

STUDIES ON WHEAT ENDOSPERM

III.* GALACTOSE-RICH POLYSACCHARIDES†

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Abstract

Galactose-rich polysaccharides have been found in the finely divided material passing 75- μ m nylon-mesh sieves during the isolation of wheat endosperm cell walls in 70% aqueous ethanol. The galactose-containing polysaccharides may be separated from arabinoxylans by gel filtration. Their possible location in endosperm cells is discussed.

The polysaccharides of wheat endosperm cell walls, isolated from wheat flour in 70% ethanol, are composed chiefly of arabinoxylans together with some glucomannan and cellulosic β -glucan (Mares and Stone 1973a). Polysaccharides containing galactose are present only in minor amounts and galactose represents less than 3% of the total monosaccharides in the isolated cell walls. This result was unexpected since Preece and Hobkirk (1953) and Kundig *et al.* (1961) had shown that there was a relatively high proportion of galactose (10–20%) in the water-soluble polysaccharides of wheat flour.

In an attempt to account for the absence of galactose-rich polysaccharides in the cell wall fractions, the material not retained on the nylon bolting cloth in the wet-sieving steps in the wall isolation procedure (fractions S1, S2, and S3; see Fig. 1, Mares and Stone 1973a) was examined. The insoluble material in each fraction was recovered by centrifugation and extracted with distilled water at 40°C. The extracts were treated with salivary amylase, reduced in volume, dialysed, and freeze-dried (see Mares and Stone 1973a). The monosaccharide composition of each fraction was determined following hydrolysis with 0.5M HNO₃. Fraction S1 contained 37% arabinose, 34.5% xylose, and 29.5% galactose by weight. The corresponding composition of fraction S2 was arabinose 36%, xylose 50%, and galactose 14%, whilst that of fraction S3 was arabinose 39%, xylose 55%, and galactose 6%. The ratio of galactose to xylose decreased in the successive wet-sieving filtrates (S1 to S3) obtained as the wall preparation was purified. In duplicate experiments the total yield and relative amount of each fraction (S1, S2, and S3) obtained from a given weight of flour varied considerably, presumably due to differences in the extent of disruption of the walls and the degree of agitation during sieving.

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The molecular size distribution of the polysaccharides extracted from fraction S1 with water was examined by chromatography on Sepharose 4B. The elution profile is shown in Figure 1. The eluted fractions were pooled to give fractions S1(a) and S1(b) which were dialysed against distilled water, evaporated to dryness, and analysed for monosaccharide composition. Fraction S1(a) contained 36% arabinose, 64% xylose, and no galactose, whilst fraction S1(b) comprised 34% arabinose, 21% xylose, and 45% galactose by weight.

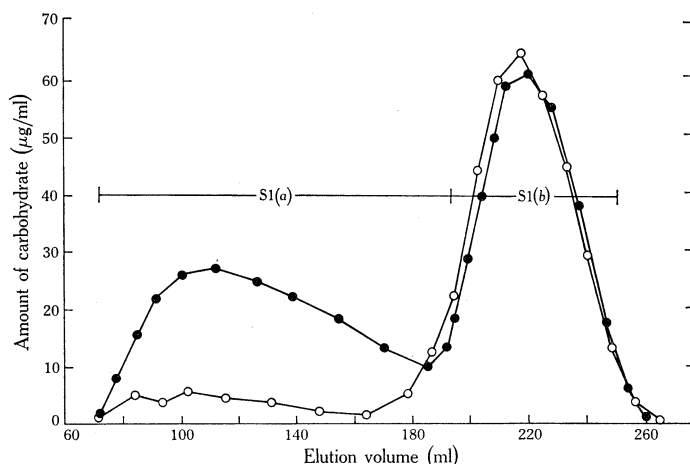


Fig. 1.—Sepharose 4B chromatography of the galactose-rich fraction isolated from wheat flour fines (fraction S1; see Mares and Stone 1973a, Fig. 1). Fractions pooled for monosaccharide analysis are designated S1(a) and S1(b). ● Water extract of fraction S1. ○ Fraction of the water extract of S1 soluble in ammonium sulphate (70 g/100 ml).

Fraction S1(a) was an arabinoxylan which resembled the water-soluble arabinoxylan extracted from isolated cell walls in xylose : arabinose ratio and in apparent molecular-size range (0.5×10^5 – 5×10^6) (Mares and Stone 1973b). Protein and carbohydrate estimations of the pooled fractions S1(a) and S1(b) indicated that S1(a) contained no detectable protein, whereas S1(b) contained approximately 9% by weight of protein and 85–90% carbohydrate. Fraction S1(b) contained all the galactose present in S1 and was smaller than the arabinoxylan, with an apparent molecular size in the range 1 – 8×10^4 .

Ammonium sulphate (70 g/100 ml) was added to a solution of fraction S1 and the precipitate and soluble fraction collected and analysed (Table 1). The Sepharose 4B elution profile of the fraction soluble in ammonium sulphate (Fig. 1) resembled that of the S1(b) fraction, and the monosaccharide composition of these two fractions was also similar.

The results suggest that the water-soluble galactose-rich polysaccharides of wheat flour are not closely associated with endosperm cell walls which had been isolated in 70% ethanol. They are, however, present in the filtrate from the first wet-sieving step in the cell wall isolation procedure. This finding supports the view that they do not represent part of the endosperm wall (or that they are very readily

dissociated from it), since the filtrate contains mostly starch granules and aggregates of protein but very few endosperm cell walls. During the purification procedure the wall fragments retained on the sieve are freed from attached starch and protein, and more wall fragments pass the sieve in the second and third wet-sieving steps. There is a corresponding increase in the xylan content of the filtrate and the proportion of galactose in the fractions decreases.

TABLE 1
AMMONIUM SULPHATE FRACTIONATION OF FRACTION S1* FROM WET-SIEVING FILTRATE

Fraction	Yield (% by wt.)	Monosaccharide composition (% by wt.)				Xyl:Ara
		Ara	Xyl	Gal	Glu	
70% ammonium sulphate-insoluble precipitate	57	36	64	—	—	1.78
70% ammonium sulphate-soluble fraction	43	34	21	45	—	0.62

*Fraction described by Mares and Stone (1973a, Fig. 1).

The galactose-rich polysaccharide appears to be of lower molecular weight than the arabinoxylan and can be separated from part of the arabinoxylan by ammonium sulphate fractionation. Galactose-rich polysaccharides appear to have a wide distribution in cereals as shown in the results obtained by Preece and Hobkirk (1953) using ammonium sulphate fractionation of the water-soluble polysaccharides from rye, wheat, oats, barley, and maize flours. In each of these species they found a component which was soluble in saturated ammonium sulphate and had a composition which was very similar in each case, containing approximately 20% glucose, 10% xylose, 50% arabinose, and 20% galactose.

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