dry and wet-ground samples, respectively. The values being greater than one indicate that the concentration range used may be favouring association between the polymer molecules. For water-extracted polysaccharide, values were 2 and 5 dl g^{-1} for N^- and F^+, respectively. The viscosity

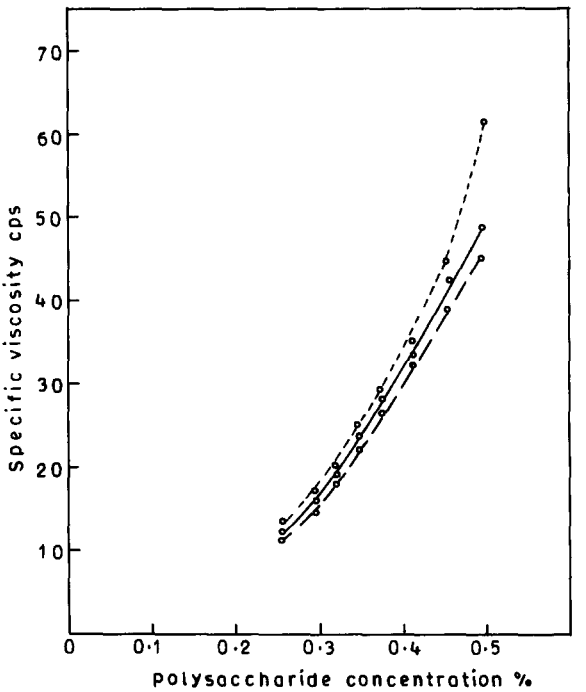

Fig. 3. Variation of specific viscosity with concentration for polysaccharides from native and fermented black gram (wet-ground) dhal. ---, F^+, ——, F^- and – – –, N^- samples, respectively.

average molecular weights were $120 \pm 3 \times 10^3$ for all these samples. These were in good agreement with that for N^- from dry or wet-ground flour, as determined by gel filtration on Sephadex G-100 (Ramadas Bhat *et al.*, 1987). Only F^+ from dry-ground flour showed a lower (63×10^3) value.

However, $[\eta]_0$ for all these preparations varied from 250 ± 15 dl g^{-1}, except F^+ from dry-ground flour which was around 190 dl g^{-1}. The values were rather low for the water-extracted polysaccharide (24 and 20 dl g^{-1} for N^- and F^+). The slope values ranged from 2·1 to 2·5 for these indicating that the concentrations used in these studies may be $C^* < C < C^{**}$ (Launay *et al.*, 1986).

Entanglement density

It is seen from Fig. 4 that when compared with N^-, F^- showed a marginal increase in $\bar{n}_e$ with concentration for the dry or wet-ground samples. But F^+ showed a marginal decrease in $\bar{n}_e$ for the wet-ground

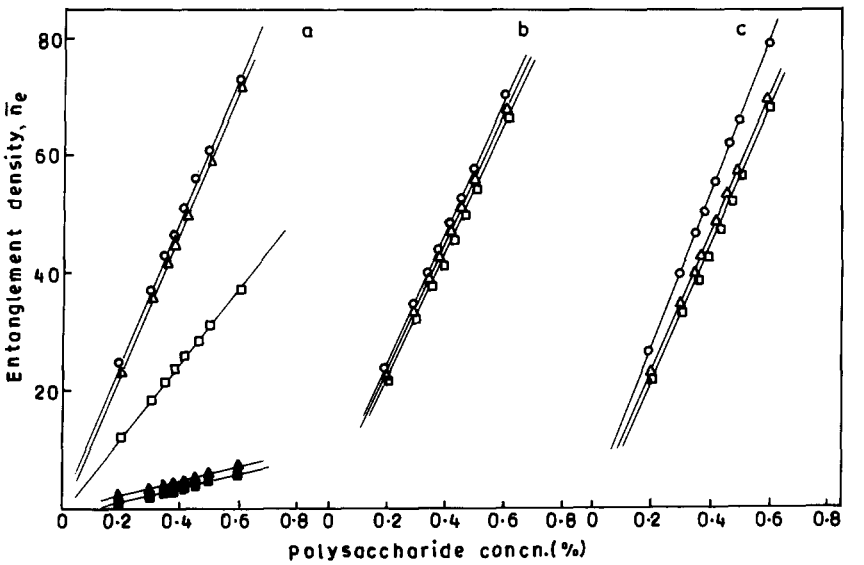

Fig. 4. Variation of entanglement density, $\bar{n}_e$ with concentration for polysaccharides from native and fermented black gram flour. (a) Dry-ground dhal; (b) wet-ground dhal; (c) wet-ground whole seeds. △, N^-; ○, F^- and □, F^+, water-extracted polysaccharide, respectively.

samples. From the dry-ground flour, water-extracted polysaccharide showed a slightly lower value for F^+, but the TCA polysaccharide showed significantly lower values. The data indicate that the ability of the polysaccharide to hydrate or swell and form an entangled network is highest in N^- from dry-ground flour but rather low in F^+. But F^- and F^+ had values comparable to N^- from wet-ground dhal and much greater than that of F^+ from dry-ground dhal.

Galactose content and viscosity relationship

In order to see whether the galactose content has any relationship with viscosity or consistency index, galactose content versus $[\eta]_0$ or $[K]_0$ were plotted and it is seen from Fig. 5 that both of them showed similar increase with increase in galactose content. The period of soaking (3–4 h for dhal and 7–8 h for seeds) prior to grinding may also play some role in determining the composition of the polysaccharide as its galactose content nearly doubled from dry to wet-ground dhal and the values were found to increase by another 100% in F^+ samples of dhal or seeds.

The data obtained from these studies are useful in giving an explanation to the observations made by convention. Usually dry-ground flours

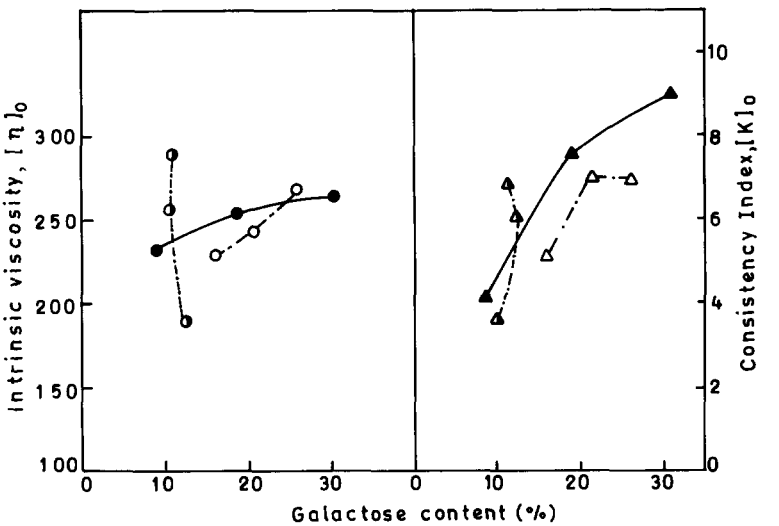

Fig. 5. Relationship between galactose content and viscosity or consistency index of the polysaccharide from native and fermented black gram flour. ◑/▲, dry-ground dhal; ○/△, wet-ground dhal; and ●/▲, wet-ground whole seeds.

are not used for making the fermented products such as '*idli*', although 'instant mixes' have been made (from dry-ground flours) for this product during the past one or two decades. The superiority of '*idli*' made from wet-ground flour may be due to the higher hydration capacity and viscosity of the polysaccharide in the batter, which facilitates greater entrapment of air during wet grinding and also better entrapment of the gas produced during subsequent autofermentation of the batter. This results in a product with soft and spongy texture and very low bulk density, which is a highly desirable quality in '*idli*'.

CONCLUSION

The results of this study indicate that the mucilaginous polysaccharide obtained from dry or wet-ground flour of black gram shows changes both in composition and properties after autofermentation. The relationship of $[\eta]_0$ or $[K]_0$ to galactose content indicated that it may influence the solubility/viscosity of the polysaccharide. The presence of NaCl may be beneficial as it may extract some pectin-type polysaccharide, and also solubilize some proteins, and both these may enhance the apparent viscosity of the batter. The role of α-galactosidase during autofermentation is not clearly known. Preliminary work has shown the polysaccharide to be heterogenous with different fractions

varying in composition and molecular weight. Further attempts to purify the polysaccharide to homogeneity and study the relationship between composition/structure and biological activity/functional properties are in progress.

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REFERENCES

Ashwell, G. (1966). In *Methods in Enzymology*, Vol. 8, ed. E. F. Neufeld & V. Ginsburg. Academic Press, New York, pp. 86–87.

Dey, P. M. & Brinson, K. (1984). In *Advances in Carbohydrate Chemistry and Biochemistry*, Vol. 42, ed. R. S. Tipson & D. Horton. Academic Press, New York, pp. 265–382.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). *Anal. Chem.*, **28**, 350.

Jarvis, M. C. (1982). *Planta*, **154**, 344.

Knutson, C. A. & Jeanes, A. (1968). *Anal. Biochem.*, **24**, 470.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. Biol. Chem.*, **193**, 265.

Launay, B., Cuvelier, G. & Martinez-Reyes, S. (1984). In *Gums and Stabilizers for the Food Industry*, Vol. 2, *Application of Hydrocolloids*, ed. G. O. Phillips, D. G. Wedlock & P. A. Williams. Pergamon Press, London, pp. 79–99.

Launay, B., Doublier, J. L. & Cuvelier, G. (1986). In *Functional Properties of Food Macromolecules*, ed. J. R. Mitchell & D. A. Ledward. Elsevier Applied Science Publishers, London, pp. 1–78.

Nelson, N. (1944). *J. Biol. Chem.*, **153**, 375.

Owens, H. S., Lotzkar, H., Schultz, T. H. & Maclay, W. D. (1946). *J. Am. Chem. Soc.*, **68**, 1628.

Ramadas Bhat, U. & Tharanathan, R. N. (1986). *Cereal Chem.*, **63**, 376.

Ramadas Bhat, U., Salimath, P. V. & Tharananthan, R. N. (1987). *Carbohydr. Res.*, **161**, 161.

Reddy, N. R. & Salunkhe, D. K. (1980). *Cereal Chem.*, **57**, 356.

Saulnier, L. & Thibault, J. F. (1987). *Carbohydr. Polym.*, **7**, 329.

Savardekar, J. S., Slonekar, J. & Jeanes, A. (1965). *Anal. Chem.*, **37**, 1602.

Susheelamma, N. S. & Rao, M. V. L. (1974). *J. Sci. Food Agric.*, **25**, 605.

Susheelamma, N. S. & Rao, M. V. L. (1978). *J. Agric. Food Chem.*, **26**, 1434.

Susheelamma, N. S. & Rao, M. V. L. (1979). *J. Food Sci.*, **44**, 1309.

Suzuki, H., Li, S. C. & Li, Y. T. (1970). *J. Biol. Chem.*, **245**, 781.

Swenson, H. A. (1963). In *Methods in Carbohydrate Chemistry*, Vol. III, ed. R. L. Whistler, J. N. Green & J. N. BeMiller. Academic Press, New York, pp. 84–91.