

# Estimation of Milling Efficiency: Prediction of Flour Refinement by the Measurement of Pericarp Fluorescence<sup>1</sup>

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## ABSTRACT

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Millstreams from pilot-scale millings of commercially grown wheat representing different crop years and several Canadian wheat classes were used to evaluate the measurement of flour refinement by fluorescence imaging of pericarp tissue. Flour fluorescence was measured using a No. 09 filter combination (excitation 450–490 nm, emission >520 nm) specific for pericarp. Pericarp fluorescence was able to discriminate between four groups of divide flours (first patent, second patent, straight grade, and clears) prepared from No. 1 Canada Western Red Spring (CWRS) millstreams. However, the measurements were not fully sensitive to the small variations in flour refinement among the flours within each divide flour group. The relationships of fluorescence measurements to flour ash content, grade color, and the tristimulus brightness component ( $L^*$ ) for

millings of Grain Research Laboratory harvest survey No. 1 CWRS eastern and western prairie composites from three successive crop years were completely homogeneous between crop years and growing location. Comparisons of millstreams from composites of the No. 1 grade of Canada Western Red Winter, Canada Prairie Spring (red), and Canada Western Utility wheat classes showed highly significant relationships between pericarp fluorescence and flour ash, flour grade color, and  $L^*$ . The relationships between pericarp fluorescence and the flour refinement indices for the different wheat classes were heterogeneous, however, indicating that the prediction of ash content or flour color established from millstreams obtained from one wheat class cannot be extrapolated to other wheat classes.

The use of fluorescence procedures for the prediction of flour refinement has been reported for both aleurone and pericarp measurements (Munck et al 1979, Symons and Dexter 1991) but has primarily concentrated on the measurement of aleurone particles or ferulic acid in flour samples (Fulcher et al 1972, Jensen et al 1982, Fulcher et al 1987, Pussayanawin et al 1988). Flour pericarp fluorescence has been used for the determination of fiber in rye flour (Kissmeyer-Nielson et al 1985) and more recently for the prediction of flour ash content and flour grade color in Canada Western Red Spring (CWRS) wheat millstreams (Symons and Dexter 1991). Recent interest also has been shown in the use of the Commission Internationale de L'Eclairage 1976  $L^*$ ,  $a^*$ , and  $b^*$  color space (tristimulus) coordinates for the measurement of flour refinement (Allen et al 1989, Dexter and Symons 1989, Symons and Dexter 1991). Pericarp fluorescence of CWRS millstreams has been related to the tristimulus brightness component ( $L^*$ ) (Symons and Dexter 1991).

The objective of this study was to establish the reliability of the estimation of flour refinement by pericarp fluorescence imaging. The relationships of pericarp fluorescence to ash content, grade color, and  $L^*$  for individual millstreams from pilot-scale millings of a No. 1 CWRS wheat export composite sample were used to predict each flour refinement index of divide flours. To evaluate the effects of crop year and location on pericarp fluorescence measurements, Grain Research Laboratory (GRL) eastern and western prairies No. 1 CWRS harvest survey composite samples from three consecutive crop years were compared. The effect of wheat class on pericarp fluorescence measurements was investigated by analyzing millstreams from Canada Western Red Winter, Canada Prairie Spring (red), and Canada Western Utility wheat classes.

## MATERIALS AND METHODS

### Wheat

The wheat used for prediction of divide flour refinement was a composite of No. 1 CWRS rail carlots unloaded at terminal elevators in Thunder Bay during the 1987–88 crop year as previously described by Symons and Dexter (1991). Other wheats used included the eastern and western prairies No. 1 CWRS

composite samples from the 1987, 1988, and 1989 GRL harvest surveys, a commercially grown sample of No. 1 Canada Western Utility (CWU) from the 1987 harvest, an export cargo composite of No. 1 Canada Western Red Winter (CWRW) wheat from the 1988–89 crop year, and a No. 1 Canada Prairie Spring (CPS) (red) composite sample from the GRL 1989 harvest survey.

### Milling

Wheats were prepared for milling as described by Dexter and Tipples (1987) and tempered in a Monarch mortar mixer (Black 1980). Millings were performed on the GRL pilot mill (Black 1980) using the modified hard wheat millflow described by Symons and Dexter (1991).

The No. 1 CWRS rail carlot composite was milled repeatedly over a one-year period while mill settings were altered to achieve a range of mill performance (Symons and Dexter 1991). Individual millstreams were retained, and divide flours were prepared according to common Canadian commercial milling practice (Panter 1988). The divide flours included a first-patent flour (60% of total flour comprising the best quality reduction streams), a second-patent flour (30% of total flour comprising the best quality break flours and intermediate quality reduction streams), a low-grade (clear) flour (the remaining 10% of total flour), and a straight-grade flour.

All of the other wheats were milled singly and individual millstreams were retained for analysis.

### Flour Refinement Measurements

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

Flour ash content (CV = 1.2%) was determined by the standard AACC (1983) method 08-01. A Simon color grader series IV (Henry Simon, Stockport, UK) was used for flour grade color determinations (SE = 0.15 units) 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  $L^*$  values (Francis 1983). The CV for  $L^*$  measurements was 0.381%.

### Fluorescent Imaging

The imaging and calibration procedures were previously described in detail (Symons and Dexter 1991).

Flour samples were loaded into a Dickey-john near-infrared cell that had been taped to a standard 2.5- × 10-cm microscope slide for ease of attachment to the microscope stage. For fluores-

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cent imaging, the sample holder was placed under a 10× Neofluor objective mounted on an Axiophot microscope (Carl Zeiss, Canada). Epi-illumination was from an HBO 50 burner through a No. 09 filter combination (excitation 450–490 nm, barrier >520 nm). The image was detected with a JVC BY110u color camera (JVC, Professional Division, Toronto, Canada) and, for measurement, the red, green, and blue (RGB) video signals were passed to an AT-IBAS image processing system (Kontron Elektronik, Eching, Germany) via a video multiplexer. For each flour sample, 25 images were captured using the 09 filter combination.

### Experimental Design

For the development of a calibration curve for the prediction of each flour refinement index for the divide flours, all millstreams from four millings of the No. 1 CWRS composite were fully randomized, with the divide flour samples and each replicate measured as a block in a fully randomized block design where each block represents a replicate. Three replicates were run. The divide flours were taken from 22 millings, not exclusive of the four calibration millings, to produce a range of flour refinement within each divide flour group.

Since system limitations had been previously identified (Symons and Dexter 1991), millstreams from the CWRS new crop composites and the other three wheat classes reported here also were intermixed randomly in each block. This design allowed direct comparison between all of the samples run and, in particular, direct comparison of the regression equations between the different wheat classes.

### Statistics

All statistics were calculated using the procedures of the SAS (1988) software system v6.04. Regression equations were compared for homogeneity using the fit for residuals from each regression line to the regression line for the combined populations (Neter et al 1990).

## RESULTS AND DISCUSSION

### Prediction of CWRS Divide Flour Refinement

Recently we demonstrated from repeated millings of the No. 1 CWRS rail carlot composite that the relationships of relative pericarp fluorescence of millstreams to flour ash content, grade color, and  $L^*$  are homogenous between millings and independent of flour age (Symons and Dexter 1991). The measurement of flour pericarp fluorescence is rapid and not affected by particle size (Symons and Dexter 1991). Therefore, the technique has potential for on-line monitoring of the refinement of key millstreams and blends.

Divide flours are custom-created from blends of several specific millstreams, usually to exacting refinement specifications (Panter 1988). Accurate measurement of divide flour refinement is important in determining commercial milling performance by maintaining the efficiency of the blending process, thereby ensuring maximum yield of the more valuable, highly refined products.

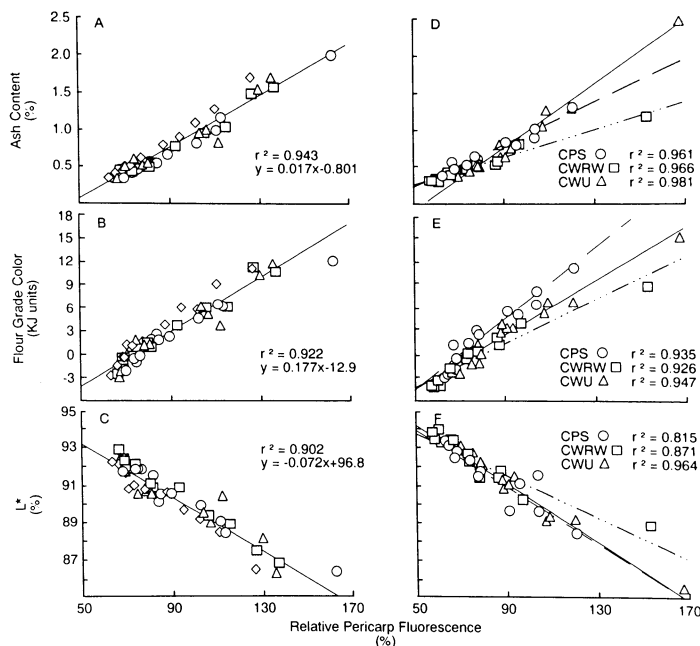
To test the ability of pericarp relative fluorescence to predict divide flour refinement, eight samples each of first-patent, second-patent, straight-grade, and clear flours were selected from over 30 replicate millings of the No. 1 CWRS rail carlot composite to provide a range of flour ash content, flour grade color, and  $L^*$  within each flour type. These divide flours were imaged in triplicate intermixed with all millstreams from four other millings. The four millstream sets were run to reestablish the regression between pericarp fluorescence measurements and flour ash content (Fig. 1A), flour grade color (Fig. 1B), and tristimulus  $L^*$  value (Fig. 1C), as previously reported (Symons and Dexter 1991). The  $a^*$  and  $b^*$  tristimulus color coordinates were not considered, as they were previously of little value in estimating flour refinement because of sensitivity to flour particle size and moisture content (Symons and Dexter 1991).

Established flour refinement indices such as flour grade color and ash content must be used with caution when comparing wheats

of diverse origin (Shuey 1976, Barnes 1986). However, they are very effective in ranking flours from a single wheat according to degree of refinement (Ziegler and Greer 1971). When the regression equations established from the four CWRS millstream sets were used to predict the refinement indices of the divide flours, each divide flour group was discriminated (Table I). The wide range of flour refinement for the second-patent flours prevented this group from being fully discriminated from the first-patent and straight-grade flours due to the overlap of flour refinement. The ranking of the individual flours within each divide group by relative fluorescence was not completely in agreement with the flour refinement indices.

Within each divide group, the prediction of flour refinement varied. The best prediction of flour refinement was within the second-patent (ash: SE of  $Y$  estimate = 0.034,  $r^2 = 0.87$ ; grade color: SE of  $Y$  estimate = 0.539,  $r^2 = 0.66$ ;  $L^*$ : SE of  $Y$  estimate = 0.231,  $r^2 = 0.62$ ) and clear flour (Table II) groups. This reflects the range of refinement in these two groups in contrast to the first-patent and straight-grade flours. To explore further the ability to predict flour refinement, the flours from first and second patents plus the straight-grade flours were combined to represent a range of high-quality commercial flours with a uniform distribution of refinement (group 1, Table II). The clear flours were used to represent a distinct group of low-grade flours (group 2, Table II). For group 1, the high-quality flours, a significant relationship of predicted to actual refinement was found for each flour refinement index (Table II). For group 2, the clear flours, the prediction of flour ash content appears to be best; however, color measurements are known to be affected by flour protein, which ranged from 13.7 to 15.8%.

The choice of experimental design could in part explain this apparent imprecision. Each replicate was measured on a separate day in the block design used to minimize the effects of instrumental variation (Symons and Dexter 1991) on the results. Therefore, by design, the experimental approach was for accuracy rather than precision. The predicted values for flour ash, grade color, and  $L^*$  also exhibited bias, both positive and negative, depending on refinement index and flour type (Table I). The prediction of



**Fig. 1.** Relationships of relative pericarp fluorescence values to flour refinement indices brightness ( $L^*$ ), grade color, and ash. A–C, All flour streams from four replicate millings of a No. 1 Canada Western Red Spring (CWRS) wheat. Replicate millings are indicated by different symbols. D–F, Three classes of Canadian wheats. CPS, Canada Prairie Spring; CWRW, Canada Western Red Winter; CWU, Canada Western Utility.

$L^*$  using pericarp fluorescence appeared to exhibit less bias than prediction of flour ash content and grade color.

These data verify the potential of pericarp fluorescence for monitoring flour refinement. The apparent inability of the procedure to detect small differences in refinement within each flour type may be attributable to instrumental limitations documented previously (Symons and Dexter 1991) or to differences in the flour characteristics (flour ash content, flour grade color, and  $L^*$ ) being measured by each method. Currently we are attempting to improve our precision and increase the sensitivity of our system to flour refinement. The differences in bias of the pericarp fluorescence measurements to flour ash content, grade color, and  $L^*$

indicate that although these tests are highly significantly correlated to each other, they are sensitive to different flour characteristics. For example, flour grade color is influenced by protein content independently of bran contamination (Barnes 1986).

#### Effects of Wheat Class on Pericarp Fluorescence

Wheats of differing class have differing characteristics, including variable hardness, chemical composition, and grain morphology, that influence milling performance (Ziegler and Greer 1971). These differences might be expected to affect the relationships between traditional flour refinement indices and pericarp fluorescence values. As a result, these relationships were established for repre-

**TABLE I**  
Predicted Flour Ash Content, Flour Grade Color, and  $L^*$  of Divide Flours from Several Millings of a No. 1 Canada Western Red Spring Composite<sup>a</sup>

Flour <sup>b</sup>	Pericarp Fluorescence	Ash		Color		$L^*$	
		Actual	Predicted	Actual	Predicted	Actual	Predicted
First-patent flours							
A	59.91	0.377	0.244	-1.7	-2.33	92.42	92.47
B	62.09	0.384	0.282	-1.1	-1.94	91.93	92.31
C	64.55	0.366	0.325	-2.4	-1.51	92.40	92.13
D	64.92	0.367	0.331	-2.2	-1.44	92.40	92.10
E	64.96	0.381	0.332	-2.2	-1.44	92.55	92.10
F	67.57	0.400	0.377	-1.7	-0.98	91.99	91.91
G	68.37	0.371	0.391	-1.7	-0.83	92.22	91.85
H	70.76	0.372	0.433	-2.2	-0.41	92.42	91.68
Mean	65.39 c	0.377 c	0.339 c	-1.90 c	-1.36 c	92.29 c	92.07 c
Second-patent flours							
E	65.39	0.427	0.339	-0.5	-1.36	91.81	92.07
H	69.25	0.434	0.407	-0.6	-0.68	91.91	91.79
G	69.62	0.482	0.413	0.0	-0.61	91.82	91.76
I	70.96	0.472	0.437	0.2	-0.38	91.13	91.67
J	71.67	0.508	0.449	0.5	-0.25	91.30	91.62
K	73.54	0.499	0.482	0.5	0.08	91.41	91.48
F	74.85	0.555	0.505	1.8	0.31	90.96	91.39
L	81.76	0.582	0.625	1.3	1.53	90.93	90.89
Mean	72.13 bc	0.495 b	0.457 bc	0.40 b	-0.17 bc	91.41 b	91.58 bc
Straight-grade flours							
M	73.30	0.529	0.478	0.1	0.04	91.47	91.50
N	75.24	0.566	0.511	1.1	0.38	91.04	91.36
O	76.06	0.507	0.526	0.7	0.52	91.41	91.30
P	76.76	0.549	0.538	1.3	0.65	91.04	91.25
Q	78.96	0.497	0.576	0.5	1.04	91.49	91.09
H	79.89	0.519	0.593	0.7	1.20	91.50	91.02
R	80.29	0.567	0.600	1.6	1.27	91.17	90.99
S	81.85	0.517	0.627	0.8	1.55	91.42	90.88
Mean	77.79 b	0.531 b	0.556 b	0.85 b	0.83 b	91.32 b	91.17 b
Clear flours							
A	98.19	1.062	0.912	7.9	4.43	88.51	89.70
L	99.84	0.925	0.941	4.9	4.72	89.54	89.58
T	106.28	0.975	1.053	5.4	5.86	89.32	89.11
I	107.22	0.973	1.070	7.0	6.02	88.75	89.04
U	108.38	1.308	1.090	8.5	6.23	88.03	88.96
V	109.07	1.205	1.102	7.7	6.35	88.50	88.91
D	120.07	1.201	1.294	7.8	8.29	88.55	88.12
F	134.01	1.385	1.538	9.2	10.75	88.09	87.11
Mean	110.38 a	1.129 a	1.125 a	7.30 a	6.58 a	88.66 a	88.82 a

<sup>a</sup>Regression equation for prediction was derived from the combined millstreams of four additional independent millings. Different letters following mean values for each column indicate significant differences for the means (Student Newman-Keuls  $P = 0.05$ ).

<sup>b</sup>Each letter indicates a different milling.

**TABLE II**  
Regression Analysis Results for the Prediction of Flour Ash Content, Grade Color, and  $L^*$

	Ash		Color		$L^*$	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Standard error of $Y$ estimate	0.059	0.145	0.661	1.75	0.300	0.762
$r^2$	0.730	0.562	0.666	0.374	0.593	0.292
Degrees of freedom	22	6	22	6	22	6
Standard error of $X$ coefficient	0.163	0.321	0.101	0.448	0.118	0.539

<sup>a</sup>Group 1 includes first- and second-patent and straight-grade flours; group 2 includes clear flours. Raw data are shown in Table I.

sentative commercial samples of CWRW, CPS (red), and CWU concurrent with the analysis of the CWRS samples.

All three classes gave highly significant correlations between pericarp relative fluorescence and flour ash content, flour grade color, and  $L^*$  (Fig. 1D-F). The relationships between pericarp fluorescence and flour ash, flour grade color, and  $L^*$  were heterogeneous between the three wheat classes, and heterogeneous to the No. 1 CWRS rail carlot composite as well (Fig. 1A-C). These results demonstrate that each wheat class has its own, perhaps characteristic, relationship between pericarp fluorescence and traditional flour refinement indices. Apparently pericarp fluorescence is measuring different properties of the flour from those determined by flour ash content, grade color, or  $L^*$ . In addition the difference in relative position of the regression lines for the three wheat classes showing the relationship of pericarp fluorescence to ash content (Fig. 1D) compared with flour grade color and  $L^*$  (Fig. 1E and F) verifies that flour ash content and flour color have some degree of independence for a diverse wheat population. In view of the established limitations of flour ash content and flour color for predicting flour refinement for wheats of diverse origin (Shuey 1976, Barnes 1986), the observed heterogeneity of the relationships between pericarp fluorescence and established flour refinement indices for Canadian bread wheat classes provides evidence that pericarp fluorescence may be a more objective flour refinement estimator. Certainly one would expect that the direct quantization of biological tissue should relate better to flour characteristics than the use of indirect markers such as flour ash content.

#### Effect of Crop Year and Growing Location on CWRS Pericarp Fluorescence

While it has been demonstrated that repeated millings of a single CWRS wheat produce homogenous relationships between pericarp fluorescence and flour refinement indices (Fig. 1A-C) (Symons and Dexter 1991), the measurement protocol would be more useful if it were stable for different wheat samples within a wheat class and, preferably, from year to year. Flour ash content and/or flour color are well established as flour refinement indices in many markets because they are so readily standardized. As a result, traditional refinement indices, despite their limitations, are likely to continue to be used as quality specifications by flour processors.

To determine the effects of growing region on measurements of pericarp fluorescence, No. 1 CWRS composites from eastern and western prairies from the 1987, 1988, and 1989 harvests were milled, the refinement of individual millstreams was determined, and the millstreams were analyzed for pericarp fluorescence concurrently with the flours from the CWRS rail carlot composite and the other wheat classes. For all three crop years the relationships of pericarp fluorescence to flour ash content, grade color, and  $L^*$  for eastern and western composites were homogenous. The results for the 1987 harvest are shown as an example in Figure 2A-C.

The eastern and western prairies composites not only represent different environments and different soil conditions, but also differences in varietal distribution. Therefore the homogeneity of the relationships of pericarp fluorescence to flour refinement indices for the two growing regions demonstrates that the relationships of pericarp fluorescence to flour ash content (Fig. 2A), flour grade color (Fig. 2B), and  $L^*$  (Fig. 2C) will be stable between different CWRS wheat lots within a crop year.

A further demonstration of the reliability of the relationships between pericarp fluorescence and flour refinement indices was the complete homogeneity of the relations of pericarp fluorescence to all three refinement indices for three consecutive crop years (Fig. 2D-F) and to the CWRS calibration millings. These three crop years represent even greater differences in growing conditions and greater shifts in varietal distribution than do the eastern and western prairies composites from a given crop year.

#### CONCLUSIONS

Our system for the prediction of flour refinement by pericarp

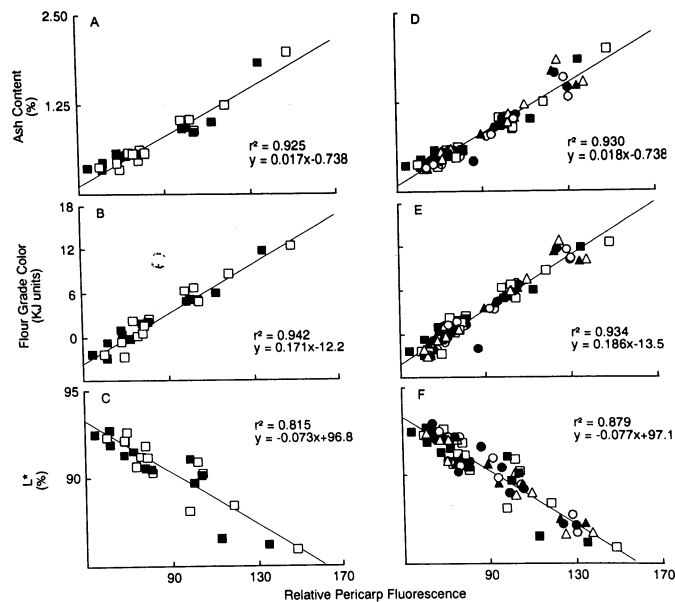


Fig. 2. Relationships of relative pericarp fluorescence values to flour refinement indices for all flour streams from A-C, 1978 composites of eastern (□) and western (■) prairies No. 1 Canada Western Red Spring (CWRS) wheat, and D-F, 1987, 1988, and 1989 composites of No. 1 CWRS wheat. Different symbols indicate years.

fluorescence has some limitations in sensitivity to very small differences in divide flour characteristics. However, the discrimination of each divide flour group was distinct. The gross variation in flour refinement indices within a divide flour group were distinguished. The pericarp fluorescence method gave homogenous results for replicated millings of No. 1 CWRS wheat and across CWRS wheat lots from different locations and from different years. The apparent strength of the relationships of pericarp fluorescence and flour refinement indices between crop years provides convincing evidence that stable relationships can be maintained among different lots within a wheat class. This is a very promising characteristic of the fluorescence procedure. Differences in the relationship of pericarp fluorescence to flour ash, grade color, and  $L^*$  were found between different classes of Canadian wheats. Thus, the measurement is sensitive to the different characteristics of the wheat classes.

We are pursuing further the effects of milling procedure on pericarp fluorescence by using different mills and are additionally exploring various methods to improve the sensitivity of our measurement system.

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