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Fluorescence Detection and Measurement of Ferulic Acid in Wheat Milling Fractions by Microscopy and HPLC

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Ferulic (4-hydroxy-3-methoxycinnamic) acid is known to occur in high concentrations in the aleurone cell walls of wheat kernels, to a lesser extent in the seed coat and embryo, and in only trace amounts in the starchy endosperm. High-performance liquid chromatography (HPLC) is used to quantitatively examine the distribution of ferulic acid and, thus, its morphological host among milling fractions. Fluorescence photomicrographs corroborate the HPLC data and show that ferulic acid is a meaningful indicator of the nonendosperm tissues in the milling process. The application of this specific and sensitive method allows inferences regarding the efficiency of physical separation at selected steps of the milling process. A high correlation exists between HPLC and microspectrofluorometry techniques for determining ferulic acid in a reasonable range. The comparative results establish the potential for rapid ferulic acid determination of bran carryover in flour during milling.

The aim of dry milling of wheat is to separate the bran and germ from the starchy endosperm. Recently, we developed a sensitive analytical liquid chromatographic method chemically specific for bran in order to allow assessment of the efficiency of separation by milling (Pussayanawin and Wetzel, 1987). The rationale for this approach and its application are presented in this report. The various tissues of wheat are comprised of different structures and chemical constituents that ultimately determine the nutritional value and functional properties of the milling end products. Several chemical compounds in wheat can be used as precise indicators of selected botanical parts of the seed: pericarp, testa, aleurone, embryo, endosperm. Besides proteins, carbohydrates, and lipids, cereal grains also contain lesser quantities of noteworthy organic compounds such as vitamins, phenolics, aromatic amines, and amino acids. All of these components are synthesized and stored in specific tissues, making three major grain fractions (bran, germ, endosperm) chemically and morphologically distinct from each other.

Ferulic (4-hydroxy-3-methoxycinnamic) acid autofluoresces in the blue region of the spectrum, and prior to this work, fluorescence microscopy had been used to localize ferulic acid in cereal kernels (Fulcher et al., 1972; Fulcher and Wong, 1979; Fulcher, 1982; Smart and O'Brien, 1979). Ferulic acid was found in high concentration in the aleurone cell walls and also in the seed coat and embryo of wheat, but not in significant quantities in the starchy endosperm of the mature grains (Fulcher, 1982). The measurement of botanical parts by using the fluorescence characteristics of pericarp, aleurone, and endosperm was previously done on wheat fractions where data were evaluated by a statistical model (Jensen et al., 1982; Jensen and Martens, 1982). The model was initially calibrated against fluorescence data for manually dissected botanical parts and synthesized mixtures with known compositions. The resulting profile of 10 portions successively removed from the outside to the inside of the kernel suggested that the determination of ferulic acid by fluorescence measurements might be a desirable way of establishing the purity of endosperm separated during milling.

In the milling process, the efficiency of separation also needs to be determined by measuring the quality of the

Table I. Pilot Milling (Kansas State University) Fractions in Order of Mill Flow[a]

break system	residue system	reduction system
prebreak	tailings (purifier)	fine-sizing redn (top)
1st break	2nd quality stock	fine-sizing redn (bottom)
2nd break	suction recovery	coarse-sizing redn (top)
3rd break		coarse-sizing redn (bottom)
4th break		1st middlings (top)
5th break		1st middlings (bottom)
bran duster		2nd middlings (top)
		2nd middlings (bottom)
		3rd middlings
		4th middlings
		5th middlings
		6th middlings

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resulting product (flour). Ferulic acid esterified to the water-soluble pentosans of wheat flour was postulated to be involved in the oxidative gelation mechanism (Hoseney and Faubion, 1981). This phenomenon may change the rheological (mixing) properties of the dough. Furthermore, the free phenolic acids may contribute to an objectionable flavor of flour and other milling fractions (Maga and Lorenz, 1973). In the present study, we evaluate ferulic acid, determined by high-performance liquid chromatographic (HPLC) and quantitative optical procedures, as an index of bran carryover in the products of a milling operation.

EXPERIMENTAL SECTION

Wheat Samples. Hard red winter wheats, representing the 1984 and 1985 crop years, were grown and sampled at known locations in Kansas, Colorado, Nebraska, Oklahoma, and Texas. Two samples from 1984 and two samples from 1985 were milled individually on the KSU pilot mill. Table I shows different milling fractions produced from three systems of the pilot mill. Representing 15 varieties from the 1984 crop year, 16 samples were milled individually on the Miag Multomat mill. Moisture and mineral content (ash) were gravimetrically determined by AACC approved methods 44-19 and 08-01; protein content was determined by the Kjeldahl procedure 46-10 (American Association of Cereal Chemists, 1981). After foreign material and broken kernels had been removed, each wheat was sampled for physical wheat tests. The weight in grams per 1000 kernels was determined with an electronic seed counter, on a 40-g sample. Wheat was sized by sifting 200-g samples for 1 min on a Ro-Tap sifter, and the amount over the no. 7 Tyler standard sieve was calculated as the percentage of large kernels. Pearling value was determined as the percent of original sample remaining over a 20-mesh wire after the sample was abraded for 1 min in a Strong-Scott Laboratory Barley Pearler (Strong-Scott Manufacturing Co., Minneapolis, MN).

HPLC Determination of Ferulic Acid. Ferulic acid was determined by the previously described high-performance liquid chromatographic procedure developed in our laboratory (Pussayanawin and Wetzel, 1987). Samples of ground wheat or flour were dispersed in 0.2 N sulfuric acid and hydrolyzed for 30 min in a boiling water bath. After hydrolysis, the extracts were incubated in a 55 °C water bath for 60 min with an α-amylase preparation to clarify the extract. After centrifugation, the clear extracts were then transferred into volumetric flasks, diluted to volume with distilled water, and filtered through a Millipore filter (HATF 01300; Millipore Corp., Bedford, MA).

Determination was performed on a HP 1084B liquid chromatograph (Hewlett-Packard, Avondale, PA). The HPLC system included a FS 970 fluorescence detector (Kratos Analytical Instruments, Ramsey, NJ) and was operated with an excitation wavelength of 312 nm and a filter passing wavelength beyond 418 nm on the emission side of the flow cell. Separation was performed with a 100 mm × 4.6 mm (i.d.) column packed with ODS-Hypersil (5 μm; Shandon Southern Instruments, Inc., Sewickley, PA). A percolumn (Upchurch Scientific Inc., Oak Harbor, WA) packed with 10-μm ODS packing material was used to protect the analytical column. The mobile phase was a mixture of 12% methanol and a sodium citrate buffer. A buffer solution was prepared by adjusting 0.01 M citric acid to pH 5.40 with concentrated sodium hydroxide solution. The flow rate was 1.0 mL/min.

Fluorescence Microscopy. Fluorescence photomicrographs of all mill fractions were obtained by dispersing aliquots of product in fluorescence-free immersion oil on glass slides. The mixtures were mounted on the slides under cover glasses and examined with a fluorescence microscope (Carl Zeiss, West Germany) equipped with a IIIRS epiilluminating condenser and HBO 100-W mercury arc illuminator. The instrument was fitted with a fluorescence filter system containing an exciter filter (λ_{max} 365 nm) and barrier filter (λ_{max} >418 nm) that optimize ferulic acid fluorescence. Photomicrographs were obtained with high-speed (ASA 400) 35-mm film.

Microspectrofluorometry. A UMSP80 microspectrofluorometer (Carl Zeiss) was used to quantitate ferulic acid fluorescence in the flour samples. The instrument was also equipped with an epiilluminating condenser, HBO 100-W mercury illuminator, and 365/418-nm fluorescence filter combination. Each flour sample was "piled" on a standard glass microscope slide and flattened with another slide to produce a sample approximately 2-cm width × 2-cm length × 2-mm depth. Samples were placed under a 10× Neothear objective on the UMSP80, and the mean fluorescence was determined from intensity measurements at 25 random points at the surface of each specimen. A uranyl glass standard was used as the full-scale fluorescence intensity standard.

RESULTS AND DISCUSSION

Fluorescence Microscopy of Bran Contamination in Milling Fractions. Because the aleurone layer contains high concentrations of ferulic acid, the cell walls of the layer (and of the scutellar portion of the germ) are readily detectable by fluorescence microscopy (Fulcher et al., 1971), and the technique affords a rapid method for at least qualitative assessment of ferulate-containing structures in flours. Consequently, fluorescence micrographs of fractions of the hard red winter wheat variety Newton, milled on the Kansas State University Ross Walking Mill, illustrate the extent to which nonendosperm tissues (as shown by aleurone cell wall fragments) contaminate various millstreams (Figure 1).

Not surprisingly, there is remarkable agreement between the extent to which aleurone cell wall fragments are detected and the ferulic acid content of the flour fraction. Figure 1a, for example, provides a view of the 1BK fraction (23.1 μg/g of ferulic acid) with occasional aleurone cell wall fragments and rather large endosperm particles. Similarly, the 1M fraction, containing somewhat lower ferulate levels (17.6 μg/g), shows apparently smaller flour particles and infrequent, small, aleurone cell wall pieces (Figure 1b). In contrast, the coarser fractions 3M, 5BK, 1T, and 5M show a concomitant increase in aleurone cell wall fragment size and frequency (Figure 1c–f), which parallels the marked increases in ferulic acid content of these fractions (63.9, 85.1, 135.9, and 359.6 μg/g of ferulic acid, respectively). In crude terms, at least the fluorescence microscope is capable of providing a rapid assessment of aleurone cell wall, and consequently bran content, in different flours.

Distribution of Ferulic Acid in Milling Fractions. Table II shows the distribution of ferulic acid and ash in 23 flour milling fractions of four different HRW wheats milled on the KSU pilot mill. Wheats A and B of the 1985 crop were milled on the same day in the summer of 1985. Wheats C and D were harvested in 1984 and were milled on the same day in the summer of 1984. A significant difference of ferulic acid values was observed between streams of a high-bran contamination and those of low-bran contamination. The range of ferulic acid values (high exceeding low by 10–20-fold) was greater than the corresponding range of ash values. This could be explained by the fact that the endosperm ash is approximately 20–26% of the total wheat ash (Hinton, 1959) and the ferulic acid

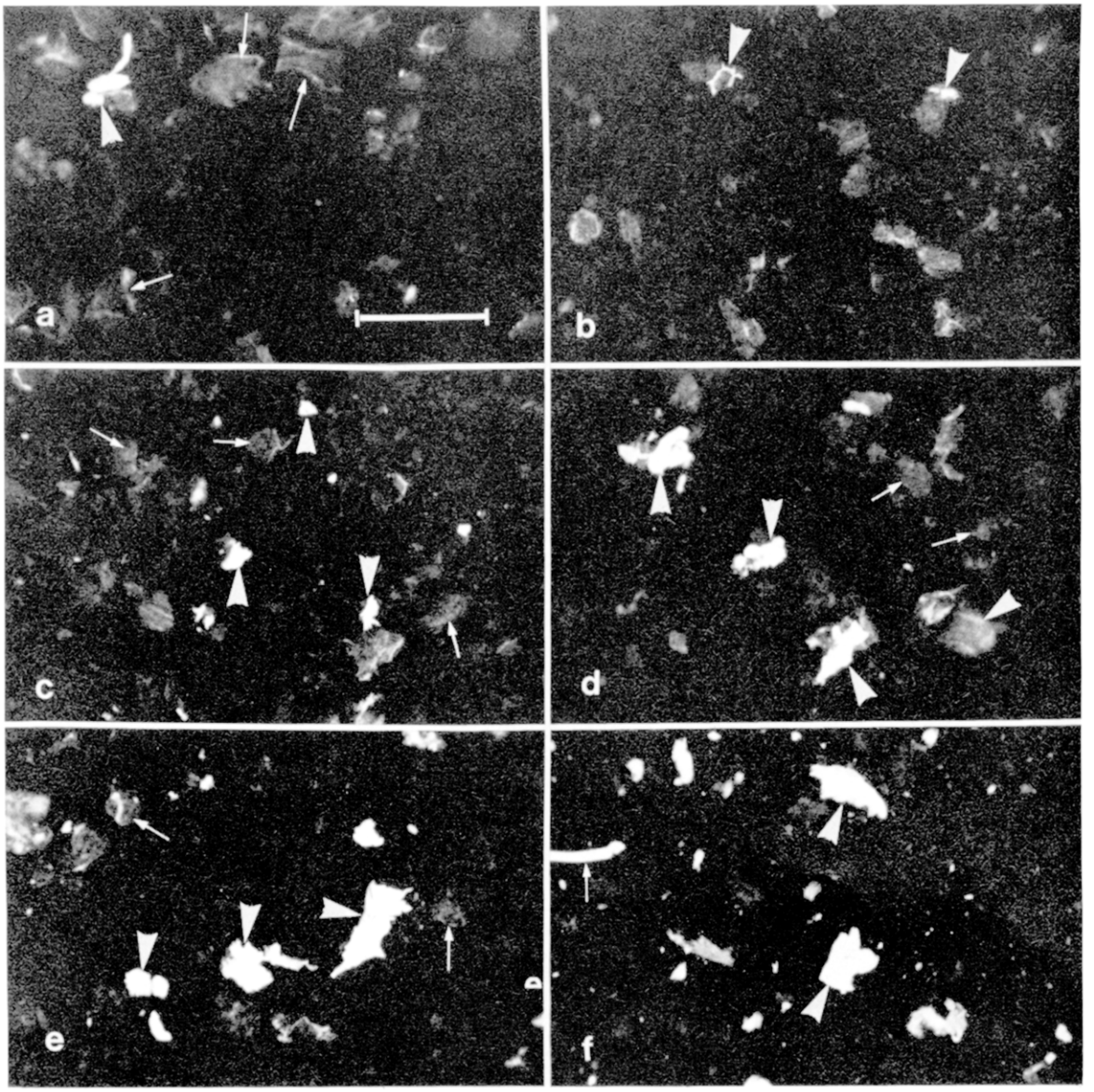

Figure 1. Fluorescence micrographs of flour suspensions in glycerol, excitation maximum 365 nm, emission maximum 450 nm. The brightest particles in each micrograph represent portions of ferulic acid rich aleurone cell walls. Micrographs were taken to represent a typical sample of each flour stream examined. Bar on 1a = 175 μm. All micrographs taken at equivalent magnifications. (a) First break (1BK) flour, 23.1 μg/g of ferulic acid. Occasional aleurone cell wall fragments are visible (large arrow) amid relatively large endosperm cell wall particles that also contain some ferulic acid specific fluorescence in the thin cell walls (small arrows). (b) First midds (1M) flour, 17.6 μg/g of ferulic acid. Only small aleurone cell wall fragments are occasionally visible (arrow), and remaining flour particles are not noticeably reduced in average diameter. (c) Third midds (3M) flour, 63.9 μg/g of ferulic acid. A significantly higher frequency of contaminating fluorescent particles (large arrows) is visible among the relatively fine-size particles (small arrows) typical of reduction flours. (d) Fifth break (5BK) four, 85.1 μg/g of ferulic acid. As in (a), the particle sizes are typically fairly large, with a signficantly higher frequency of fluorescent aleurone wall fragments (large arrows) reflecting the increased levels of ferulate. Some endosperm particles are also evident (small arrows). (e) First tailings (1T) flour, 135.9 μg/g of ferulic acid. Aleurone wall fragments (large arrows) also contaminate the millstream significantly, and fewer midendosperm particles are visible (small arrows). (f) Fifth midds (5M) flour, 359.6 μg/g of ferulic acid. A typical very coarse fraction with high levels of contaminating aleurone cell walls (large arrows), occasional hairs (trichomes) derived from the kernel surface (small arrow), and only fine midendosperm particles.

of the endosperm cell wall is less than 5% of the total ferulic acid of the kernel.

The higher ash content of the first two break (1BK, 2BK) flours compared to the first two middlings reduction flours (1M, 2M) shows that the gradient in mineral content decreases from outer to inner layers of the endosperm. The high ash content of the break flours is due to a higher proportion of the peripheral endosperm cells (Kent and Jones, 1952), which have higher ash and protein contents than the central endosperm cells (Hinton, 1959). The ferulic acid contents of the 1BK and 2BK flours are slightly different from those of the 1M and 2M flours. The ferulic acid level of the 4BK flours, which have a dark color, is slightly different from that of the 3M flours, but the ash content of the 4BK flours is consistently high. This suggested that the ash in the 4BK flours is mainly from the minerals of the endosperm, rather than the bran. The 4BK flours may contain particles from the subaleurone layer, which is high in mineral and protein contents. Our data indicate that within the intact kernel the distribution gradient of ferulic acid in the endosperm is much less than the gradient of mineral content.

Flours from coarse sizing reductions are relatively higher in ferulic acid content than the fine sizings flours. When the flours from the sizings reductions are separated into top and bottom flours, the flours collected at the bottom

Table II. Distribution of Ferulic Acid and Mineral Content in Milling Fractions (KSU Pilot Mill)

	wheat A		wheat B		wheat C		wheat D	
fraction[a]	FA	ash	FA	ash	FA	ash	FA	ash
PBK	33.3	0.51	24.4	0.38	34.4	0.37	31.8	0.42
1BK	21.1	0.45	16.9	0.35	16.1	0.27	16.5	0.36
2BK	21.2	0.42	17.3	0.41	13.9	0.31	16.7	0.38
3BK	23.9	0.43	24.4	0.41	21.8	0.37	23.1	0.43
BR	24.0	0.40	26.3	0.35	21.5	0.32	27.0	0.37
4BK	37.0	0.52	38.1	0.51	33.2	0.50	39.6	0.60
5BK	72.1	0.82	81.6	0.89	70.7	0.75	92.0	0.87
BSD	287	1.9	266	1.9	231	1.5	283	1.9
SUC	55.0	0.54	44.2	0.48	40.8	0.51	48.4	0.52
1T	52.1	0.62	69.6	0.67	93.3	0.72	88.2	0.73
2Q	41.0	0.48	45.1	0.43	51.8	0.48	45.5	0.49
CST	20.6	0.35	20.5	0.30	26.1	0.36	22.5	0.38
CSB	24.3	0.32	28.3	0.29	40.5	0.35	31.6	0.34
FST	15.7	0.32	19.4	0.29	18.2	0.29	19.3	0.31
FSB	17.7	0.29	21.5	0.27	18.5	0.26	19.8	0.30
1MT	13.9	0.28	15.0	0.25	15.4	0.28	17.3	0.29
1MB	14.2	0.29	16.6	0.26	25.3	0.28	20.8	0.29
2MT	12.8	0.30	15.9	0.26	17.5	0.28	19.2	0.30
2MB	21.3	0.30	24.3	0.26	29.6	0.29	19.8	0.31
3M	33.9	0.35	32.1	0.30	48.1	0.40	45.9	0.41
4M	72.6	0.55	76.2	0.54	128	0.69	118	0.69
5M	180	1.1	118	1.0	195	1.2	210	1.5
6M	733	3.3	434	2.1	795	3.4	789	3.4

[a] These streams correspond to those named on Table I: BK = break; BSD = bran duster; SUC = suction; 1T = first tailing; 2Q = quality reduction; M = middlings reduction; CST and CSB = coarse-sizings top and bottom; FST and FSB = fine-sizings top and bottom. FA measured in micrograms/gram and ash in percent.

sieves (CSB, FSB) consistently have higher ferulic acid contents than the ones collected at the next higher sieves (CST, FST). This observation is consistent with the visual determination (Pekar test). The darker appearance of the bottom fractions is due to a scattering effect of the larger particles. The evidence suggested that the flours of larger particle size collected at the bottom sieves may contain aleurone fragments, since the aleurone tissues are more difficult to grind, and will be larger in size when compared to flour particles after a reduction process. The less sensitive ash determination fails to discriminate between these two significantly different materials.

Changes in Ferulic Acid with Increasing Extraction Rate. Miller's curves were generated on the basis of cumulative ferulic acid, expressed as a function of total percent flour (Figure 2A). The shape of the curves has general significance, since it will give some indication of the miller's skill. The change in ferulic acid is gradual up to 55% extraction, becomes progressively greater in the range 55–65%, and becomes very much greater above 65%. In the first region, where the flours are considered to have high endosperm purity, the streams generally include the first three break flours (1BK, 2BK, 3BK), the first and second midds flours (1M, 2M), and the sizings flours (FST, FSB, CST, CSB). The second region includes the third midds (3M) and fourth break (4BK) flours and the flours from the residue system (SUC, 1T, 2Q). In the last region, the flours are high in bran (aleurone layer) and germ fragments. These include flours from the last break (5BK), bran duster (BSD), and the low-grade flours (5M, 6M).

When the miller's curves were produced on the traditional basis of ash (Figure 2B), the first region where the gradual change in ash content occurs is shorter than the first region of ferulic acid curves. This gradual change ended at approximately 45% extraction. The wider range (45–65%) of the second region of the ash curves resulted from the contribution of endosperm ash by the break flours (1BK, 2BK, 3BK, 4BK). When the total flour corresponding to a given extraction rate is sold in the form of separate grade, the specification based on the ash curves will necessitate some high-protein streams being diverted

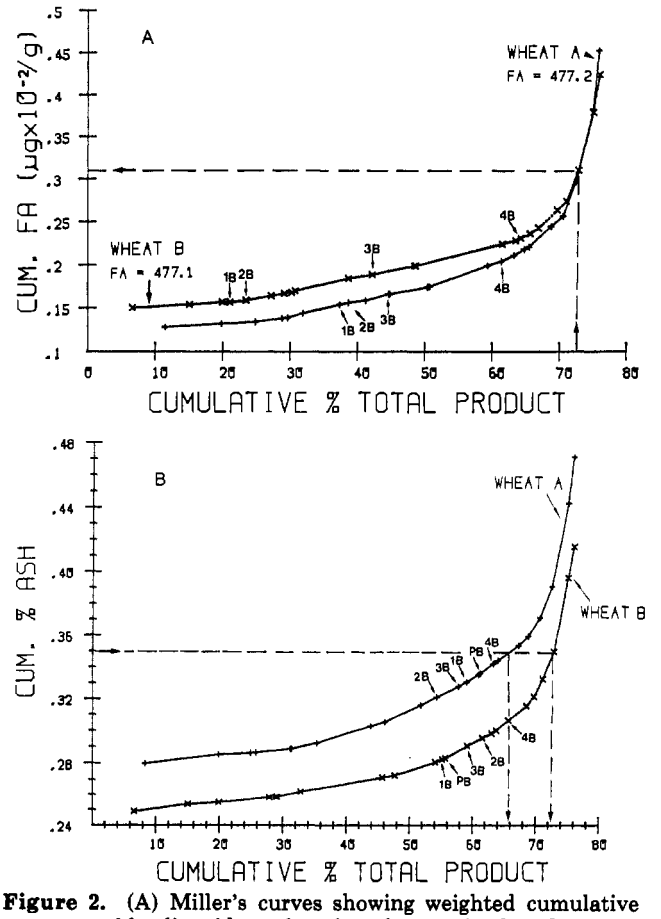

Figure 2. (A) Miller's curves showing weighted cumulative carryover of ferulic acid as a function of extraction based on total mill output for two different wheats with break flours identified (KSU pilot mill). Wheat A stream in order of increasing ferulic acid: 2MT, 1MT, 1MB, FST, FSB, CST, 1BK, 2BK, 2MB, 3BK, BR, CSB, PBK, 3M, 4BK, 2Q, 1T, SUC, 5BK, 4M, 5M, BSD, 6M. Wheat B streams in order of increasing ferulic acid: 1MT, 2MT, 1MB, 1BK, 2BK, FST, CST, FSB, 2MB, 3BK, PBK, BR, CSB, 3M, 4BK, SUC, 2Q, 1T, 4M, 5BK, 5M, BSD, 6M. (B) Miller's curves based on cumulative ash for the same two wheats shown in Figure 2A (KSU pilot mill).

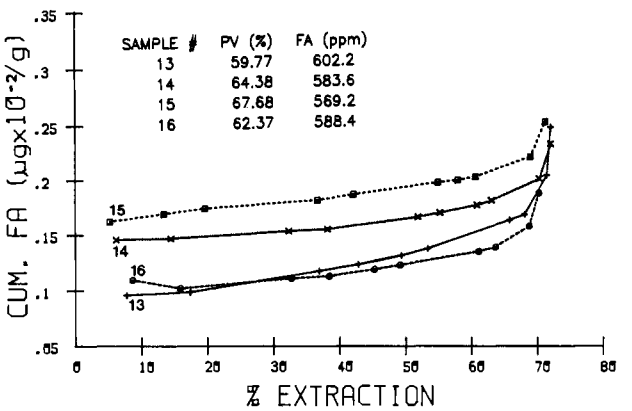

Figure 3. Miller's curves showing weighted cumulative carryover of ferulic acid as a function of extraction based on total mill output (Miag Multomat mill) for four wheats (PV = pearling value, %; FA = ferulic acid, μg/g).

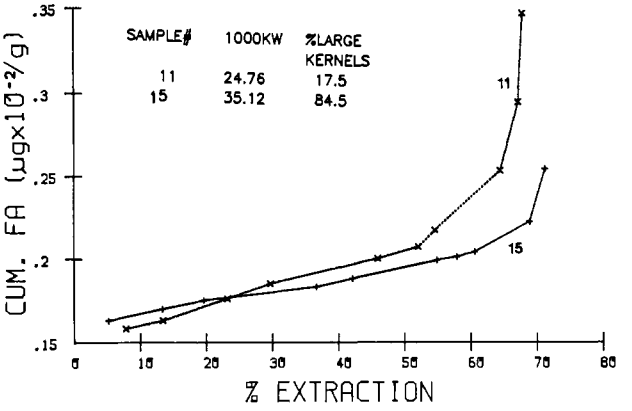

Figure 4. Typical miller's curves based on cumulative ferulic acid for large-kernel wheat and small-kernel wheat (Miag Multomat mill).

to lower priced (clears) grade. Comparing the ferulic acid and ash curves in each milling experiment, the ash curves tend to separate because of the differences in their endosperm mineral content. The threshold levels of the ferulic acid curves tend to stay close together, since the endosperm contains very small quantities of ferulic acid. These threshold levels apparently have no relation to the ferulic acid content of whole wheat. This large effect of the differences in the endosperm mineral content of each wheat has cost the milling industry a fortune through indiscriminate application of ash specifications. For example, when an ash content of 0.35% was used as a specification of high-quality flour, wheat B produced 72% high-grade flour and wheat A produced only 66% high-grade flour (Figure 2B). From ferulic acid curves (Figure 2A), there was no difference between actual bran contamination (based upon the selective determination of ferulic acid content) of the two wheats at 72% extraction. The high mineral content of the endosperm of wheat A would cause the industry to lose 6% of higher priced flours because of misclassification.

In general we observed that the ferulic acid contents of flours of similar extractions from different wheats varied relatively little whereas the mineral content showed considerable variation. In addition to the stream analysis of millings represented here in Table II and Figure 2A,B, cumulative ferulic acid and ash curves were also generated from five different milling experiments with the Miag Multomat mill involving three, four, two, three, and four wheats each, respectively. In these experiments, which involved a total of 16 millings, most of the cumulative ash curves tended to separate similar to those in Figure 2B. This is not unusual because of the variation among the wheats in the mineral content of their endosperms. From observations based on these 16 different wheats (Pussayanawin, 1986), the threshold level of ferulic acid curves appeared to have no relation to the ferulic content of the whole wheat. Figure 3 includes cumulative ferulic acid curves for one of these milling experiments involving wheats 13, 14, 15, and 16. In the few cases where we observed differences in the threshold level of ferulic acid curves, it was interesting to note that within each milling experiment the curves with the high-threshold ferulic acid levels belonged to the wheats with high pearling values. A typical example is wheat 15 in Figure 3. The pearling value is the percent of original sample remaining over a 20-mesh wire after pearling for 1 min in a Strong-Scott Laboratory barley pearler. The action of the pearler is to shear the bran coat from the endosperm. The pearling value has been believed to indicate the degree of hardness of the endosperm (Taylor et al., 1939). Observed differences in the threshold level of cumulative ferulic acid curves for milling wheat of similar ferulic acid content within such controlled milling experiments indicate a difference in the ease of separation of the endosperm from the other parts of the kernel by the milling. Our results suggest that flour milled from wheats of the same class with a high pearling value tend to have more bran contamination consistent with the typical data in Figure 3.

The significant difference in the shape of ferulic acid curves between small-kernel and large-kernel wheats is shown in Figure 4. The smaller the kernel, the higher the level of bran and germ entering the milling system. In the milling of small-kernel wheats, the amounts of the later reduction flours produced were low but their bran contamination was higher than in the millings of large-kernel wheats. For small-kernel wheats, the change in ferulic acid was gradual up to 55% extraction and then progressively greater above this point. Large-kernel wheats showed a gradual rise in ferulic acid up to 65% extraction. The rising slope from the lowest extraction to 60% extraction differed between large- and small-kernel wheats. The large variation of ferulic acid content of the 4 and 5 midds flours between different wheats reflected by cumulative ferulic acid beyond 65% extraction apparently is not related to the ferulic acid content of the parent wheats. (Note the last two points respectively of each curve in Figure 4.)

Comparative HPLC and Microspectrofluorometry for Ferulic Acid Quantitation. Although the fluorescence microscope in its usual configuration is able only to provide a qualitative assessment of ferulic acid content in millstreams (inferred from the presence of aleurone cell wall fragments), quantitative assessments are also possible by adapting a spectrophotometer to a typical fluorescence microscope. With use of the UMSP80 as such an instrument, it was possible, therefore, to evaluate the potential of optical detection methods as a rapid screening method for bran content without the requirement for hydrolysis, extraction, and chromatography. A comparison of ferulic acid values obtained on a set of millstreams by the HPLC procedure and by the microspectrofluorometer is shown in Table III. The data show that the sensitivity of the fluorescence (microscopic) method is somewhat decreased due either to the quenching effects of other bran components (e.g., oil, pericarp phenolic compounds) or to a self-absorption phenomenon in the fractions. Nonetheless, a high correlation exists betwen the microspectrofluorometry and HPLC methods (excluding the 5M flours, a correlation coefficient of 0.97 was obtained). The procedure is complete in less than 1 min for each sample, and

Table III. Comparative HPLC and Microspectrofluorometry Techniques for Ferulic Acid Determination

sample[a]	HPLC ferulic acid, μg/g	rel fluorescence intens mean	std dev	std error
		Wheat 6		
1BK	15.5	33.84	2.01	.39
2BK	15.6	32.16	2.59	.52
grader	21.4	35.64	3.01	.60
3BK	47.6	44.64	4.10	.82
1M	11.7	30.80	2.39	.48
2M	16.1	34.20	3.28	.66
3M	29.8	39.40	4.31	.86
1MR	12.4	31.61	2.52	.50
4M	85.4	53.08	8.93	1.79
5M	348.5	77.96	11.39	2.28
		Wheat 7		
2BK	12.6	32.01	1.47	.29
4M	73.4	47.52	5.65	1.13
5M	396.9	76.08	9.98	1.99
		Wheat 8		
2BK	14.6	32.52	2.26	.45
5M	484.2	77.08	6.63	1.33
		Wheat 9		
5M	574.9	85.16	12.58	2.52

the high correlation between the two methods establishes the potential for development of rapid, sensitive assays of ferulic acid (and hence bran) content of flour streams using strictly optical systems. Furthermore, the extremely low ferulic acid content of the endosperm makes it possible to establish an absolute value for judging the purity of flours, while the sensitivity and specificity of the HPLC procedure provide an authentic basis for measuring bran carryover and for standardizing future rapid quality control methods for ferulic acid.

Registry No. Ferulic acid, 1135-24-6.

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An Analytical Method for Residues of Imazalil in Tomatoes and Bell Peppers after Postharvest Application and Storage

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A method using gas–liquid chromatography with electron capture detection was developed for residues of the fungicide imazalil in tomatoes and bell peppers. Recoveries from fortified controls ranged from 90.1 to 95.7% for tomato samples and from 73.6 to 79.2% for pepper samples. Both fruits were dipped in aqueous solutions containing 50, 100, or 250 mg/L imazalil, and the residues were determined after various storage times. The fruits were stored at 10 °C for 3 days and 21 °C thereafter to simulate commercial shipping and storage. Residues of imazalil in tomatoes for the three concentrations of dip were 0.24, 0.45, and 0.84 mg/kg 2 h after dipping and 0.13, 0.25, and 0.53 mg/kg after 15 days of storage. Similarly, residue levels in bell peppers were 0.51, 0.55, and 1.51 mg/kg 2 h after dipping and 0.05, 0.11, and 0.28 mg/kg after 12 days of storage.

The use of imidazole-based compounds to control many fungal and some bacterial disorders in both agricultural and medical fields has steadily increased in recent years. Medical uses of imidazole containing antifungal drugs have recently been reviewed by Beggs et al. (1981). The present work concerns the agricultural uses of one imidazole-based compound, imazalil, 1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1*H*-imidazole, which has been shown to have remarkable activity on penicillium citrus fruit rots and significant activity on many other fruit rots such as alternaria rot, stem end rot, and sour rot (Laville et al., 1977). Subsequently imazalil has been found to be effective in controlling postharvest alternaria rot of tomatoes and peppers (Spalding, 1980). The analysis of imazalil by gas–liquid chromatography (GLC) and high-pressure liquid chromatography (HPLC) has been reported by several

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