

# Relationship of Flour Aleurone Fluorescence to Flour Refinement for Some Canadian Hard Common Wheat Classes<sup>1</sup>

S. J. SYMONS and J. E. DEXTER<sup>2</sup>

## ABSTRACT

Cereal Chem. 70(1):90-95

Millstreams from pilot-scale millings of commercially grown wheats from the Canadian hard common wheat classes Canada Western Red Spring, Canada Prairie Spring, Canada Western Red Winter, and Canada Western Utility were used to evaluate the potential of fluorescence imaging of aleurone tissue as a flour refinement indicator. Flour aleurone fluorescence was measured using UV excitation (excitation 365 nm, barrier >420 nm). Aleurone fluorescence is acknowledged to be due to ferulic acid, which is highly concentrated in aleurone cell walls. In the current study, for every wheat class examined, aleurone fluorescence was highly correlated with ferulic acid content for every millstream with the exception of bran finisher flour. Bran finisher flour gives a moderate aleurone

fluorescence despite a high level of ferulic acid. For all wheat classes, break flours gave a distinctly lower aleurone fluorescence than did reduction flours of comparable ash content and color; the former have a lower aleurone content on the basis of ferulic acid content. Aleurone fluorescence has good potential for on-line monitoring of mill performance because it is strongly related ( $P < 0.01$ ) to the ash content and brightness of reduction flours, a primary determinate of mill efficiency, for all wheat classes. The relationships of aleurone fluorescence to flour ash content and flour color were homogeneous for Canada Western Red Spring wheats from three crop years and two locations but were heterogeneous between wheat classes.

Flour derived from essentially pure starchy endosperm is highly valued because of its brightness and superior processing capability (Ziegler and Greer 1971). Therefore, the goal of millers who wish to produce a white flour is to achieve the maximum efficiency of separation of the starchy endosperm from other wheat kernel tissues. Flour ash content and flour grade color determinations are used widely to measure the degree of flour refinement. An increase in flour ash content is indicative of less refined flour because the starchy endosperm has a much lower ash content than does aleurone or pericarp tissue (Hinton 1959, Morris et al 1946). The duller appearance of a less refined flour is reflected by a higher flour grade color value (Kent-Jones and Martin 1950).

Neither ash content nor grade color are completely effective flour refinement indices because they do not measure the contamination of the flour by nonendosperm tissue directly but rather measure related factors. Fulcher et al (1972) demonstrated that the bluish white autofluorescence of the thick aleurone cell walls seen under UV excitation (filter combination III) was identical to that of pure ferulic acid crystals. This strongly suggested that ferulic acid, which is highly concentrated in aleurone tissue, was the compound responsible for the fluorescence. Munck et al (1979) suggested that quantifying fluorescence compounds, such as ferulic acid, which are highly concentrated in a particular grain tissue, could provide a direct measure of flour refinement. Subsequently, Jensen et al (1982) used fluorescence to estimate aleurone, pericarp, and germ contamination of flour. Fulcher et al (1987), Wetzel et al (1988), Pussayanawin and Wetzel (1987), and Pussayanawin et al (1988) reported that the fluorescence of ferulic acid in flour can be used to estimate aleurone contamination. Pericarp content of flour also can be measured by fluorescence (Kissmeyer-Nielson et al 1985; Munck et al 1979; Symons and Dexter 1991, 1992).

In contrast to flour ash content and grade color determinations, computerized fluorescence methods for the measurement of flour refinement have the potential of providing a rapid, nondestructive analytical system for flour quality determination. A commercial system (Dipix, Ottawa, Ontario, Canada) for the rapid measurement of flour quality using the fluorescence characteristics of flour has been reported (Wetzel et al 1990). These features make fluorescence procedures promising for on-line monitoring of mill

performance. Another promising rapid, nondestructive procedure to estimate flour refinement is the use of reflected-light colorimeters to measure the Commission Internationale de l'Eclairage (CIE) 1976  $L^*$  brightness coordinate of flour (Allen et al 1989; Dexter and Symons 1989; Symons and Dexter 1991, 1992).

The objective of this study was to further investigate the potential of estimating flour refinement by fluorescence imaging using UV-light excitation to detect aleurone tissue. Individual millstreams from pilot-scale millings of a commercially grown No. 1 Canada Western Red Spring (CWRS) wheat sample were used to establish relationships of fluorescence intensity to flour ash content, flour grade color, and  $L^*$  and to flour ferulic acid content. The robustness of the relationships within the CWRS wheat class was evaluated for millstreams from CWRS harvest survey samples from two locations for three successive years. The effect of wheat class on the relationships was evaluated for millstreams from commercially grown samples of the Canada Western Red Winter (CWRW), Canada Prairie Spring (CPS), and Canada Western Utility (CWU) wheat classes.

## MATERIALS AND METHODS

### Wheat

All of the wheats used in this study have been described previously (Symons and Dexter 1992). The No. 1 CWRS control is a composite of rail carlots unloading at terminal elevators during the 1987-1988 crop year. The 1987-1989 harvest samples were the Grain Research Laboratory (GRL) No. 1 CWRS harvest survey composites from the western and eastern prairies. The No. 1 CWU, No. 1 CWRW, and No. 1 CPS wheats all were grown commercially in 1989.

### Milling

Wheat was prepared for milling as described by Dexter and Tipples (1987), tempered as described by Black (1980) to optimum moisture according to hardness (16.3% for CWRS and CWU; 15.5% for CWRW; 15.2% for CPS) and milled using the GRL pilot mill (Black 1980) hard wheat mill flow described by Symons and Dexter (1991).

### Flour Refinement Measurements

Flour ash content was determined in triplicate by the standard AACC (1983) method 08-01. A Simon color grader series IV (Henry Simon, Stockport, UK) was used for triplicated flour grade color determinations as described in the instruction manual.

Tristimulus color coordinate measurements were performed in duplicate with a Minolta Chroma Meter CR-231 (Meyer Instruments Ltd., Cornwall, ON) on dry flour loaded in a Dickey-

<sup>1</sup>Contribution No. 684 of the Canadian Grain Commission, Grain Research Laboratory, 1404-303 Main St., Winnipeg, Manitoba R3C 3G8.

<sup>2</sup>Canadian Grain Commission, Grain Research Laboratory, 1404-303 Main St., Winnipeg, Manitoba, Canada R3C 3G8.

john near-infrared reflectance (NIR) cell. Color readings were expressed by CIE 1976  $L^*$  (lightness),  $a^*$  (red/green chromaticity), and  $b^*$  (yellow/blue chromaticity) color space coordinates (Francis 1983). Only  $L^*$  values are reported because  $a^*$  and  $b^*$  do not relate well to flour refinement (Symons and Dexter 1991).

### Fluorescence Imaging

The fluorescence imaging procedures were described in detail previously (Symons and Dexter 1991). Flour samples were loaded into a Dickey-john NIR cell. For fluorescence imaging, the sample was scanned using a  $\times 10$  Neofluor objective on an Axiophot microscope (Carl Zeiss, Canada). Epi-illumination used an HBO 50 burner through a No. 02 filter combination (excitation 365 nm, barrier  $>420$  nm). The image was detected with a JVC BY110u color camera (JVC, Toronto, Canada) and, for measurement, the green video signal was passed to an AT-IBAS image processing system (Kontron Elektronik, Eching, West Germany) via a video multiplexer. Of the video sources available to us, the signal from the green video channel of the BY110u camera was found to provide a wide dynamic range within the detection limits of the saticon tube. In contrast, the blue video detector, which was highly sensitive to the blue/white aleurone fluorescence signal, was frequently saturated by the same fluorescence signal. The red, green, blue (RGB) camera was selected with knowledge of potential limitations but with the ability to switch rapidly between the three video tubes (RGB) for sample measurement. This facility was required as both aleurone and pericarp fluorescence (Symons and Dexter 1991 1992) measurements were made for each flour sample and each tissue required the use of different filters and video detectors. For each flour sample, 25 images were captured. Flour fluorescence measurements were standardized against a uranyl glass standard.

A fully randomized block design was used for fluorescence measurements. Each block contained all millstreams from all wheats in random order. Each block was considered a single replicate, and all measurements were completed in a single day. Three replicates were run. This design eliminated day-to-day variability (Symons and Dexter 1991) and allowed direct comparison between all of the imaged samples. The coefficient of variation of relative aleurone fluorescence values is about 7%.

### Ferulic Acid Analysis

Each flour was extracted in duplicate by the procedure of Sosulski et al (1982) with minor modifications (Hatcher 1990) to isolate total (free and bound) ferulic acid. Each extract was analyzed in duplicate with a Waters (Milford, MA) 860 chromatography data system using a Supelco (Bellefonte, PA) LC-18 analytical column under conditions described by Hatcher (1990). The coefficient of variation of total ferulic acid content was 12.1%.

### Statistics

All statistics were calculated using the procedures of the SAS (1988) software system version 6.04. Comparison for heterogeneity of regression slopes was by analysis of covariance type I errors (SAS 1986).

## RESULTS AND DISCUSSION

### Aleurone Fluorescence, Ferulic Acid Content, and Refinement Indices of CWRS Flour Streams

As seen in Table I, the first three break (B) flours from the No. 1 CWRS rail carlot composite were higher in ash content and duller in color (higher grade color and lower  $L^*$ ) than were the first three middlings (M) flours and the two sizing (S) flours, which together make up the prime quality reduction flour. In agreement with our previous report (Symons and Dexter 1991), it was not possible to differentiate B1, B2, and B3 from S1, M1, or M2 by aleurone fluorescence. S2 and M3 actually exhibited a slightly stronger fluorescence than did B1, B2, and B3.

Fluorescence microscopy has provided strong evidence that ferulic acid, which is highly concentrated in aleurone cell walls, is responsible for aleurone fluorescence (Fulcher et al 1972, 1987).

In the current study, the aleurone fluorescence of B1, B2, and B3, comparable to that of the highly refined reduction flours, was consistent with ferulic acid contents. B1, B2, and B3 contained ferulic acid levels comparable to S1, S2, M1, and M2 and less ferulic acid than M3.

The only flour to exhibit an anomaly between aleurone fluorescence and ferulic acid content was the bran finisher (BF) flour, which gave a moderate fluorescence despite an extraordinarily high ferulic acid content (Table I). When the BF flour was excluded, ferulic acid content of the remaining flour streams exhibited a very strong relationship ( $r^2 = 0.94$ ) to aleurone fluorescence, comparable to that demonstrated by Fulcher et al (1987) and Pussayanawin et al (1988) for American hard red winter wheat millstreams.

Hard wheat break flours are derived from the outer region of the starchy endosperm (Kent and Jones 1952), which is high in ash content (Hinton 1959; Morris et al 1945, 1946) but low in ferulic acid content (Fulcher et al 1972). Break flours also are higher in pericarp tissue than are prime quality reduction streams (Symons and Dexter 1991, 1992), and pericarp is also high in ash content (Hinton 1959, Morris et al 1946) but low in ferulic acid (Fulcher et al 1972). Therefore, the relatively high ash content of break flours was not related exclusively to aleurone contamination, accounting for the low aleurone fluorescence of break flours relative to reduction flours of comparable ash content.

The B4 flour exhibited an aleurone fluorescence and ferulic acid content consistent with reduction (middling and sizing) flours of comparable ash content and color (Table I). However, in contrast to the other break flours, the B4 flour from our standard hard wheat pilot mill flow (Symons and Dexter 1991) was not derived from grinding of coarse bran-rich material but rather from finer bran-rich material from B3, the purifiers, and the sizings. Therefore, B4 flour was similar in composition to a low-grade reduction stream, as evident from its moderate protein content.

It is well known that the flour grade color of the break flours is not consistent with their functionality. Break flours show superior functionality compared to reduction flours of comparable flour ash and color content. Break flours have improved dough and baking quality in comparison to similar reduction flours (Holas and Tipples 1978, Black et al 1981, Preston et al 1982). The relatively low value for aleurone fluorescence, when compared to ash or grade color, for the break flours suggests that aleurone fluorescence is a better indicator of flour functionality than are either of the traditional quality measurements.

There is no obvious explanation for the consistent anomalous aleurone fluorescence of BF flour except to note that the BF

TABLE I  
Aleurone Fluorescence, Ferulic Acid Content, Refinement Indices, and Protein Content of Flour Streams from a Pilot-Scale Milling of a Composite of No. 1 Canada Western Red Spring Wheat Rail Carlots<sup>a</sup>

Flour Stream	Aleurone Fluorescence (%)	Ferulic Acid ( $\mu\text{g/g}$ )	Ash (%)	Grade Color Units	$L^*$ (%)	Protein (%)
Break 1	33.9	25.0	0.49	0.9	91.0	14.3
Break 2	33.6	22.5	0.49	0.9	90.8	15.7
Break 3	34.0	25.0	0.55	1.5	90.7	18.5
Break 4	45.8	116.3	1.03	6.1	88.8	12.7
Bran finisher	39.2	130.0	1.56	10.7	86.7	21.0
Sizing 1	33.6	18.8	0.39	-1.9	92.3	12.8
Sizing 2	35.6	25.0	0.35	-0.5	92.3	12.2
Middlings 1	33.5	25.0	0.35	-2.5	92.8	11.8
Middlings 2	33.8	27.5	0.40	-1.4	92.2	13.0
Middlings 3	36.2	36.3	0.43	-0.2	92.0	12.4
Middlings 4	42.5	81.3	0.77	3.7	90.8	12.7
Middlings 5	45.3	125.0	0.95	6.0	89.3	12.8
Middlings 6	47.0	177.5	1.47	11.3	87.4	14.6
Straight-grade	36.1	42.8	0.52	0.8	91.4	13.5

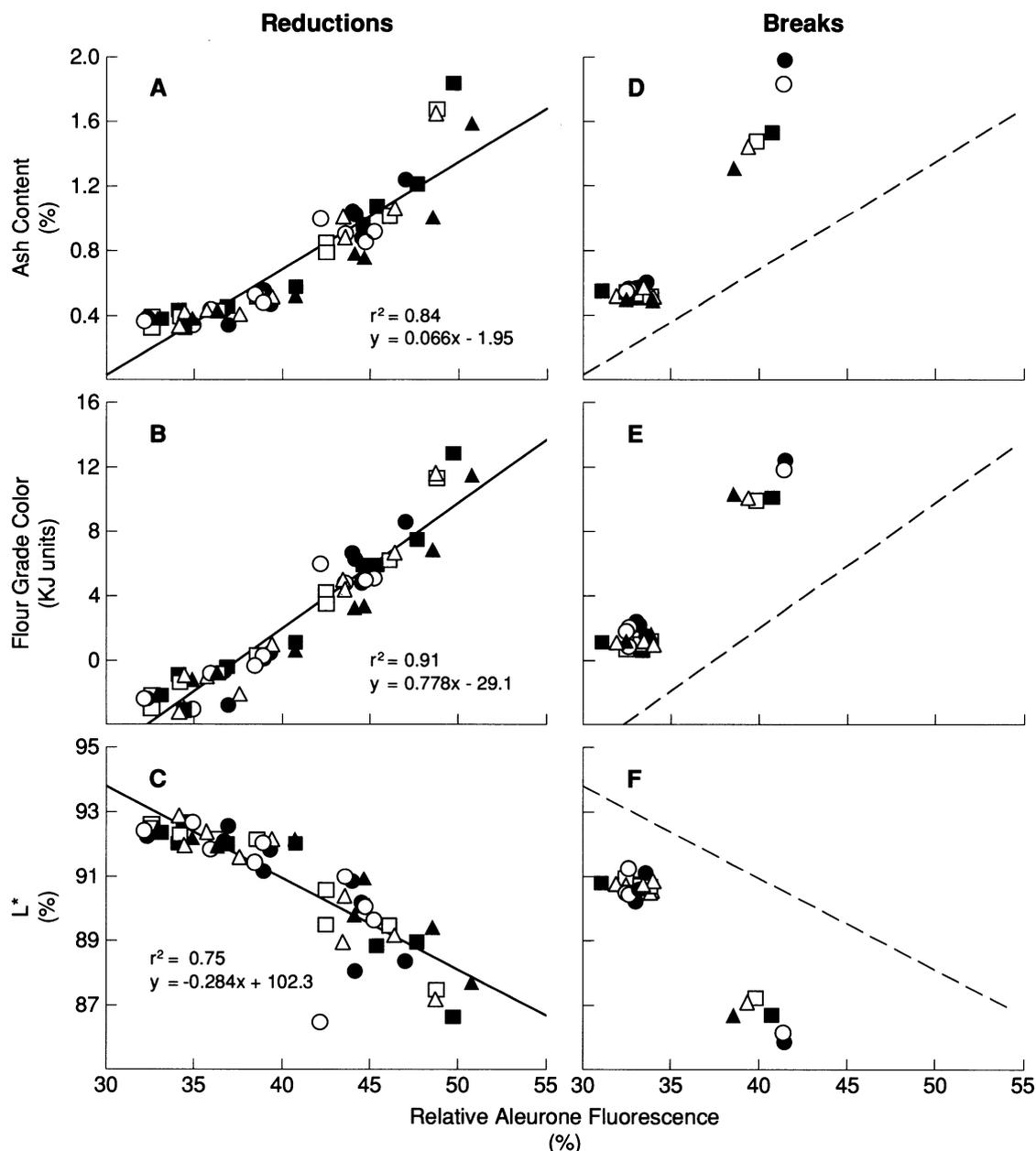
<sup>a</sup>Expressed on "as is" moisture basis.

flour is produced by impacting rather than by grinding. The preparation of flour by impacting may result in contamination of the flour by seed coat tissues that either quench or restrict fluorescence. Fulcher et al (1987) and Pussayanawin et al (1988) also found high levels of ferulic acid in BF flour but did not report BF flour aleurone fluorescence. BF flour is derived largely from the starchy endosperm-bran interface and, therefore, is rich in aleurone tissue. Regardless, the anomalous BF flour aleurone fluorescence observed with our system is of little concern for monitoring flour refinement because BF flour is a minor stream and usually either is relegated to low-grade flour or is used for industrial or feed purposes.

Although the relationship of ferulic acid content to ash content is distinct for break flours and reduction flours (Table I), flour ferulic acid content was strongly related to ash content ( $r^2 = 0.89$ ) for the complete set of millstreams from the CWRS carlot composite milling. This relationship verifies the potential of ferulic acid as an alternative to ash content as a flour refinement index

(Pussayanawin et al 1988). Ferulic acid content also was strongly related to grade color ( $r^2 = 0.91$ ) and  $L^*$  ( $r^2 = 0.83$ ). When protein content was included with ferulic acid as an independent variable, the relationships to all flour refinement indices improved ( $r^2$  to ash = 0.98; to grade color = 0.94; to  $L^*$  = 0.94) because B1, B2, and B3, which have ferulic acid contents comparable to the most refined prime reduction flours, are higher in protein content (Table I).

The relationships of aleurone fluorescence to ash content ( $r^2 = 0.60$ ), grade color ( $r^2 = 0.66$ ), and  $L^*$  ( $r^2 = 0.42$ ), although significant ( $P < 0.05$ ) when all flour streams are included (Table I), are too weak to be effective for estimating flour ash content or brightness reliably. When only the reduction flours and B4 flour are selected, the relationships of aleurone fluorescence to the flour refinement indices become much stronger ( $r^2$  to ash = 0.89; to grade color = 0.93; to  $L^*$  = 0.92), indicating that for reduction flours, aleurone fluorescence has excellent potential as a flour refinement indicator. The clear differentiation by



**Fig. 1.** The relationships of relative aleurone fluorescence values to flour ash content (A, D), grade color (B, E), and  $L^*$  (C, F) for reduction flour streams (A-C) and break flour streams (D-F) from composites of Grain Research Laboratory harvest surveys of No. 1 Canada western red spring wheats from 1987 (circles), 1988 (triangles), and 1989 (squares) from the eastern (open symbols) and western (closed symbols) prairies. The regression lines for the relationships for reduction flours are shown for reference with the break flour plots.

aleurone fluorescence of BF flour from B1, B2, and B3 holds promise that break flour refinement also may be related to aleurone fluorescence. However, conclusive evidence of its effectiveness requires a break flour sample set with a more uniform distribution of refinement than that exhibited by B1, B2, B3, and BF.

#### Effects of Crop Year and Growing Location

The robustness of the relationships of aleurone fluorescence to flour refinement indices for CWRS wheat millstreams was investigated for eastern prairies and western prairies No. 1 CWRS composites from the 1987, 1988, and 1989 GRL harvest surveys (Fig. 1). The distinct relationships of aleurone fluorescence to break and to reduction flours and the anomalously low fluorescence of BF flour were confirmed for all of the CWRS harvest survey wheats.

Ferulic acid contents from selected streams of the eastern prairie composites from each harvest year (Table II) confirmed the trends observed earlier for the carlot composite sample (Table I). In all cases, BF flour exhibited a high ferulic acid content consistent with heavy aleurone contamination but a relatively moderate aleurone fluorescence. The lower aleurone fluorescence of the

break flours compared to reduction flours of lower ash content and greater brightness was consistent with relative levels of ferulic acid within the streams. When B1, B2, B3, and BF were excluded, the relationships of aleurone fluorescence to all of the refinement indices were very strong ( $r^2 > 0.8$ ), providing confirmation of the excellent potential of aleurone fluorescence for monitoring the refinement of CWRS reduction flours (Fig. 1A-C). The relationships were also homogeneous ( $P > 0.8$ ) among the samples. This provides compelling evidence that the relationships of aleurone fluorescence to reduction flour refinement are robust and not influenced by environment. Therefore, the relationship of CWRS reduction flour aleurone fluorescence to flour ash content, flour grade color, and  $L^*$  will be stable between different CWRS wheat lots within a crop year and between crop years.

#### Effect of Wheat Class

Established flour refinement indices such as color tests and ash content are effective for ranking flours from wheats of similar class and origin (Ziegler and Greer 1971). However, they must be used with caution when comparing wheats of contrasting class and diverse origin (Ziegler and Greer 1971). Therefore, it was of interest to determine the relationships of aleurone fluorescence to flour ash content and flour color for Canadian hard common wheat classes other than CWRS.

Analyses of individual millstreams from CPS, CWU, and CWRW wheats are shown in Tables III-V. The three wheat classes

**TABLE II**  
Aleurone Fluorescence, Ferulic Acid Content, and Flour Refinement Indices for Selected Flour Streams from Pilot-Scale Millings of Eastern Prairies No. 1 Canada Western Red Spring Wheat Samples from the 1987, 1988, and 1989 Harvest<sup>a</sup>

Flour Stream	Aleurone Fluorescence (%)	Ferulic Acid ( $\mu\text{g/g}$ )	Ash (%)	Grade Color Units	$L^*$ (%)
1987					
Break 3	33.6	25.9	0.60	1.5	91.1
Bran finisher	41.4	231.7	1.98	12.4	85.9
Middlings 1	37.0	23.5	0.35	-2.8	92.6
Middlings 3	39.3	52.4	0.47	0.5	91.8
1988					
Break 3	32.4	27.5	0.50	1.2	90.7
Bran finisher	38.5	127.9	1.31	10.3	86.7
Middlings 1	34.5	19.7	0.32	-2.9	92.7
Middlings 3	40.7	52.9	0.53	0.6	92.1
1989					
Break 3	31.1	24.9	0.55	1.1	90.8
Bran finisher	40.7	144.6	1.54	10.1	86.7
Middlings 1	34.4	27.3	0.33	-3.1	92.7
Middlings 3	40.8	62.8	0.58	1.1	92.0

<sup>a</sup> Expressed on "as is" moisture basis.

**TABLE III**  
Aleurone Fluorescence, Ferulic Acid Content, Protein Content, and Flour Refinement Indices for Flour Streams from a Pilot-Scale Milling of Commercially Grown No. 1 Canada Prairie Spring Wheat<sup>a</sup>

Flour Stream	Aleurone Fluorescence (%)	Ferulic Acid ( $\mu\text{g/g}$ )	Ash (%)	Grade Color Units	$L^*$ (%)	Protein (%)
Break 1	34.8	ND <sup>b</sup>	0.64	3.1	91.4	11.1
Break 2	34.6	43.2	0.60	2.6	91.7	11.6
Break 3	32.9	ND	0.57	1.2	92.4	13.0
Break 4	43.4	ND	0.83	5.6	89.5	11.0
Bran finisher	37.4	160.0	1.29	11.2	88.3	15.2
Sizing 1	34.5	ND	0.43	-2.5	93.0	9.6
Sizing 2	37.6	ND	0.45	-1.9	92.7	10.0
Middlings 1	37.6	33.2	0.39	-3.0	93.3	9.4
Middlings 2	36.3	ND	0.47	-0.5	92.8	10.6
Middlings 3	40.4	ND	0.53	1.5	92.2	10.3
Middlings 4	41.0	148.2	0.89	6.5	91.5	10.7
Middlings 5	42.3	ND	0.80	5.2	91.0	10.6
Middlings 6	44.1	ND	1.01	8.2	89.5	11.1

<sup>a</sup> Expressed on "as is" moisture basis.

<sup>b</sup> Not determined.

**TABLE IV**  
Aleurone Fluorescence, Ferulic Acid Content, Protein Content, and Flour Refinement Indices for Flour Streams from a Pilot-Scale Milling of Commercially Grown No. 1 Canada Western Utility Wheat<sup>a</sup>

Flour Stream	Aleurone Fluorescence (%)	Ferulic Acid ( $\mu\text{g/g}$ )	Ash (%)	Grade Color Units	$L^*$ (%)	Protein (%)
Break 1	37.1	ND <sup>b</sup>	0.64	4.0	90.7	11.8
Break 2	37.5	38.8	0.64	3.5	91.0	12.7
Break 3	38.7	ND	0.81	2.9	91.2	14.6
Break 4	47.4	ND	1.05	5.4	89.0	12.6
Bran finisher	43.8	290.7	2.42	15.2	85.5	17.2
Sizing 1	36.7	ND	0.45	-1.2	92.7	11.1
Sizing 2	39.3	ND	0.52	-1.1	92.2	11.7
Middlings 1	37.4	21.3	0.37	-2.4	93.0	11.1
Middlings 2	38.9	ND	0.51	0.1	92.0	12.3
Middlings 3	41.4	48.8	0.54	1.3	92.0	11.9
Middlings 4	43.6	ND	0.76	3.5	91.4	11.9
Middlings 5	46.5	ND	1.26	6.8	89.3	12.8
Middlings 6	48.0	ND	1.29	6.8	89.1	12.7

<sup>a</sup> Expressed on "as is" moisture basis.

<sup>b</sup> Not determined.

**TABLE V**  
Aleurone Fluorescence, Ferulic Acid Content, Protein Content, and Flour Refinement Indices for Flour Streams from a Pilot-Scale Milling of Commercially Grown No. 1 Canada Western Red Winter Wheat<sup>a</sup>

Flour Stream	Aleurone Fluorescence (%)	Ferulic Acid ( $\mu\text{g/g}$ )	Ash (%)	Grade Color Units	$L^*$ (%)	Protein (%)
Break 1	33.8	39.4	0.53	2.2	91.3	12.2
Break 2	31.8	ND	0.47	0.1	92.2	13.1
Break 3	31.7	ND	0.46	-0.6	92.6	13.6
Break 4	41.6	ND	0.80	4.0	90.1	12.1
Bran finisher	39.6	152.3	1.17	8.8	88.8	15.5
Sizing 1	30.8	ND	0.32	-4.0	93.7	10.0
Sizing 2	30.2	ND	0.31	-4.2	93.3	11.0
Middlings 1	34.4	22.6	0.30	-4.1	93.2	10.6
Middlings 2	32.1	ND	0.34	-3.3	93.9	10.6
Middlings 3	33.4	ND	0.39	-2.3	93.3	11.8
Middlings 4	35.3	ND	0.43	-1.8	93.3	11.4
Middlings 5	42.5	ND	0.53	0.4	91.3	11.2
Middlings 6	41.8	90.0	0.58	1.2	91.7	11.2

<sup>a</sup> Expressed on "as is" moisture basis.

<sup>b</sup> Not determined.

all exhibited the same distinctly lower aleurone fluorescence of break flours compared to reduction flours of comparable ash content and color and the anomalously low aleurone fluorescence of BF flour that were observed for the CWRS wheats (Tables I and II, Fig. 1).

The ferulic acid content patterns for selected millstreams from the three wheats (Tables III-V) were in general agreement with the CWRS results (Tables I and II). The lower aleurone fluorescence of the break flours was at least partially attributable to lower ferulic acid content compared to reduction flours of comparable ash content and color. The BF flours contained very high levels of ferulic acid despite exhibiting moderate aleurone fluorescence.

The strength of the relationships of aleurone fluorescence to the flour refinement indices for each of the CWRW, CPS, and CWU wheat samples was similar to that observed for the CWRS samples. When only reduction flours (including B4) were considered, aleurone fluorescence correlated strongly ( $P < 0.01$ ) to ash content, grade color, and  $L^*$  ( $r^2$  ranged from 0.73 to 0.93) for each wheat. This provides strong evidence of the broad applicability of aleurone fluorescence for estimating the degree of refinement of hard common wheat reduction flours.

The relationships of aleurone fluorescence to the ash content and color of reduction flours for the CPS (Table III), CWU (Table IV), and CWRW (Table V) wheats were tested for homogeneity to each other and to the previously discussed CWRS wheats (Table I, Fig. 1). Heterogeneity was found between the wheat classes ( $P = 0.05$ ) for the relationships to all three flour refinement indices. Rather than being discouraging, the heterogeneity of the flour aleurone fluorescence relationships offers encouragement that it may be a more objective flour refinement index than are existing flour refinement indices for comparing flours from diverse wheats.

## CONCLUSIONS

The aleurone fluorescence measurement system reported here cannot distinguish between break flours and reduction flours of lower ash content and brighter color. Break flours are derived primarily from the outer part of the starchy endosperm, which is high in protein content and high in ash content. Therefore, break flours have aleurone content comparable to reduction flours of lower ash content and brighter color, as reflected by comparable ferulic acid contents.

Despite the limitations identified in this study, aleurone fluorescence measurement has potential for on-line monitoring of mill performance. Prime quality patent flours, which command a premium price in divide flour markets, are composed of the best quality reduction flours. Therefore, the key to improving milling efficiency and profitability by on-line monitoring is to maximize the yield and the degree of refinement of the best quality reduction flours. Aleurone fluorescence was strongly related to the refinement of reduction flours for all of the Canadian common wheat classes examined.

Results from three different imaging approaches to the measurement of flour fluorescence—bran particle area measurement (Wetzel et al 1990), microspectrofluorimetry (Fulcher et al 1987, Pussayanawin et al 1988, Wetzel et al 1988), and the system reported here—all indicate the excellent potential for the rapid determination of flour refinement. Although flour refinement has been strongly related to flour ferulic acid content using high-performance liquid chromatography and microspectrofluorimetry methods, we focused on the comparison of flour fluorescence measurements to ash and color determinations. While it may be argued that fluorescence measurement of flour provides a superior quality control specification, flour ash content and color grade, despite their limitations, are used in most markets today. These traditional indicators of flour quality have proven to be relatively effective, and the acceptance of a fluorescence method would certainly be hastened if a relationship between this and traditional methods was demonstrated.

## ACKNOWLEDGMENTS

We gratefully acknowledge the expert technical assistance of N. M. Edwards, R. J. Desjardins, J. Burrows, C. M. Panting, and L. C. M. van Schepdael. D. W. Hatcher provided valuable advice and assistance in the measurement of ferulic acid.

## LITERATURE CITED

- ALLEN, H. M., OLIVER, J. R., and BLAKENEY, A. B. 1989. Use of a tristimulus color meter in a wheat breeding programme. Pages 185-189 in: Proc. Aust. Cereal Chem. Conf., 39th. T. Wescott, Y. Williams, and R. Ryker, eds. Royal Australian Chemical Institute: Victoria.
- AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC. Method 08-01, approved April 1961, revised October 1981. The Association: St. Paul, MN.
- BLACK, H. C. 1980. The GRL pilot mill. Assoc. Oper. Millers Tech. Bull. (Sept.) 3834.
- BLACK, H. C., PRESTON, K. R., and TIPPLES, K. H. 1981. The GRL pilot mill. I. Flour yields and analytical properties of flour streams milled from Canadian red spring wheats. Can. Inst. Food Sci. Technol. 14:315.
- DEXTER, J. E., and SYMONS, S. J. 1989. Milling efficiency: The relevance of flour ash content and flour color and the potential application of near infrared reflectance spectroscopy and fluorescent imaging. Pages 73-100 in: Proc. Symp. Wheat End-Use Properties: Wheat and Flour Characterization for Specific End-Uses. H. Salovaara, ed. International Association for Cereal Chemistry: Budapest, Hungary.
- DEXTER, J. E., and TIPPLES, K. H. 1987. Wheat milling at the grain research laboratory. Part 2. Equipment and procedures. Milling 180(7):16.
- FRANCIS, F. J. 1983. Colorimetry of foods. Pages 105-122 in: Physical Properties of Food. M. Peleg and E. B. Bagley, eds. Avi Publishing: Westport, CT.
- FULCHER, R. G., O'BRIEN, T. P., and LEE, J. W. 1972. Studies on the aleurone layer. I. Conventional and fluorescence microscopy of the cell wall with emphasis on phenol-carbohydrate complexes in wheat. Aust. J. Biol. Sci. 25:23.
- FULCHER, R. G., PUSSAYANAWIN, V., and WETZEL, D. 1987. Detection and localization of phenolic compounds in cereal grains using computerized microspectrophotometry. Pages 69-72 in: Proc. Aust. Cereal Chem. Conf., 37th. L. Murray, ed. Royal Australian Chemical Institute: Victoria.
- HATCHER, D. W. 1990. Polyphenol oxidase and phenolic acids: Their levels and interaction in color production in Canadian wheat flours. Ph.D. diss. University of Manitoba: Winnipeg, Canada.
- HINTON, J. J. C. 1959. The distribution of ash in the wheat kernel. Cereal Chem. 36:19.
- HOLAS, J., and TIPPLES, K. 1978. Factors affecting farinograph and baking absorption. I. Quality characteristics of flour streams. Cereal Chem. 55:637.
- JENSEN, S. A., MUNCK, L., and MARTEN, H. 1982. The botanical constituents of wheat and wheat milling fractions. I. Quantification by autofluorescence. Cereal Chem. 59:477.
- KENT, N. L., and JONES, C. R. 1952. The cellular structure of wheat flour. Cereal Chem. 29:383-398.
- KENT-JONES, D. W., and MARTIN, W. 1950. A photoelectric method of determining the colour of flour as affected by grade, by measurements of reflecting power. Analyst 75:127.
- KISSMEYER-NIELSON, A., JENSEN, S. A., and MUNCK, L. 1985. The botanical composition of rye and rye milling fractions determined by fluorescence spectrometry and amino acid composition. J. Cereal Sci. 3:181.
- MORRIS, V. H., ALEXANDER, T. L., and PASCOE, E. D. 1945. Studies of the composition of the wheat kernel. I. Distribution of ash and protein in center sections. Cereal Chem. 22:351.
- MORRIS, V. H., ALEXANDER, T. L., and PASCOE, E. D. 1946. Studies of the wheat kernel. III. Distribution of ash and protein in central and peripheral zones of whole kernels. Cereal Chem. 23:540.
- MUNCK, L., FEIL, C., and GIBBONS, G. C. 1979. Analysis of botanical components in cereals and cereal products—A new way of understanding cereal processing. Pages 27-40 in: Cereals for Food and Beverages. G. E. Inglett and L. Munck, eds. Academic Press: New York.
- PRESTON, K. R., KILBORN, R. H., and BLACK, H. C. 1982. The GRL pilot mill. II. Physical dough and baking properties of flour streams milled from Canadian red spring wheats. Can. Inst. Food Sci. Technol. 15:29.
- PUSSAYANAWIN, V., and WETZEL, D. L. 1987. High-performance

liquid chromatographic determination of ferulic acid in wheat milling fractions as a measure of bran contamination. *J. Chromatogr.* 391:243.

PUSSAYANAWIN, V., WETZEL, D. L., and FULCHER, R. G. 1988. Fluorescence detection and measurement of ferulic acid in wheat milling fractions by microscopy and HPLC. *J. Agric. Food Chem.* 36:515.

SAS INSTITUTE, INC. 1986. SAS System for Linear Models. SAS Institute Inc.: Cary, NC.

SAS INSTITUTE, INC. 1988. SAS User's Guide: Statistics. Release 6.03 Ed. SAS Institute Inc.: Cary, NC.

SOSULSKI, F., KRYGIER, K., and HOGGE, L. 1982. Free, esterified, and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereal and potato flours. *J. Agric. Food Chem.* 30:337.

SYMONS, S. J., and DEXTER, J. E. 1991. Computer analysis of fluorescence for the measurement of flour refinement as determined by flour ash content, flour grade color, and tristimulus color mea-

surements. *Cereal Chem.* 68:454.

SYMONS, S. J., and DEXTER, J. E. 1992. Estimation of milling efficiency: Prediction of flour refinement by the measurement of pericarp fluorescence. *Cereal Chem.* 69:137.

WETZEL, D. L., PUSSAYANAWIN, V., and FULCHER, R. G. 1988. Determination of ferulic acid in grain by HPLC and microspectrofluorimetry. Pages 409-426 in: *Frontier of Flavor Proc. Int. Flavor Conf.*, 5th. G. Charalambous, ed. Elsevier Science Publishers: Amsterdam.

WETZEL, D. L., POSNER, E. S., and ELLIS, S. K. 1990. Bran determination in commercial mill streams and intermediates with a digital fluorescence imaging instrument. *Cereal Foods World* 35:848.

ZIEGLER, E., and GREER, E. N. 1971. Principles of Milling. Pages 115-199 in: *Wheat Chemistry and Technology*. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

[Received December 16, 1991. Accepted July 13, 1992.]

## Comparative Effects of Wheat Flour Protein, Lipid, and Pentosan Composition in Relation to Baking and Milling Quality<sup>1</sup>

ROBERT GRAYBOSCH,<sup>2</sup> C. JAMES PETERSON,<sup>2</sup> KENNETH J. MOORE,<sup>2</sup>  
MARK STEARNS,<sup>3</sup> and DANA L. GRANT<sup>4</sup>

### ABSTRACT

*Cereal Chem.* 70(1):95-101

Variation in milling, baking, and dough-handling properties among 58 hard wheat (*Triticum aestivum*) flours was examined in relation to the variation in flour protein and lipid concentrations and composition and to the variation in water-soluble pentosan concentrations. Simple correlations showed no single biochemical component capable of explaining more than 41% of the variation in any given quality parameter. Similarly, no single biochemical component was highly related to all quality attributes. Canonical analyses, a multivariate statistical approach, revealed that the measured biochemical components were able to explain more than 90% of the variation in major quality attributes such as dough-

handling and loaf characteristics. Flour protein concentration was found to be the primary factor contributing to variation in both dough strength and loaf characteristics. Once the primary effects of protein concentration were established, flour polar lipid concentrations showed substantial positive contributions to dough handling. Loaf textural features largely were unrelated to protein concentrations; however, glutenin concentrations, water-soluble pentosans, and flour lipids showed positive relationships. Assay of numerous biochemical components together with multivariate approaches may be needed to develop effective predictive models for observed variation in wheat end-use quality.

Understanding the biochemical basis for variation in hard red wheat quality could enable the development of rapid, predictive tests for end-use quality. Rapid biochemical tests capable of predicting wheat quality would enable millers to identify and composite grain samples based on quality potential. Biochemically based predictive tools would allow bakers the opportunity to make adjustments to bakery formulations and equipment settings before flour lots reached the bakery floor. Wheat breeders need to identify which key biochemical components affecting quality are genetically controlled and amenable to alteration through selection. Identification of biochemical components that are highly influ-

enced by environment would assist in the development of wheats with enhanced quality and stability over diverse environmental conditions.

Attempts to explain the biochemical basis for flour quality variation of hard wheats grown in North America are numerous. Orth and Bushuk (1972), Khan et al (1989), and Graybosch et al (1990) have examined the role of flour protein composition as measured by the relative amounts of protein in various solubility classes. The effects of lipid variation in relation to quality were studied by Pomeranz et al (1966), Hosney et al (1969), and Chung et al (1980). Shogren et al (1987) analyzed the contribution of pentosan composition. In each study, significant relationships were established between quality variation and the biochemical factor of interest. Thus, numerous biochemical components must contribute to quality variation. No single biochemical component, however, has been shown to explain more than a portion of the quality variation observed among large numbers of flour samples. Also, few studies have examined the relative effects of protein, lipid, and pentosan composition in a common set of flour samples.

During the process of baking, wheat flour becomes a complex biological system in which numerous flour biochemical components interact with each other and with added ingredients to determine quality characteristics. A thorough understanding of the biochemical basis for wheat flour quality variation will require

<sup>1</sup>Paper 9818 Journal Series, Agricultural Research Division, University of Nebraska, Lincoln. Mention of a trademark or proprietary product does not constitute a guarantee or warranty by the United States Department of Agriculture and the University of Nebraska and does not imply its approval to the exclusion of other products that also may be suitable.

<sup>2</sup>USDA-ARS, University of Nebraska, Lincoln 68583.

<sup>3</sup>Campbell-Taggart, Inc., Dallas, TX 75266.

<sup>4</sup>Department of Agronomy, University of Nebraska, Lincoln 68583.