Computer Analysis of Fluorescence for the Measurement of Flour Refinement as Determined by Flour Ash Content, Flour Grade Color, and Tristimulus Color Measurements[1]

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ABSTRACT

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Millstreams from repeated pilot-scale millings of a commercially grown No. 1 Canada western red spring wheat were used to measure flour refinement when selecting for aleurone and pericarp tissue by fluorescence. Measurements were averaged from 25 full-field fluorescence images using UV excitation for aleurone and blue excitation for pericarp. Pericarp fluorescence was strongly correlated ($r^2 > 0.9$) to the tristimulus color coordinate L* (brightness) of the flour, flour ash content, and flour grade color. The regression equations for pericarp fluorescence to all the above refinement indexes were homogeneous for replicate millings performed over a one-year period and were not influenced by flour age. Aleurone fluorescence was also significantly correlated ($P < 0.05$) to the same flour refinement indexes. With the aleurone measurement procedure, millstreams were segregated into two groups, which made the estimation of flour refinement using aleurone more complex than for pericarp using our system. Variability in the fluorescence measurements and flour refinement indexes associated with operators, experimental design, equipment, flour moisture, and flour particle size were investigated. Tristimulus a* and b* values were highly sensitive to moisture changes in the flour, and b* values were sensitive to flour particle size. Tristimulus L* values were slightly sensitive to particle size but insensitive to flour moisture, while relative fluorescence measurements were insensitive to both flour moisture and particle size. Instrumental variability was associated with our fluorescence methods.

The principle aim of wheat milling is to separate the bran (pericarp, testa, and aleurone) and germ (embryo) from the flour (starchy endosperm). The efficiency of the separation of these components is defined as flour refinement, with a highly refined flour consisting of almost pure starchy endosperm. Both the degree of flour refinement and the intrinsic quality of the wheat from which the flour was milled affect the end-use quality of the flour (Ziegler and Greer 1971). Components from the germ, aleurone, testa, and pericarp can have significant effects on the processing qualities of the flour produced. As a result, the price of flour in many countries is related to the degree of flour refinement.

The degree of flour refinement is often estimated by either flour ash content or flour color measurement. Ash content is lowest in the middle of the endosperm and highest in the aleurone (Morris et al 1945, Hinton 1959). The bran layers contain 15–20 times as much ash as the central endosperm (Ziegler and Greer 1971). A limitation of ash content is that flour ash is strongly influenced by wheat class and growing conditions and must be used with caution as a flour refinement indicator when the origin of the wheat is unknown (Shuey 1976). This technique is readily standardized but is tedious.

A more rapid alternative method for the determination of flour refinement is the measurement of visible light reflected from flour-water pastes through a green filter. This is the preferred method in countries such as the United Kingdom, where nutrients that influence ash content (e.g., calcium carbonate and iron) are added to flour. The green filter makes this procedure highly sensitive to bran and other contaminating constituents present in the flour that influence paste grayness, while sensitivity to yellow xanthophyll pigments is low. The flour color grader developed in the United Kingdom (Kent-Jones and Martin 1950, Kent-Jones et al 1950) is a popular instrument of this type. In the United States, the green Agtron flour measurement system is widely used (Patton and Dishaw 1968).

Flour-water paste reflectance readings are closely related to the ash content of flour streams from individual wheats (Patton and Dishaw 1968, Ziegler and Greer 1971), and simplicity and speed make flour color determination preferable to flour ash determination. However, flours from different wheats may not be comparable when measured by the flour color grader because of contributions from the endosperm. Thus, flour grade color cannot be regarded as an accurate indicator of bran content between different wheats (Barnes 1986).

The use of a green filter for color measurement was criticized by Croes (1961), who suggested that a preferred method for the measurement of a flour-water paste would include contributions from brightness, yellowness, and whiteness. These three components of reflected light are measured with a tristimulus reflectometer.

Ash content or color methods for measuring flour refinement are not fully satisfactory because only an approximate indication of endosperm contamination is given. Additionally, only an after-the-fact estimation of mill performance is provided, and this is not necessarily related to baking performance (Dexter and Symons 1989).

In addition to the measurement of flour-water pastes, Shuey and Skarsaune (1973) found that the Agtron in the green mode could be used to nondestructively measure the quality of dry flour. More recently, in line with the recommendations of Croes (1961), dry flour measurement of the Commission Internationale de L'Eclairage (1986) 1976 L*, a*, b* color space (tristimulus) coordinates was evaluated for measuring flour refinement because it is rapid, simple, and precise (Allen et al 1989, Dexter and Symons 1989).

Another possible alternative to current flour refinement indexes was introduced in 1979 by Munck et al, who reported that the autofluorescence of the different wheat seed tissues could be used for the rapid quantitative analysis of wheat kernel constituents within flour. Spectrofluorometric analysis of well-documented milled samples identified a characteristic peak for aleurone fluorescence at an excitation wavelength of 320 nm and at emission wavelengths in excess of 375 nm (Jensen et al 1982). Further work with fluorescence spectral data using partial least-squares regression determined that rye flour ash correlated with aleurone autofluorescence ($r = 0.89$), while pericarp autofluorescence (excitation 450 nm, emission 540 nm) best correlated with rye flour fiber content ($r = 0.97$) (Kissmeyer-Nielson et al 1985).

More recently, wheat flour ash was shown to correlate with the ferulic acid content of wheat flour streams (Pussayanawin et al 1988). The fluorescence spectrum of wheat aleurone cell walls was comparable to that for ferulic acid crystals when observed under identical conditions (Fulcher et al 1972). When measured with a microspectrofluorimeter, the relative fluorescence

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of millstreams from several hard red winter wheats correlated with ferulic acid concentration (Fulcher et al 1987, Pussayanawin et al 1988), leading to the suggestion that the relative fluorescence of flour (excitation 365 nm, emission 420 nm) has potential as a rapid method for a sensitive assay of ferulic acid, and hence bran, using strictly optical systems.

The objective of this study was to investigate the measurement of flour refinement by a rapid fluorescence imaging system and tristimulus color coordinates. These data were compared with traditional flour ash content and flour grade color measurements.

MATERIALS AND METHODS

Wheat

The wheat used was a fully blended composite of No. 1 Canada western red spring (CWRS) with 12.5% guaranteed protein content and No. 1 CWRS with 13.5% guaranteed protein content from rail carlots unloaded at Thunder Bay terminal elevators in the fall of 1988. The blended wheat (13.5% mb) had a test weight of 81.7 kg/hl, protein content of 13.1%, ash content of 1.61%, kernel weight of 32.2 mg, and a falling number of 410 sec.

Milling

Wheat was prepared for milling as described by Dexter and Tipples (1987), tempered to 16.3% moisture content in a Monarch mortar mixer (Black 1980), and milled using the Grain Research Laboratory (GRL) pilot mill (Black 1980). The hard wheat mill flow described by Black (1980) was modified recently and is presented in Figure 1.

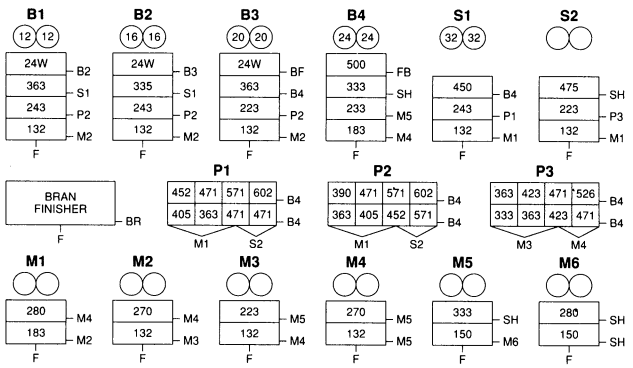

Fig. 1. Grain Research Laboratory pilot mill hard wheat flow. Nitex sieve apertures given in microns. 24 wire (W) aperture 730 microns. Roll fluting expressed as corrugations per inch. B = break, S = sizing, M = middling, P = purifier, F = flour, BF = bran finisher, SH = shorts, BR = bran, FB = fine bran.

The wheat was milled repeatedly for one year. Variability in the yield and degree of refinement of all millstreams was achieved by deliberately changing break-roll gaps and altering purifier settings among millings. The range of mill performance is illustrated by cumulative ash curves (Shellenberger and Ward 1967) representing the worst and the best results achieved (Fig. 2).

Milled products of identical refinement with variable particle sizes were prepared by capturing stock from the first purifier destined for the first middling rolls (Fig. 1). A portion of this material was reduced four times on the reduction roll stand of the GRL Ross research mill (Scanlon and Dexter 1986). The roll stand is equipped with 254-mm diameter smooth rolls with a lightly frosted finish. A portion of all recovered stock was retained after each grinding pass. The particle size profile of the samples was determined by sieving the material on a Buhler MLU 300 laboratory plan sifter (Buhler-Miag (Canada) Ltd., Don Mills, ON) for 2 min using 8XX (183 μm), 10XX (132 μm), and 12XX (91 μm) sieves.

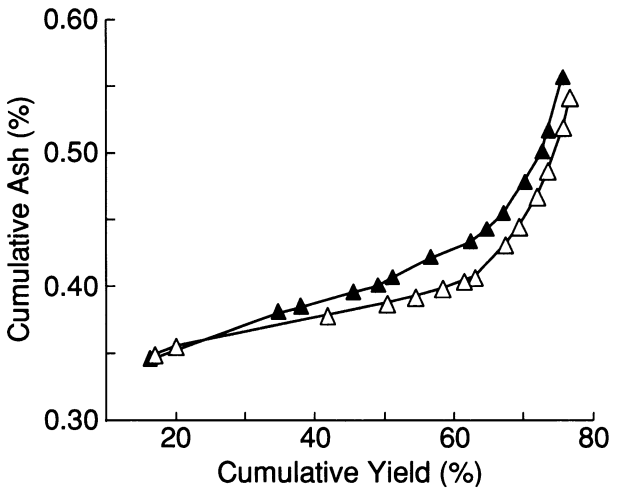

Fig. 2. Cumulative ash curves that illustrate the maximum range of mill performance among replicate millings of a No. 1 Canada western red spring wheat achieved by manipulating mill settings. The flatter curve (△) is indicative of better milling performance than the steeper curve (▲). Ash content expressed on 14% moisture basis; flour yield expressed as proportion of clean wheat on constant moisture basis.

TABLE I
Effect of Moisture Content on Flour Refinement Indexes and Standardized Fluorescence Values for Four No. 1 CWRS-13.5 Millstreams[a,b]

Stream	Moisture (%)	Ash (%)	Grade Color (K-J units)	Tristimulus Units L (%)	a	b	Fluorescence Aleurone (%)	Pericarp (%)
Middling 1	10.3	0.355	−2.47	92.85	−0.44	7.34	35.28	45.20
	12.0	0.348	−2.56	92.95	−0.56	7.72	36.05	44.60
	12.9	0.345	−2.61	92.84	−0.71	8.45	34.78	43.58
	14.2	0.342	−2.44	92.88	−0.86	9.14	34.55	42.95
Sizing 1	10.9	0.402	−1.77	92.53	−0.28	6.80	37.03	48.60
	12.3	0.391	−1.89	92.49	−0.44	7.41	35.85	47.65
	13.3	0.387	−1.84	92.54	−0.61	8.24	34.40	46.28
	14.2	0.386	−1.89	92.54	−0.78	9.25	33.15	45.50
Break 1	10.8	0.515	1.41	90.97	−0.17	7.54	36.73	56.48
	12.2	0.506	1.31	91.08	−0.26	8.01	36.13	52.55
	13.5	0.511	1.23	91.05	−0.40	8.78	34.70	52.60
	14.5	0.498	1.17	91.02	−0.57	9.63	33.75	51.48
Bran flour	10.7	1.671	11.86	86.28	0.36	11.82	41.38	97.60
	11.8	1.653	11.92	86.34	0.32	12.08	40.90	95.70
	12.9	1.640	11.96	86.29	0.28	12.25	39.68	94.00
	14.0	1.628	11.99	86.26	0.28	12.21	38.68	93.20

[a] Canada western red spring wheats with 13.5% guaranteed protein content.
[b] All values are means of quadruplicate analyses.

Flours of variable moisture content at a constant degree of refinement were obtained by bench drying a portion of each of four millstreams selected to represent the full range of flour refinement (Table I) and then recombining the dried material with the corresponding original material in varying proportions. The mixtures were sealed in airtight containers and stored for one week before analysis to ensure uniform moisture distribution.

Moisture content of all flours and millstreams was determined in duplicate with a rapid moisture meter (C. W. Brabender Instruments, South Hackensack, NJ) as outlined in the instruction manual.

Flour Refinement Measurements

All flour refinement analyses were performed in at least duplicate unless otherwise noted.

Flour ash content was determined by the standard AACC method 08-01 (AACC 1983). A Simon Colour Grader Series IV (Henry Simon, Stockport, U.K.) was used for flour grade color determinations as described in the instruction manual.

Tristimulus color coordinate measurements were performed with a Minolta Chroma Meter CR-231 (Meyer Instruments Ltd., Cornwall, ON) on dry flour loaded in a Dickey-john near-infrared reflectance cell. Color readings were expressed by Judd-Hunter values for L* (lightness), a* (red-green chromaticity), and b* (yellow-blue chromaticity) (Francis 1983).

Fluorescence Imaging

Flour samples were loaded into the near-infrared reflectance cell, which had been taped to a standard microscope slide (2.5 × 10 cm) for ease of attachment to the microscope stage. For fluorescence imaging and photography, the sample holder was placed on a motorized stage under a 10x Neofluor objective mounted on an Axiophot microscope (Carl Zeiss, Canada). Epi-illumination was from an HBO-50 burner through a No. 02 filter combination (excitation 365 nm, barrier >420 nm) or a No. 09 filter combination (excitation 450-490 nm, barrier >520 nm). The image was directed either to the eyepieces or to the color camera (BY110u, JVC Professional Division, Canada) using beam splitters.

For measurement, the red, green, and blue (RGB) video signals generated by the camera were passed to an image processing system (AT-IBAS, Kontron Electronik, Eching, Germany) via a video mutliplexer. The image was stored as a 480 × 512 pixel array in video memory until required for measurement. For calibration purposes, a standard uranyl glass surface (Carl Zeiss, Canada) was used. The standard was kept at a constant focal distance by a machined mount attached to the 10x objective.

The uranyl glass standard was measured five times using the 02 filter system immediately before and after the measurement of each flour sample. The five images of the standard were averaged for the red and green color signals each time the standard was measured. For each flour, 25 images were captured (our imaging system could store 25 images in video memory) using either the 02 or 09 filter combinations.

The mean gray level was determined for each flour image. This level represents the overall brightness of the field fluorescence. These measurements were recalculated to relative fluorescence by using the following formula:

$$(\text{field mean gray value}/\text{gray value of standard}) \times 100\%$$

For the determination of pericarp, field mean gray values were determined from the red image of the flour using the 09 filter block, and the gray value of the standard was given by the red image of the uranyl glass standard. For the determination of aleurone, the green image of the flour using the 02 filter block and the green image of the standard were used.

Statistics

All statistics were calculated using the procedures of the SAS (1988) software system, version 6.04.

RESULTS

Effect of Flour Moisture Content on Refinement Measurements and Relative Fluorescence

For a system measuring flour quality, the effects of flour moisture content must be fully documented. For meaningful comparison between samples, analytical measurements such as flour ash content were corrected to a constant moisture content (Table I). Both a* and b* were also affected by flour moisture content for all millstreams examined. In contrast, L* and flour grade color were independent of flour moisture content.

Relative fluorescence values for aleurone and pericarp estimation decreased with increasing moisture content for all flours examined. These differences are likely not caused by variability in packing within the Dickey-john cell at different moisture contents because L* was not affected. It is possible that these differences were the result of quenching by water.

Effect of Flour Particle Size Distribution on Refinement Measurements and Relative Fluorescence

The particle size distribution of reground samples of stock destined for the first middling rolls is shown in Table II. Sample A represents unground material with a relatively course particle size distribution, and samples B–E are successive grinds of diminishing particle size distribution. A slight reduction in moisture content was noted as a result of the regrinding process. Flour ash when corrected for moisture content differences remained constant, confirming complete recovery of stock during regrinding.

Flour grade color appeared to be unrelated to flour particle size (Table II). All tristimulus color coordinates were influenced by particle size, with L* and b* values being more strongly influenced than a* values. In contrast, aleurone and pericarp relative fluorescence values were relatively stable over the complete particle size range, except for a slight but significant ($P < 0.05$) difference between flours A and E for aleurone fluorescence.

Repeatability of Relative Fluorescence Results for Millstreams from Replicate Millings

Six millings of the No. 1 CWRS wheat that represented the complete range of mill performance were chosen for preliminary experiments establishing the relationship between relative fluorescence and flour refinement indexes. Pericarp and aleurone relative fluorescence values of all millstreams from each milling

TABLE II
Effect of Particle Size on Flour Refinement Indexes (Standardized Fluorescence Values for Reground Stock from the First Purifier Destined for the First Middling Rolls)

	Sample				
Property	**A**	**B**	**C**	**D**	**E**
Particle size[a]					
Percent held on					
8XX	64.6	46.6	28.0	18.0	8.5
10XX	24.7	25.2	26.0	19.0	12.1
12XX	8.1	15.3	22.5	20.0	17.1
Pan	2.5	12.9	23.5	43.0	62.4
Moisture content, %[a]	14.0	14.0	13.9	13.7	13.6
Ash content, %[b]	0.329	0.335	0.335	0.340	0.340
Grade color, K-J units[b]	−2.74	−2.81	−2.79	−2.88	−2.88
Tristimulus units[b]					
L	90.28	90.98	91.60	92.02	92.43
a	−0.98	−0.99	−0.96	−0.96	−0.94
b	13.37	12.34	11.42	10.46	9.74
Standardized fluorescence, %[c]					
Aleurone	34.8	34.5	34.6	33.5	32.9
Pericarp	64.2	65.7	64.5	65.4	65.7

[a] Determined singly.
[b] Values are means of duplicate analyses.
[c] Values are means of triplicate analyses.

were determined in quadruplicate. The relationships between relative fluorescence results, triplicated determinations of ash content (as-is moisture basis), flour grade color, and L* for the six millings were determined and tested for homogeneity. Values for a* and b* were not considered because of a relatively narrow range among millstreams and a sensitivity to moisture content and particle size distribution (Tables I and II).

For all six millings, strong linear relationships ($r^2 > 0.9$) were noted between relative fluorescence and flour ash content (Fig. 3A and D), flour grade color (Fig. 3B and E), and L* values (Fig. 3C and F). The results were homogenous ($P > 0.05$) for all six millings, for both pericarp (Fig. 3A–C) and aleurone (Fig. 3D–F).

The six millings were a few days to nine months old when they were analyzed, additionally confirming that storage does not influence aleurone or pericarp fluorescence results.

Aleurone fluorescence measurements gave lower r^2 values for flour ash content (Fig. 3D), flour grade color (Fig. 3E), and L* values (Fig. 3F) than for pericarp fluorescence. This was the result of a distinct subgroup of millstreams formed from the bran flours and some break flours, where the relative fluorescence was lower than expected for their ash content. The explanation for the formation of two groups of millstreams is under investigation.

Effects of Operators on Fluorescence Measurements

The possibility that fluorescence measurements may differ among operators, perhaps due to differences in loading the Dickey-john sample cell, was investigated using a Latin square

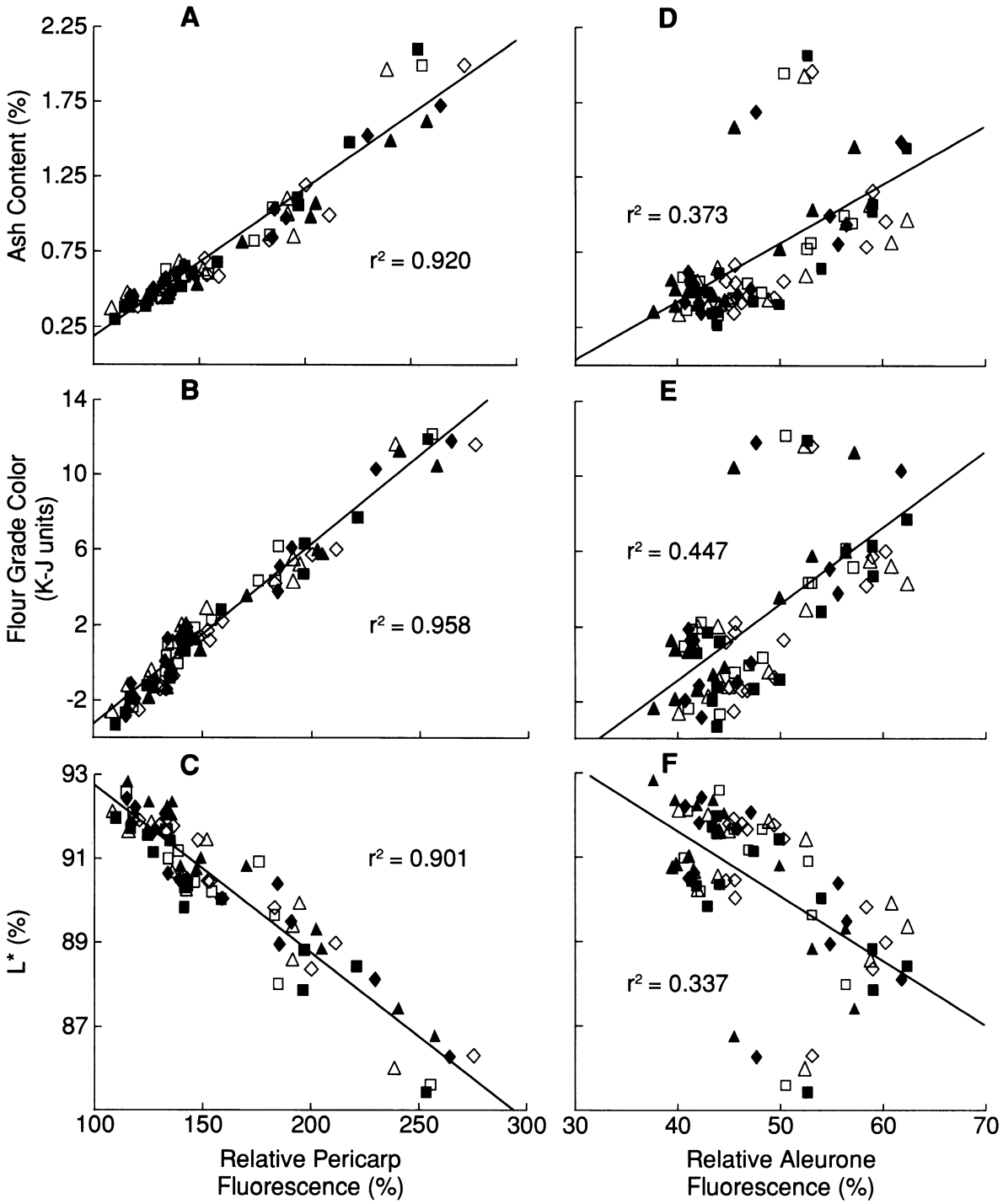

Fig. 3 Relationships of relative pericarp fluorescence values (**A–C**) and relative aleurone fluorescence values (**D–F**) to flour refinement indexes for all flour streams from six replicate millings of a No. 1 Canada western red spring wheat. The different symbols represent replicate millings.

design experiment. Each of three operators replicated measurements on four millstreams of variable refinement in an early, a middle, or a late shift on each of three days. For relative pericarp fluorescence, a day-to-day effect was observed, but no effect attributable to operator or time of day was found (Table III).

For relative aleurone fluorescence, a small ($P < 0.05$) day-to-day variation was noted for day 2, and a small ($P < 0.05$) operator effect was noted for operator 1 (Table IV).

The small effects identified for relative pericarp and relative aleurone fluorescence, while statistically significant, are of little practical concern. The operator and day-to-day effects accounted for only 2.6 and 3.4%, respectively, of the variance observed for relative pericarp fluorescence (Table III) and 0.07 and 7.9% for relative aleurone fluorescence (Table IV). Thus, these effects are minor compared with the effects of the four flour samples. Preliminary experiments (not shown) indicated that the coefficient of variation for each set of 25 flour images was typically 4–6%.

Instrumental Limitations—Burner Variability and Filter Deterioration

Slight variability between days using the same burner (Tables III and IV) can be attributed to switching the HBO-50 burner on and off. Of more practical significance, after 150 hr of use, when burners are replaced, a large shift in fluorescence values may be found for aleurone and pericarp fluorescence. This is illustrated in Figure 4 for a single milling measured in duplicate using two different HBO burners on the same day.

For each burner, relative pericarp fluorescence was strongly related to flour ash (Fig. 4A), grade color (burner 1, $r^2 = 0.97$; burner 2, $r^2 = 0.97$), and L* value (burner 1, $r^2 = 0.94$; burner 2, $r^2 = 0.95$). This relationship is represented in Figure 3A–C. A similar difference between burners for aleurone measurements was also found (Fig. 4B). For pericarp and aleurone fluorescence, the standardized values between burners were very highly correlated, allowing direct comparison between data from any burner. The differences were not due to misalignment of the burner or optics since great care was taken to ensure complete alignment and peak performance each time a burner was changed.

An important instrumental consideration is the decrease with use in transmission of the 02 filter system to very low fluorescence measurements. The filter system must be periodically checked and replaced to ensure peak performance and reliable results.

DISCUSSION

Two fluorescence filter systems (02 and 09) were chosen for flour analysis. Since both of these systems are broad band excitation, many components of the flour could contribute to the measured fluorescence signal. The terms "aleurone" and "pericarp" are used, as these are the tissues that are most readily identified using each filter system, respectively.

The use of millstreams from repeated millings of the same wheat was chosen to verify the robustness of the relationship between whole field fluorescence measurement and flour ash content, flour grade color, and L*. Those relationships for replicated millings over a one-year period were found to be homogeneous regardless of mill performance.

Relative aleurone fluorescence measurements, while giving a significant ($P < 0.05$) relationship to flour ash content, flour grade color, and L*, also showed segregation into two groups of millstreams (Figs. 3D–F and 4B) that appear to be related to the break and reduction roll systems in the mill. Thus, the two groups we found may be the result of our imaging system or flour components other than those measured by fluorescence contributing to the flour ash, grade color, or L* determinations. The measurement system used here differs from that previously reported for American hard red winter wheat, in which microspectrofluorimetry was used to relate flour ferulic acid content to aleurone fluorescence (Fulcher et al 1987, Pussayanawin et al 1988). We recently found evidence that the relationship of aleurone fluorescence measurement to the flour extraction rate of experimentally milled CWRS varied among samples (Dexter and Symons 1989). The current study has demonstrated that our fluorescence measurements were also influenced by the equipment

TABLE III
Effects of Operator and Day of Analysis on Pericarp Standardized Fluorescence Values (%)

Operator[a]	Day 1	Day 2	Day 3	Mean[b]
1	119.9	120.1	96.2	112.0 a
2	116.5	124.1	102.8	114.5 a
3	115.3	126.4	98.5	113.1 a
Mean[b]	117.2 b	123.5 a	98.9 c	

[a]Each operator analyzed four flours in duplicate each day.
[b]Means followed by different letters are significantly different (LSD = $P < 0.05$).

TABLE IV
Effect of Operator and Day of Analysis on Standardized Aleurone Fluorescence Values (%)

Operator[a]	Day 1	Day 2	Day 3	Mean[b]
1	36.1	37.0	36.8	36.6 b
2	37.2	37.8	37.1	37.3 a
3	37.2	37.8	36.7	37.1 a
Mean[b]	36.8 b	37.5 a	36.8 b	

[a]Each operator analyzed four flours in duplicate each day.
[b]Means followed by different letters are significantly different (LSD = $P < 0.05$).

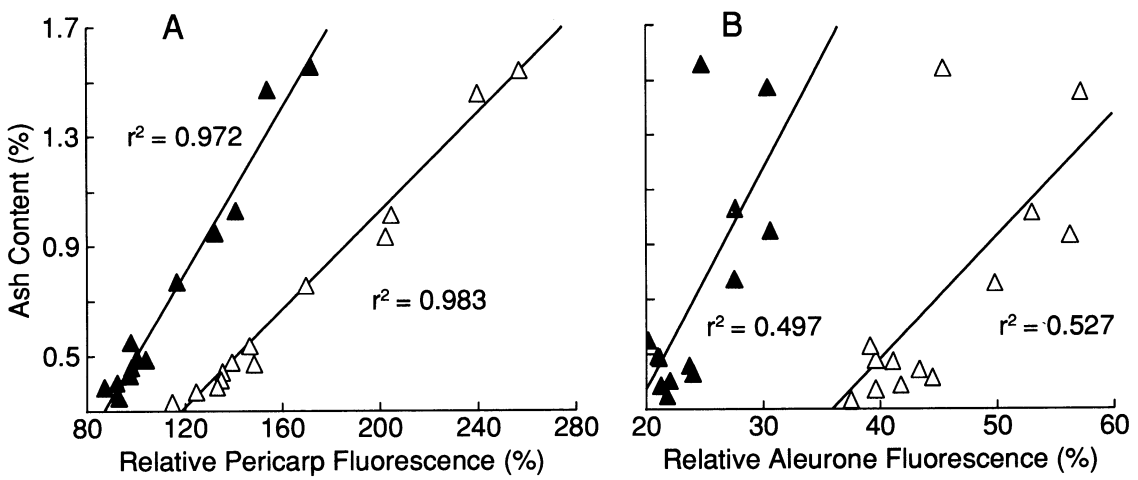

Fig. 4. Effect of changing mercury burners on relative pericarp fluorescence values (**A**) and relative aleurone fluorescence values (**B**) for flour from a single milling of No. 1 Canada western red spring wheat.

used (light source, filter systems, optics, and detector [camera]). We are currently undertaking a detailed study of aleurone relative fluorescence to fully investigate these and other effects.

In comparison to the measurement of relative aleurone fluorescence, the measurement of relative pericarp fluorescence to estimate wheat flour refinement has received little attention. The fluorescence measurement system used in this study showed a very high degree of sensitivity to pericarp fluorescence, and the results were highly correlated with flour ash content, flour grade color, and L* values (Figs. 3A–C and 4A).

On the basis of these preliminary results, the measurement of pericarp fluorescence has potential for rapid estimation of flour refinement. Pericarp fluorescence has been used in conjunction with additional fluorescence measurements to create a multi-wavelength model for the prediction of flour refinement (Pedersen 1987). We are currently investigating the stability of pericarp fluorescence measurements as a flour refinement estimator for a diverse population of Canadian wheats representing all major wheat classes and different crop years.

The high degree of sensitivity of pericarp fluorescence made the measurements highly sensitive to instrumental effects and other influences. The slight variability found from day to day suggests that the uranyl glass standard did not correct for all the variability encountered. The uranyl glass standard was measured with the 02 filter block, and pericarp fluorescence was measured with the 09 filter block. An assumption that changes in one part of the excitation spectrum would be equivalent to changes in another part would seem invalid on the basis of our experience. The uranyl glass standard was not fluorescent when using the 09 filter block. Standardization for fluorescence work is often achieved by fluorochromes embedded in a clear plastic matrix (Guilbault 1973). The standard is normally related to the compound under investigation. For our measurements, several components of the wheat kernel were contributing to the pericarp fluorescence measurement, and hence the preparation of such a standard was not practical. The flour itself cannot be used as a standard because of its heterogeneity, while the dissection of a wheat kernel into its constituent parts to create a fluorescence reference (Jensen et al 1982) would be tedious and impractical for a long-term study such as ours. The stability of a tissue standard is also uncertain. A consideration that should also be made is that the sample may not respond in the same way as the standard, either to changes in the light source or as a result of changes in sample concentration. The response of the sample with changing concentration may not always be linear (Jensen et al 1982). The selection of an adequate standard would correct for all deviations identified in this study as attributable to instrumentation and sample heterogeneity.

Variability in fluorescence measurements resulting from operator, particle size, or moisture were very small or insignificant, especially when compared with the large differences between millstreams. The fluorescence imaging procedure used at the GRL is operator-independent (Tables III and IV) for practical purposes. Slight effects of particle size were noted, but these differences could be accounted for by the change in moisture content of the flour sample as a result of regrinding. The insensitivity of the fluorescence procedure to particle size is in contrast to the current rapid analytical methods using near-infrared reflectance (Posner and Wetzel 1986), a technique that is highly sensitive to particle size. However, the fluorescence system was affected by the moisture content of the flour (Table I), possibly the result of quenching by the additional water present. These slight changes in fluorescence require that flour ash be considered on an "as-is" moisture basis for the most precise refinement prediction. In an operational system, the moisture content of flour from a given millstream should not vary greatly. As a result, for continuous monitoring of refinement changes within a fixed millstream, the sensitivity of fluorescence measurements to moisture content would not be limiting.

While flour fluorescence is not greatly influenced by flour particle size or moisture content, tristimulus a* and b* coordinates are very sensitive to these characteristics, which limits their value for the estimation of flour refinement. Additionally, a* and b* values have been shown to be influenced by the sample cell used (Allen et al 1989). The L* value was not sensitive to moisture content and was only moderately sensitive to particle size. In addition, measurement of L* is rapid, simple, and precise, meriting further investigation as a flour refinement estimator.

Much of the interest in the measurement of flour quality using fluorescence procedures is based on the desire to have rapid and accurate flour refinement determinations. Flour fluorescence measurements would seem to have good potential for meeting these requirements. Both aleurone and pericarp fluorescence measurements, as made by our system, provided highly reproducible data on the degree of flour refinement. Pericarp fluorescence of millstreams was linearly related to flour ash, color grade, and L*. For our system, which uses a method different from that previously published (Fulcher et al 1987, Pussayanawin et al 1988), standardized aleurone fluorescence segregated millstreams into two groups.

Although the GRL flour imaging system has a few technical aspects that require further investigation, under our controlled laboratory conditions we were able to compare and correct data for these technical variations. Flour fluorescence procedures are rapid, accurate, and reproducible and have potential as a method of measuring flour refinement.

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