Pigment Characterization in Grain Sorghum. I. Red Varieties[1]

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ABSTRACT

The pigments in three red varieties of sorghum grain, B 378, Tx 415, and RS 671, were found mainly in the stylar area, the epidermis, cross cell and tube-cell layers of the pericarp. The mesocarp in general was not colored. Upon chromatographic purification of the extracts from each variety, three major pigments, two yellow and one orange, were isolated. Color reactions, R_f values,and spectral measurements indicated the two yellow pigments to be similar to apigeninidin-5-glucoside and kaempferol-3-rutinoside-7-glucuronide, respectively. An unidentified orange pigment having anthocyanin properties was also isolated.

Sorghum grains with varying degrees of pigmentation in the pericarp and/or integument have not been well accepted for human consumption (1,2). In addition, the pigmentation can cause an undesirable off-color in the processed product (3)[2,3]. Studies of the locations of these pigments in various types of sorghum grains have been reported by Swanson (4). Pigmentation may occur in the epidermal and hypodermal cells of the pericarp and/or the nucellar layer. Sanders (5) also reported the presence of orange pigmentation in the integument of grains of the Early Hegari variety. Blessin et al. (6) reported orange pigmentation in the pericarp of the Martin variety of sorghum. However, no cellular differences in pigmentation were reported by these workers. Solubility of unidentified leucoanthocyanins in water was

[1]Presented at the joint AACC-AOCS Annual Meeting, Washington, D.C., April 1968.

[2]Maclay, W. D. Paper given at conference on grain sorghum research and utilization, Amarillo, Texas, 1959, p. 63.

[3]Watson, S. A. Paper given at conference on grain sorghum research and utilization, Amarillo, Texas, 1961, p. 48.

observed by Blessin et al. (7). Chemical properties of the pigments such as R_f values and the absorption spectra in a light red variety of sorghum have been reported by Nakayama (8). The compounds were not identified. Fisetinidin has been tentatively identified as one of the reaction products resulting from treatment of anthocyanogens with 12N hydrochloric acid at room temperature in several varieties of yellow milo and red kafir (6). Pelargonidin (and probably eriodictyol) has been found to be the product of acid hydrolysis of three anthocyanogen-type flavonoids in seed coats of commercial sorghum (9). Acylated cyanidin-3-glucoside, apigeninidin, and luteolinidin and their 5-glucosides, and an unidentified acid-stable form have been identified in the first internodes, coleoptiles, and roots of the Wheatland variety (10). A number of substances of the coumarin, cinnamate, and caffeic acid types and phenolic constituents similar to chlorogenic acid and leucoanthocyanins were also detected from sorghum pearlings of the RS 610 sorghum hybrid (11). Because of the scarcity of information on the chemical and histological nature of pigmentation in these grains, it was the purpose of this research to investigate the location and the chemical properties of pigments in several red varieties of sorghum grains. The results summarized in this report are for the varieties B 378, Tx 415, and RS 671. Data on other grains with different colors and pigmentation will be reported in the future.

MATERIALS AND METHODS

Sorghum grains of the red varieties B 378, Tx 415, and their hybrid RS 671 were used in this study because of their heavy pigmentation and their use for human consumption (Table I) (2).

Location Study

Grains were cut longitudinally with a freezing microtome. Sections of 10 μ were mounted in Canadian balsam and examined under a microscope. Treatment with saturated lead acetate was also made in order to precipitate the anthocyanin pigments and assist in the identification of the different cell layers.

Extraction of the Pigments

Sorghum grains were cleaned in a laboratory air cleaner to remove the glumes and other foreign matter. Whole kernels from each variety were extracted with diethyl ether overnight at room temperature to remove the waxy surface coating. The ether was then discarded and the grains were extracted in darkness with methanol acidified with concentrated hydrochloric acid (HCl-MeOH 1% v./v.) (12). The alcohol extraction was repeated again. Each combined pigment extraction was concentrated in a rotary evaporator under vacuum at 40°C. The crude pigment concentrate was stored in a freezer until used.

TABLE I. NAMES AND VISUAL COLORS OF THE RED VARIETIES OF SORGHUM GRAIN USED FOR ANALYSES

Name	Visual Color
B 378	Red with a shade of yellow, some black spots
Tx 415	Light red with a shade of yellow
RS 671	Red with a shade of yellow, some black spots

TABLE II. SOLVENT SYSTEMS USED FOR PURIFICATION AND CHARACTERIZATION OF PIGMENTS IN SORGHUM GRAINS[a]

Code	Chemicals Used	Composition by Volume
BAW	1-Butanol:acetic acid:water	4:1:5 upper phase
BAW-HCl	1-Butanol:acetic acid:water:conc.HCl	60:10:20:1
BuOH-HCl	1-Butanol:2N HCl	1:1 upper phase
HAc-HCl	Acetic acid:conc. HCl:water	15:3:85
1% HCl	Conc. HCl:water	3:97
15% HAc	Acetic acid:water	15:85
H_2O	Distilled water	

[a]All the solvent systems used in this study were freshly prepared.

Chromatographic Separation and Purification of the Pigments

Pigments were separated from the crude concentrate on Whatman No. 3MM chromatographic paper as descending bands developed in 1-butanol:2N HCl (Table II). The major bands were eluted with HCl-MeOH (1% v./v.) and condensed in a flash evaporator under vacuum at 40°C. These partially purified pigment concentrates were rechromatographed three to four times with different solvent systems suggested by Harborne (13) and Stafford (10) for the isolation of anthocyanins. The composition of the solvent systems is presented in Table II.

Characterization of the Pigments

Each purified pigment eluant was chromatographed in descending manner on Whatman No. 1 chromatographic paper with different solvent systems (Table II). Pelargonidin chloride obtained from the Aldrich Chemical Co., Inc., Milwaukee, Wisconsin, was used to standardize the environment and technique. Examination of the purified pigments on filter paper under visible and ultraviolet lights, with or without ammonia, was also performed. Spectral measurements of the purified pigments in methanol containing 0.01% conc. HCl (HCl-MeOH 0.01% v./v.) were also recorded.

RESULTS AND DISCUSSION

Location Study

Examination of the sections of sorghum grain varieties B 378, Tx 415, and RS 671 indicated orange pigmentation in the stylar and pericarp areas (Table III). Figure 1 illustrates the different location of pigmentation in the pericarp. Treatment with lead acetate was an aid in identifying the location of the pigments. The results agreed with those of Blessin et al. (6). Investigation of the pericarp structure revealed that only the epidermal area, the cross cells, and the tube-cell layers contained orange pigmentation. The mesocarp area was not pigmented

TABLE III. LOCATIONS OF PIGMENTATION IN THE THREE RED VARIETIES OF GRAIN SORGHUM

Varieties	Stylar Area	Epicarp	Mesocarp	Cross Cell Tube Cell
B 378	orange	orange	no pigment[a]	orange
Tx 415	orange	orange	no pigment	orange
RS 671	orange	orange	no pigment[a]	orange

[a]Mesocarp was pigmented if black or brown spots were visible.

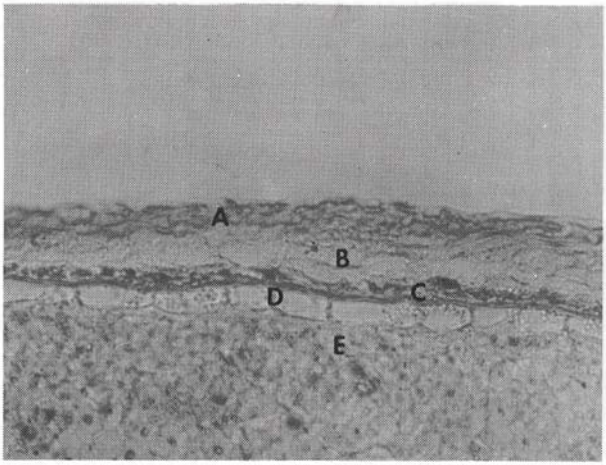

Fig. 1. Different locations of pigmentation in the pericarp of red sorghum grain RS 671 (300X). A, epicarp; B, mesocarp; C, cross-cells and tube-cell layers; D, aleurone layer; E, endosperm.

except that black and brown spots were visible. All three varieties exhibited similar pigment locations.

Characterization of the Pigments

Three major pigments, two yellow (Y-1 and Y-2) and one orange (O-1), were obtained from each variety by the chromatographic separation and purification process. Other pigments were also present but were not purified because they were in such minor quantity. Color changes due to different treatments are presented in Table IV. Pigments from all three varieties reacted similarly. The color reactions suggested that Y-1 was a yellow anthocyanin in nature and resembled that reported by Stafford (10) for apigeninidin pigments. Y-2 belonged to the flavone and flavonol group and O-1 was of the anthocyanin type (13). R_f values of the yellow pigments in several solvent systems (Table V) suggested Y-1 as apigeninidin-5-glucoside and Y-2, kaempferol-3-rutinoside-7-glucuronide (13). The orange pigment was immobile in HAc-HCl and moved to the solvent front in BAW. Measurement of the absorption spectra of these pigments in HCl-MeOH 0.01% v./v.

TABLE IV. COLOR REACTIONS OF PURIFIED PIGMENTS

Pigment	Color			
	Without NH_3		With NH_3	
	Visible light	U.V. light	Visible light	U.V. light
Y-1	yellow	fluorescent yellow	bright red	fluorescent red
Y-2	yellow	dull purple	greenish yellow	dull purple
O-1	orange	orange	purple	purple
Apigeninidin-5-glucoside[a]	yellow	fluorescent yellow	bright red	fluorescent red
Kaempferol glucosides[b]	yellow	dull purple	greenish yellow	dull purple

[a]Stafford, H. A.; see ref. 10.

[b]Harborne, J. B.; see ref. 13.

TABLE V. R_f VALUES AND SPECTRAL DATA OF THE YELLOW PIGMENTS IN GRAIN SORGHUM VARIETIES B 378, Tx 415, AND RS 617

Pigments	R_f Values X 100					Wave Length
	BAW	1% HCl	HAc-HCl	15% HAc	H_2O	Absorption Maxima millimicrons
Y-1	42	22	55	...	...	477
Y-2	26	...	...	87	94	360
Apigeninidin-5-glucoside[a]	41	22	55	...	...	477
Kaempferol-3-rutinoside-7-glucuronide[a]	26	...	...	85	93	....

[a]Harborne, J. B.; see ref. 13.

(Table V) also indicated a resemblance to those reported by Stafford (10), Harborne (12), and Geissman (14). The orange pigment had an absorption maximum at 480 mμ, suggesting the possibility of a polymer of the apigeninidins.

Addition of 5% AlCl (v./v.) in ethanol to these pigments did not induce a spectral shift, indicating that either the 3- or 5-position was not free. This further supported the assumption that Y-1 was apigeninidin-5-glucoside and Y-2 was kaempferol-3-rutinoside-7-glucuronide. It was unfortunate that authentic compounds of these two pigments were not available for direct comparison study. It is hoped that identification of these pigments can be confirmed in the near future.

Acknowledgment

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