Comparison of Flavonoids in Bran of Four Classes of Wheat[1]

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Cereal Chem. 66(6):516-518

ABSTRACT

Flavonoids from bran of hard red spring, hard red winter, durum, and white wheats were extracted with diluted NaOH solution (pH 11). They were purified by chromatography on Amberlite XAD-2 resin and a Sephadex G-15 column followed by paper chromatography with two different solvents. The properties of the hard red winter, durum, and white wheat flavonoids were identical in chromatography R_f values, ultraviolet spectra, and fluorescent color to those of Len, a variety of hard red spring wheat in which the flavonoid was earlier purified and identified as 6,8-di-C-glycosylapigenins. Finally, after partly purifying on the Amberlite XAD-2 column, the flavonoid contents of nine wheat varieties were measured by ultraviolet spectrometry at 405 nm.

Flavonoids are plant pigments widely distributed in nature. A mixture of two di-C-glycosylflavones was isolated from bran of hard red spring (HRS) wheat (Len variety) and identified as 6-C-pentosyl-8-C-hexosyl- and 6-C-hexosyl-8-C-pentosylapigenins by ultraviolet (UV) spectroscopy, mass spectroscopy, and ^{13}C-nuclear magnetic resonance spectroscopy (Feng et al 1988). In this paper, the work was extended to the characterization of flavonoids in bran of other wheat classes using the di-C-glycosylapigenins fraction identified in HRS wheat bran (Len) as a standard. Also the quantity of flavonoid in varieties of different wheat classes was measured by UV spectroscopy.

MATERIALS AND METHODS

Wheat Samples

Bran samples from four classes of wheat were used in this study. Varieties of three wheat classes grown at Hettinger, ND, in 1986 were Len, Stoa, and Alex (HRS class); Roughrider and Agassiz (hard red winter [HRW] class); and Rugby, Lloyd, and Vic (durum class). The white wheat used in this study was a gift of unknown variety. The bran samples were cleaned and ground by the methods used before (Feng et al 1988).

Isolation and Characterization of Bran Flavonoid Fraction

The extraction and purification procedures for bran flavonoids from four classes of wheat were the same as those used previously (Feng et al 1988) except for omission of the paper chromatographic step with *tert*-butanol/acetic acid/water and the thin-layer chromatography step.

Characterization of Flavonoids

The purified flavonoid fraction from three classes of wheat (HRW, durum, and white) was characterized by directly comparing chromatography R_f values, UV spectra, and fluorescent color with those of the purified flavonoid from Len variety HRS wheat. Paper (Whatman 3MM) and polyamide thin-layer plates (Alltech Associates, Deerfield, IL) were used as adsorbents. The solvents, 15% acetic acid, ethyl acetate/formic acid/water (66:14:20, v/v), and *t*-BuOH/acetic acid/H_2O (3:1:1, v/v) were used for paper chromatography and H_2O/methanol/butanone (4:3:3, v/v) was used for polyamide thin-layer chromatography. The flavonoids from the four classes of wheat were cochromatographed ascendingly each time.

The reagents and procedures used for obtaining UV spectra of flavonoids were those described by Mabry et al 1970. The UV spectra were determined on flavonoid solution and the nonflavonoid phenol fraction by scanning from 225-525 nm at a speed of 1,200 nm/min on a Beckman DU-7 spectrophotometer using methanol as a blank.

The fluorescent color of the wheat flavonoid fraction with and without ammonia treatment was also determined. The dry paper chromatograms were first viewed under UV light (366 nm) alone; the fluorescent color was noted, and then the paper was exposed to NH_3 for 2 sec and the color again recorded.

Quantative Analysis of Flavonoids in Bran

For a flavonoid standard curve, 10-mg samples of purified flavonoids from Len bran (Feng et al 1988) were dissolved in 100 ml of spectroscopic methanol, and from this 10-100 μg/ml solutions were prepared. Using a 1-ml absorption cell, one drop of NaOMe (Mabry et al 1970) was added to 0.5 ml of flavonoid solution, and light absorption was determined at 405 nm using methanol as a blank. The standard curve of light absorption versus flavonoid concentration (μm/ml) is shown in Figure 1.

For the determination, wheat bran (2 g) was extracted 12 hr twice with 80 ml of water, pH 11 (NaOH). The combined extracts were acidified to pH 5 with acetic acid and centrifuged to remove the precipitate that was formed. The supernatant was applied to a 2.8 × 6 cm column of Amberlite XAD-2 (Eastman Kodak Co., Rochester, NY). The column was washed with 300 ml of water and then 10 ml of methanol. The flavonoid fraction was then eluted with 50 ml of methanol. The light absorption at 405 nm by the flavonoid was determined after adding one drop of NaOMe to 0.5 ml of solution. The quantity of flavonoid in each sample was calculated from the standard curve (Fig. 1). The nonflavonoid phenols that are also adsorbed and eluted from the Amberlite XAD-2 column were separated from the flavonoid fraction by paper chromatography with ethyl acetate/formic acid/

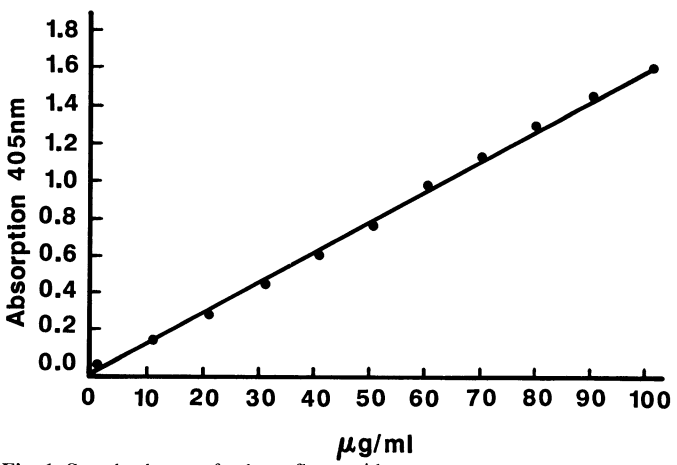

Fig. 1. Standard curve for bran flavonoids.

[1]Published with the approval of the Director of the Agricultural Experiment Station as Journal Series 1778.

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This paper was prepared for electronic processing.

water (66:14:20, v/v) and 15% acetic acid. The UV spectrum of this fraction was used in developing the quantitative method.

RESULTS AND DISCUSSION

Flavonoids in Wheat Classes

Chromatographic properties. The flavonoids in four classes of wheat were chromatographed side by side on paper using three different solvents and on polyamide plates as described in the Methods section. For each chromatographic system the R_f values of 0.57, 0.31, 0.36, and 0.76, respectively, were identified for the four classes of wheat. The high R_f value of 0.57 for the paper chromatograph with 15% acetic acid indicates a glycosyl flavonoid, because the flavonoid aglycones have a much lower R_f value (Mabry et al 1970). This indicates that the flavonoids in the HRW, durum, and white wheats are the same as those

TABLE I
Quantitative Analysis for Wheat Bran Flavonoids[a]

Class and Variety	Mean (μg/g)
Hard red spring	
Len	149.1
Alex	245.4
Stoa	289.0
Hard red winter	
Roughrider	288.2
Agassiz	271.1
Durum wheat	
Rugby	405.7
Lloyd	358.4
Vic	291.2
White wheat[b]	320.3
Overall mean	290.9

[a]Single extraction analyzed in duplicate.
[b]Variety not known.

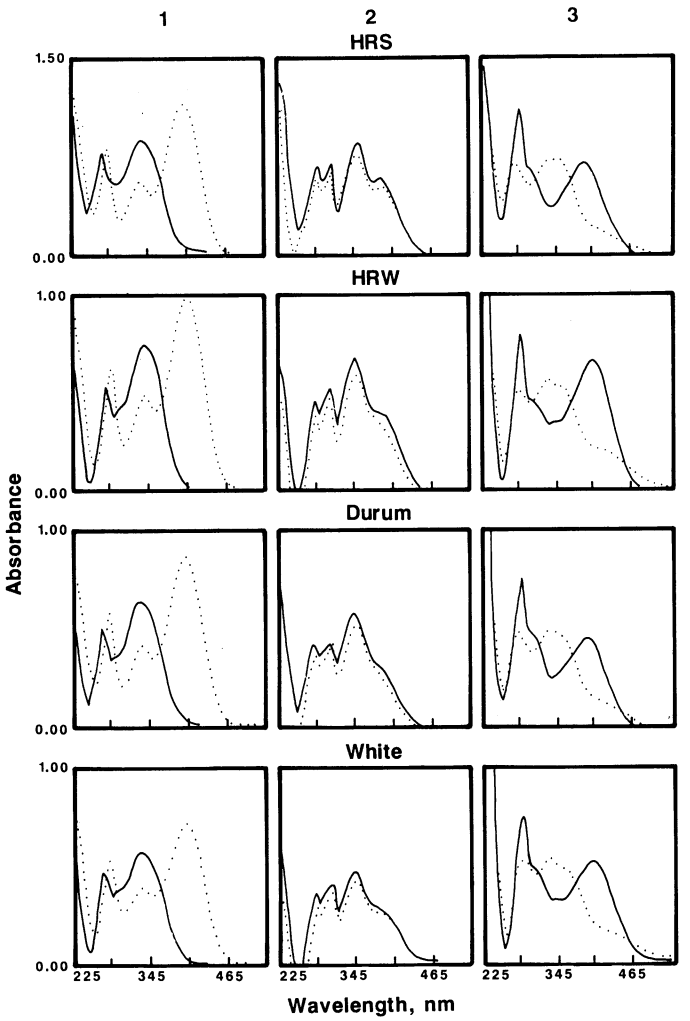

Fig. 2. Ultraviolet spectra of flavonoids from four classes of wheat. The varieties are the same as in Table I. Column 1: methanol (—), methanol + NaOMe (· · ·). Column 2: methanol + $AlCl_3$ (—), methanol + $AlCl_3$ + HCl (· · ·). Column 3: methanol + NaOAc (—), methanol + NaOAc + H_3BO_3 (· · ·).

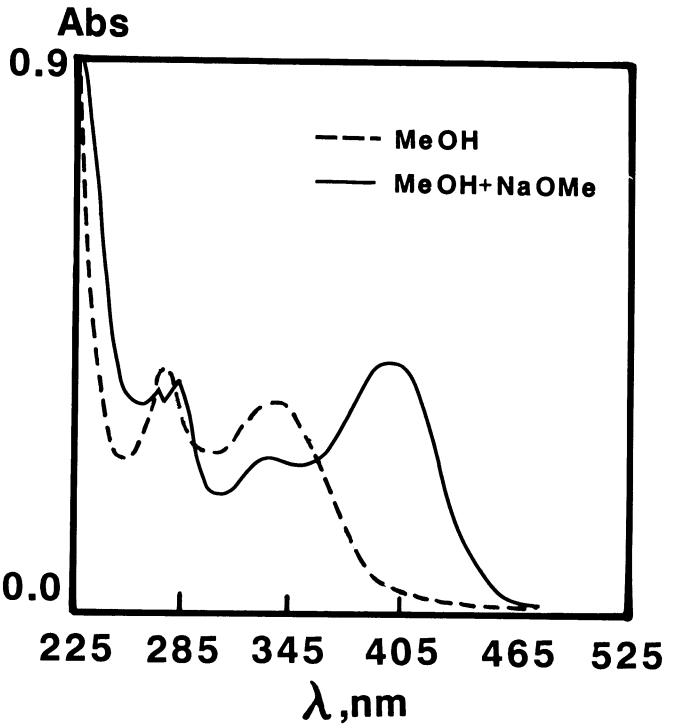

Fig. 3. Ultraviolet spectra of purified bran flavonoids in methanol and methanol plus sodium methoxide.

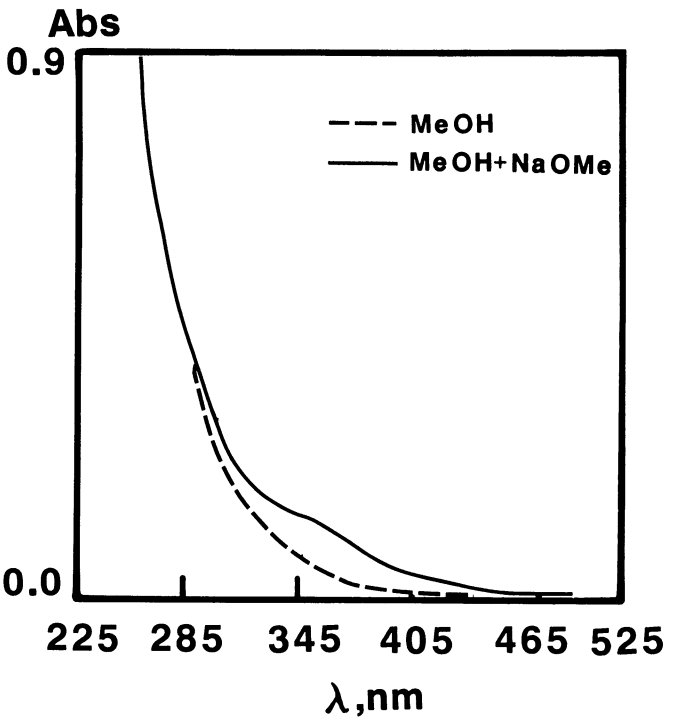

Fig. 4. Ultraviolet spectra of nonflavonoid phenols in methanol and methanol plus sodium methanol also eluted with flavonids from an Amberlite XAD-2 column.

in Len bran flavonoids, reported to be a mixture of 6-C-pentosyl-8-C-hexosyl- and 6-C-hoxosyl-8-C-pentosylapigenin (Feng et al 1988).

UV spectra in methanolic reagents. In Figure 2 the UV spectra in methanol (solid line, column 1) and five methanolic shift reagents (column 1, dotted line, and both lines in columns 2 and 3) for flavonoid fractions from the other HRW, durum, and white classes of wheat were almost identical to those of purified flavonoids from the Len (HRS) bran. Flavonoids are identified by the presence or absence of spectra shifts that occur in the presence of these shift reagents; this is explained in detail in the first paper (Feng et al 1988). The spectra were also identical to those of apigenin (Mabry et al 1970). The flavonoids in each class apparently have an apigenin aglycone as found for bran of Len wheat by UV spectra and ^{13}C-NMR (Feng et al 1988).

The fluorescent colors of bran flavonoids. The appearance of a flavonoid under UV light with and without ammonia (NH_3) treatment can be used to differentiate types of flavonoids. The fluorescent color is related to the flavonoid structure and can be used as a guide in the preliminary identification of flavonoids (Mabry et al 1970). For all four wheat classes the color was dark purple, which turned to yellow green on exposure of the flavonoid spot to NH_3 gas. This indicates that the flavonoid in all four classes of wheat is a flavone with 5-OH and 4′-OH (Mabry et al 1970), as found in apigenin.

Thus, R_f values, UV spectra, and the color under UV light with or without NH_3 indicate that the flavonoids in HRW, durum, and white wheat are probably 6,8- diglycosylapigenins as found in the bran of Len variety HRS wheat (Feng et al 1988).

Quantitative Analysis for Flavonids

The flavonoids in bran give an absorption peak at 396 nm when alkaline NaOMe is added to a methanol solution of the flavonoid (Fig. 3). This absorption peak was used to measure the quantity of flavonoid. The wavelength of 405 nm was chosen to decrease interference from nonflavonoids, which have a peak tailing off into the 396 nm range (Fig. 4). The results of quantitative analysis for flavonoids in wheat are given in Table I. Varieties in the same wheat class were found to vary in the level of wheat flavonoids. However, varieties of durum and white wheat contained higher average amounts of flavonoids than the average for varieties of HRS and HRW wheat. However, the results in Table I show only the relative amounts of flavonoids in wheat bran. On repeated extractions of the bran sample with pH 11 water (NaOH), each extraction continued to extract more flavonoids. The rate of extraction, however, decreased after two 12-hr extractions, as used here, because 60% of the flavonoid extracted was extracted in the first two of the seven extractions.

ACKNOWLEDGMENTS

We thank Gordon Rubenthaler of USDA at Pullman, WA, for the gift of a sample of bran from white wheat.

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[Received February 27, 1989. Revision received May 25, 1989. Accepted June 21, 1989.]