

Grain Sorghum Condensed Tannins. II. Preharvest Changes¹

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ABSTRACT

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Condensed tannins from three varieties of bird-resistant grain sorghum were analyzed over 10 weeks before and at harvest to determine differences in α -amylase inhibitory activity and changes in composition of the condensed tannins. Composition of the condensed tannin fraction was determined by gel filtration on Sephadex LH-20. Compositional differences were noted among condensed tannins from the three grain varieties and among

tannins extracted from the same variety harvested at different dates. For all varieties, enzyme inhibition determined on a per kernel basis rose gradually until six weeks before harvest. Thereafter, one variety maintained a constant level of inhibition; the other two varieties lowered their inhibition levels but at different rates.

Changes in grain sorghum tannins with maturity of the grain have been the subject of debate. Although Mabbayad (1974) referred to studies reporting both constant tannin levels and decreasing tannin levels with maturity, his own data suggested that tannin levels increase as grain matures. The conflicting reports might be a result of different analytical methods. Mabbayad also presented evidence that tannin content may vary with growing conditions.

In this investigation we made no attempt to determine tannins per se. Changes in the condensed tannin fraction were determined by gel filtration, and quantitation was based on inhibition of α -amylase and the data expressed either per unit weight or per kernel of grain.

METHODS AND MATERIALS

Three hybrid varieties of bird-resistant sorghum grain (G-516 BR, G₁-459 BR, and Funk 79) and one unidentified, nonbird-resistant hybrid were grown in 1974 on land near Manhattan, KS. Eight rows of each variety were planted in the same field. Beginning when the grain was in the soft-dough stage (September 4, 1974) and through harvest (November 16, 1974), samples were gathered each week at random from the middle six rows of each group.

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Sampling involved collection of several heads of grain. After air drying and threshing on a single head thrasher, the samples were stored at 4°C.

The condensed tannins were determined as percentage inhibition of porcine α -amylase by the Phadebas α -amylase determination described previously (Davis and Hosenev 1979). The condensed tannins were isolated and fractionated on Sephadex LH-20 also as described previously (Davis and Hosenev 1979).

RESULTS AND DISCUSSION

Enzyme inhibition for samples of each variety of grain sorghum determined at 10, 8, 6, and 4 weeks preharvest and at harvest are given in Figs. 1 and 2. Gel filtration elution patterns determined for the isolated condensed tannin fractions of each bird-resistant variety at six weeks preharvest and at harvest are shown in Figs. 3 and 4. Thousand-kernel weights calculated from the averages of five (20-kernel) samples of each variety each week are shown in Fig. 5.

Elution patterns of the condensed tannins from the three bird-resistant varieties six weeks before harvest were quite similar (Fig. 3). All showed a peak eluting at approximately 55 ml and another peak eluting at between 90 and 100 ml. The ratio of peak heights was similar for G-459 BR and BR-79, but the peaks of G-516 BR were nearly equal in height. The elution patterns of the harvest samples (Fig. 4) present some interesting changes from those of the six-week preharvest samples: The first peak (55 ml) in both G-516 BR and G-459 BR increased relative to the second peak; in G-459 BR peak 2 was less separated from peak 1 than it was in the six-week samples; the second peak of G-516 BR eluted at about 10 ml lower volume in the harvest sample than in the six-week sample; BR 79 showed the most drastic changes—the first peak decreased in height relative to the second peak and eluted at 10 ml greater volume than it did in the six-week preharvest sample, and the

second peak increased in height relative to the first and eluted at about 35 ml greater volume than in the six-week sample.

Void volume for the column (determined with Blue dextrin) was 48 ml, which indicated that the first peak noted for all but the BR 79 harvest sample consisted of condensed tannins excluded from the gel. Porter and Wilson (1972) noted that Sephadex G-25 in 50% aqueous acetone was not an ideal gel filter for some materials used to calibrate a column. They believed, however, that the separation of condensed tannins (from wood) was not affected. Until condensed tannin samples of known molecular weight are available, the problem of molecular weight determination by gel filtration will be difficult to resolve.

If it is assumed that condensed tannins are separated by size

during gel filtration, the enzyme-inhibition data (Table 1) support the statement of Goldstein and Swain (1963) that molecular size is not the sole determinant of protein binding by condensed tannins. Condensed tannins extracted from grain harvested at different dates and fractions of the tannins collected from gel filtration showed that two varieties (G-516 BR and G-459 BR) had higher inhibition values for the lower elution volume (high molecular weight) fraction. Though the BR 79 sample had comparable activity, its active fraction eluted much later (lower molecular weight). This is especially interesting in view of the differences noted among the elution patterns of the three samples. Calculated on a weight basis, α -amylase inhibition, determined on 20-mg samples of ground grain for each bird-resistant variety of 10, 8, 6, 4, and 0

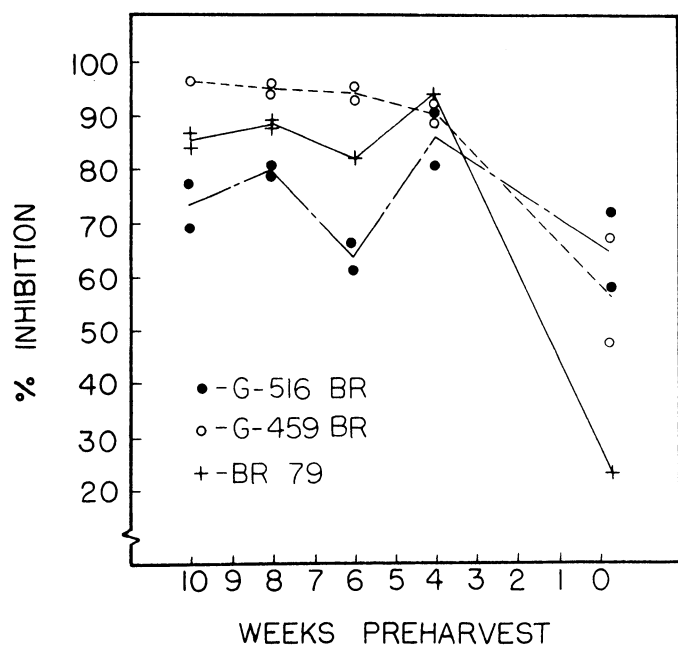


Fig. 1. Percent inhibition of α -amylase by ground grain (20 mg) from three varieties of grain sorghum at different stages of maturity.

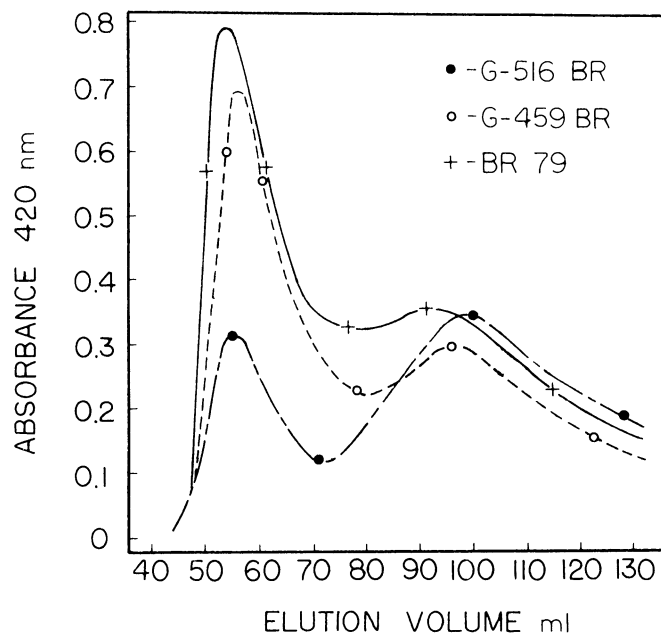


Fig. 3. Elution profile of condensed tannin extracted from three varieties of grain sorghum collected six weeks preharvest. The column was Sephadex LH-20 eluted with 50% acetone.

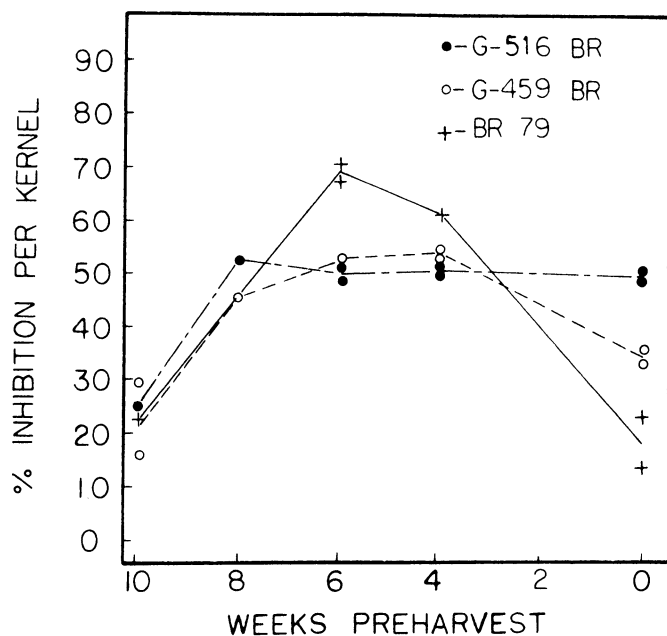


Fig. 2. Inhibition per kernel (percent inhibition of α -amylase \times average kernel weight/sample weight) for condensed tannin extracted from three varieties harvested at different stages of maturity.

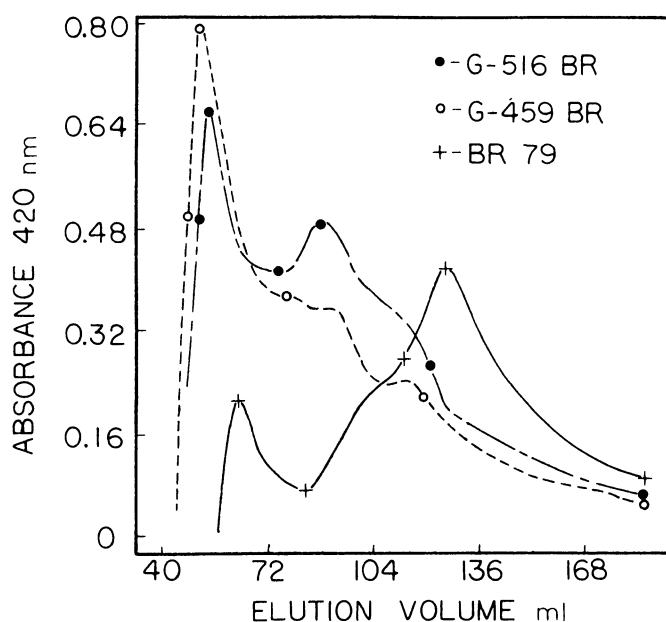


Fig. 4. Elution profile of condensed tannins extracted from three varieties of grain sorghum collected at harvest. Column was Sephadex LH-20 eluted with 50% acetone.

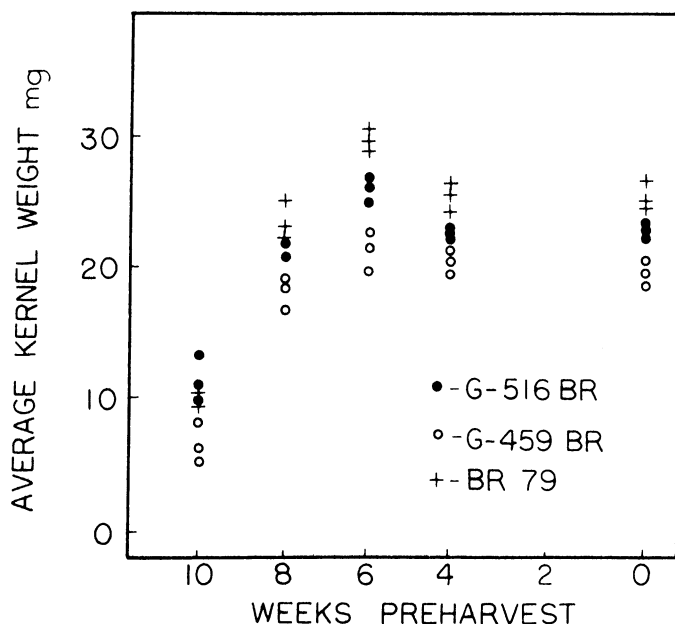


Fig. 5. Average kernel weight for three varieties of grain sorghum harvested at different stages of maturity.

weeks before harvest, was relatively constant for the BR 79 and G-459 BR samples up to four weeks preharvest (Fig. 1). During the last four weeks of maturation, the BR 79 sample dropped from 95% inhibition to 23%; the G-459 BR sample dropped from greater than 90% inhibition to 58%; and the G-516 BR sample showed a zigzag pattern, with final inhibition levels about the same as those of the 10-week preharvest sample. When percentage inhibition was calculated on a per kernel basis, all three varieties showed a gradual increase in inhibition activity through the six week preharvest and then declined; the BR 79 sample's activity decreased much more than that of the other two (Fig. 2).

TABLE I
Percent Inhibition of Condensed Tannin Eluted from
Sephadex LH-20 (0.0238 mg/ml)

Sample	Elution Volume (ml)	% Inhibition
GR 516 BR (Harvest)		
Original solution	...	78.3
Peak 1	49-57	92.3
Peak 2	80-110	83.9
GR 459 BR (Harvest)		
Original solution	...	83.2
Peak 1	49-61	92.7
Peak 2	81-113	61.4
BR 79 (Harvest)		
Original solution	...	81.0
Peak 1	61-79	36.0
Peak 2	101-158	86.3

The nonbird-resistant variety was checked at 0, 6, and 10 weeks preharvest. The samples gave less than 10% inhibition.

The decline in inhibitory activity over the last four weeks before harvest for the G-459 BR and BR 79 samples, together with data (Table I) showing that the isolated condensed tannins were about equal in inhibitory ability, suggests that the amount of condensed tannin per kernel of grain sorghum declines as the grain approaches harvest.

LITERATURE CITED

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