THE LOCATION AND EFFECT OF POLYPHENOLIC COMPOUNDS IN GRAIN SORGHUM

by

Mary Elizabeth Glover

Agronomy

Submitted in Partial Fulfillment of the Requirements of the

University Undergraduate Fellows Program

1976 - 1977

Approved by:

Lloyd W. Rooney.

L. W. Rooney

ABSTRACT

The Location and Effect of Polyphenolic Compounds in Grain Sorghum

Mary Elizabeth Glover

Texas A&M University

Directed by: Dr. L. W. Rooney

A review of tannins and their relation with brown sorghums is presented. Sorghums high in tannins cause a decrease in the digestibility of the grain, thereby lowering animal utilization. Other polyphenolic compounds often cause off coloring in food products making them undesirable. This study was conducted to try and determine the location of the polyphenolic compounds in the sorghum kernel.

Samples used included kernels with a white pericarp with a testa, a white pericarp without a testa, a red pericarp with a testa, and a red pericarp without a testa. Kernels with a partial testa were also viewed under light microscopy. However, due to a shortage of this type no other work was done with them. Of the others, both pearled and unpearled forms were tested.

Stains used were sodium hydroxide, ferric chloride, and the nitroso reaction. The unpearled samples showed little or no color. The tannins appear to be located in the testa layer which does not appear to have an organized structure.

ACKNOWLEDGMENTS

The author expresses sincere appreciation to Dr. Rooney and Don Sullins for their guidance and assistance. Without their helpful direction the paper could have never been completed.

The author is very grateful to all the people at the Cereal Quality Laboratory for their assistance.

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

Grain sorghum (Sorghum bicolor (L.) Moench) is an important food and feed crop all over the world. Sorghum can be grown in the hotter, drier areas, where other grains would not be as dependable. Throughout most of Africa and Asia, the grain is a valuable food for the people. In the United States, however, sorghum is primarily used for livestock consumption. Sorghums are divided into three main market classes: white, yellow, and brown.

Brown sorghums have a pigmented subcoat. This subcoat layer is referred to as the testa, and was originally the inner and outer integuments or the ovule wall. At this time, the testa was composed of two layers of cells with a definite structure. However, as the kernel matured, these cells were crushed against the ovary wall by the endosperm and the embryo. As the cells walls are disrupted, the testa begins to appear as a continuous layer with no real structure (4, 16).

The presence of a testa is controlled by two genes (B_ B_). Both genes must be in the dominant condition for the testa to remain. If either gene is recessive, the cells disintegrate and are reabsorbed. Sorghums with partial testa have been found. The testa of the partial type occurs sporadically around the kernel (11). The genetics of this type have not been fully understood. In sorghums with complete testa,

---

This paper follows the style of Crop Science.

the thickness varies from one variety to another. Testa thickness differs in individual kernels. The thickest region is generally at the crown, and the thinnest over the embryo (4, 16).

The pigmentation of the testa is controlled by yet another gene. Sorghums have been found with a definite layer between the pericarp and the aleurone cells, but the layer was colorless (11). The presence of this unpigmented layer indicates that at least one other gene is involved. The unpigmented testa is not common. Brown sorghums have a pigmented testa.

A high concentration of tannins causes the pigmentation of the testa. Tannins are "naturally occuring compounds" of high molecular weight (17). Tannins are polyphenolic compounds, which can form "crosslinks between proteins and other macromolecules" (17). For centuries tannins have been used for preserving animal skins as leather. The tannins used for preserving were usually obtained from oak bark (5, 17, 18).

Tannins occur in two main forms: hydrolyzable and condensed. The hydrolyzable tannins are carbohydrates attached to phenolic rings. Enzymes and dilute acids readily break off the carbohydrates and the compound loses its tannin qualities. The condensed tannins are much more complex and often classified as either anthocyanogens and leucoanthocyanins. Condensed tannins are much more resistant to breakdown by both enzymes and acid (15, 18).

The astringent tastes found in many foods are due to the presence of

tannins (5, 17, 18). Tannins of this type produce a drying or contracting sensation in the mouth. The polyphenolic compounds interfere with the ability of the saliva to lubricate the mouth. The tannins also cause the lining of the tongue to contract. Generally, this feeling is undesireable, but in tea, wine and cider the sensation is very beneficial (5). Many immature fruits contain large amounts of tannins (5, 21). As the fruit ripens, the tannins are not reduced in the actual amount present. Instead, the compounds are probably polymerized, thus reducing the astringent taste present in the immature fruit (18).

Tannins affect the taste of a substance, but more important, tannins bind with the protein in food. The complexing of proteins with tannins causes an interference with enzymes. Cellulase and polyphenol oxidase enzymes are inhibited by certain polyphenolic substances. The higher melecular weight tannins have been shown to interfere with pectic enzymes (21). Miller and Kneen found that tannins reduce the activity of amylase (8). The exact method of complexing with the proteins and enzymes is not known (16).

Tannins, as stated before, are present in the testa layer of brown sorghums. Blessin found anthocyanogens in the tip of the seed and in the pericarp (1). Tannins are usually not found in the endosperm. Nip and Burns found that the presence of tannins in the pericarp was limited to the epicarp and the cross and tube cells (9). Tannins have also been found in the glumes of sorghum. These polyphenols are thought to leach

into the kernel in the presence of high moisture (12). Most of the tannins are found in the testa layer. Brown sorghums contain 1.3 to 2% tannin, while other sorghums average about .2 to .4 percent (4).

Sorghums with testa, because of their high tannin content, have become a controversial subject. This market class of sorghums has both good and bad qualities. Because of the astringent taste, brown sorghums are labeled bird resistent. Birds usually consume grain before the kernel is completely ripened. Brown sorghums have the most astringent taste at this time. If other food is available, the birds will reject this high tannin type. This rejection is very noticeable in the yields obtained in the Southeastern United States and in parts of Africa where birds have been a problem (2, 4, 16).

Molds, fungi and other microorganisms are also deterred by brown sorghums. This is probably due to the interference by tannins with the enzymes of the organism (1, 3, 6). Tannins also tend to retard pre-harvest seed germination (16). The polyphenolic compounds may be complexing with the enzymes which activate germination in the embryo.

The interference by tannins with enzymes reduces the dry matter digestibility. The intake of food high in polyphenolic compounds is often depressed. Even if the intake is not suppressed, growth and feed conversion are decreased (8, 18). This decrease has been shown with chicks, rats and swine (3, 10). In ruminants the intake is not suppressed. If extra protein is consumed along with the high tannin grain, good weight

gains are still achieved. However, the overall utilization of the feed by the animal is decreased ten to twenty percent.

Tannins can produce off-colors in various sorghum products. The polyphenols are thought to migrate into parts of the endosperm during periods of high humidity. Rainy weather before harvest and the steeping process during the wet milling process produce the two most humid conditions. The migration of the polyphenols causes a staining of the starch which is very undesireable. Another way to cause off-colored sorghum is to subject the grain to basic conditions such as sodium hydroxide. The starch then turns yellow to pale green (8).

Tannin content is influenced by pericarp color, presence of a testa, extent of the testa, plant color, and the environment while development occurs (7). The actual influence of the environment on the level of tannins is not really known. In a study of Tipton et. al., six different hybrids were grown during two separate years at several locations. The year, location and the hybrid grown were all found to affect the amount of tannin found in the kernel. Each hybrid had a specific level of maturity of the caryopsis at which the kernel had the highest tannin.

Brown sorghums, despite the bad qualities, offer benefits that can not be overlooked. Many African tribes have perfered brown sorghums for their beer because of the astringent taste. More important, however, brown sorghums can be very useful in areas with large bird populations. The decision between a twenty percent reduction in utilization and a forty

percent loss to birds is much easier to make if only animals are involved. But off colored food products are usually not accepted by people at all.

An efficient method for removing all tannins during processing is needed. With the discovery of this process, the brown sorghums could be mold and bird resistent in the field and yet, perfectly acceptable in either food or feed products.

The purpose of this paper was twofold.

1. To compare the location of polyphenolic compounds in sorghums of different genotypes by the use of light microscopy.

2. To develop histological techniques for the determination of the location and concentration of polyphenolic precursors in the endosperm of sorghum. Hopefully, this information will be beneficial to developing a process to enable the use of brown sorghums.

MATERIALS AND METHODS

Samples

The four samples were from the Sorghum Conversion program number SC112 grown at the Texas Agricultural Experiment Station at Lubbock, Texas in 1972. The phenotypes were a white pericarp without a testa, a white with a testa, a red pericarp without a testa and a red pericarp with a testa. The fifth sample was grown at the Texas Agricultural Experiment Station at Renner, Texas in 1976. The kernels have a partial testa, which only covers the endosperm and the embryo sporadically. Dr. Fred Miller just found the sample; therefore, the supply of seed was rather small.

Sectioning

The slides were prepared as described by Jensen (6). Five tenths gram of deashed gelatin (Eastman Kodac) was placed in 100 ml of water and allowed to swell for 4 hrs. The solution was heated to dissolve the gelatin, and then fifty mg of Chromium potassium sulfate (Allied Chemical) was added. The entire solution was filtered with glass wool. The slides went through a cleaning process before treatment. The process consisted of dipping the slides into two beakers of distilled water, wiping with a clean cheesecloth, dipping into two beakers of 95% ethyl alcohol. The clean slides were then ignited over an open flame and allowed to burn dry. The slides were cooled and dipped into the

autoradiographic gelatin and allowed to dry in a vertical position (6).

Kernels from each of the five samples were cut in half and mounted on wooden dowels with epoxy glue and allowed to dry overnight. The kernels were carefully filed to smooth the surface prior to sectioning. A Spencer Rotary Microtome equiped with a glass knife was utilized for sectioning. The dowels were mounted in the microtome and sections four microns thick were cut from the specimen. As the sections were cut, careful use of a needle prevented the sections from curling. The sections were then transfered to autoradiographic coated slides with the aid of a camel hair brush. This sectioning process is essentially that of Wolf (22).

The sections were placed on a small droplet of water on the treated slides. Two needles were used to reposition the section and prevent curling. Excess water was removed with filter paper. The sections were then checked for folded edges under a stereoscope. The rest of the water was removed by placing the slide on a slide warmer at 38°C for 1 hr.

Milling

A method of Rooney and Sullins (13) was used to remove the pericarp and testa. This method consisted of a Strong - Scott Barley Pearler that was modified by substituting a wire brush for the carborundum wheel. The spinning wire brush wears away the outer layers of the kernel. Each sample was pearled for thirty seconds (13).

A sample of each grain type was ground in a Udy Cyclone Sample mill. The pearled kernels were handpicked before grinding to reduce the amount of testa left in the sample.

Staining

Microscopic sections were stained with the ferric sulfate reaction. This consisted of 0.5 - 1% ferric chloride in 0.1N HCl.

All samples with the exception of the microscopic sections were strained with 5N sodium hydroxide.

Flour samples were also treated with the nitroso reaction. Equal volumes of 10% sodium nitrate, 20% urea, and 10% acetic acid were added to the samples. After three to four minutes, a double volume of 2N NaOH was added.

Photography

Sections were observed with a Zeiss Universal Research Microscope equipped with Nomarski differential interference contrast and bright field optics. Photomicrographs were taken with a Zeiss C - 35 - matic camera. Film used for color photography was High Speed Ektachrome with an ASA of 160. Black and white photography utilized Plus - X Panchromatic film with an ASA of 125.

Gross morphology pictures were taken with a Zeiss Tessovar Stereoscope equipped with a Pentax K2 automatic 35mm camera. High Speed Ektachrome and Plus X were again the films used. Light was

achieved by a fiberoptic system.

RESULTS AND DISCUSSION

Microscopy

The major components of a sorghum kernel were labeled in figure 1. The same important areas were easily identified by the use of light microscopy.

The testa appeared as a pigmented strip just above the aleurone layer. In many cases, as in Figure 2B, the testa (T) was two-toned. The darker color was generally closest to the pericarp. This darker layer was a reddish brown, while the lower one was a yellowish brown. No real structure could be observed in the testa layer. The scanning electron microscope photo, figure 2D showed the testa as a solid, non-cellular bond.

The presence or absence of a testa did not seem to affect the aleurone layer. In both figure 2A and 2B, the aleurone layer (A) was a single celled strip with little or no pigmentation. In figure 2C, however, the aleurone layer is highly pigmented. This section came from a kernel with a red pericarp and without a testa. However, the kernel had dark spots scattered about. Just to either side of the darkened part of the layer pictured in figure 2C, were normal unpigmented areas.

In a few places, the pigment of the testa seemed to diffuse into a small part of the cross and tube cells in the pericarp. However, the color did not spread past this area and did not affect the rest of the pericarp. The mesocarp area appeared stained periodically, but the

Fig. 1. General morphology of the sorghum kernel.

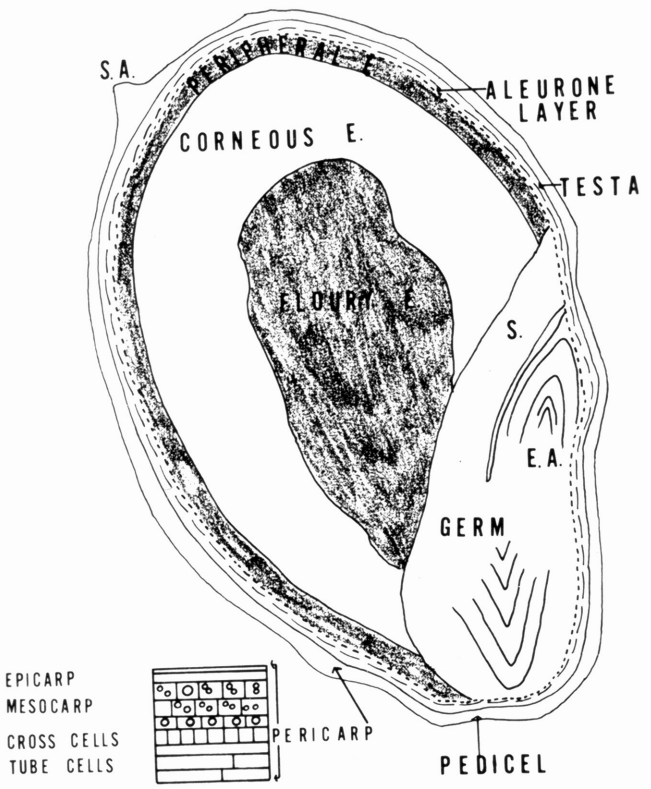
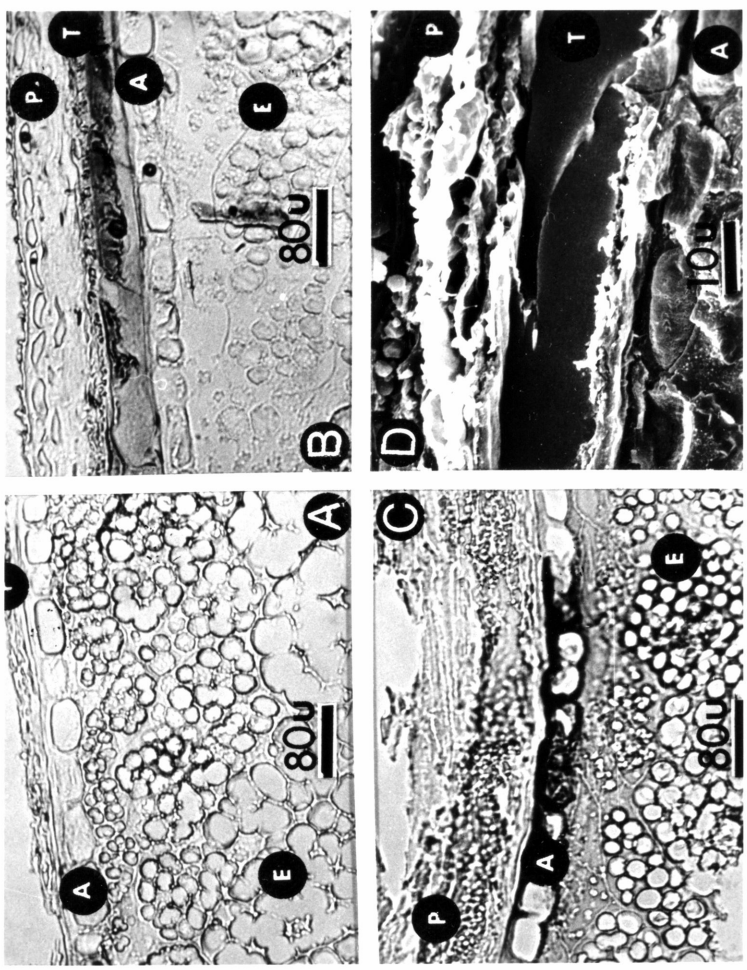

S.A.
PERIPHERAL E.
ALEURONE LAYER
CORNEOUS E.
TESTA
FLOURY E.
S.
E.A.
GERM
EPICARP
MESOCARP
CROSS CELLS
TUBE CELLS
PERICARP
PEDICEL

Fig. 2. Cross sections of sorghum kernel. (A) White pericarp without testa. P, pericarp; A, aleurone; E, endosperm. (B) White pericarp with testa. T, testa. (C) Red pericarp without testa. (D) Scanning electron photomicrograph of the testa (1000x).


A
E
80u
A
B
P
T
A
E
80u
C
P
A
E
80u
D
P
T
A
80u

color was probably artifacts from sectioning. Such a contamination occured in the endosperm near the center of figure 2B.

The color of the outer pericarp was primarily due to pigments in the epicarp region. The differences in color could not be portrayed with the black and white pictures, but color photos showed the different areas fairly well. The pigments were in a very thin band just above the mesocarp. This area appears in figure 2B, but has accidentally been removed by sectioning in figure 2A.

Partial Testa Sorghums

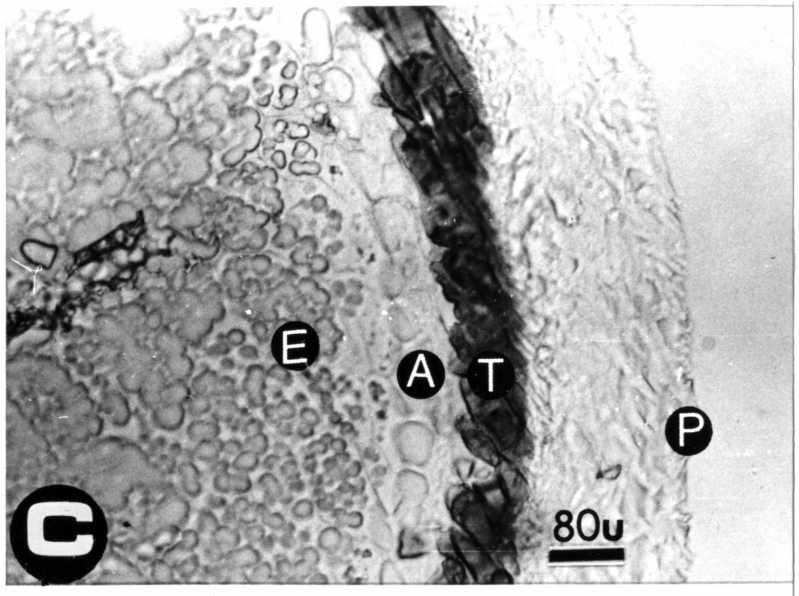
Each kernel with the partial testa was unique in that no set pattern existed in the way the testa occured. The two kernels in figure 3A exemplified this. The areas without testa (TA) on the lighter kernel were more sporadic around the grain. The darker kernel had more solid areas with testa (TP). The lighter kernel had a stained area (SA) which occured in the area without a testa.

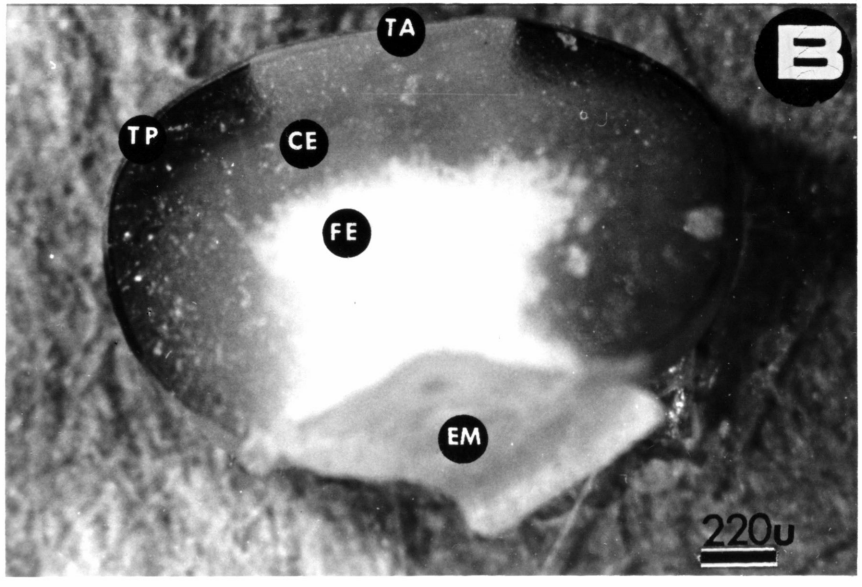
In figure 3B the testa appeared in two main areas. In these areas, the dark testa reflected through the peripheral and corneous endosperms causing them to appear stained. However, when sections were removed the areas were not pigmented.

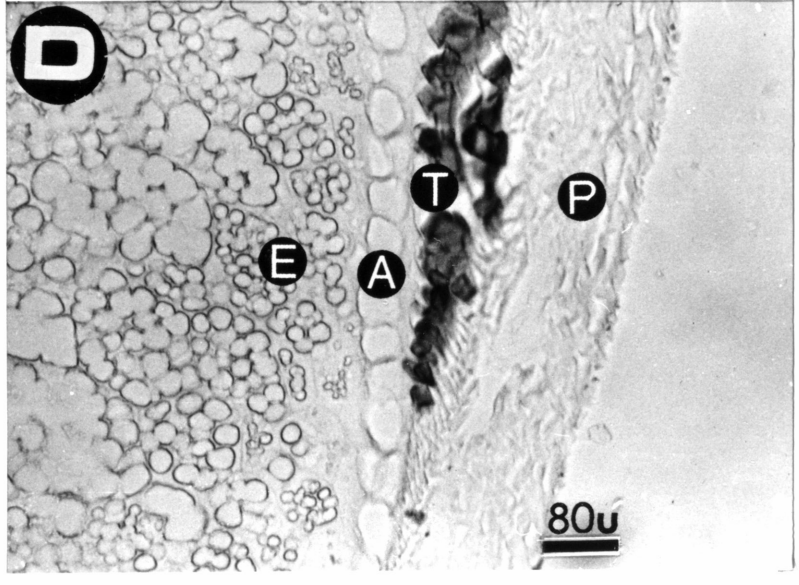
Figures 3C and 3D were sections from a partial testa section. In 3C the testa started out as a rather thick layer, about half the size of the pericarp. However, as the testa continued, the layer became first thinner, then less compacted, and finally faded away. The rest of the

Fig. 3. Kernels with partial testa. (A) Whole kernel. TA, testa absent; TP, testa present; SA, stained area. (B) Half kernel. CE, corneous endosperm; FE, floury endosperm; EM, embryo. (C & D) Cross sections of area where testa ends. P, pericarp; T, testa; A, aleurone; E, endosperm.


TA
TP
SA
TA
TP
960u
A


E
A
T
P
80u
C


TA
B
TP
CE
FE
EM
220u


D
T
P
E
A
80u

kernel remained unchanged. The testa varied in thickness in several areas of the kernel.

Milling

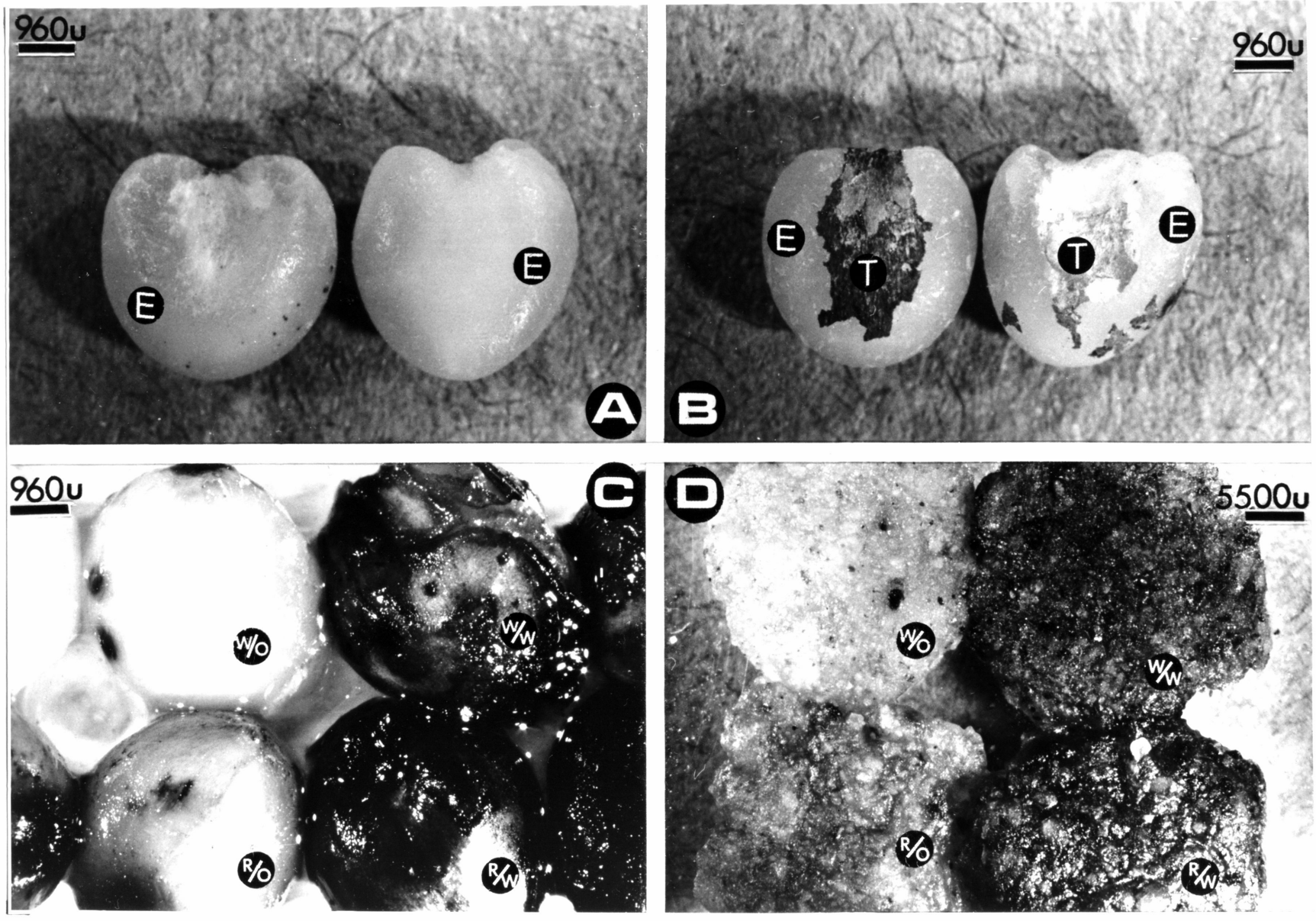
The two samples without testa had most of the pericarp removed as in figure 4A. However, the two samples with testa, figure 4B, presented problems. The curved areas of the grain protected the testa from the scraping action of the brush. If the kernels were left in the pearler longer, more testa was removed, but a higher percentage of broken kernels resulted. The remaining testa could be removed by hand scraping.

Hand scraping was not always successful, however. Many times after the testa was removed, stains remained that went fairly deep into the endosperm. These spots occured more frequently on sorghums with testa. A few of the spots are visible in figure 3A.

The flour samples obtained from the four pearled samples were all an off-white. The samples had been handpicked to keep the stained spots and the testa artifacts to a minimum. All four of the flours were exactly the same.

The unpearled flours were not all identical. The sample with a white pericarp and no testa was a very light tan. The sample with a red pericarp and no testa was a light tan with red particles scattered throughout. The two samples with testa were a light brown and the particles were a darker color. These two samples were almost identical.

Fig. 4. (A) White pericarp, without testa after pearling. E, endosperm. (B) White pericarp, with testa after pearling. T, testa. (C) Whole kernels forty minutes after 5N NaOH treatment. W/O, white pericarp, without testa; W/W, white pericarp, with testa; R/O, red pericarp without testa; R/W, red pericarp with testa. (D) Ground grain forty minutes after 5N NaOH treatment.


960u
E
E
A
960u
E
T
T
E
B
960u
C
W/O
W/W
R/O
R/W
D
5500u
W/O
W/W
R/O
R/W

Staining

The sodium hydroxide gelatinized the starch that came in contact and produced a greenish color. The results were visible to the naked eye and happened rather quickly.

Sodium hydroxide on whole grain produced the four kernels pictured in figure 4C. The white pericarp without a testa (W/O) had stains on the side and in the tip or stylar region, but was primarily off-white. The stains on the side were the type described before which go into the endosperm. The alkali treatment tended to diffuse the pigments and therefore, decrease the intensity of the spots. The red pericarp without a testa (R/O) was also lightly colored. The two samples with testa had very dark coloring. The white pericarp with testa (W/W) became a chocolate brown, while that with a red pericarp (R/W) turned almost black.

The kernels which had been pearled showed little color change after alkali treatment. The four sample remained white except for the stained areas. The color in the pigmented areas diffused over a larger area similar to the spots in figure 3C.

Half kernels of the pearled and unpearled acted very similar to the whole kernel counterparts. The gelatinization of the starch occured more quickly because the alkali was applied in a much closer contact than with whole kernels. This gelatinization clouded the colors produced by the alkali and tannins; therefore, the intensity of the colors was greatly lessened.

The unpearled flour samples treated with sodium hydroxide appeared as those in figure 4D. Once again, the two samples without testa (W/O, R/O), were barely discolored. The two with testa (W/W, R/W), on the other hand, turned very dark. The two flour samples with testa were harder to distinguish than the whole grain counterparts. This was probably because the extra pigment from the red pericarp was very small when diluted with all the endosperm of the kernel. The whole grain samples were treated on the outer portion where the pigments were concentrated.

The pearled flour samples treated with alkali showed little differences. All four turned an off-white color with a slight greenish tinge.

The nitroso reaction and the ferric sulfate reaction were unsuccessful. The reactions worked when tested on pure tannins. Perhaps the concentration of tannin in the sorghum samples was not enough to give visible signs of presence. Another error could have been in the application technique. The sections and flour samples might not have been the proper forms to treat. The complete failure of these two reactions could not be stated fairly without more experimentation with concentrations and applications.

CONCLUSIONS

In the four samples used, the largest percent of tannins occured in the testa. The pericarp color, if dark, did have a small effect on the total amount. However, the pericarp was almost insignificant when considered in relationship with the whole kernel.

The stain spots were present not as remnents of a testa, but as darkened areas in the aleurone layer. This stained area appeared much darker than any of the testas observed.

No tannin precursors were found in the endosperm. This does not conclude that precursors were not present. Instead, new stains or methods for observing tannins were needed. The precursors were either not in enough concentration or did not act completely as tannins.

LITERATURE CITED

1. Blessin, C. W., Van Etten, C. H., and R. J. Dimler. 1968. Cereal Chemistry 40:241.

2. Doggett, H. 1970. Sorghum. Longmans, London.

3. Featherston, W. R. and J. C. Rogler. 1975. Influence of tannins on the utilization of sorghum grain by rats and chicks. Nutritional Reports Int. Vol. II No. 6. p. 491-497.

4. Freeman, J. E. 1970. Development and structure of the sorghum plant and its fruit. p. 28-72. In J. S. Wall and W. R. Ross (ed.) Sorghum production and utilization. Avi Publishing Co., Inc., Westport, Conn.

5. Geissman, T. A. 1962. The chemistry of flavonoid compounds. MacMillan Co., New York.

6. Jenson, W. A. 1962. Botanical histochemistry.

7. Maxson, E. D., Clark, L. E., L. W. Rooney and J. W. Johnson. 1972. Factors affecting the tannin content of sorghum grain as determined by two methods of tannin analysis. Crop Science 12:233-235.

8. _____, and L. W. Rooney. 1973. Evaluation of methods for tannin analysis in sorghum grain. Cereal Chemistry 49:719.

9. Nip, W. K. and E. E. Burns. 1968. Pigment characterization in grain sorghums. (Abstr.) Cereal Sci. Today 13:119.

10. Noland, P. R., Bangus, C. A. and R. N. Sharp. 1970. Comparisons of bird-resistant grain sorghum in swine finishing rations. Animal Sci. Res. Conference. p. 37-40.

11. Quinby, J. R. and J. H. Martin. 1954. Sorghum improvement. Advan. Agron. 6:305-309.

12. Rooney, L. W. and L. E. Clark. 1968. The chemistry and processing of sorghum grain. Cereal Sci. Today 13:258-261, 264-265, 285-286.

13. _____ and R. D. Sullins. 1969. A laboratory method for milling small samples of sorghum grain. Cereal Chemistry 46:486-490.

14. _ _ _ _ 1973. A review of the physical properties, composition and structure of sorghum grain as related to utilization. p. 316-342. In Y. Pomeranz (ed.) Industrial uses of cereals. Am. Association of Cereal Chemists, Inc., St. Paul.

15. Strumeyer, D. H. and M. J. Malin. 1975. Condensed tannins in grain sorghum: isolation, fractionation and characterization. J. Agric. Food. Chem. 23:909-914.

16. Sullins, R. D. and L. W. Rooney. 1975. The role of microscopy in nutritional evaluation of cereals. Proceedings of Twenty-third Meeting of the American Association of Feed Microscopists. College Park, Maryland.

17. Swain, T. 1965. The tannins. p. 552-580. In J. Bonner and J. E. Varner. (ed.) Plant biochemistry. Academic Press, New York.

18. Sykes, A. H. 1970. Grain sorghum in poultry nutrition. U. S. Feed Grains Council, London.

19. Tipton, K. W., Mabbayad, B. B., Marshall, J. G., Rabb, J. L., Robinson, D. L. and L. W. Sloane. 1974. Tannin investigations of brown-seeded grain sorghum hybrids. Partial summary of Ph. D. Dissertation by B. B. Mabbayad Louisiana State University, Baton Rouge, Louisiana.

20. Wall, J. S. and C. W. Blessin. 1970. Composition of Sorghum Plant and Grain p. 118-166. In J. S. Wall and W. R. Ross (ed.) Sorghum production and utilization. Avi Publishing Co., Inc., Conn.

21. Williams, A. H. 1962. Enzyme chemistry of phenolic compounds. Proceedings of the Plant Phenolics Group. Macmillan Company, New York.

22. Wolf, M. J. and U. Khoo 1970. Mature cereal grain endosperm: rapid glass knife sectioning for examination of proteins. Stain Technology 45: 277-283.