

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

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### Introduction

Sorghum containing condensed tannins has dominant  $B_1B_2$  genes that produce a thick, pigmented testa layer in the kernel upon maturation (Blakely et al. 1979, Earp and Rooney 1986). This layer varies in thickness, intensity and color. The presence of this pigmented layer indicates that the kernel contains condensed tannins that reduce the feed efficiency of livestock rations. These “bird proof” sorghums are readily consumed by birds and other livestock when provided in feed rations; however, more ration is required to produce the same amount of daily gain. Thus feed efficiency is reduced significantly. The decrease in feed efficiency depends on the livestock species, the method of feeding and other factors.

The best method of determining condensed tannins in sorghum is the vanillin-HCl method when the blanks are subtracted to eliminate background non-tannin materials. However, it requires significant time and is not readily applied in routine grading of sorghum. Efforts by the National Sorghum Grain Producers Association have nearly eliminated tannin sorghum production in the United States. However, a bleach test is used to look for tannin sorghums during grading (Waniska et al. 1992).

The Clorox® 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) (USDA 1987) to test samples during grading for the presence of tannin sorghum, since the color of red and tannin sorghums are similar, especially when sorghum is weathered. This test uses Clorox bleach and KOH to remove the pericarp and turn the pigmented testa layer dark black. It is relatively simple, quick, inexpensive and usually provides an accurate indication of the relative percentage of tannin sorghum kernels during grading and assigning class. The test should always be run along with standard check samples of tannin and non-tannin sorghums to confirm that the test is working properly since the Clorox loses its strength over time.

Under certain circumstances, sorghums without a pigmented testa will turn dark or black after bleaching, which can lead to erroneous conclusions that a sorghum contains tannins. The false positives occur when sorghum is extensively weathered or molded in the field prior to harvest. The anthocyanin pigments from the glumes and pericarp migrate deep into the endosperm and can form a colored layer that can be confused with a pigmented testa. In addition, the kernel produces anthocyanins and other pigments in response to insect bites and infection by molds prior to and after maturation. These non-tannin pigments stain the outer layers so intensely that a dark color remains on the kernel after bleaching. These kernels are sometimes classed as tannin sorghums by inspectors who do not have much experience with grading of sorghum or never see the relation of grain weathering and stained kernels after bleaching.

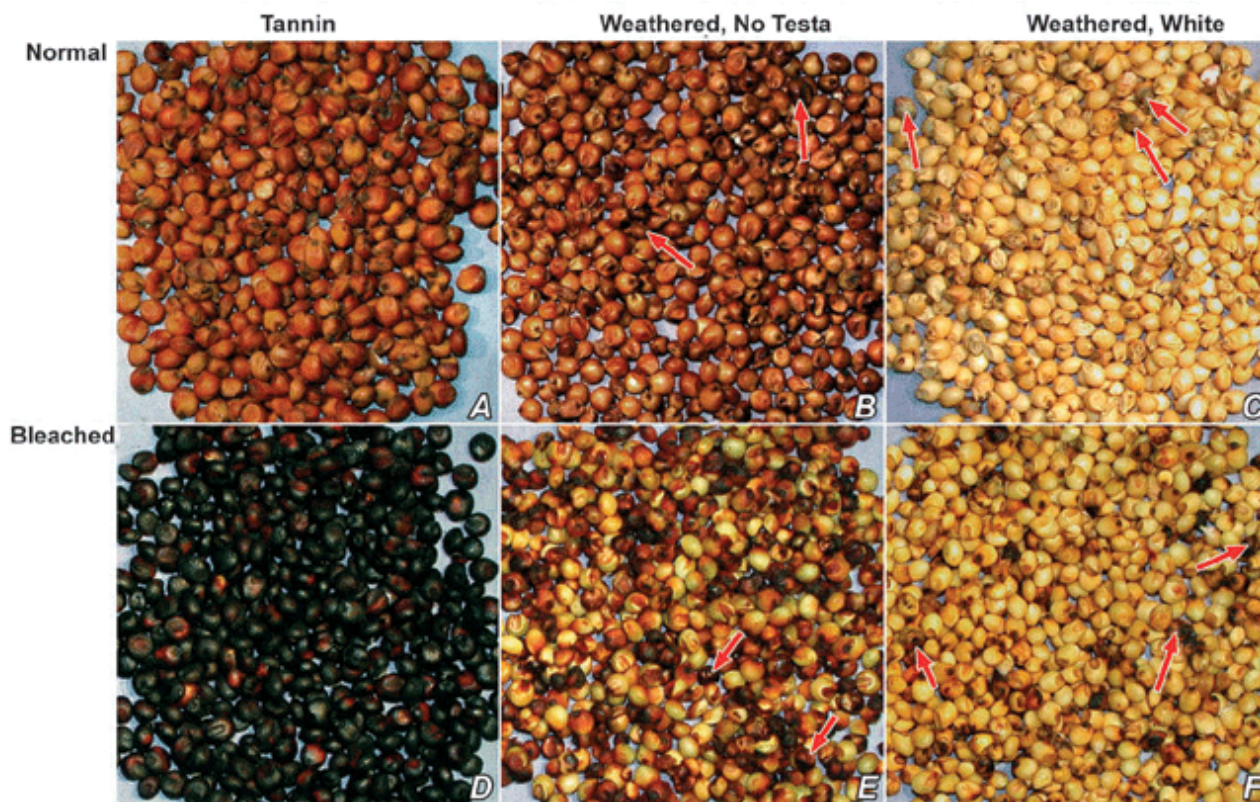
The FGIS-GIPSA procedures clearly indicate that weathered sorghum kernels should not be counted as tannin sorghums. This is stated in their procedures as follows: “sorghum kernels injured due to mold, insect, and weather damage may exhibit dark spots similar to those depicted above and should not be confused with bleached tannin sorghum.” Thus experienced grain inspectors know to look for stained sorghum kernels but relatively inexperienced inspectors may not be aware of this situation and that can cause erroneous and confusing marketing problems when off-colored, stained sorghum is called tannin sorghum.

We have observed this situation on many occasions during the past. It can cause sorghum to be improperly classed as mixed sorghum, which is unfortunate since there is no evidence to suggest that these pigments decrease feeding value. This can be clarified by using the vanillin-HCl method to quantitatively analyze for condensed tannins by removing the absorbance of the blank, which eliminates the inherent background material (Price et al. 1978).

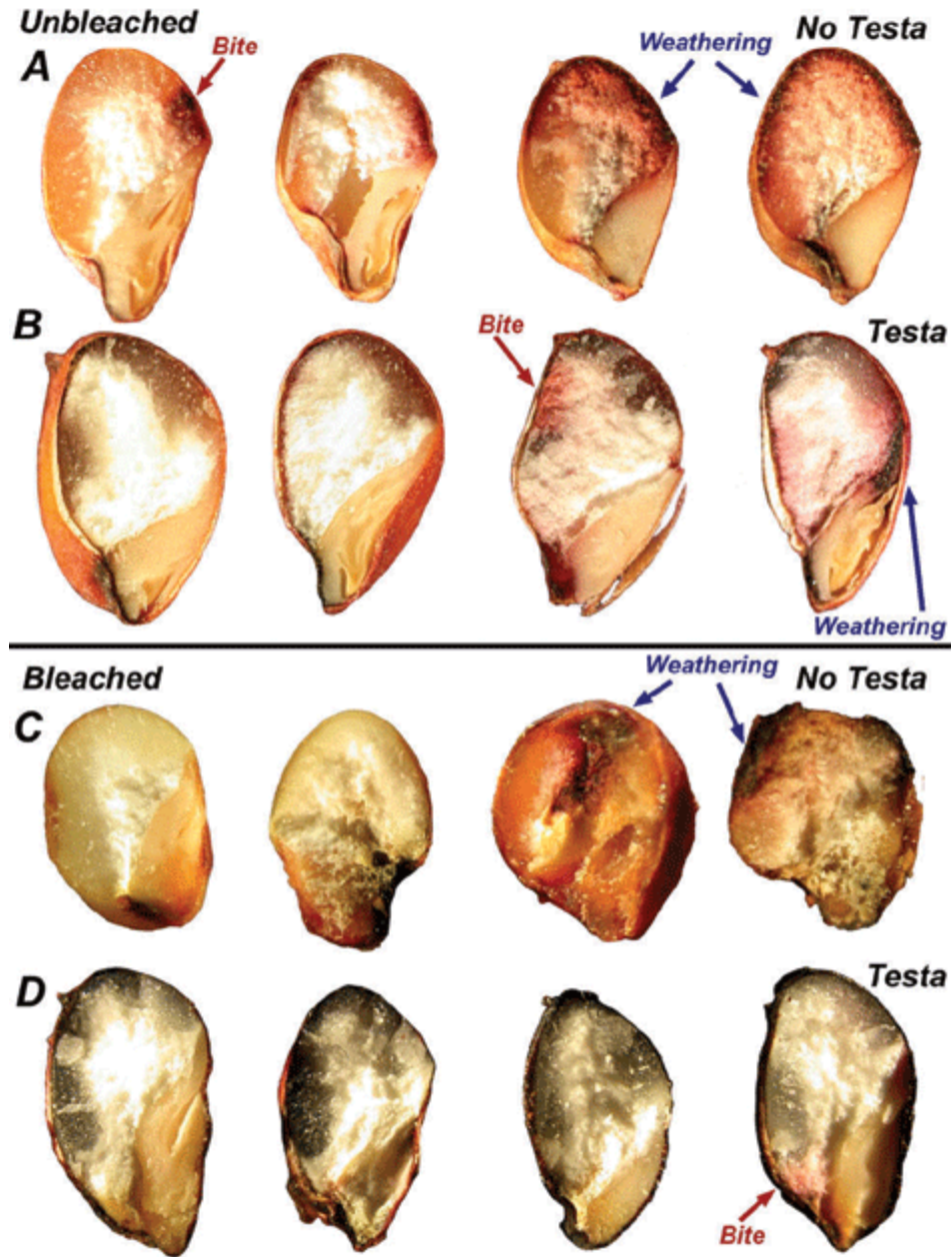
The objective of this presentation is to document what these kernels look like before and after bleaching compared to non-tannin and tannin sorghums.

### Materials and Methods

Commercial sorghum samples that had been graded as containing more than 3% tannin sorghum, thus becoming mixed sorghum, were obtained from an elevator and swine feeding operation. The samples included a red and a white pericarp sorghum. Both had purple plant color and glumes.



**Figure 1.** Photos of sorghum (A, B, C) and bleached sorghum (D, E, F). The tannin sorghum contains a pigmented testa that stains black upon bleaching with Clorox. The weathered red and white grains, both without a testa, had some kernels that appeared dark and off colored. The arrows in the figures point to stained kernels of sorghum (Fig. 1B, C) and to the bleached stained kernels (Fig. 1E, F).



**Figure 2.** A, B. Unbleached half kernels of non-tannin (A) and tannin (B) sorghum showing the effect of weathering on appearance and apparent pigmented layers under the pericarp. The arrows indicate where damage has occurred. C, D. Bleached half kernels of non-tannin (C) and tannin (D) sorghums. Arrows indicate weathering and damage. The non-tannin kernels are hard to distinguish from those with tannins because of the pigments leached into the kernel that give the appearance of a pigmented testa.



**Bleach test.** Standard procedures according to FGIS-GIPSA were used and standard sorghum and tannin sorghums were included as checks during all bleaching procedures. All analyses were repeated at least three times with excellent precision.

Photos were taken of longitudinal kernels selected to represent the dark weathered kernels and the normal appearing kernels from each sample.

Condensed tannins were determined by the vanillin-HCl method of Price et al. (1978) with the blanks subtracted to eliminate non-tannin positives (Hahn and Rooney 1984). Catechin was used as a standard. The modified Folin-Ciocalteu method of Kaluza et al. (1980) was used to determine phenols; absorbance was measured at 600 nm, and gallic acid was used as a standard.

**Microscopy.** Kernels that appeared dark were selected and sectioned by hand and viewed with a Zeiss light microscope to determine if a pigmented testa was present. Half kernels of a normal and a weathered kernel that was representative of the false-positive grains were viewed

with a JEOL scanning electron microscope.

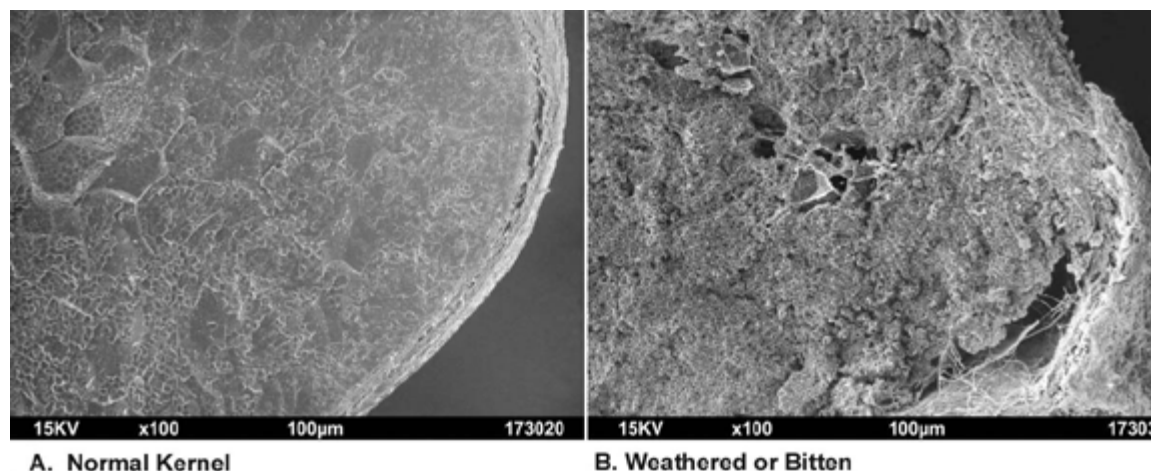
## Results and Discussion

Hand sectioning of the suspected false positive samples indicated that no testa was present (not shown). Photos of standard sorghum samples and the commercial samples that produced false positives are presented (Fig. 1A–C) along with longitudinal sections of the same kernels (Fig. 2A, B). The pigments have diffused from the purple glumes, from the red pericarp and in some cases are the result of insect bites or damage (Fig. 2A, B arrows). The severely weathered, insect damaged or molded kernels in the bleached samples (Fig. 1E, F) appear dark but the intensity of the darkness is significantly less than those with a pigmented testa (Fig. 1D). The bleached white damaged kernels are in general less dark than the damaged kernels from the red sorghum. After bleaching the sorghum samples, the interior damage to the weathered grains became more

**Table 1. Tannins<sup>1</sup> (catechin) and phenols in sorghum samples.**

Sample analysis	Brown ATx623 × SC103	White weathered	White non-weathered	Red weathered	Red non-weathered
<b>Tannins</b>					
Blank	0.051	0.150	0.013	0.225	0.072
Abs.– Blank	0.180	–0.004	0.001	0.006	0.002
mg/100 mg	2.3	–0.02	0.01	0.07	0.03
St. dev.	0.11	0.08	0.00	0.06	0.02
<b>Phenols</b>					
mg/100 mg	1.26	0.38	0.28	0.22	0.11
St. dev.	0.05	0.01	0.03	0.02	0.02

1. (absorbance of sample – blank) at 500 nm; results are means of triplicates.



**Figure 3.** Scanning electron microscopy photos of A) non-weathered and B) weathered sorghum kernels.

apparent in the longitudinal views and internal staining was visible on the outer surface (Fig. 2C, arrows indicate damage). Aside from the dark testa layer, the tannin sorghums underwent very little damage and the entire outer appearance was black (Fig. 2D, Fig. 1D).

When a sorghum kernel undergoes weathering or suffers an insect bite, the resulting damage to the endosperm can be seen in the SEM photos (Fig. 3A, B). The seed responds to damage by producing enzymes that degrade the starch in the endosperm (Fig. 3B), as well as releasing anthocyanins into the damaged areas. This is why the heavily weathered kernels in Fig. 2A and B usually have a more floury, less defined endosperm structure where the pigments are released.

The damaged commercial white and red sorghums have only trace levels of catechins, which indicates that they did not contain condensed tannins (Table 1). The weathered samples had significantly higher vanillin-HCl blank readings than non-weathered sorghums, indicating they had higher levels of background pigmentation. The total phenol analysis confirmed that the weathered kernels had significantly higher levels of phenols. Hence, the false positives from the bleach test (Fig. 1) were mainly due to pigments that leached deep inside the endosperm and could not be removed by the bleaching procedure. Caution is thus required when interpreting bleach test data alone. Appropriate standards should be incorporated when suspected false positives are noticed. Analysis via the vanillin-HCl method can be used to confirm that the sample does not contain tannin sorghums. The kernels can also be dissected and visually evaluated for the presence of a testa layer, which can be difficult without sufficient experience.

Fortunately the problem of false positive tannin sorghums does not occur routinely and causes only limited problems. However, when it happens, improper classification is significant. With vigilance and a working knowledge of the methods available, these situations can be minimized.

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