



# False Positives for Tannin Sorghum in Non-Tannin Sorghum Using the Bleach Test

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## ABSTRACT

Weathered and damaged sorghums without a pigmented testa will either turn dark or black after bleaching. This can lead to erroneous, false positive results indicating that a sorghum kernel contains tannins. Weathered red and white sorghum varieties were evaluated for tannin and phenol contents, and physical characteristics were evaluated using SEM and macro photography. Hand-sectioning of the suspected false positive samples indicated that no testa was present in the kernels. Severely weathered, insect-damaged or molded kernels in the bleached samples appeared dark, but the intensity of the darkness was significantly less than those with a pigmented testa. Damaged commercial white and red sorghums had increased levels of phenols but they did not contain condensed tannins. SEM revealed extensive damage of the endosperm in the stained areas. False positives from the Bleach test were mainly due to pigments that leached deep inside the endosperm and could not be removed by the bleaching procedure. Therefore, additional methods, such as visual inspection, are necessary to confirm suspected tannin content in sorghums. This is important to the sorghum industry because samples are sometimes erroneously classified as tannin sorghums, which causes rejection or reduced market value.

## INTRODUCTION

- Sorghums with B1\_B2\_ss and B1\_B2\_Ss genes, which produce a pigmented testa layer, contain condensed tannins (Figure 1).
- The presence of the pigmented testa layer reduces the feed efficiency of livestock rations.

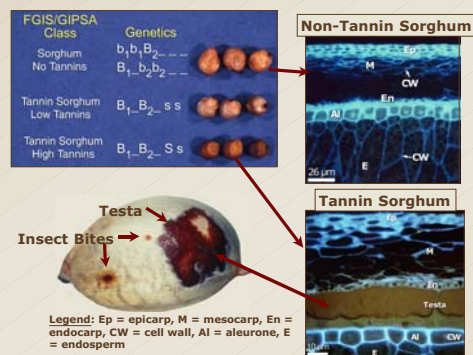


Figure 1. Structures of Non-Tannin and Tannin Sorghum Kernels

- The Vanillin-HCl method is the best method for determining condensed tannins in sorghum; however, this method requires significant time and is not readily applied in routine grading.
- The Chlorox Bleach Test is used by the United States Department of Agriculture's Federal Grain Inspection Service-Grain Inspection, Packers and Stockyard Administration (USDA-FGIS-GIPSA) to test for the presence of tannin sorghum during grading. This method uses Chlorox and KOH which remove the pericarp and turn the pigmented testa layer black.
- The Chlorox Bleach Test is simple, quick, and inexpensive, and it is relatively accurate in terms of determining the percentage of sorghum kernels during grading and classification.
- Non-tannin sorghums that have been damaged due to weathering, insect bites, or mold infection will turn dark after bleaching since anthocyanin pigments from the glumes and pericarp migrate into the endosperm and form a colored layer that can be mistaken for a pigmented testa.
- Classifying these damaged sorghums as mixed sorghums can be unfortunate since this can cause rejection or market value.

## OBJECTIVE

- To document what the damaged sorghum kernels look like before and after bleaching compared to non-tannin and tannin sorghums.

## MATERIALS & METHODS

- Commercial Sorghum samples containing more than 3% tannin (brown) sorghum were obtained. These samples included a red and white pericarp sorghum grown on plants with purple secondary plant and glume color.
- The Bleach Test was performed using the standard procedures according to FGIS-GIPSA. Standard and tannin sorghums were included as checks in the procedures.
- Photos of longitudinal kernels representing the dark, weathered kernels and the normal-appearing kernels were taken from each sample.
- The Vanillin-HCl method was used to determine condensed tannins content with catechin used as a standard. Absorbance was measured at 500 nm.
- The Folin-Ciocalteu method was used to determine phenol content using gallic acid as a standard. Absorbance was measured at 600 nm.
- Dark kernels were sectioned by hand and viewed with a Zeiss Light Microscope to determine the presence of a pigmented testa.

- Half kernels of normal and weathered kernels representing false positive grains were viewed with a JEOL scanning electron microscope (SEM).

## RESULTS

- Tannin sorghums underwent little damage during weathering. The outer appearance was black after bleaching for normal and weathered (not shown) samples (Fig. 2D, Fig. 3D).
- Severely weathered, insect-damaged, or molded kernels (Fig. 2 E, F) appeared darker after bleaching compared to normal red or white sorghums. The intensity of darkness after bleaching was less than those with a pigmented testa layer (Fig. 2 D).

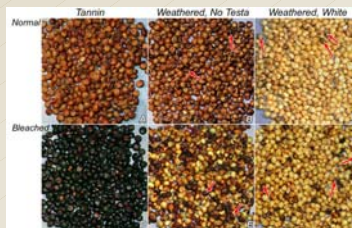


Figure 2 A-F. Standard sorghum samples and the commercial samples that produce false positives. Arrows in figure point to stained kernels of sorghum (B, C) and to the bleached stained kernels (E, F).

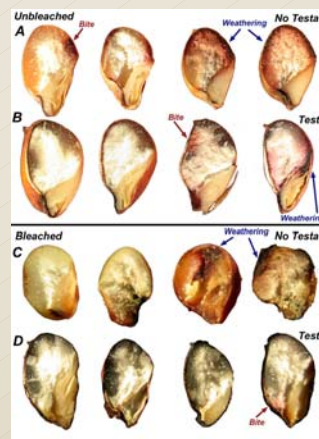


Figure 3. Longitudinal sections of sorghum kernels exhibiting several kinds of deterioration.

- The interior damage of weathered kernels became obvious after bleaching (Fig. 3).
- Stress-induced pigmentation of the endosperm corresponded to dark regions on the outer surface (Fig. 3C, arrows indicate damage).

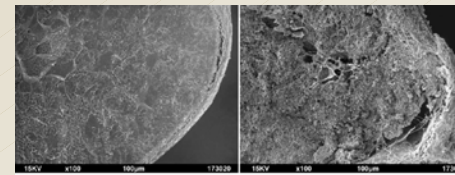


Figure 4. SEM photos of A) normal sorghum and B) sorghum kernel having endosperm damage due to weathering or insects.

- Enzymes that are produced as a result of kernel damage degrade the starch in the endosperm (Fig. 4B). As a result, the endosperm becomes more floury (Fig. 3A, B) and less defined in addition to releasing anthocyanins in the damaged areas.

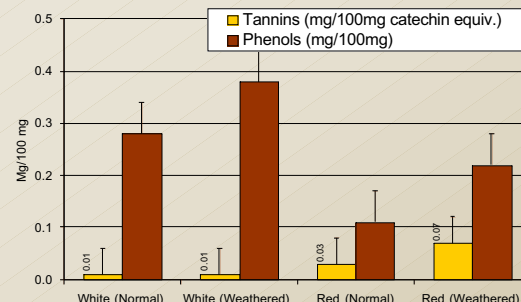


Figure 5. Tannin (catechin) and phenol contents in normal and weathered sorghums.

- Tannin sorghum contained 2.3 mg/100 mg catechin equiv. and 1.26 mg/100 phenols.
- White and red sorghums had trace levels of catechins; they did not contain condensed tannins (Fig. 5).
- Weathered white and red sorghums had higher levels of phenols than the normal sorghums (Fig. 5).

## CONCLUSION

- False positives from the Bleach Test were mainly due to pigments that migrated deep inside the endosperm and could not be removed by the bleaching procedure.
- Other methods of analysis such as the Vanillin-HCl method and kernel dissection for visual inspection should be used when suspected false positives are noticed.

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