# The Botanical Constituents of Wheat and Wheat Milling Fractions. I. Quantification by Autofluorescence[1]

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## ABSTRACT

A method is described for quantifying pericarp, aleurone, and endosperm in wheat milling fractions by their autofluorescence characteristics. Fluorescence data from wheat fractions are evaluated by a statistical model initially calibrated against manually dissected botanical parts and synthesized mixtures with known compositions. The composition of mixtures of pure botanical components could be quantified by their autofluorescence. The method was tested on decortication fractions from a winter wheat milled in an abrasive decorticator. A high correlation existed between pericarp and fiber and between endosperm and starch, whereas a significantly lower correlation existed between aleurone and ash. Autofluorescent quantification of the botanical components of wheat flour streams may be more relevant than standard chemical analyses such as ash for monitoring the physical separation of these components in wheat milling processes.


Dry milling of wheat aims at separating the different botanical parts of the seed: pericarp, testa, aleurone, embryo, and endosperm. On one hand, the tissues have different structure and mechanical properties important in milling; on the other, they have widely different chemical compositions that ultimately determine the nutritional value (Munck 1981) and properties of the end products (eg, for baking) (Pomeranz 1980a,1980b). Thus, an analytical method that gives highly accurate measurements of the botanical components of various mill streams is important in optimizing their mechanical separation in milling processes and in product development.

Ash and color analyses are widely used to monitor wheat milling. The measurements are quite effective if the aim is to monitor the removal of whole bran (pericarp, testa, and aleurone) from the endosperm. This is normally the goal for a conventional roller-milling system. These analyses are not satisfactory, however, if the aim is to separate, for example, aleurone from pericarp and testa, because the pericarp, testa, germ, and aleurone are rich in ash (Hinton 1959) and contribute to color grade (Chen and Geddes 1945).

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Several chemical components in wheat, alone or in combinations, can be used as precise indicators of botanical parts. Niacin is an excellent indicator of aleurone because 82% of the wheat kernels' niacin is concentrated in the aleurone layer (Heathcote et al 1952). In a similar way, other vitamins, amino acids, minerals, and carotenoids may be used as indicators of the botanical constituents. Unfortunately, most of these analyses are lengthy to perform and are, therefore, only of theoretical interest.

We have proposed an alternative method (Munck et al 1979a, 1979b) in which the specific autofluorescence of pericarp, aleurone, and endosperm can be used as indicators of these three botanical constituents. However, the complicated autofluorescence energy transfer systems observed in cereal fractions need to be further defined and evaluated with an appropriate statistical model to quantify the botanical composition. We have further developed the autofluorescence method toward a quantification of the botanical constituents in wheat-flour streams.

## MATERIALS AND METHODS

### Raw Material

A sample of a winter wheat (variety Solid) harvested in 1979 was used. To obtain a sample with uniform seed size and density, the wheat was processed in the Carlsberg Research Mill. The sample (500 kg) was cleaned and separated in a Cimbria Unigrain Seed Cleaner, type Super 03 (Cimbria A/S, Thisted, Denmark). A main fraction of 258 kg was obtained by using sieve units at 2.8 and 3.5 mm. This fraction was separated further by a Delta Gravity

Separator type 51 (Cimbria A/S, Thisted, Denmark). Four fractions were obtained with varying density, and the fractions with the largest density (42 kg) were used. The sample was stored at 2°C for later use.

### Dissection of Wheat Kernels

The germ (embryonic axis plus scutellum) from individual dry kernels was gently removed using forceps and stored at 2°C. Degermed kernels were softened for dissection on a wet sand bed and kept in the dark at 2°C for 4 hr. Individual kernels were dissected under a microscope (×40). The outer pericarp was readily removed with a scalpel and the pigment strand gently drawn out. The pericarp remaining attached to the testa layer was then removed by working a scalpel between the testa and cross cell layer.

The testa layer could not be removed from the underlying aleurone cells in a similar way. Wheat kernels were therefore split along the crease, and the endosperm, including the subaleurone layer, was removed by careful scraping with a scalpel. All the excised endosperm was collected, leaving shells of aleurone plus testa. The testa layer was removed from the underlying aleurone cells by prolonged, careful scraping, leaving shells of pure aleurone cells. The progress in the separation was easy to follow because of the characteristic reddish-brown color of the testa layer.

The purity of the fractions obtained was evaluated with fluorescence microscopy (Bacic and Stone 1981, Fulcher and Wong 1979). All separated botanical parts, except germ and endosperm, were examined with a Reichert-Jung Univar microscope (Reichert-Jung, Vienna, Austria) with the filter combinations given in Table I.

### Decortication and Sieving Procedures

Fifteen kilograms of wheat sample was successively decorticated in a Schule laboratory vertical decortication machine (type LVSM, F. H. Schule GmbH, Hamburg, W. Germany) consisting of nine abrasive stones (silicium carbide). The adaptability and functions of the machine were previously outlined (Zwingelberg 1980). Ten fractions were produced varying from an accumulated decortication rate of 1.1 to 34.7% (w/w). (Table II).

**TABLE I**
**Spectral Characteristics of Fluorescence Filter Combinations for Microscopy**

| Combinations | Exciter Filter[a] (nm) | Barrier Filter[b] (nm) | Identification of[c] | | | |
|---|---|---|---|---|---|---|
| | | | Pericarp | Aleurone | Testa | Endosperm |
| I | 450–490 | LWP 515 | +++ | + | ++ | + |
| II | SWP 500 | LWP 515 | +++ | + | ++ | + |
| III | SWP 405 | LWP 418 | + | +++ | ++ | + |

[a]SWP = short wave pass.
[b]LWP = long wave pass.
[c]+++ = easy, ++ = possible, and + = difficult to identify.

A subsample of 50 g of the decortication fractions was separated further by sieving in an Alpine Luftstrahlsieb 200 (Alpine GmbH, Augsburg, W. Germany) as described in Table II.

Whole wheat and pearled wheat kernels were milled in a Udy Cyclone mill (Udy Analyzer Co., Boulder, CO) through a 1.0-mm sieve and the flour thoroughly mixed after milling.

### Determination of Moisture, Fiber, Ash, and Starch

The moisture content of the milled wheat samples was measured by oven drying 2 hr at 130°C. Analyses of crude fiber and ash were made according to AACC (1979) and ICC (1977) methods, respectively. Starch was measured after enzymatic degradation by amyloglucosidase (E. Merck GmbH, Darmstadt, W. Germany) and the liberated glucose estimated with a glucose-peroxidase reagent (Boehringer, Mannheim, GmbH, W. Germany).

All results were based on duplicate determinations, and the results for fiber, ash, and starch are reported on a dry basis.

### Autofluorescence Measurements

*Equipment.* All experiments were performed with a scanning spectrofluorometer (Jasco FP 550, Japan Spectroscopic Co. Ltd.). The output signal from the spectrofluorometer was transferred to a Hewlett Packard 9825T calculator. Details and specifications of the fluorescence system were previously published (Jensen and Aastrup 1981).

*Sample Preparation.* A subsample of flour of about 100 mg was

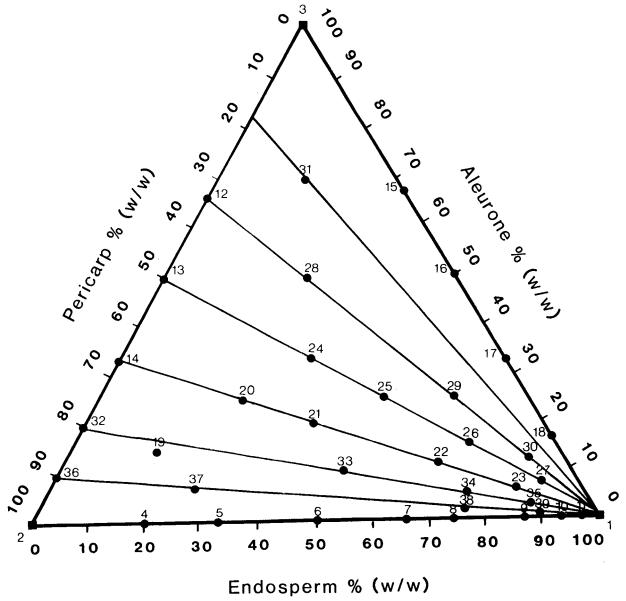


**Fig. 1.** Triangular concentration of pericarp, aleurone, and endosperm showing the 39 calibration samples (total concentration, 2 mg/ml).

**TABLE II**
**Sequence of Decortication and Sieving of Wheat Fractions 1–50**

| | Main Fraction | Accumulated Decortication Rate | Sieving Fractions | | | |
|---|---|---|---|---|---|---|
| | | | <32μ | 32μ << 63μ | 63μ << 125μ | >125μ |
| Decortication Fractions | 1 | 1.1 | ← | 11 | → | 12 |
| | 2 | 5.1 | 13 | 14 | 15 | 16 |
| | 3 | 6.9 | 17 | 18 | 19 | 20 |
| | 4 | 10.3 | 21 | 22 | 23 | 24 |
| | 5 | 12.7 | 25 | 26 | 27 | 28 |
| | 6 | 19.3 | 29 | 30 | 31 | 32 |
| | 7 | 26.3 | 33 | 34 | 35 | 36 |
| | 8 | 28.7 | 37 | 38 | 39 | 40 |
| | 9 | 30.8 | 41 | 42 | 43 | 44 |
| | 10 | 34.7 | 45 | 46 | 47 | 48 |
| Wheat | 49 | ... | ... | ... | ... | ... |
| Decorticated wheat | 50 | ... | ... | ... | ... | ... |

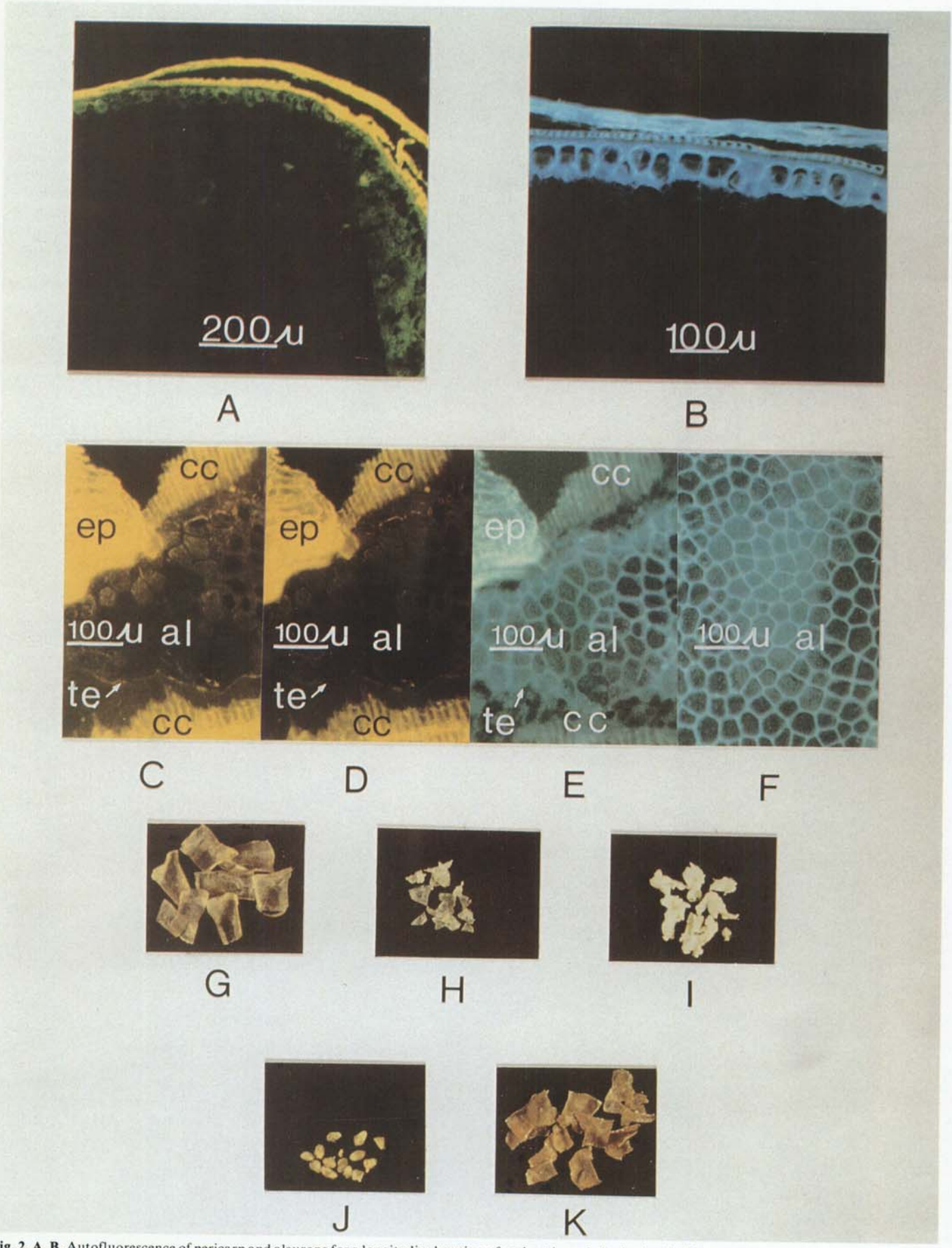


**Fig. 2. A, B,** Autofluorescence of pericarp and aleurone for a longitudinal section of a wheat kernel obtained with filter combinations I and III, respectively. **C–F,** Identification of the different botanical parts. al = aleurone, cc = cross cells, ep = outer pericarp, and te = testa, using (**C**) filter combination II, (**D**) filter combination I, and (**E**) filter combination III. **F,** A shell of purified aleurone cells obtained with filter combination III. **G–K,** Photographs in normal light of manually dissected: **G,** pericarp; **H,** aleurone; **I,** endosperm; **J,** germ; and **K,** aleurone plus testa.

milled in a ball mill (F. K. Retsch GmbH, W. Germany) for 15 min to obtain a homogenous fine flour. The sample was dried in a vacuum oven at 50° C to constant weight and stored in a dessicator until use.

Twenty milligrams dry weight was taken for the fluoroscence measurement, and a 10-ml, 3:1 mixture of fluorescence-free glycerol (E. Merck GmbH, Darmstadt, W. Germany), and distilled water was added. The suspension was mixed vigorously on a Whirli-mixer and transferred to a 10 × 10-mm quartz cuvette. Fluorescence spectra were obtained according to the specifications given in Table III. The spectra were recorded in the order A-B-C, and each spectrum was stored as 56 digital values on an HP 9825T data tape. For calculation, the 3 × 56 = 168 digital values were reduced to 3 × 18 = 54, culminating in one spectrum consisting of 54 wavelength variables.

The manually dissected wheat fractions were treated as described for the milling fractions. Fluorescence spectra were characterized and obtained at different concentrations for the individual constituents: pericarp, aleurone, and endosperm. The estimation was limited to calculating the content of pericarp, aleurone, and endosperm because of a lack of specific information regarding germ and testa.

A set of 36 calibration mixtures was made with various combinations of pericarp, aleurone, and endosperm (total concentration 2 mg/ml) (Fig. 1). Fluorescence spectra were obtained according to Table III.

Background due to the spectra of 75% glycerol was subtracted from all the recorded spectra.

*Quantification.* The relationship between fluorescence emission and the concentration of a fluorescent compound (FRP) is given as:

$$F = Io \cdot Qrel\ (1 - 10^{-\xi \cdot L \cdot C}). \quad (1)$$

where F = intensity of fluorescence emission, Io = intensity of incident light, Qrel = quantum yield, $\xi$ = extinction coefficient, L = path length, and C = concentration of FRP.

At high concentrations, fluorescence is no longer proportional to the concentration of the FRP. Transformation of the nonlinear equation 1 to equation 2 provides a linear calibration curve relating fluorescence over an extended range of concentration:

$$C = 1/\xi \cdot -\log\left[1 - \frac{F}{(Io \cdot Qrel)}\right] \quad (2)$$

for L = 1.

For the present experiments, K = Io · Qrel and $\xi$ were estimated by the method of Bjork and Dahlqvist (1969). The criterion used for an optimal solution (choice of K) was a maximum correlation between concentration and the logarithmic expression of equation 2.

**TABLE III**
**Specifications of Recorded Fluorescence Spectra**

| Spectrum | Excitation (nm) | Emission Range (nm) | Relative Gain |
|---|---|---|---|
| A | 275 | 296–464 | 1 |
| B | 350 | 371–539 | 7 |
| C | 450 | 471–639 | 60 |

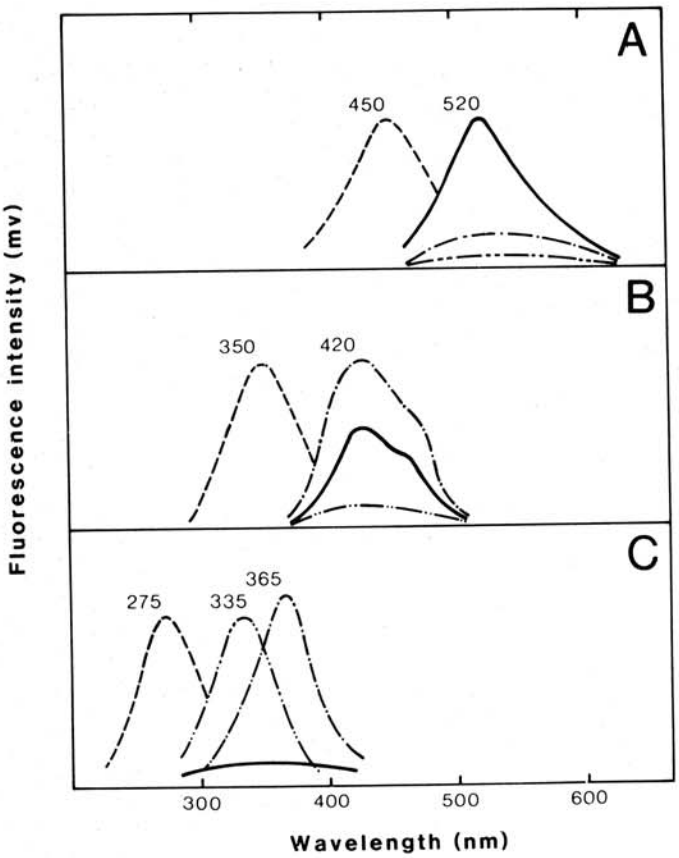


**Fig. 3.** Excitation (---) (**A**, pericarp; **B**, aleurone; **C**, endosperm) and emission spectra of manually dissected pericarp (——) aleurone (— · —) and endosperm (— ·· —) at excitation: A, 450 nm; B, 350 nm; C, 275 nm.

### Statistical Calibration Procedures

To predict the concentration of botanical components from the autofluorescence spectra of an unknown sample, a mathematical relationship between spectral data and concentrations must be established in samples with known concentrations by a multivariate calibration procedure.

Because the fluorescence signals from mixtures of botanical components did not behave additively, models less rigorous than Beers law needed to be used to relate fluorescence data to component concentration.

Two different approaches were tested. Quantitative K nearest neighbors interpolation (KNN) (Jensen 1982, Wold and Sjostrøm 1977) was chosen as a virtually model-free exploratory method, whereas partial least squares (PLS) (Wold et al 1980) regression on latent variables was chosen as a soft linear analytical model. The PLS regression method gave the best relationship.

The PLS approach to the prediction of the concentration of botanical components from fluorescence spectra is similar to the indirect, multivariate calibration of near-infrared reflectance data to chemical data (Williams 1975). In the PLS regression, however, the 54 fluorescence variables are transformed to a small set of latent variables (PLS-factors), which are related to the botanical component concentration. In contrast to the multiple linear regression technique used in near-infrared reflectance calibration, the PLS-regression allows extensive multicolinearity and noise in the variables and utilizes all variables in the final solution (Martens 1982).

## RESULTS

### Fractionation of Kernels by Manual Dissection

The characteristic autofluorescence of pericarp and aleurone is shown in Fig. 2A–B, for a longitudinal section of a wheat kernel. With filter combination I (Table I), both the outer pericarp and the inner pericarp exhibited a yellowish-green color. The chemical basis for this autofluorescence has, however, not yet been established. In contrast, ferulic acid has been shown to be responsible for the bluish-white autofluorescence of the thick cell walls of the aleurone seen under ultraviolet excitation (filter combination III) (Fulcher et al 1972). With this filter combination, pericarp also exhibited a blue autofluorescence, whereas a slight autofluorescence was observed from the cell walls of the endosperm.

Manual dissection of germ, outer pericarp, and endosperm was done readily (Fig. 2C–F). Progress in the separation of cross cells

from the underlying testa layer was followed using filter combinations I and II. The cross cells, often somewhat rectangular, were clearly visualized as a compact layer with a nonfluorescent testa layer below (Fig. 2C–E). Each shell of purified aleurone plus testa cells was investigated under the microscope. Adjacent starchy endosperm of subaleurone could be identified by a decrease in fluorescence intensity. For the purification of aleurone cells, the adjacent testa cells were observed as black sheets under ultraviolet excitation (Fig. 2E). In blue excitation, testa cells were identified as two layers crossing each other (Fig. 2C–D). The dissection resulted in shells of purified aleurone cells as seen in Fig. 2F.

Photographs of the manually dissected fractions are shown in Fig. 2G–K. Pericarp and germ are yellow because carotenoid and flavanoid pigments are present. The retention of these pigments in the flour is generally considered undesirable because of consumer demand for a white product, ie, pure endosperm. Sheets of aleurone plus testa are reddish-brown, whereas purified aleurone cells are almost white. This demonstrates that aleurone cells contribute only slightly to color grade if mixed with endosperm.

### Excitation and Emission Spectra of Pericarp, Aleurone, and Endosperm

The finely milled, manually dissected fractions suspended in glycerol displayed fluorescence spectra as shown in Fig. 3A–C. The pericarp fraction showed a broad emission peak at 520 nm when optimally excited by light at 450 nm (Fig. 3A). When excited by light of this wavelength, endosperm and aleurone fractions exhibited weak emission at 520 nm. These results confirm the qualitative results obtained with the fluorescence microscope. For the aleurone fraction, a main emission peak was found at 420 nm with a shoulder at 470 nm when optimally excited by light at 350 nm (Fig. 3B). The peaks observed at 420 and 470 nm are characteristic for ferulic acid (Fincher 1976). The pericarp fraction shared the same characteristic emission spectrum, thus indicating the presence of ferulic acid, but at a lower level (about 50% that of aleurone), whereas the emission for endosperm was weak.

When excited by light at 275 nm, the endosperm and aleurone fractions were characterized by strong emission peaks at 335 and 365 nm, respectively (Fig. 3C). The excitation band at 275 nm is typical for proteins containing tryptophan (Konev 1967). In addition, tyrosine and phenylalanine also exhibit autofluorescence. Because the quantum yield of these amino acids is low compared to that of tryptophan, the major fluorescence response is from tryptophan.

### Fluorescence Intensity Related to Concentration of Pericarp, Aleurone, and Endosperm

*Individual Components at Different Concentrations.* The relationship between endosperm concentration and fluoroscence intensity read at 335 nm was in accordance with the theoretical equation 1, providing an asymptotic approach to an upper limit (Fig. 4). The linear correlation coefficient between F and C was r = 0.957. Iterative transformation of the data according to equation 2 (Fig. 5) improved the linear correlation coefficient to r = 0.999, confirming the nonlinear relationship between endosperm concentration and fluorescence intensity. The optimum solution was reached at Io · Qrel = 1,665 mV.

Similar relations were found for pericarp and aleurone (Table IV). The linear correlation coefficients were improved from 0.961 to 0.998 and from 0.916 to 0.998 for pericarp and aleurone, respectively, by transformation of the data according to equation 2.

These results confirm that quantitative fluorescence of suspended samples of each botanical constituent behave separately in accordance with the fluorescence theory (equations 1–2).

*Mixtures of Pericarp, Aleurone, and Endosperm.* Considering the 39 calibration samples of constant total component concentration shown in Fig. 1, the fluorescence intensity of the botanical constituents was rather complex, showing a nonadditive relationship. This situation is illustrated two-dimensionally in Figs. 6–8, where individual wavelengths out of the 54 wavelength variables were selected to maximize the differences between pericarp, aleurone, and endosperm. In Fig. 6, the fluorescence intensity read at excitation 350 nm and emission 420 nm (aleurone primary, pericarp secondary) is related to the fluorescence intensity read at excitation 275 nm and emission 375 nm (aleurone primary, endosperm secondary). The triangular configuration of Fig. 1 and the order of the calibration points are retained in Fig. 6, but the positions of the calibration samples are significantly shifted. Calibration samples 13, 24, 25, 26, and 27 had a 50:50 proportion of pericarp and aleurone at progressively increasing content of endosperm. These samples lie on a curve running from sample 13 (50% pericarp, 50% aleurone) to sample 1 (100% endosperm). However, the position of sample 13 is shifted towards 100% pericarp compared to Fig. 1, and sample 24 (1/3 pericarp, 1/3 aleurone, 1/3 endosperm) is shifted towards the zero endosperm line running from pure pericarp to pure aleurone. These results

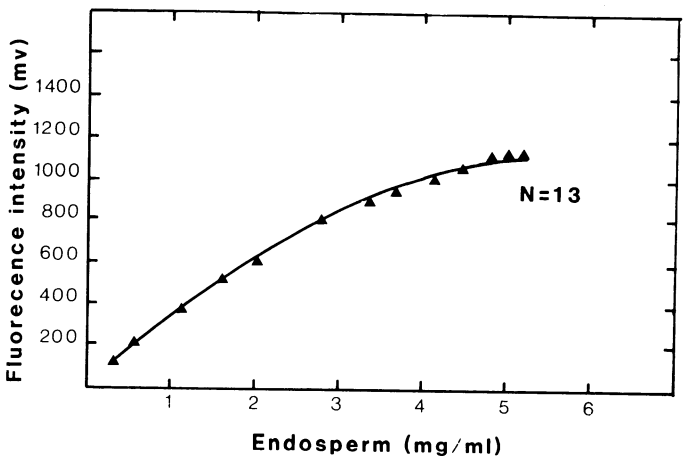


**Fig. 4.** Relationship between endosperm concentration and fluorescence intensity (read at 335 nm).

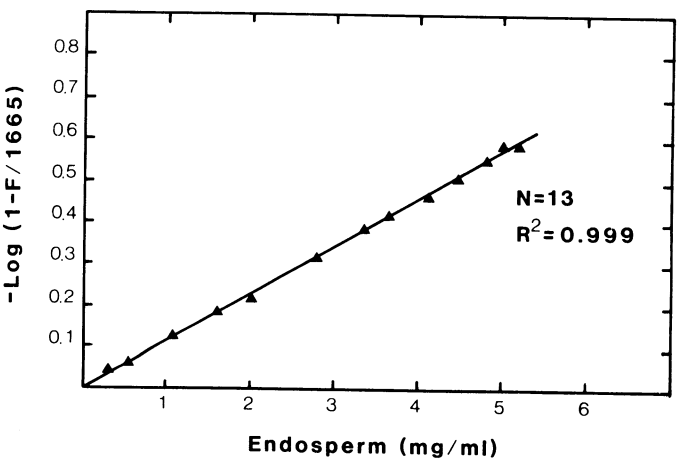


**Fig. 5.** Relationship between endosperm concentration and fluorescence intensity (read at 335 nm) transformed according to equation 2. F = fluorescence intensity in millivolts.

**TABLE IV**
**Regression Results for the Botanical Components Analyzed Separately**

| | Number | *r[a] | Io · Qrel Estimated (mV) | **r[b] | Excitation/ Emission Wavelengths (nm) |
|---|---|---|---|---|---|
| Pericarp | 10 | 0.961 | 2729 | 0.998 | 450/520 |
| Aleurone | 12 | 0.961 | 1350 | 0.998 | 350/420 |
| Endosperm | 13 | 0.957 | 1665 | 0.999 | 275/335 |

[a] * = correlation coefficient between C and F.
[b] ** = correlation coefficient between C and $-\text{Log}[1 - F/I_o \cdot \text{Qrel}]$.

demonstrate that the fluorescence signals of the botanical constituents interfere greatly with one another. Generally, most of the calibration samples were shifted towards 100% pericarp, compared to the triangular concentration diagram (Fig. 1).

A similar tendency is observed for two other two-dimensional delineations. In Fig. 7, the fluorescence intensity read at excitation 275 nm and emission 320 nm (endosperm primary, aleurone secondary) is related to fluorescence intensity read at excitation 275 nm and emission 375 nm (aleurone primary, endosperm secondary). In Fig. 8, the fluorescence intensity read at excitation 450 nm and emission 520 nm (pericarp primary, aleurone secondary) is related to fluorescence intensity read at excitation 350 nm and emission 420 nm (aleurone primary, pericarp secondary).

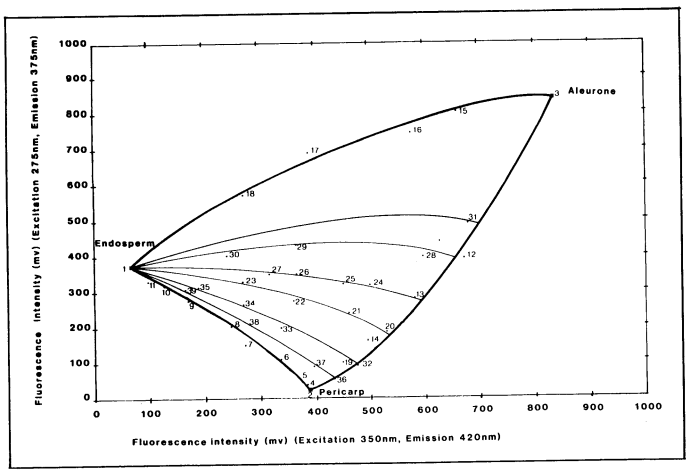


**Fig. 6.** Two-dimensional plots of the 39 calibration samples. Fluorescence intensity at excitation 350 nm (emission 420 nm) related to fluorescence intensity at excitation 275 nm (emission 375 nm).

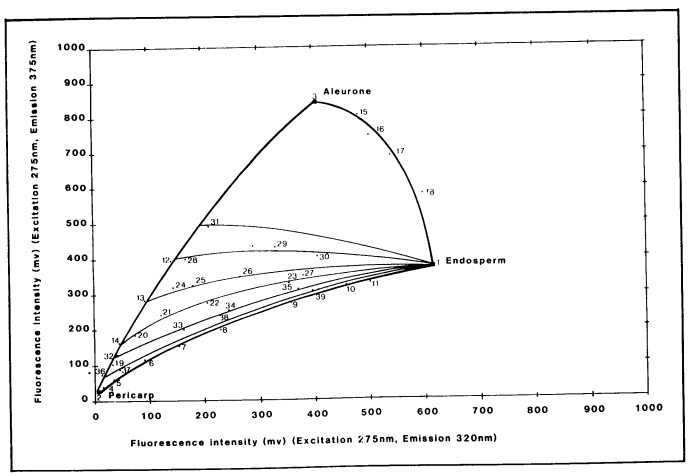


**Fig. 7.** Two-dimensional plots of the 39 calibration samples. Fluorescence intensity at excitation 275 nm (emission 320 nm) related to fluorescence intensity at excitation 275 nm (emission 375 nm).

## Evaluation of the PLS Method

The potential of the PLS method was evaluated with respect to the predictive ability on independent samples. The regression was performed on 19 of the 39 calibration samples, chosen at random, and the remaining 20 samples were used for testing. For pericarp, aleurone, and endosperm, the standard error of prediction was 4.5, 3.4, and 7.9, respectively, and the squared correlation coefficients between known and predicted compositions were 0.994, 0.982, and 0.984, respectively.

## Botanical Composition of Wheat and Wheat Fractions

The botanical composition (pericarp, aleurone, and endosperm) of wheat and wheat fractions was estimated using the PLS-calibration.

*Whole and Pearled Wheat.* The percentages of pericarp, aleurone, and endosperm for whole wheat were found to be 9.7, 6.7, and 80.2%, respectively, in good agreement with the results obtained by manual dissection (Table V). Pericarp, however, had a slight bias toward overestimation. Both the fluorescence method and manual dissection yielded results that agreed with previously published data (Hargin and Morrison 1980, Hinton 1959).

For pearled wheat, a pericarp and aleurone content of 0.8 and 2.6%, respectively, was found. This implies a retention of 8% for the pericarp and 38% for the aleurone situated in the crease and thus inaccessible to decortication.

*Decortication and Sieving Fractions.* For the decortication fractions, the majority of pericarp was removed until an accumulated decortication rate of 10.3% (fraction 4 in Table II) was reached (Fig. 9).

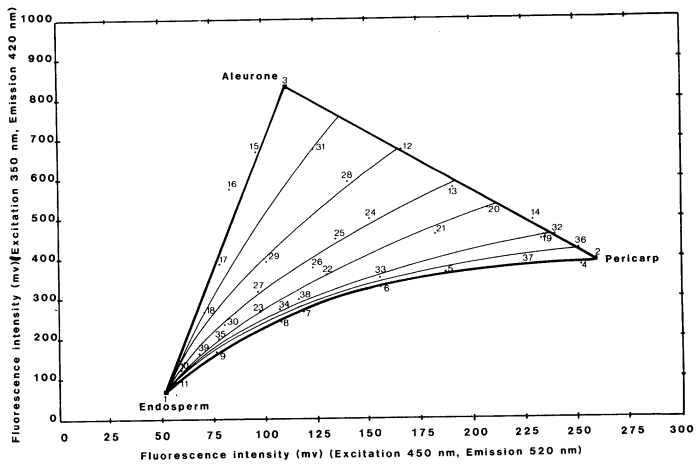


**Fig. 8.** Two-dimensional plots of the 39 calibration samples. Fluorescence intensity at excitation 450 nm (emission 520 nm) related to fluorescence intensity at excitation 350 nm (emission 420 nm).

**TABLE V**
**Weight Distribution of the Botanical Constituents in Wheat and Pearled Wheat Kernels**

| Weight (percent dry basis) | Whole Wheat | | | | Pearled Kernels |
|---|---|---|---|---|---|
| | Fluorescence Method[a] | Manually Dissected[b] | Hargin and Morrison (1980) | Hinton (1959) | Fluorescence Method |
| Pericarp | 9.7 ± 1.0 | 8.2 ± 0.4 | 8.4 | 7.4 ± 9.5 | 0.8 |
| Aleurone | 6.7 ± 0.7 | 6.5 ± 0.4 | 8.4 | 6.4 ± 7.0 | 2.6 |
| Endosperm | 80.2 ± 2.8 | 81.8 ± 1.3 | 80.3 | 81.4 ± 84.1 | 96.6 |
| Germ | (2.2)[c] | 2.2 ± 0.2 | 3.0 | 1.8 ± 3.6 | ... |
| Testa[d] | (1.3)[c] | 1.3 | ... | ... | ... |

[a] N = 2.
[b] N = 20. Results are given as ± 1 standard deviation.
[c] Values from manual dissection used in the calculations.
[d] Calculated by difference.

The pericarp content decreased about 50% from fraction 2 to fraction 3, indicating that the outer pericarp was readily removed in the decortication process. On the other hand, the pericarp in the crease region caused delay in the pericarp release observed for fractions 4–10.

For aleurone, a maximum content of 25% was found in fraction 3, with an accumulated decortication rate of about 7%. In the following fractions, the aleurone content decreased in a way similar to that of pericarp.

The endosperm content in fraction 1 was high, about 15%. This result indicates that the separation of the botanical constituents of wheat with the vertical decortication machine was relatively poor. The percentage of endosperm increased slightly in fraction 2, followed by a sigmoidal increase approaching an asymptote equal to an endosperm content at 100%. The endosperm content in fraction 10 was 88%, as compared to 97% in the pearled kernels.

Integrating the component contents of the 10 different decortication fractions and the pearled remainder yielded a reconstituted composition of the whole wheat of 7, 6, and 86% for pericarp, aleurone, and endosperm, respectively. Thus, pericarp and aleurone were slightly underestimated and endosperm overestimated compared to the composition obtained by manual dissection (Table V).

The botanical composition (pericarp, aleurone, and endosperm) for the sieving fractions—less than 32 $\mu$ (for fraction 1 less than 125 $\mu$) and more than 125 $\mu$ is related to their corresponding accumulated decortication rate (Fig. 10). The separation of pericarp by sieving was very pronounced. Up to an accumulated decortication rate of 26.3% (fraction 7), all sieving fractions greater than 125 $\mu$ had a pericarp content about twice that in the sieving fractions less than 32 $\mu$. The pericarp content in the intermediate sieving fraction, 32 $\mu$<<63 $\mu$ and 63 $\mu$<<125 $\mu$, was observed to be between the two extremes illustrated in Fig. 10. This demonstrates that the pericarp of the milled product tends to have a greater particle size. The content of endosperm was largest in the sieving fractions less than 32 $\mu$. In contrast, only a slight separation was observed for aleurone. Even the two intermediate sieving fractions had an aleurone content close to those illustrated for the two extreme fractions.

**Botanical Composition Related to Ash, Fiber, and Starch Content**

The relationship between the botanical and chemical constituents is shown in Table VI. The correlation coefficient (r) was calculated using all 50 wheat milling samples. These results demonstrate that starch and fiber measurements were excellent indicators of endosperm and pericarp, respectively, whereas measurement of ash yielded little information regarding the botanical constituents. Endosperm and starch were highly positively correlated, with a correlation coefficient of r = 0.978, whereas pericarp and starch provide a negative correlation coefficient of r = ÷ 0.942. An opposite tendency was observed for fiber with a positive correlation coefficient between fiber and pericarp of r = 0.974. The correlation between ash content and the botanical parts was relatively low but was highest for aleurone with a positive coefficient of r = 0.891.

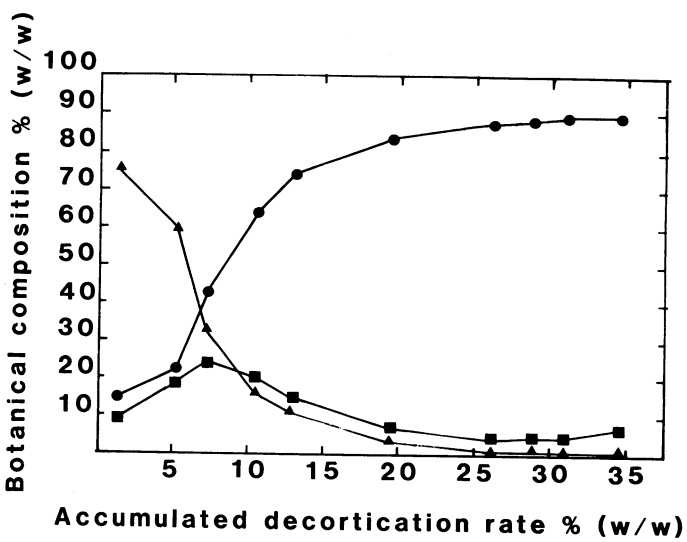


**Fig. 9.** Botanical profile of the decortication fractions, predicted by partial least squares modelling of their autofluorescence spectra. ▲ = pericarp, ■ = aleurone, and ● = endosperm.

## DISCUSSION

The method used was developed using the autofluorescence characteristics of pericarp, aleurone, and endosperm to measure percentages of these components in wheat milling fractions. The spectrofluorometric analyses of manually dissected botanical parts demonstrate that the yellowish-green autofluorescence observed for pericarp was specific and in accordance with the microscopic observations. Studies of the autofluorescence of ferulic acid showed only traces of this acid in the endosperm, whereas the content in pericarp was about half that in the aleurone layer, a

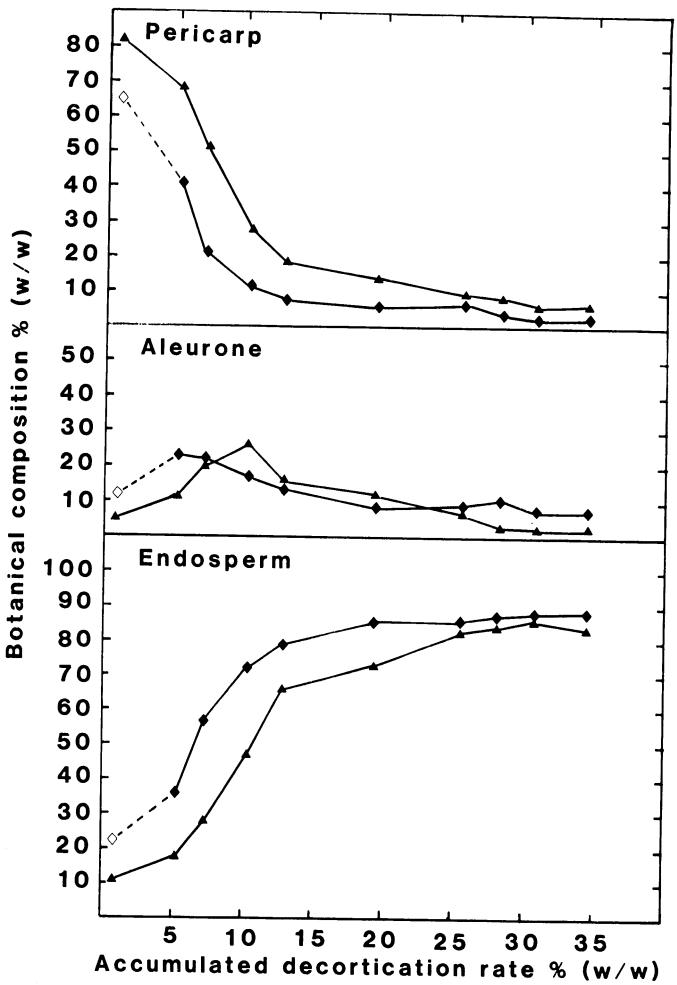


**Fig. 10.** Botanical profile of the sieving fractions predicted by partial least squares modelling of their autofluorescence spectra. ◆ = less than 32 $\mu$ (for decortication fraction 1 less than 125 $\mu$) and ▲ greater than 125 $\mu$.

**TABLE VI**
**Correlation Coefficients Between Botanical and Chemical Constituents (N = 50)**

| | Ash | Fiber | Starch |
|---|---|---|---|
| Pericarp | 0.370 | 0.974 | −0.942 |
| Aleurone | 0.891 | 0.468 | −0.741 |
| Endosperm | ÷0.541 | −0.966 | 0.978 |

distribution comparable to that of ash (Hinton 1959). Separate measurement of ferulic acid may, therefore, be a potential substitute for the ash test if such an analysis is really required in milling. The chemical shift from 365 to 335 nm in the fluorescence observed for tryptophan in aleurone compared to that in endosperm might be explained by the proportion of different proteins in aleurone and endosperm.

The autofluorescence of tryptophan in endosperm (emission maximum 335 nm) and in aleurone (emission maximum 365 nm) (Fig. 3) was highly affected by the presence of pericarp, which caused a decrease (Fig. 1 compared to Figs. 6 and 7). This effect might be explained by light scattering and quenching rather than by secondary absorption because the excitation maximum was as high as 450 nm for the yellowish-green autofluorescence of pericarp (Fig. 3). At 275 nm excitation, mixed endosperm and aleurone showed an emission peak for tryptophan between the two extremes (335 and 365 nm) and the intensity increased with an increasing content of aleurone (Fig. 7). These results differ from the results previously published by Munck et al (1979a) because their aleurone fraction contained pericarp.

For the autofluorescence of ferulic acid, mixtures of the botanical components exhibited an almost linear emission (Figs. 6 and 8). This indicates negligible energy transfer from the 420-nm emission peak of ferulic acid to the 450-nm excitation peak of pericarp. Analytically, ferulic acid fluorescence may therefore be an attractive quality. As with ferulic acid, the autofluorescence at 520 nm of pericarp was only slightly affected by the presence of endosperm or of aleurone (Fig. 8). The chemical basis for the yellowish-green autofluorescence of pericarp has not yet been established. An excitation maximum at 450 nm indicates that the responsible component or components are carotenoids rather than phenolic compounds (Williams and Fleming 1973).

The method presented describes a new way of analyzing various fractions of milling. The autofluorescence data of manually dissected botanical constituents from a winter wheat (Solid) was effective in evaluating the botanical composition of mixed constituents. The botanical profiles from fractions of wheat obtained in a decorticator were in accordance with the morphological sequence through the wheat kernel: pericarp - aleurone - endosperm. No specific separation was observed in the sieving fractions for aleurone cells. This indicates that the aleurone layer in the present decortication experiments tends to have a broad particle size distribution, whereas particles of pericarp and endosperm were coarse and fine, respectively.

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