Effect of Processing on Flavonoids in Millet (*Pennisetum americanum*) Flour

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ABSTRACT

Pigments of pearl millet flour had maximum absorbance at 355 nm. The absorbance and *C*-glycosylflavanol content varied among 17 millet varieties. Absorbance was 0.72 and 0.91 mg/100 g, and *C*-glycosylvitexin equivalent was 76.6 and 275.7 mg/100 g for Ghana 16152 and Ancateurs samples, respectively. Increasing levels of decortication from 0 to 50% progressively reduced both absorbance and flavanol concentration, but rates differed among samples. Cooking also reduced the absorbance and flavanol content of flour more than did steeping in acid or sour milk.

Cereal Chem. 68(2):180–183

Pearl millet (*Pennisetum americanum*), also known as cattail millet, candle millet, *bjara* (India), *dikhum* (Sudan), or *jero* (Nigeria), is widely grown as a food grain in Africa and Asia. The peripheral areas of the seed contain a gray, pH-sensitive pigment consisting of *C*-glycosylflavones and other phenolics (Reichert et al 1980). The major flavones in the pigments have been identified as *C*-glycosylvitexin, vitexin, and glycosylorientin (Reichert 1979). Osman (1981), Osman and Fatah (1981), and Osman et al (1983) linked millet diets with the high incidence of endemic goiter in Sudan. Klopfenstein et al (1983) suggested that the goitrogenic effect of a millet diet might be due to the grain's high mineral content, but later studies (Gaitan et al 1986, Birzer et al 1987) confirmed the goitrogenicity of millet flavones.

Traditional processing of millet for food in Nigeria involves the removal of some of the outer layers of the kernel by pounding the grain in a mortar. The decorticated grain is steeped overnight in water containing tamarind bean extract or sour milk to bleach pigments. The grain is subsequently sun-dried and pounded in the mortar to reduce particle size. The bleached flour is then cooked into *tuwo,* a stiff porridge.

The objective of this study was to evaluate 1) the effects of variety, decortication, and steeping in water, 0.2*N* HCl, or sour milk for various time intervals on the absorbance and *C*-glycosylflavanol concentration of millet flour and 2) the effect of cooking on absorbance and *C*-glycosylflavanol content of a cooked porridge (*tuwo*).

MATERIALS AND METHODS

Samples

Seventeen millet varieties were evaluated in the study. Varieties Ghana 16151 and Ghana 16152 had straw-colored pericarps (Table I). The other 15 varieties have been described (Reichert et al 1980).

Physical and Chemical Analyses

Thousand-kernel weight was determined by weighing 100 seeds (five replicates). Grain color was evaluated visually. Endosperm texture was determined by cutting several kernels in half longitudinally and visually estimating the ratio of corneous to floury endosperm. A variety with more than 90% corneous endosperm was rated 1; a variety with less than 10% corneous endosperm was rated 5. The abrasive hardness index was determined as described by Reichert et al (1984). Moisture was determined in a forced-air oven at 130°C (AACC 1983).

Kernel Dissection

Millet grain, Nigerian composite $(S_1)C_1$ variety, was carefully dissected to produce reasonably pure fractions of endosperm, germ, and pericarp.

Ground Millet Samples

Grain equilibrated to 10.7% moisture was decorticated to remove 10, 20, 30, 40, and 50% of the weight in a Tangential Abrasive Dehulling Device (TADD) (Reichert et al 1986). The decorticated grain was reduced to pass through a 0.25-mm mesh screen in a Udy mill.

Flour Samples

Nigerian composite $(S_1)C_1$ variety was used for the preparation of flours. The grain was decorticated to remove 8% of the weight in the TADD. Decorticated grain (20 g) was steeped in 50 ml of distilled water for 4, 9, and 16 hr. The steep water was decanted and the grain washed three times with 50 ml of water. The washed grain was spread out to dry for 48 hr on a table in the laboratory. Decorticated gran (20 g) was also steeped in 50 ml of 0.2*N* HCl for 5, 10, and 20 min, followed by washing with 50 ml of water and drying at 20 ± 1°C for 48 hr (three times).

Traditional Preparation of Flour

A millet sample (100 g) was steeped in 150 ml of water for 30 min. The water was decanted and the grain pounded in a mortar for 15 min. Bran was washed from the grain three times with 150 ml of water. The grain was dried in a forced-air oven at 60°C for 2 hr. The yield of decorticated, oven-dried grain was 80 ± 2%. Sour milk (2.5 ml of cultured buttermilk, Dairy Producers Co-op Ltd., Regina, SK), 15 g of oven-dried grain, and 150 ml of water was steeped for 4, 8, and 16 hr and then

TABLE I
Physical Properties of Millet Samples

Variety	Grain Color	1,000-Kernel Weight (g)	Endosperm Texture[a]	Abrasive Hardness Index (sec)
Market	Gray	12.1 c[b]	3.0	2.3
Yellow	Yellow-green	7.4 l	3.0	3.8
Purple	Brown	5.6 p	5.0	2.7
White seed	Straw	8.4 k	3.0	3.1
Senegal dwarf (synthetic)	Yellow-green	9.7 g	3.0	2.7
Nigerian composite $(S_1)C_1$	Gray	11.1 d	3.0	2.4
HB-3	Gray	4.4 q	1.5	2.4
Anceteurs	Brown	7.2 lm	1.5	2.8
Deep slate	Gray	7.1 lm	1.5	3.1
Serere 2A-9	Gray	12.4 b	3.0	2.9
Maiwa composite	Yellow	9.7 gh	5.0	2.0
pHB 14	Gray	9.0 ij	1.0	2.9
Brown	Light brown	9.3 i	3.0	2.9
World composite $(S_1)C_1$	Brown	11.4 d	3.0	3.4
Ex-Borno $(S_1)C_1$	Gray	10.8 ef	3.0	3.9
Ghana 16151	Straw	15.2 a	3.0	2.8
Ghana 16152	Straw	16.7 a	4.5	...

[a] Ratio of corneous to floury endosperm.
[b] Means with the same letters are not different ($P = 0.05$).

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air-dried at 20 ± 1°C. The grain was reduced in a Udy mill fitted with a 0.25 mm mesh screen.

Sour Milk Treatment

A 2.5-ml portion of cultured buttermilk (pH 4.4) and 150 ml of water was added to 20 g of millet (8% TADD dehulled). This mixture (pH 4.6) was steeped for 4, 8, and 16 hr before rinsing three times with 50 ml of water and drying at 20–21°C. The dry grain was reduced as described earlier.

Preparation of *Tuwo*

Water (3.0 ml) was added to 2.5 g of millet flour in a small beaker to make a slurry. The slurry, plus 3.0 ml of rinse water, was added to 5 ml of boiling water. This mixture was stirred continuously with a glass rod for 2 min. The stiff porridge, *tuwo,* was immediately cooled in an ice bath for 30 min and lyophilized overnight.

Absorbance Measurements

Ground millet flour (Market variety, 0–50% decorticated, 10.7% moisture content) was scanned on a model 139 UV VIS spectrophotometer (Hitachi Perkin-Elmer, Hitachi Ltd., Tokyo) (Reichert 1979). Maximum absorbance from 320 to 500 nm was observed at 355 nm with a model 139-0640 reflectance attachment. Therefore, the absorbance of millet flour and dried, finely ground *tuwo* were conducted at the 355-nm wavelength.

Determination of *C*-Glycosylflavanol Content

C-Glycosylflavanol concentration was determined by the method of Reichert (1979), which was modified for small samples (0.1 g). Ground millet (100 ± 2 mg) and hexane (10 ml) were added to screw-cap test tubes. The capped tubes were heated in a water bath at 60°C for 20 min and were shaken manually at 5-min intervals. The tubes were subsequently centrifuged at 2,000 × g for 5 min and the supernatants carefully decanted. The extraction process was repeated to give an oil-free residue, which was heated in an oven at 80°C for 1 hr to remove residual hexane. The flour was then extracted twice with methanol (10 ml) at 75°C for 20 min. Sodium methoxide (5 ml of a 2.5% w/v solution) was added to the methanol extract, and absorbance was measured using a Beckman model 25 spectrophotometer at 387 nm (Reichert 1979). *C*-glycosylflavanol content was calculated as the glycosylvitexin equivalent per 100 g of millet on a dry weight basis.

RESULTS AND DISCUSSION

Significant varietal differences were observed in kernel weight, texture, and hardness of the millet samples (Table I). Thousand-kernel weight ranged from 4.4 g (variety HB-3) to 16.7 g (Ghana 16151). The abrasive hardness index ranged from 2.0 to 3.9 sec, and endosperm texture ranged from 1.0 to 5.0 among the varieties. Grain color ranged from different shades of gray to a few brown and straw-colored varieties. The perceived color of millet grain has been attributed to pericarp color and thickness, endosperm, and aleurone pigmentation (McDonough and Rooney 1989).

The whole-grain millet, Nigeria composite $(S_1)C_1$ variety, contains 128.4 mg of *C*-glycosylvitexin equivalent per 100-g sample. The pericarp (7.4% of the whole grain by weight) contained 973.3 mg of *C*-glycosylvitexin equivalent per 100 g, or 50% of the amount in the whole grain of this variety. Millet germ (12% of whole grain) contained 216 mg/100 g, or 19%, while the endosperm (79.6% of whole grain by weight) contained 54.8 mg of *C*-glycosylvitexin equivalent per 100 g, or 30.4% of the total *C*-glycosylflavanol content of the grain. Thus, 70% of the *C*-glycosylflavanol content of this millet was in the pericarp and germ fractions. This explains the large reduction in *C*-glycosylflavanol content after decorticating millet 10% (Reichert 1979). (Gaitan 1990) observed that the bran fraction contained greater amounts of *C*-glycosylflavanol than the endosperm. Therefore, any process that removes the pericarp and germ fractions should effectively reduce the *C*-glycosylflavanol content of millet.

C-Glycosylflavanol Content of Millet Varieties

The *C*-glycosylflavanol content of millet varieties ranged from 76.6 mg in Ghana 16152, with a straw-colored pericarp and white endosperm, to 275.7 mg *C*-glycosylvitexin equivalent per 100-g sample in Ancateurs, with a brown pericarp and gray endosperm (Table II). Flavanol content is related ($r = 0.77$, $P < 0.01$) to appearance of the grain, with the straw-colored grains having the fewest and the brown grains the most flavanols (Fig 1). Differences in the appearance of millet may be due to differences in pericarp color and the extent of pigmentation of the aleurone and the endosperm (McDonough and Rooney 1989).

Osman (1981), Osman and Fatah (1981), and Osman et al (1983) reported a high incidence of goiter in the millet-consuming population of Sudan, which has not been observed in the millet-consuming population of Nigeria. Varietal differences in the *C*-glycosylflavanol content of millet, the method of processing millet

TABLE II
***C*-Glycosylflavanol Concentration (mg/100 g)[a] in Whole and Decorticated Millet**

Sample	Level of Decortication, %					
	0	10	20	30	40	50
Market	137.1	100.0	56.6	45.5	37.0	25.2
Yellow	199.0	120.6	61.8	44.9	43.1	33.9
Purple	182.7	94.1	61.7	46.3	38.2	32.8
White seed	142.4	75.3	29.4	17.2	16.1	10.4
Senegal dwarf (synthetic)	153.9	131.5	63.2	48.0	38.7	31.3
Nigerian composite $(S_1)C_1$	128.3	108.7	55.8	40.8	36.0	37.1
HB-3	175.2	146.6	67.7	52.2	43.7	33.0
Ancateurs	275.7	202.4	90.3	59.2	47.7	38.0
Deep slate	142.2	106.8	72.6	46.1	32.4	27.8
Serere 2A-9	138.6	96.3	51.5	37.7	26.5	18.9
Maiwa composite	133.9	104.7	58.6	47.6	36.6	24.8
pHB 14	150.5	72.0	47.8	34.3	24.4	30.6
Brown	210.8	108.3	70.5	53.5	38.5	17.8
World composite $(S_1)C_1$	148.1	116.4	95.7	37.6	24.6	22.1
Ex Borno $(S_1)C_1$	138.9	97.1	61.1	45.8	31.5	24.7
Ghana 16151	109.1	62.0	33.7	22.9	...	...
Ghana 16152	76.6	57.7	31.2	...	...	...
Mean	157	106	64	43	37	27
Least significant difference[b]	3.3	3.0	3.6	2.0	1.4	1.6

[a] Milligrams of *C*-glucosylvitexin equivaient per 100 g of sample, db.
[b] Significant at 95% confidence interval.

into food, and the amounts of millet foods consumed may affect the incidence of goiter in millet-consuming populations.

Effect of Decortication on *C*-Glycosylflavanol Content of Millet Flour

The *C*-glycosylflavanol content of the 17 millet samples decreased with increasing levels of decortication (Fig 2). The sharp decrease in flavanol concentration when 0–10% of the grain was decorticated may be attributed to removal of the pericarp tissue, which contains the greatest concentration of flavanols in the kernel. Reichert (1979) reported a similar observation consistent with the distribution of *C*-glycosylflavanols in millet grain. Decorticating the grain 20% would remove the aleurone, the germ, and part of the peripheral endosperm, which contain relatively greater amounts of flavanols than does the inner endosperm. Varietal differences in the amounts of flavanols removed during each subsequent 10% decortication of the millet samples (Table II) may be due to differences in pericarp color, the presence and depth of pigmentation in the endosperm, endosperm texture, and kernel shape.

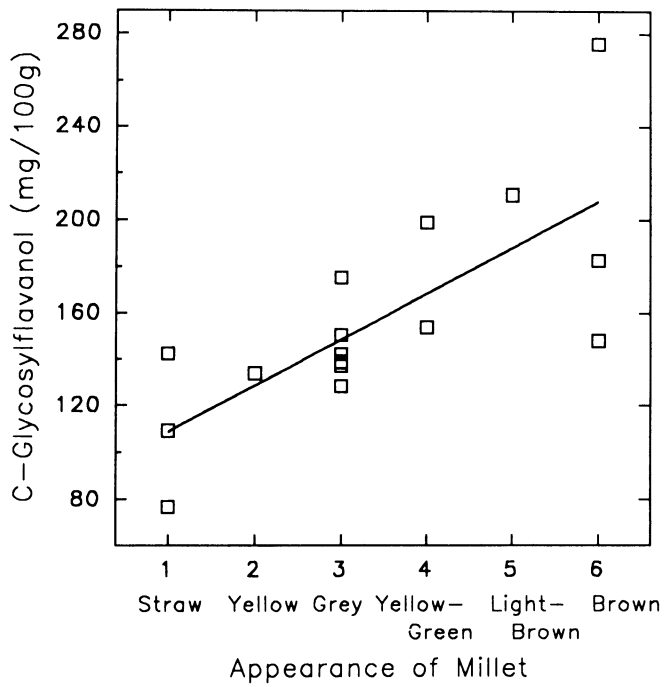

Fig. 1. Relationship of millet appearance and flavanol content.

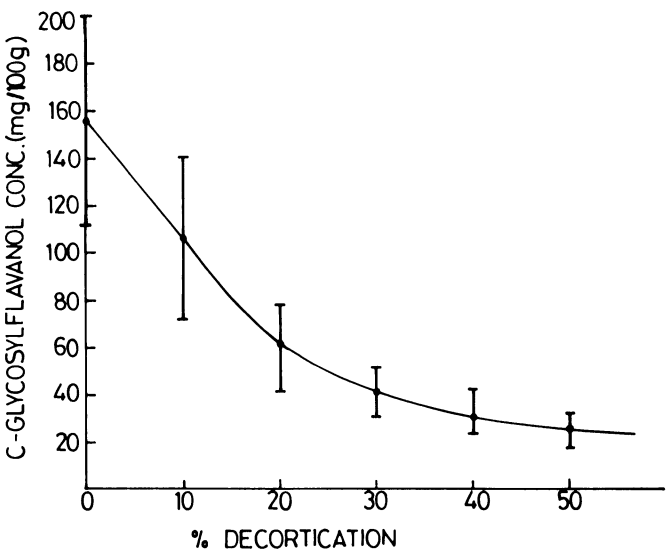

Fig. 2. Effect of decortication on flavanol concentration in millet.

Effect of Decortication on Absorbance of Millet Flour

Absorbance of dry millet flour showed a peak at 355 nm (Fig. 3). Reichert (1979) reported maximum absorbance for unextracted millet flour paste (pH 10) at 350–450 nm. Flour from 50% decorticated millet had a lower absorbance than did whole-grain flour. However, the absorbance maxima for both flours were the same (Fig. 3), indicating similar absorbing compounds. Progressive decortication from 0 to 50% reduced absorbance of the flours progressively (Fig. 4), but at different rates for different varieties. This agrees with the observation made for the flavanol concentration of millets.

Absorbance of traditionally milled flour (decorticated 20%) was similar to that of flour decorticated 8% by dry milling in the TADD (Table III). Decortication of the grain by the traditional milling process did not reduce flour absorbance as much as decortication using the TADD. This may be due to the greater amounts of kernel breakage during pounding and the lack of selective abrasion of the outer pigmented layers of the kernel that characterize milling in the TADD. Also, steeping before pounding may have leached the very soluble pigments from the pericarp into the endosperm.

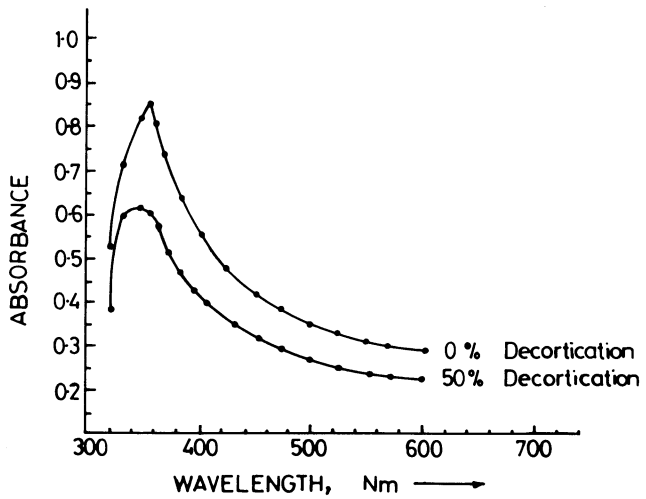

Fig. 3. Absorbance scan of millet flour.

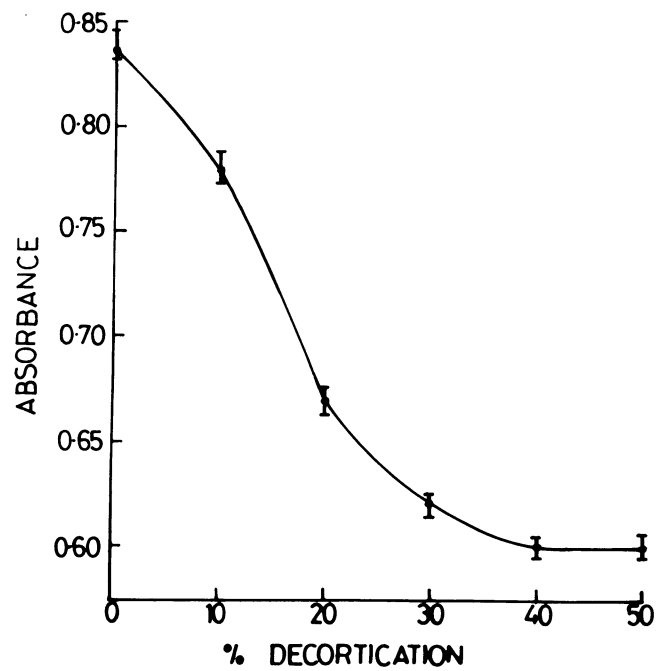

Fig. 4. Effect of decortication on absorbance of millet flour.

TABLE III
Effects of Steeping and Cooking on Absorbance and *C*-Glycosylflavanol Content of Millet Flour

Treatment and Percent of Decortication	Steeping Time, hr	Absorbance at 355 nm		Flavanols, mg/100 mg	
		Raw	Cooked	Raw	Cooked
No steeping					
0	0	0.80	0.65	128.4	90.2
8	0	0.79	0.45	100.8	43.0
20[a]	0	0.77	0.55	95.5	68.2
Water steeping					
8	4	0.72	0.57	63.9	49.5
	8	0.74	0.59	66.4	57.8
	16	0.73	0.66	70.2	60.3
HCL (0.2*N*) steeping					
8	0.08	0.70	0.55	98.9	70.2
	0.17	0.69	0.56	99.5	78.3
	0.33	0.72	0.56	94.5	64.7
Sour milk					
8	4	0.80	...	88.3	...
	8	0.80	...	86.4	...
	16	0.80	...	91.8	...
20[a]	4	0.76	0.55	95.5	68.2
	8	0.76	0.54	67.1	66.1
	16	0.77	0.53	84.3	81.0
Least significant difference[b]		0.03	0.11	3.55	2.61

[a]Traditionally decorticated to remove 20% of kernel.
[b]$\alpha = 0.05$.

Effect of Steeping on Absorbance and *C*-Glycosylflavanol Content of Millet Flour

Steeping of decorticated (8%) millet in water significantly decreased pigment absorbance in the flour, but steeping in sour milk did not affect flour absorbance (Table III). Reichert and Young (1979) reported a lower absorbance of pigments in millet flour that was steeped in sour milk than of that steeped in water. The differences in observations may be due to varietal differences of the millets. The souring process was also slightly different in the two studies. Acid bleaching of pigments using 0.2*N* HCl decreased flour absorbance. Reichert (1979) attributed this loss of flour to the dissociative effect of acid on the metal in the metal-flavanol complex that produces the gray pigments of millet.

A short steeping time corresponded to lower *C*-glycosylflavanol content in the flour for all the steeping solutions (Table III). This was probably due to the leaching of the water-soluble flavanols into the endosperm during prolonged steeping. Water steeping reduced flavanol concentration the most. The acid medium (0.2*N* HCl) decreased flour absorbance similar to that observed using the water medium with relatively shorter times. However, the acidic condition did not reduce the flavanol concentration to the same extent as did the water medium. Decreased absorbance of acid-bleached flour may be due to the dissociation of cations from the *C*-glycosylflavanol-ion complex more than to a decrease in flavanol content. On the other hand, steeping in sour milk decreased *C*-glycosylflavanol content of flour but did not decrease flour absorbance.

Effect of Cooking on Absorbance and Concentration of *C*-Glycosylflavanol Compounds

Cooking significantly reduced the absorbance and concentration of *C*-glycosylflavanol in millet except for one traditionally milled flour treated with sour milk (Table III). The reduction in absorbance with cooking may be due to conversion of the flavanols into intermediate compounds through cleavage of the middle ring of the flavanols (Gaitan 1990). These intermediate compounds, with absorption maxima different from 355 nm, may not be detectable by the assay method used for the flavanols.

CONCLUSIONS

The *C*-glycosylflavanol content of millets varies with the cultivar. Therefore, selection of varieties with low flavanol content and desirable food properties may reduce the incidence of goiter in millet-consuming populations. Dry decortication was more effective in reducing flavanol concentration in millet flour than steeping the grain in water, acid, or sour milk before decortication. Steeping grain for a short period removes more pigments than does the traditional overnight steeping practice.

ACKNOWLEDGMENTS

This research was funded through a research fellowship grant from the CIDA/NSERC. The author is grateful to R. D. Reichert, in whose laboratory the research was done, for supplying the millet samples, and to Don Schwab, of the National Research Council of Canada, Plant Bio-technology Institute, Saskatoon, SK, for technical assistance.

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[Received June 5, 1989. Accepted November 9, 1990.]