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POLYPHENOLS IN CEREALS AND LEGUMES

Proceedings of a symposium held during the 36th annual meeting of the
Institute of Food Technologists, St. Louis, Missouri, 10 – 13 June 1979

Editor: Joseph H. Hulse

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Polyphenols in *Pennisetum* Millet¹

R. D. Reichert², C. G. Youngs², and D. A. Christensen³

Polyphenols are responsible for an undesirable gray discoloration in millet flour that creates a problem of consumer acceptance. Bleaching these pigments in dilute acid markedly improved the aesthetic quality of the grains. The major pH-sensitive pigments were identified as glucosylvitexin, glucosylorientin, and vitexin in the relative proportion of 29:11:4. These compounds are concentrated in the peripheral layers of the kernel. Nutritional studies have shown that these phenolic compounds are not as noxious as tannins present in the testa layer of some sorghum varieties.

Pearl millet (*Pennisetum typhoides*) plays a very important role in the agriculture of many developing countries. It is widely grown in Africa and Asia as a food grain. Although yields of millet are generally low in comparison to those of other grains, millet will yield better than most cereals under adverse heat and limited rainfall conditions.

Considerable confusion exists in the millet literature with regard to millet species. Table 1 lists common names and scientific names of the most well-known species (Rachie 1974). In some places the name *Pennisetum typhoides* has been changed to *Pennisetum americanum* and this has added to the confusion. This presentation will deal exclusively with the *Pennisetum* millet that is most commonly referred to as pearl or bulrush millet.

The Millet Colour Problem

Our interest in millet polyphenols is based mainly on aesthetic rather than nutritional considerations. Polyphenols in the grain are responsible for a gray pigmentation which some populations in the world find objectionable.

This problem was discovered by workers who began to mechanize grain processing in

Maiduguri, Nigeria. The objective of this IDRC-supported work was to replace the traditional mortar and pestle method of dehulling and grinding grain with mechanical dry-milling equipment (Anonymous 1976). A mill was established at Maiduguri incorporating a grain cleaner and destoner, a dehuller (Reichert and Youngs 1976), a hammermill, sifter, and a packaging facility. The grains which were initially processed included maize, sorghum, and millet. Consumer acceptance was excellent for maize and sorghum flour. However, millet flour met with so much consumer resistance that the mill eventually had to stop processing millet. The gray colour of the millet flour produced by this mill was responsible for the consumer resistance, because the traditionally prepared product was creamy-white in colour.

A survey (Rolston 1975) of millet processing in this area revealed that villagers did not process their millet by a completely dry process. Following mortar and pestle dehulling, the grains were often soaked overnight in water containing tamarind pods or sour milk. This additional processing had a remarkable whitening effect; the original gray colour disappeared completely.

The objective of this paper is to review progress towards understanding this problem, and to suggest some possible solutions. Some of this work has been fully described in Cereal Chemistry (Reichert 1979; Reichert and Youngs 1979). Results are also reported from a preliminary rat-feeding experiment in which millet fractions following dehulling and containing different concentrations of polyphenols were compared with similar fractions obtained by dehulling high- and low-tannin sorghum grains.

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Table 1. Common and scientific names of millet species (Rachie 1974).

Common name	Scientific name
1. Bajra, pearl millet, bulrush millet, cattail millet, dukhn, cumbo, sajja, dark millet, candle millet	<i>Pennisetum typhoides</i> Stapf. and Hubb. → <i>Pennisetum americanum</i> (L.) K. Schum.
2. Italian or foxtail millet	<i>Setaria italica</i> Beauv.
3. Proso or common millet	<i>Panicum miliaceum</i> Linn.
4. Little millet	<i>Panicum miliare</i> Lam.
5. Ragi or finger millet	<i>Eleusine coracana</i> Gaertn.
6. Koda or ditch millet	<i>Paspalum scrobiculatum</i> Linn.
7. Japanese barnyard millet	<i>Echinochloa frumentacea</i> (Roxb.) Link.
8. Jungle rice or shama millet	<i>Echinochloa colona</i> (L.) Link.
9. Australian millet	<i>Echinochloa decompositum</i>
10. Browntop millet	<i>Brachiaria ramosa</i> (Linn.) Stapf.
11. Teff	<i>Eragrostis tef</i> (Zucc.) Trotter
12. Fonio or hungry rice	<i>Digitaria iburua</i> Stapf.
13. Fonio or hungry rice	<i>Digitaria exilis</i> Stapf.
14. Adlay or Job's tears	<i>Coix lachryma-jobi</i> Linn.

Material and Methods

Grain Samples and Processing

The 16 millet varieties analyzed for polyphenols were the same as those previously used (Reichert and Youngs 1979). The millet, low-tannin sorghum (LTS), and high-tannin sorghum (HTS) used in the nutritional trial were obtained from Senegal in 1977. These varieties were Souna III, Sorgho CE90, and Sorgho X3055, respectively. A long-season variety of millet was obtained from Maiduguri, Nigeria in 1974. All other work reported here was done using a commercial, short-season variety obtained from Maiduguri, Nigeria in 1975.

Soaking treatments in acid, water, or sour milk were described previously (Reichert and Youngs 1979).

Small samples of millet were dehulled in a Strong-Scott barley pearler and the degree of dehulling was determined by the amount of fines passing through a 20-mesh screen (Reichert and Youngs 1976).

Fractionation of grains for the rat-feeding trial was accomplished using a Hill grain thresher at 770 rpm and a cleaning fan speed of 1800 rpm (Reichert and Youngs 1976). Grains were ground in a coffee grinder prior to preparation of diets.

Polyphenol Analysis

Tannins were measured by the vanillin-HCl method using blanks as described by Price et al. (1978).

C-glycosylflavones and alkali-labile ferulic acid (ALFA) which were previously identified and quantified in millet (Reichert 1979) by paper chromatographic and spectrophotometric meth-

ods were quantified in 16 millet varieties. Samples for spectrophotometric determination were prepared as previously described. The concentration of C-glycosylflavones was determined in the methanol extract at alkaline pH by application of the formula $C = (0.667)(A/23205)$ (Reichert 1977). The term A is the absorbance at 387 nm; 0.667 is the relative contribution of C-glycosylflavones to the absorbance (from paper chromatography); 23205 is the extinction coefficient; and C is the concentration in moles/litre. Moles/litre were converted to mg/100 g millet flour based on the molecular weight of glucosylvitexin (594.5). The concentration of ALFA was determined in the ether extract of the base hydrolyzate of methanol-extracted millet flour (Reichert 1979). The formula $C = (0.898)(A/19863)$ was used to calculate the concentration, where A is the absorbance at 317 nm; 19863 is the extinction coefficient; 0.898 represents the relative contribution of ferulic acid to the absorbance (from paper chromatography); and C is the concentration in moles/litre (Reichert 1977).

Flour-Reflectance Measurements

Reflectance properties of dry flours and flour pastes were measured in terms of absorbance units. Measurements were made using a Hitachi Perkin-Elmer spectrophotometer equipped with a diffuse reflectance attachment (Reichert 1977 and 1979).

To determine reflectance characteristics of the 16 millet varieties at alkaline pH the millet flours were diluted with wheat starch. Twelve grams of wheat starch was thoroughly mixed with 0.5 g of millet flour. Ten millilitres of 0.1 N NaOH was added and then mixed for 3 min before a reflectance measurement at 375 nm was made on the slurry.

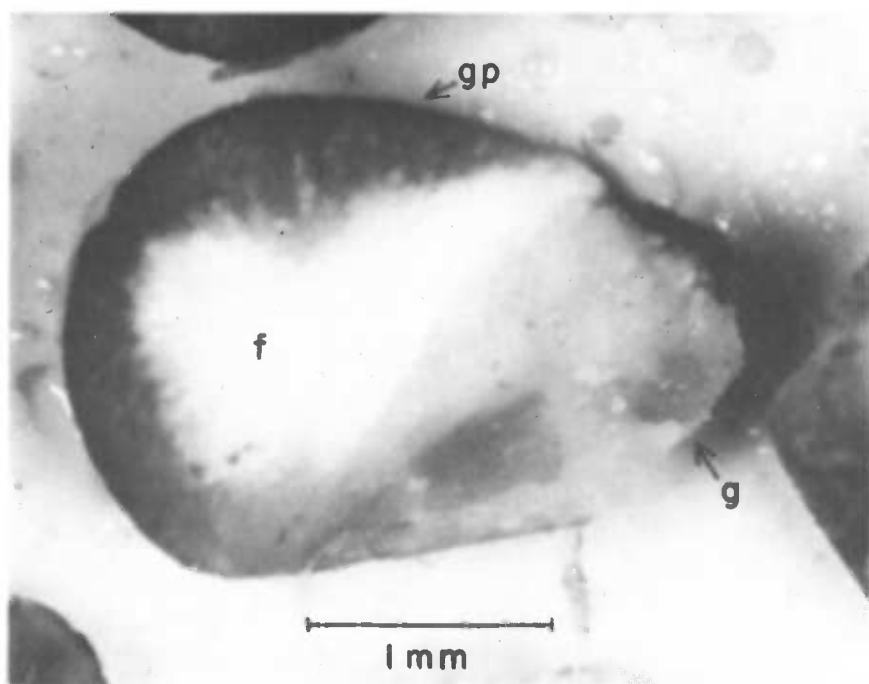


Fig. 1. Photograph of whole millet grain showing the location of gray pigmentation (gp), germ (g), and floury endosperm (f).

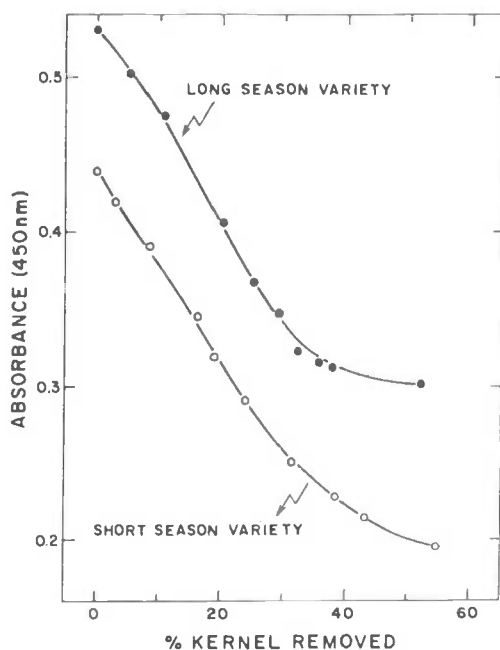


Fig. 2. The absorbance (450 nm) of dry flours prepared by progressively dehulling long- and short-season millet varieties.

Nutrition Study

Fractions from the abrasive dehulling of millet, HTS, and LTS were evaluated for nutritional quality using weanling male Wistar-strain rats. These fractions were incorporated at 60% of the feeding ration. The protein content of all diets was equalized at 23% by the addition of casein. The crude fibre content (based on crude fibre content plus added cellulose) was 6% in each diet. Methionine was supplemented to meet the requirement of the growing rat as specified by Warner and Breuer (1972). Six rats per ration were fed and watered ad libitum for 28 days. Feces collected during the last two weeks were analyzed for chromic oxide (Bolin and Lockhart 1960).

Results and Discussion

Distribution of the Gray Pigment in Millet

The gray pigment was predominantly located in the peripheral area of the seed (Fig. 1). The central portion of the seed was very white in comparison. A considerable improvement in flour colour was made by simply pearling the grain in a barley pearler (Fig. 2), in which process

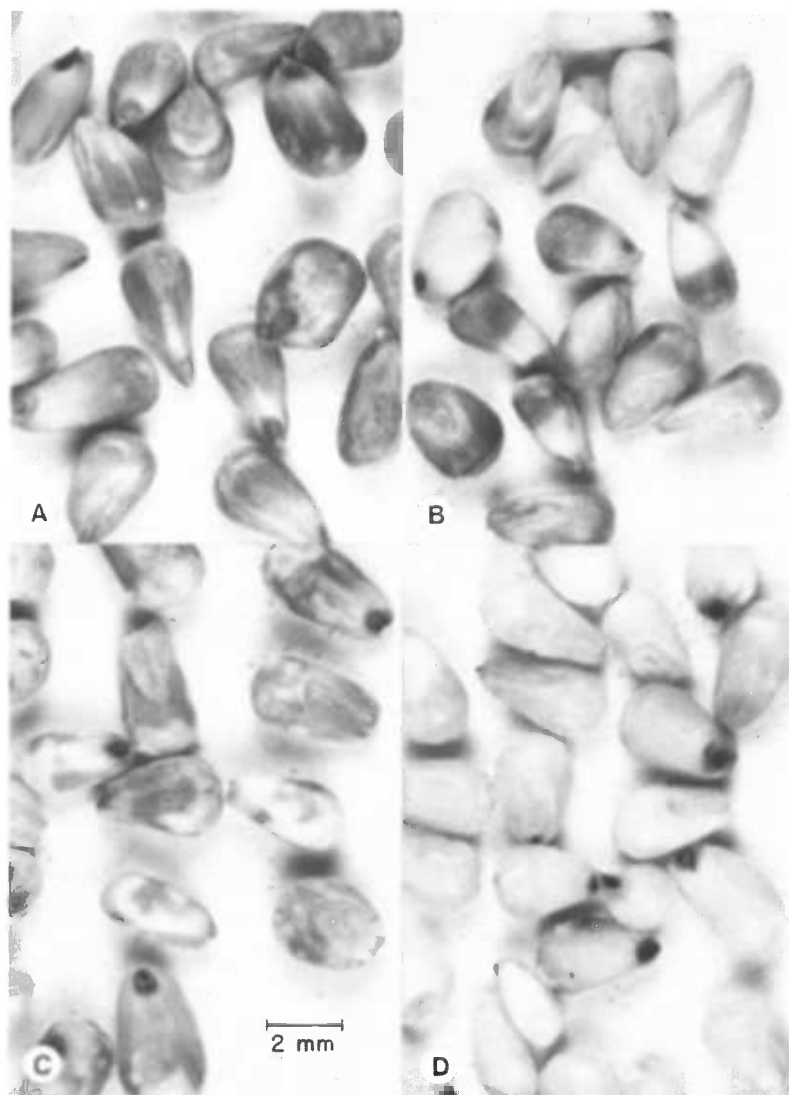


Fig. 3. Photographs of whole and dehulled millet grains demonstrating the influence of acid bleaching on exterior colour intensity. A. untreated whole millet; B. whole millet soaked in 0.2 *N* HCl for 12 h; C. scarified millet grain (2.4% removed) soaked in 0.2 *N* HCl for 20 min; D. same as C but soaked 3 h.

the outer layers are abraded off by a carborundum stone. The losses incurred in pearling a sample to an acceptable degree of whiteness were, however, in excess of 45%.

Acid Bleaching of Millet

The traditional bleaching treatments of millet grain, which involve soaking grains in sour milk

or tamarind pod solutions, lower the pH to 4.5–5.0. It was found that soaking the seeds in H_2O caused a slight reduction in colour, but that soaking in an acidic medium such as 0.2 *N* HCl caused the same dramatic colour change as did the traditional method.

Fig. 3 illustrates the dramatic improvement in grain colour achieved by soaking whole or dehulled grains in 0.2 *N* HCl. Whole millet grain

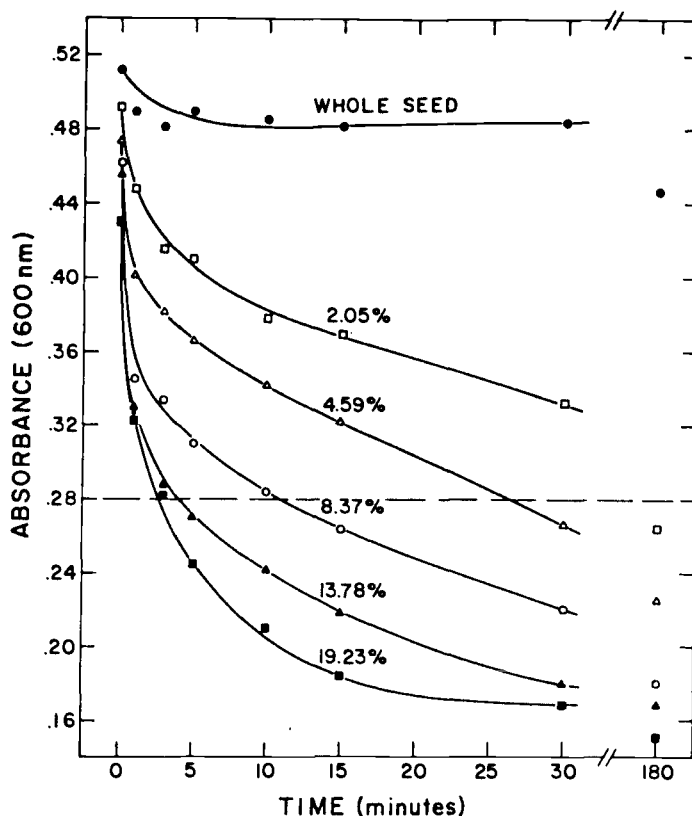


Fig. 4. Absorbance of flour pastes from millet which was abrasively dehulled to various degrees (% kernel removed) and soaked in 0.2 N HCl for various time intervals (time, min). The broken line represents the absorbance of a sample that was traditionally dehulled (mortar and pestle) and soaked overnight in a solution containing sour milk.

absorbed the acid slowly through the embryo (Fig. 3B). The progression of the acid was easy to follow because of the distinct colour boundary observed in many seeds. Some seeds required as long as 48 h for the acid to migrate to the opposite end. However, scarification of millet grain in the barley pearler allowed rapid absorption of the acid through all areas where the husk had been broken (Fig. 3C).

The relation between the degree of scarification and the rate of acid absorption into millet is illustrated in Fig. 4. Whole millet grain showed very little colour change during the 3-hour soaking in 0.2 N HCl, whereas seeds that had 19.2% kernel removed with the barley pearler whitened very rapidly. The traditionally prepared product, which was dehulled in a mortar and pestle and soaked in a solution containing sour milk, gave an absorbance value of 0.28. To

achieve this degree of whiteness, typical combinations of degree of pearling and soaking time are: 4.6% kernel removed and soaked 26 min; 8.4% kernel removed and soaked 11 min; 13.8% kernel removed and soaked 4 min; and 19.2% kernel removed and soaked 3 min.

On treatment with acid, some varieties of millet lighten in colour to a greater degree than others (Fig. 5). All varieties were soaked in 0.1 N HCl for 48 h and then air-dried. The treatment was judged to be excellent where the treated grain was white in colour. These groups included PHB-14, Serere 2A-9, Maiwa composite, white, and deep slate. Two varieties responded very little to acid bleaching and these were the purple and deep purple varieties. All other grains were yellow in colour after treatment. This indicates that the gray or yellow-gray pigment is easily bleached, whereas the yellow or brown pigment is not.

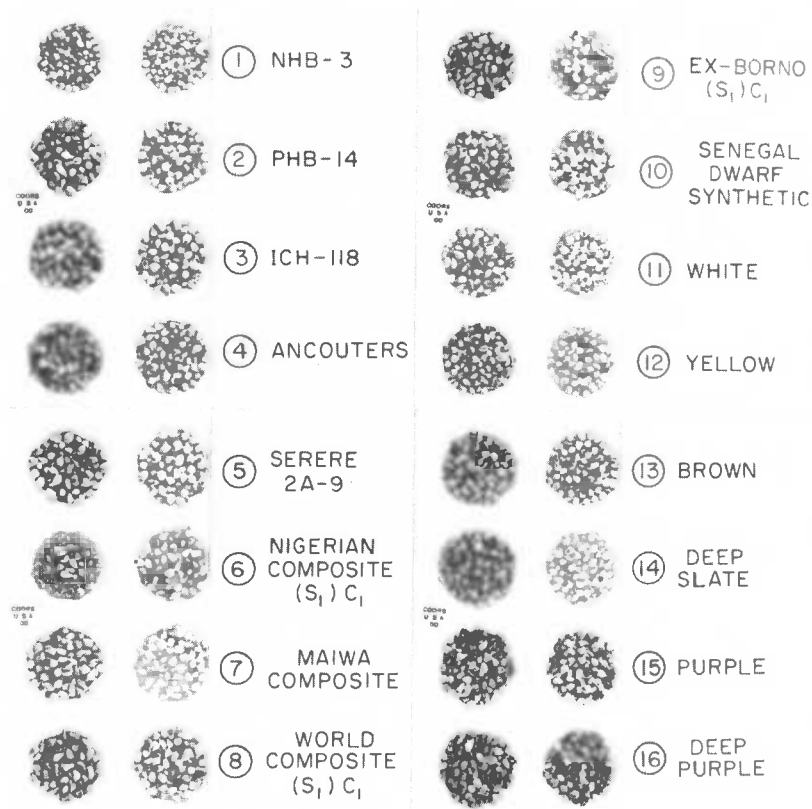


Fig. 5. A comparison of pericarp colours of 16 varieties of millet before (left) and after (right) soaking in 0.1 N HCl.

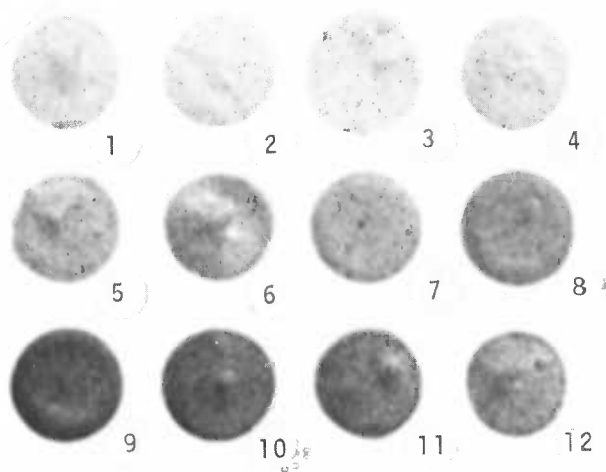


Fig. 6. Millet flour pastes at pH 1-12. Pastes from pH 1 to 4 were creamy white, from pH 5 to 7 they were gray, and from pH 8 to 12 they were bright yellow-green in colour.

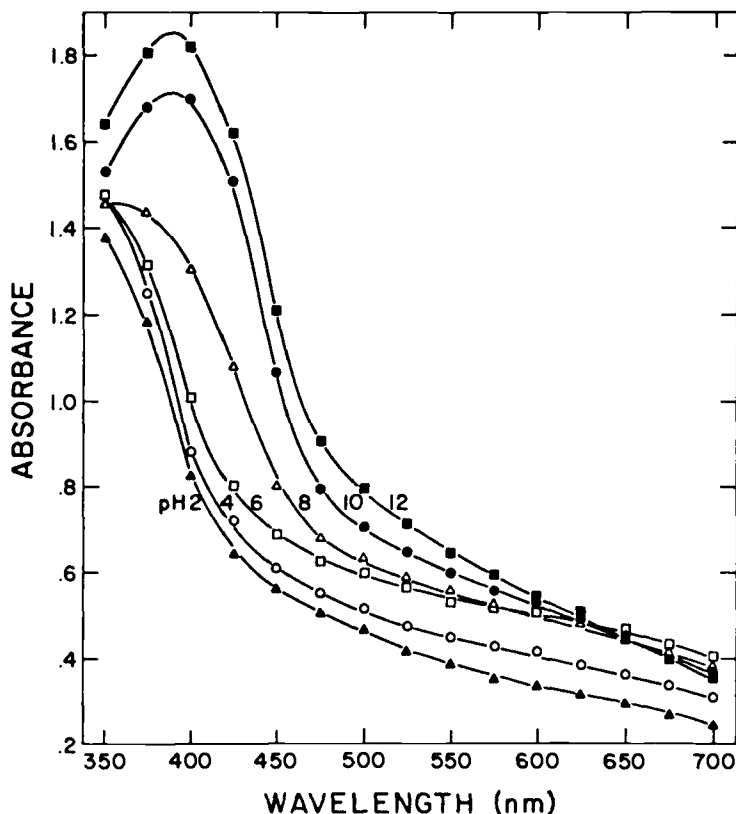


Fig. 7. Influence of pH (2-12) on absorbance of millet flour pastes at wavelengths of 350-700 nm.

pH-Sensitive Pigments in Millet

As a function of pH, millet flour pastes exhibited a range of colours (Fig. 6). Flour pastes from pH 1-4 were creamy-white, from pH 5-7 they were gray, and from pH 8-12 they were bright yellow-green in colour. Reflectance spectra of these same pastes showed the appearance of an absorption maximum at approximately 390 nm which was evident only under alkaline conditions (Fig. 7).

The pigments responsible for the yellow-green colour of millet flour pastes at alkaline pH were methanol soluble. The natural gray pigmentation in the peripheral area of the seed was also methanol soluble. This was demonstrated by simply cracking the grain to expose the endosperm and stirring the broken kernels in methanol for a few hours. After this treatment the gray pigment had disappeared completely.

Examination of paper chromatograms of methanol extracts of millet flour did not reveal the presence of any gray pigment. However, the

pigments responsible for the yellow-green colour of millet paste at alkaline pH were easily recognizable. The structures of the pigments were elucidated by UV spectrophotometric techniques using diagnostic reagents as described by Mabry et al. (1970). Sugar groups were identified by paper chromatography following acid hydrolysis (Pridham 1956). The structure of these compounds and their relative proportions in millet are shown in Fig. 8. Glucosylvitexin, glucosylorientin, and vitexin are from a class of flavonoids known as C-glycosylflavones. These compounds are characterized by having a sugar bound via a carbon-carbon bond to the flavonoid nucleus. This sugar group is not hydrolyzable by normal acid or enzymatic hydrolysis procedures.

Glucosylvitexin was the major pH-sensitive pigment in millet, and the pure compound showed an absorption maximum at alkaline pH of 395 nm. Similarly, glucosylorientin and vitexin showed absorption maxima at 405 nm and 395 nm, respectively, under alkaline conditions. This illustrated that these compounds were likely

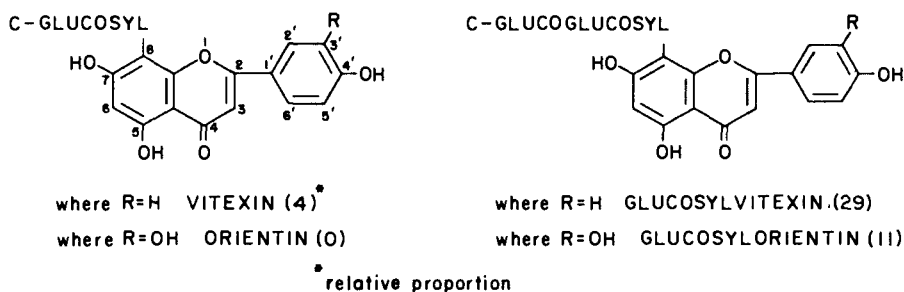


Fig. 8. Structures and relative proportions of the major C-glycosylflavones in millet.

responsible for the reflectance maximum of the millet flour paste (alkaline pH) observed at 390 nm.

Methanol-extracted millet flour also contained a substantial quantity of ferulic acid which was liberated by alkaline hydrolysis. Some of the pH sensitivity which methanol-extracted millet flour still exhibits could be due to ferulic acid esterified to glucose, quinic acid, other sugars, or an amino acid.

The concentrations of C-glycosylflavones and ALFA in whole and dehulled millet are illustrated in Fig. 9. Whole millet contained 124 mg/100 g of C-glycosylflavones and 158 mg/100 g of ALFA. When the grain was dehulled, concentrations of these phenolics decreased markedly. The correlation coefficient between C-glycosylflavones and dry flour absorbance (450 nm) of pearled grains was 0.994. Similarly the correlation coefficient between ALFA and dry flour absorbance (450 nm) was 0.991. This indicated that the decrease in colour of millet flour and the decrease in the polyphenol content were well correlated following abrasive dehulling of this millet.

To further investigate the relationship between colour and polyphenol concentration, a number of grain types were analyzed for reflectance properties and polyphenol content (Table 2). The reflectance measurement of the flour paste (natural pH of 6.3) at 450 nm is a measure of whiteness. The reflectance measurement of the flour paste (pH 11.2) at 375 nm is a measure of the degree of yellowness or yellow-green pigmentation in the paste. The pigmentation of whole millet flour pastes at alkaline pH was so intense that dilution of the paste with wheat starch was necessary to obtain reproducible and reliable reflectance values.

Considerable variation existed in the grain colour and whiteness of the flour among the various types analyzed (Table 2). This character of grain colour needs to be considered by plant breeders in the future selection of food-grade millet varieties. The C-glycosylflavone content varied from 87 to 259 mg/100 g, while the ALFA content varied from 127 to 241 mg/100 g. The values reported in the table are only approximate, as a paper chromatographic analysis was not done on all samples. The degree of yellowness of the flour paste at alkaline pH also varied considerably as shown in the last column. These pastes ranged in colour from very pale yellow for

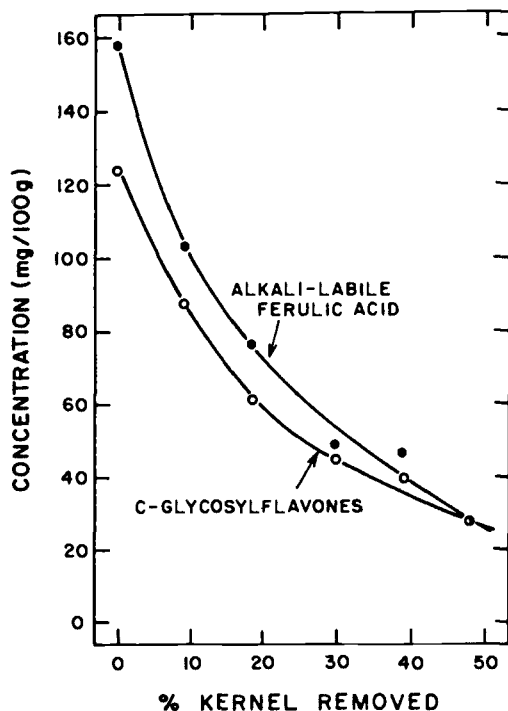


Fig. 9. Effect of the degree of kernel removal on the concentration of C-glycosylflavones and ALFA.

Table 2. Colour, polyphenol content, and reflectance properties of bulrush millet varieties.

Variety	Grain colour	ALFA ¹ (mg/100g)	C-GF ² (mg/100g)	Abs (450nm) of flour paste, pH 6.3	Abs (375nm) of flour paste, pH 11.2 ³
NHB-3	gray-yellow	175	151	0.676	0.590
PHB-14	gray	190	127	0.681	0.573
ICH-118	gray-light brown	161	87	0.667	0.534
Ancouters	brown-yellow	185	259	0.813	0.654
Serere 2A-9	gray-green	220	134	0.645	0.559
Nigerian composite (S ₁)C ₁	gray-light brown	144	111	0.639	0.532
Maiwa composite	yellow-gray	133	124	0.519	0.527
World composite (S ₁)C ₁	gray-light brown	153	113	0.674	0.540
Ex-Borno (S ₁)C ₁	gray-brown	156	124	0.670	0.573
Senegal dwarf synthetic	yellow-green	241	119	0.705	0.580
White	yellow	137	107	0.564	0.549
Yellow	yellow-green	210	172	0.707	0.630
Brown	light brown	175	139	0.775	0.637
Deep slate	gray	127	113	0.673	0.566
Purple	brown	188	158	0.780	0.610
Deep purple	dark brown	199	130	0.792	0.582

¹Alkali-labile ferulic acid.²C-glycosylflavones.³4% Flour in wheat starch adjusted to pH 11.2.

ICH-118 to a much more intense yellow-green for Ancouters.

Correlation coefficients between the concentrations of polyphenols and colour are illustrated in Table 3. As was expected, the C-glycosylflavones are responsible for much of the colour of the millet flour paste at alkaline pH. It appears, however, that the whiteness of the natural flour is not related, or only very weakly related, to the total C-glycosylflavone or ALFA content.

The gray pigmentation appears to be a result of a complex interaction rather than the result of the presence of a single compound. Indeed, the in vivo colour of a compound may be due to many factors which could include chelation of the phenolic in vivo with Cu, Fe, Al, or other metal ions; copigmentation effects which enhance colours; or a pH effect which changes the degree of ionization of the phenolic (Singleton 1972).

Table 3. Correlation of polyphenol content and millet colour.

	Flour paste absorbance	
	450nm, pH 6.3	375nm, pH 11.2
C-glycosylflavones	0.57	0.80
ALFA	0.51	0.48

Effect of Millet Polyphenols on Nutritional Quality

A preliminary experiment was conducted to determine if the dehulled millet fractions, containing different amounts of polyphenols, had any adverse effect on rats fed a diet containing 23% protein (supplemented with casein) and all other nutrients necessary for a complete ration. Fractions were prepared by abrasive dehulling in a Hill grain thresher, which gradually removes the peripheral layers of the grain. A high-tannin (HTS) and low-tannin sorghum (LTS) were similarly processed. All fractions were fed at a 60% level in the diet.

Proximate analysis of these fractions (Table 4) demonstrated a marked concentration of the fat, protein, fibre, and ash into the hull fractions. The most marked fractionation of protein occurred in millet, indicating that the protein is not as uniformly distributed throughout the seed as in sorghum.

Fractionation of the tannins, as determined by the vanillin-HCl method, occurred during processing of the HTS grain. This grain was extremely difficult to dehull because of its soft endosperm structure. Millet or LTS contained no measurable level of tannin. In fact, all varieties of millet studied so far have appeared free from tannins. The polyphenols reported in this paper (C-glycosylflavones and ALFA), however, were

Table 4. Yields, proximate analysis, and tannin analysis of grain fractions (% dry basis).

Fraction	Yield (%)	Crude fat (%)	Protein (%)	Ash (%)	Crude fibre (%)	Tannin (%)
LTS	—	2.81	13.28	1.76	2.03	0
Dehulled LTS	85.2	2.05	12.57	1.34	0.78	0
LTS hull	14.8	8.24	14.32	4.18	8.72	0
HTS	—	2.77	11.35	1.61	1.85	3.60
Dehulled HTS	57.8	1.60	12.00	1.02	1.10	1.43
HTS hull	42.2	4.52	11.41	2.47	3.62	5.85
Millet	—	5.21	11.10	1.73	1.92	0
Dehulled millet	86.6	3.86	10.50	1.26	0.77	0
Millet hull	13.4	13.52	18.26	4.56	6.75	0
Wheat flour	72	0.99	12.71	0.43	0.18	0

Table 5. Effect of dehulling on the nutritional quality of millet and high- and low-tannin sorghum.

Fraction	Feed intake (g)	Weight gain (g)	Feed/gain	Dry-matter digestibility (%)	Protein digestibility (%)
LTS	415.9 _a	179.5 _a	2.32 _a	86.8 _a	87.3 _a
Dehulled LTS	420.1 _a	187.6 _a	2.24 _a	88.2 _a	89.9 _b
LTS hull	505.6 _b	182.9 _a	2.78 _b	74.2 _b	81.6 _c
HTS	427.7 _a	176.8 _a	2.42 _a	82.9 _a	79.9 _a
Dehulled HTS	435.7 _a	188.8 _a	2.31 _a	87.6 _b	86.1 _b
HTS hull	503.0 _b	182.2 _a	2.77 _b	75.7 _c	69.5 _c
Millet	423.2 _a	183.5 _{ab}	2.31 _a	86.1 _a	88.4 _a
Dehulled millet	441.4 _a	195.5 _a	2.26 _a	88.6 _b	90.0 _a
Millet hull	439.8 _a	170.6 _b	2.58 _b	72.6 _c	77.4 _b
Wheat flour	432.3	193.6	2.21	90.4	91.6

NOTE: Each grain was analyzed separately for purposes of statistical evaluation. Values in any one column followed by different letters are significantly different.

present and the dehulled millet seed would be expected to contain approximately 40% less than the whole seed. The concentration of polyphenols in the hull fraction is about 3 times that in the whole grain.

Table 5 shows that in the feeding experiments dehulled millet was not significantly different from whole millet in feed intake, weight gain, feed/gain ratio, or protein digestibility. Values for the LTS fractions were very similar to those of millet fractions. Dehulled LTS was not significantly different from whole LTS in feed intake, weight gain, feed/gain ratio, or dry-matter diges-

tibility. However, protein digestibility was somewhat improved by dehulling. Dehulling HTS caused a marked improvement in dry-matter and protein digestibility.

From this experiment it appears that the millet fractions performed in a similar manner to the LTS fractions in all nutritional variables measured. It appears, therefore, that any problems associated with millet polyphenols are of the same general magnitude as those associated with LTS. Much work needs to be done to clarify the effect of simple nontannin phenolics on nutritional quality.

Conclusions

Polyphenols in millet grain are not as nutritionally adverse as the tannins present in the testa layer of some cultivars of sorghum. However, these polyphenols present an aesthetic problem because of a gray pigmentation in the peripheral areas of the seed. Consumer acceptance of millet flour produced by a dry-milling facility in an African country was considered significantly inferior to the traditionally accepted product, a creamy-white millet flour that villagers produced by soaking dehulled grain in solutions containing sour milk or tamarind pods.

One solution to the problem of consumer acceptance might be for the mill simply to sell dehulled millet grain to consumers who could

themselves treat the grain in whichever way they were accustomed. Consumers could return the treated and dried grain to the mill to be ground into flour.

Alternatively, the mill could set up a bleaching plant using a chemical to lower the pH. Lactic acid is responsible for lowering the pH of sour milk. Similarly, tartaric acid is responsible for lowering the pH when tamarind pods are used in the traditional process. Either of these chemicals or simply dilute HCl could be used to bleach millet. Such a plant would require a drying facility to reduce the moisture content of treated grains.

A third solution is for plant breeders to breed for varieties with less colour. This is a long-term approach, but may be the most satisfactory.