

# Influence of Structural Characteristics of Aleurone Layer on Milling Behavior of Durum Wheat (*Triticum durum* Desf.)

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## ABSTRACT

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The structure of the aleurone layer was considered for many years as a potential factor influencing wheat milling efficiency. Eight durum wheat samples of different milling values, including distinct cultivars and harvesting conditions, were employed to investigate the structural characteristics of the aleurone layer through image analysis of kernel sections. Particular attention was paid to tissue thickness and structural irregularity of its interface with the starchy endosperm. Wheat cultivar, agricultural conditions, and location of measurement within the grain had an influ-

ence similar to both thickness and irregularity of the aleurone layer. Conversely, grain weight and morphology showed no effect on these parameters. Statistical investigation demonstrated no correlation between structural characteristics and wheat milling behavior. However, the negative correlation between the extraction rate of semolina and starch content in the bran fraction, which was used as an indicator of the endosperm-aleurone dissociation extent, demonstrated the relevance of the tissue adhesion on milling efficiency.

The semolina milling value of durum wheat is defined as the yield of semolina of defined purity under industrial conditions (Abecassis 1993). According to Chaurand et al (1999), the semolina milling value is dependent on regulatory factors (ash value of semolina fraction), harvesting conditions, and intrinsic factors (i.e., factors related to the morphological and physical characteristics of the grain). Among these, the structure of the aleurone layer is presumed to play a significant role. The aleurone layer consists of living tissue, generally one cell thick, surrounding the endosperm. From a botanical standpoint, it is the outer layer of the endosperm. However, as it is removed during milling, it constitutes the innermost layer of bran. Its structural characteristics could affect milling efficiency in two distinct ways.

First, the thickness of aleurone layer, which accounts for almost one-half of the total thickness of bran coats, is likely to affect the bran-to-endosperm volume ratio, which was defined as a determining factor of the semolina yield (Moss 1973; Simmons and Meredith 1979). Variability in thickness of the aleurone layer was subjected to many studies on common wheat samples (*Triticum aestivum* L.) (Bates 1943; Shellenberger and Morgenson 1949). Significant varietal differences were reported but no relationship was established with wheat millability (Larkin et al 1951). This parameter has yet never been investigated in detail on durum wheat (*Triticum durum* Desf.) cultivars. Considering the distinct pattern of process and the difference in milling properties of the two wheat classes (Davis and Eustace 1984), it was worth investigating thickness of the aleurone layer and its relation with the tissue dissociation occurring during the durum milling process.

Second, the irregularity in shape of aleurone cells is presumed to play a relevant role in the adhesion of endosperm to bran (Bradbury et al 1956). It was especially observed that the easiest bran to free from adherent endosperm during the milling had the smoothest aleurone-endosperm profiles (Crewe and Jones 1951). Later, scanning electron microscope examination of isolated fragments of aleurone layer showed that its outer face, adjacent to the nucellar epidermis, presented a flat surface, whereas its inner face appeared more irregular with a succession of convex and concave cells and intercell depressions (Stevens 1973). Aleurone irregularity could favor adhesion or simply entangle residual fragments of starchy endosperm and thus influence the extraction rate of semolina.

The aim of this work was to identify with precision the influence of the structure of the aleurone layer on milling quality of durum wheat. Eight wheat samples, characterized by distinct milling behaviors, were employed in a detailed microscopic study to quantify by image analysis both the thickness of aleurone tissue and the structural heterogeneity of its interface with the starchy endosperm. Relationships between these structural parameters and morphological characteristics of the grains were investigated.

In addition, determination of the starch content in the various bran fractions was performed to assess the loss of endosperm in bran and evaluate the tissue dissociation caused by the milling process. Relationships between milling efficiency and structural characteristics of the aleurone layer were statistically investigated.

## MATERIALS AND METHODS

### Wheat Samples and Grain Selection

The study was performed on eight durum wheat samples. Four cultivars ('Ardente', 'Primadur', 'Nefer', 'Lloyd') with distinct morphological grain characteristics were grown in 1999 at Maugio (INRA). The influence of crop site was investigated on the Lloyd cultivar which was grown in three different locations: Maugio (S1, Southeast of France); Crambade (S2, Southwest of France); and Ouzouer (S3, Northern France). The effect of nitrogen addition was specifically studied on Nefer cultivar at Crambade crop site: Nefer N- received 50 kg/ha over the cropping period; Nefer N+ was fertilized with 100 kg/ha of nitrogen.

To select representative grains of each cultivar, 120 grains were randomly taken so as to establish the weight distribution of each batch. Seven grains were selected within each batch according to mass: three grains with mass corresponding to the average of the batch ( $m = M_{avg}$ ); two grains with mass corresponding to the average plus standard deviation of the sample ( $m = M_{avg} + \sigma$ ); and two grains with mass corresponding to the average minus the standard deviation of the sample ( $m = M_{avg} - \sigma$ ).

### Grain Embedding

Before fixation, two small holes were bored in dry grains using a miniaturized drilling machine to support the penetration of the fixation medium. Samples were fixed in 2% glutaraldehyde (0.2M phosphate buffer, pH 7.0, containing 1%, w/v, acrolein and 1%, w/v, caffeine) for 1 hr under vacuum at room temperature, then for 72 hr at 4°C. Subsequently, they were dehydrated in a graded ethanol series then impregnated for six days in a medium containing Technovit 7100 resin (100 mL; Kulzer), Technovit 7100 accelerator (1 g; Kulzer), Technovit polyethylene glycol PEG 400 (1.5 mL; Kulzer), and triethylene glycol dimethacrylate (0.5 mL). They were finally embedded in the impregnation medium (5 mL) added with

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Technovit 7100 hardener (0.35 mL). Sections (5  $\mu\text{m}$ ) were obtained with a Leica RM 2165 microtome.

### Location of Optical Measurement in Grain

Transverse sectioning was made over the entire grain length. Only sections located in the quarter ( $L/4$ ), the middle ( $L/2$ ), and the three-quarter ( $3L/4$ ) of grains were used for measurements. Measurements were made at four points of each transverse section: on the crease (C), the dorsal (D), lateral (L), and basal (B) locations (Fig. 1).

### Fluorescence Microscopy

Sections were mounted with an influence-free immersion oil. Micrographs were obtained from an Olympus fluorescence microscope equipped with Gx20 Olympus T6 (Gx20) optic and an HBO-100W mercury lamp as source of radiation for image. Observations were performed at 350 nm excitation and 420 nm emission wavelengths.

### Image Analysis

Image recording was performed using Optimas software. Aleurone layer interfaces were discretized for 300  $\mu\text{m}$  length of the layer to obtain a numeric outline. Aleurone layer thickness was determined on the median line which represents the straight line for which the sums of the areas of the profile over and under the line are equal.

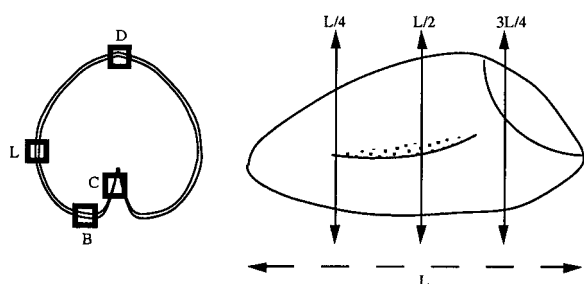


Fig. 1. Locations of measurements in the grain.

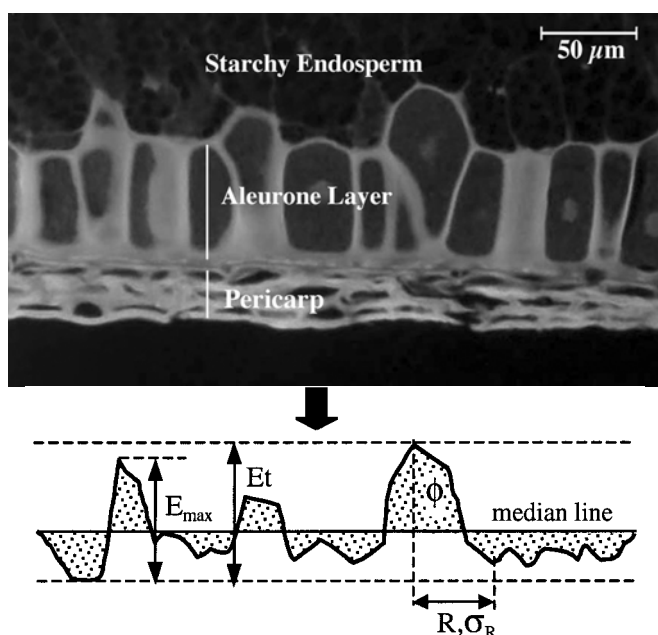


Fig. 2. Fluorescence micrograph of the transverse section of aleurone layer. Representation of irregularity parameters extracted from the profile:  $E_t$  ( $\mu\text{m}$ ) (maximum height of the profile);  $E_{\text{max}}$  ( $\mu\text{m}$ ) (maximum distance between hollow and adjacent peak);  $\phi$  ( $\mu\text{m}^2$ ) (surface ranging between the profile and the median line divided by the evaluation length);  $R$  ( $\mu\text{m}$ ) (average pitch of the undulation);  $\sigma_R$  ( $\mu\text{m}$ ) (standard deviation of  $R$ ).

The structural irregularity was expressed by five distinct parameters (Fig. 2).  $E_t$  ( $\mu\text{m}$ ) corresponded to the maximum height of the profile;  $E_{\text{max}}$  ( $\mu\text{m}$ ) corresponded to the maximum distance between a hollow and an adjacent peak;  $\phi$  ( $\mu\text{m}^2$ ) represented the area ranging between the profile and the median line divided by the evaluation length (300  $\mu\text{m}$ );  $R$  ( $\mu\text{m}$ ) was the average pitch of the undulation;  $\sigma_R$  ( $\mu\text{m}$ ) was the standard deviation of  $R$ .

According to the location of the measurement points on the grain, corrections were performed to take into account the angle of the cut against the curve of grain: real value = measured value  $\times \cos.\alpha$ ; with  $\alpha$  = angle between the direction of cut and the perpendicular of the surface plan determined using a mathematical model of grain morphology (Mabille and Abecassis 2003).

### Milling Fractions

Milling fractions were obtained from a conventional milling process performed in a semiindustrial semolina mill (150 kg/hr). Four break rolls, four sizing rolls, three plan sifter stacks, and three double-deck purifiers composed the milling unit. Clean wheat grains were tempered to 15% wb for 15 hr, and then at 17% for 3 hr before milling. After milling, 18 fractions were obtained: six purified semolina, four break flours, four sizing flours, and four feeds (coarse bran [CB], sizing fine bran [SFB], purifying fine bran [PFB], and shorts). Ash content of semolina and flour fractions was determined according to the ISO 2171-1980 standard procedure at 900°C.

### Physical Characteristics of Grains

The thousand grain weight (TGW) (g.dm) was measured according to the AFNOR standard method (V03-702). Kernel vitreousness (%) was determined according to the ISO 5532 method using a Pohl's farinator.

### Starch Determination

Starch content was determined using a commercial assay kit from Megazyme (Approved Method 76-13, AACC 2000). Bran fractions were ground for 30 sec (IKA-10). Sample was collected and 100 mg were pretreated with dimethyl sulphoxide (DMSO) at 100°C for 5 min. After incubation in the presence of thermostable  $\alpha$ -amylase (300 U) and amyloglucosidase (20 U), glucose content was determined according to the glucose oxidase-peroxidase procedure.

TABLE I  
Effects of Grain Mass, Grain Section, and Measurement Location on Thickness of Aleurone Layer<sup>a,b</sup>

		Aleurone Layer Thickness ( $\mu\text{m}$ )
Mean value		65.5
Grain mass	M	66.5
	M+ $\sigma$	65.8
	M- $\sigma$	64.3
Grain section	L/4	61.9a
	L/2	63.3b
	3L/4	71.4c
Measurement location	Dorsale face (D)	70.1a
	Lateral face (L)	71.3a
	Basal face (B)	59.4b
	Crease (C)	61.3b
Effect of grain mass		ns
Effect of section		****
Effect of measurement location		****

<sup>a</sup> Values followed by the same letter are not significantly different ( $P < 0.05$ ).

<sup>b</sup> \*\*\*\*, not significant at  $P = 0.0001$ ; ns, not significant.

## Statistical Studies

Variance analysis and principal component analysis were performed using Statgraphics software (Manugistics, Rockville, MD). Effects of grain mass ( $n = 196$ ), grain section ( $n = 196$ ), measurement location ( $n = 147$ ), wheat cultivar ( $n = 84$ ), crop site ( $n = 84$ ), and nitrogen addition ( $n = 84$ ) on the structural aleurone characteristics (tissue thickness and irregularity) were studied by variance analysis regardless of each other.

**TABLE II**  
Effects of Wheat Cultivar, Crop Site, and Nitrogen Addition on Thickness of Aleurone Layer<sup>a,b</sup>

	Aleurone Layer Thickness ( $\mu\text{m}$ )	Standard Deviation ( $\mu\text{m}$ )	
		Grain Section	Measurement Location
Ardente	58.8a	8.3	7.7
Primadur	63.0b	9.6	9.1
Nefer	65.1b	10.8	11.8
Lloyd	69.3c	9.6	7.5
S1	65.9a	9.3	7.2
S2	62.4b	10.4	9.4
S3	63.8a,b	9.9	7.9
N+	62.6	8.3	8.5
N-	65.5	9.3	7.8
Effect of cultivar	****		
Effect of crop site	*		
Effect of N addition	ns		

<sup>a</sup> Values followed by the same letter are not significantly different ( $P < 0.05$ ).

<sup>b</sup> \* and \*\*\*\* not significant at  $P = 0.05$  and  $0.0001$ ; ns, not significant.

**TABLE III**  
Effects of Wheat Cultivar, Crop Site, and Nitrogen Addition for Least Square Means of R and  $\phi$  Values for Eight Wheat Samples<sup>a,b</sup>

	$\phi$ ( $\mu\text{m}^2$ )	R ( $\mu\text{m}^2$ )
Ardente	5.2a,b	18.2a
Primadur	4.4d	18.9a,b
Nefer	4.9c,d	20.2b
Lloyd	5.5a	22.1c
S1	5.2a	19.8
S2	5.1a	21.1
S3	4.7b	18.6
N+	4.9	19.9
N-	5.1	19.8
Effect of cultivar	****	***
Effect of crop site	**	ns
Effect of N addition	ns	ns

<sup>a</sup> Values followed by the same letter are not significantly different ( $P < 0.05$ ).

<sup>b</sup> \*\*, \*\*\*, and \*\*\*\* not significant at  $P = 0.01$ ,  $0.001$ , and  $0.0001$ , respectively; ns, not significant.

**TABLE IV**  
Starch Content (% dm) of Four Feed Fractions Determined for Wheat Samples<sup>a</sup>

	Shorts	SFB	PFB	CB
Ardente	34.0	17.7	14.8	11.9
Primadur	37.9	16.9	16.5	15.1
Nefer	43.1	30.1	17.8	11.9
Nefer N+	50.7	28.2	19.4	16.9
Nefer N-	46.8	24.1	19.6	14.8
Lloyd S1	44.5	21.1	17.9	12.9
Lloyd S2	49.9	23.5	20.2	18.7
Lloyd S3	46.7	29.6	19.7	16.7

<sup>a</sup> Coefficient of variation 6%. SFB, sizing fine bran; PFB, purifying fine bran; CB, coarse bran.

## RESULTS

### Variability of Aleurone Layer Thickness in a Grain

Variability of aleurone layer thickness was studied according to grain mass, grain section, and measurement location (Table I). As previously reported on common wheat (Crewe and Jones 1951), the tissue thickness appeared unrelated to the grain mass and morphology. In contrast, high variability was observed within the grain.

Considering a transverse section, the aleurone thickness slightly increased from the dorsal to the lateral region where it was the highest. Also, the aleurone was the narrowest at the level of the grain base then thickened again in the crease where it was two cells thick. In agreement with previous observations (Morrison et al 1978), our results point out the particular structure of the ventral aleurone cells, which differed from the lateral and dorsal cells by a rectangular shape, an inferior intracellular space, and a lower inclusions content. This specificity indicated a lower involvement in the enzyme synthesis during germination (Fulcher et al 1972).

Observations on a longitudinal section showed a thickening of the aleurone layer from the basal (L/4) to the apical (3L/4) grain ends except in the part covering the embryo where aleurone cells exhibited a distinct thin structure ( $\approx 20 \mu\text{m}$ ) as already observed by Morrison et al (1978). In contrast with previous observations of common wheat (Crewe and Jones 1951) where variations in thickness were most noticeable in a longitudinal section, our results showed similar differences ( $\approx 11 \mu\text{m}$ ) in both orientations.

It must be noted that the differentiation of the aleurone cells occurs during the first stages of kernel development (15 days postanthesis) (Evers 1970; Simmonds and O'Brien 1981). Consequently, variations in aleurone thickness within the grain are functions of its compression resulting from the kernel expansion. This might indicate heterogeneous development direction of the starchy endosperm with, in particular, a preferential growth according to a longitudinal axis from the embryo to the apical end. According to a transverse section, the thinness of the tissue at the basal faces tends to confirm the bilateral development of endosperm with an expansion in flanks direction before the crease formation (Simmonds and O'Brien, 1981).

### Variability Within Wheat Samples

Variance analysis of the aleurone layer thickness was realized as a function of wheat cultivars, crop site, and nitrogen addition in Table II. Considerable variations occurred between the four wheat cultivars with a  $10.5\text{-}\mu\text{m}$  difference ( $\approx 15\%$  of tissue thickness) between Lloyd and Ardente cultivars. The influence of the crop site appeared significant with a  $3.5\text{-}\mu\text{m}$  difference between S1 and S2, but no significant effect of nitrogen addition was observed. This last observation was not in agreement with the hypothesis of a correlation between nitrogen content and area occupied by the aleurone cell (Kosina 1979).

In addition, the effects of the grain section and of the measurement location were variable according to the cultivar. In a longitudinal axis, Ardente exhibited the most homogenous tissue with a difference of only  $8.3 \mu\text{m}$  between L/4 and 3L/4 sections whereas Nefer presented a variation of  $10.8 \mu\text{m}$ . According to the transverse section, the highest variation due to the measurement location was also observed in Nefer (between lateral and basal faces) whereas Lloyd exhibited the most homogeneous layer.

In agreement with earlier reports on common wheat (Crewe and Jones 1951), variability of aleurone layer thickness within the grain appeared to be similar to the variability between wheat samples for the corresponding location.

### Structural Irregularity of the Endosperm-Aleurone Layer Interface

The structural irregularity of the interface was quantified by five numerical variables:  $E_t$  and  $E_{\text{max}}$  characterized the amplitude of

variations,  $R$  and  $\sigma_R$  instead define a periodic variation and  $\phi$  constitutes a more general expression of the irregularity taking into account the overall variation.

The correlation matrix established on the five representative variables of irregularity showed strong correlations between  $R$  and  $\sigma_R$  ( $R^2 = 0.72$ ) and between  $Et$ ,  $E_{max}$ , and  $\phi$  ( $R^2 = 0.86$  and  $0.74$ , respectively).  $R$  and  $\phi$  were thus two independent variables that can be considered sufficient to describe the irregularity of profile.

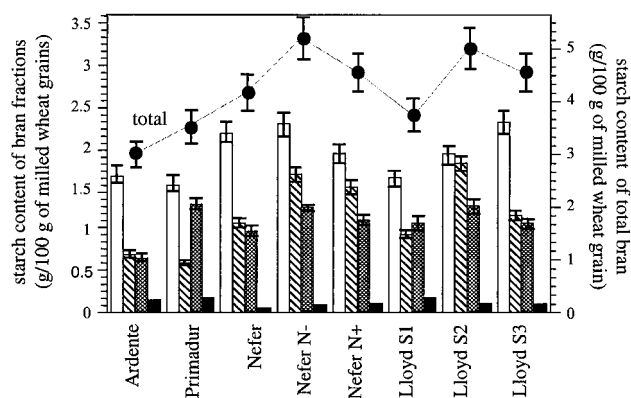
As previously observed with aleurone layer thickness measurements, significant effects of the grain section and the measurement location were observed on  $R$  and  $\phi$  ( $P < 0.01$ ). According to a transverse section,  $R$  and  $\phi$  were lower at the level of the grain base. The highest values were observed on the dorsal and lateral parts of the grain. Multiple range tests procedure (95% LSD) showed the similar classification for  $R$  and  $\phi$ :  $B \leq C \leq L, D$ . According to a longitudinal section, increasing  $R$  and  $\phi$  values were measured from the basal toward the apical end of the grain (multiple range test:  $L/4 \leq L/2 \leq 3L/4$ ). Furthermore,  $R$  and  $\phi$  were unrelated both to the tissue thickness and the grain mass ( $P > 0.05$ ).

On the other hand, variance analysis revealed distinct variabilities of  $R$  and  $\phi$  according to wheat cultivar and agricultural conditions (Table III). The  $\phi$  value was subjected to significant variations according to cultivar (20% difference between Lloyd and Primadur) and the crop site to a lower extent (9% difference between S1 and S3), whereas nitrogen addition had no effect.

The  $R$  value appeared to be related to wheat cultivar only, with difference of 18% between Ardente and Lloyd. No influence of crop site or nitrogen addition was demonstrated.

### Quantification of Starch in Bran Fractions

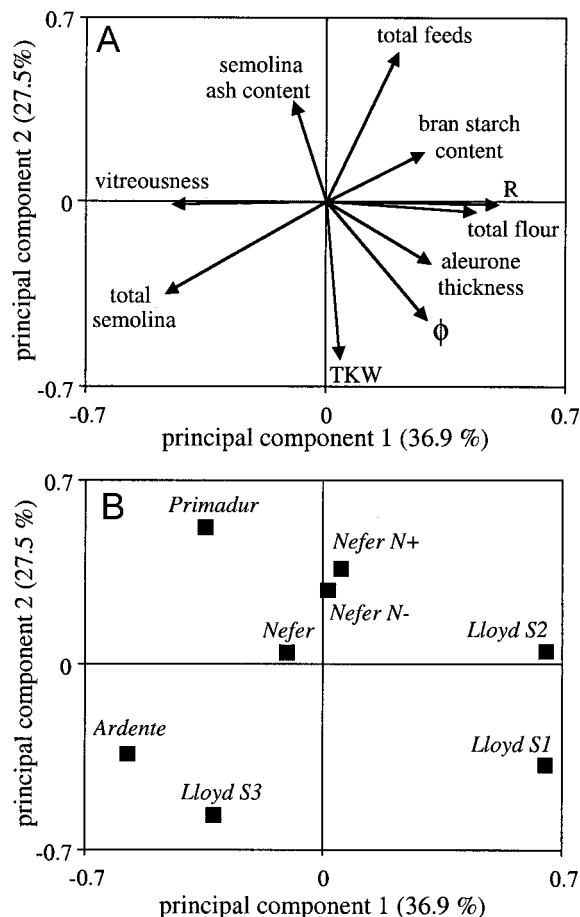
Bran starch contents (Table IV) were significantly different according to both the fraction and the wheat sample with noticeable influence of both cultivar and agricultural conditions (crop site and nitrogen addition). The shorts were the more starchy fraction and were subject to relative sample variability with a difference of 33% between Ardente and Nefer N+. The three other bran fractions contained



**Fig. 3.** Starch content (g/100 g of milled grains) of shorts (///), coarse bran (■), sizing fine bran (▨), purifying fine bran (□), and total bran fraction (●) for eight wheat samples.

less starch. The sizing fine bran (SFB) was subject to the greatest relative sample variability, with starch content about twice as high in Nefer N+ than in Primadur. The purifying fine bran (PFB) and coarse bran (CB) exhibited more consistent starch contents among the sample set, with maximal differences of 26 and 35% between samples.

In addition, a strong correlation ( $R^2 = 0.92$ ) was observed for starch content between shorts and PFB which can be explained by a common extraction mechanism during the process. In fact, shorts and PFB were produced from the same detacher system, whose purpose was to scrape endosperm particles free from small bran fragments, and their separation was achieved by a subsequent purifying step according to particle size. As a result, both fractions exhibited a proportional amount of adherent endosperm. On the other hand, the low correlation in starch content between PFB and



**Fig. 4.** Principal component analysis: **A**, representation of  $R$  ( $\mu\text{m}$ ) and  $\phi$  ( $\mu\text{m}^2$ ) variables of the structural irregularity, proportion of starch (%) in total bran fractions, milling yields (total flour, semolina, and feed extraction rate (% dm), thousand grains weight (TGW)(g.dm), and grain vitreousness (%); **B**, projection of samples.

**TABLE V**  
Extraction Rate (% dm) of Milling Fractions (total semolina, total flour, and feeds) for Wheat Cultivars and Ash Content (% dm) of Flour and Semolina<sup>a</sup>

	Total Semolina	Ash Content	Total Flour	Ash Content	Shorts	PFB	SFB	CB
Ardente	74.8	0.99	8.3	2.25	1.9	9.0	0.8	5.1
Primadur	72.5	0.97	7.9	2.01	1.5	8.9	0.9	8.2
Nefer	72.9	0.9	9.7	2.14	2.4	7.0	0.1	8.0
Nefer N+	73.3	1.01	8.7	2.72	3.2	7.8	0.3	7.2
Nefer N-	73.1	1.12	8.5	2.41	3.1	7.7	0.4	7.2
Lloyd S1	72.2	0.92	9.5	2.03	2.0	7.5	0.8	8.0
Lloyd S2	71.3	0.92	10.3	1.95	3.5	7.9	0.4	6.6
Lloyd S3	75.4	0.91	8.0	2.29	2.4	7.5	0.4	6.2

<sup>a</sup> PFB, purifying fine bran; SFB, sizing fine bran; CB, coarse bran.

CB ( $R^2 = 0.55$ ), which was generated by distinct methods during the process, demonstrated evidence of an intrinsic grain characteristic that partly controls the dissociation of endosperm from bran.

Weighting the starch content within each fraction against its respective extraction rate (Table V) permitted estimation of the total amount of starch directed to feed fractions during the milling process (Fig. 3). Considering the low proportion of SFB produced during the milling process, the total amount of starch in bran was mainly dependent on the sum of starch amounts in shorts, PFB, and CB, but it was more particularly related to the starch content of the shorts fraction ( $R^2 = 0.88$ ).

The large amount of starch in each bran fraction showed the imperfect tissue dissociation generated during the milling and emphasized the limits of the process. Considering that mill settings were identical for the eight wheat samples, the considerable variations observed between the different cultivars suggested that this technological aspect was related to intrinsic grain characteristics that must be investigated.

### Influence of Structural Characteristics of the Aleurone Layer on Wheat Milling Behavior

Principal component analysis was applied with variables  $R$  and  $\phi$ , total starch content of bran and yields in flour, semolina, and feeds (Fig. 4A). The similarity map was representative of 64.5% of the total inertia, putting the eventual correlation into perspective. For wheat milling behavior, the yield in semolina appeared consistently in contrast to that of yield in flour and feeds. As previously reported (Dexter and Matsuo 1981), the proportion of flour produced was strongly and negatively correlated to grain vitreousness.

As described above, thickness of aleurone layer appeared unrelated to grain mass and to TGW. The TGW value was more particularly related to starchy endosperm mass, explaining its negative correlation with extraction rate of feeds. In addition, thickness of aleurone layer did not significantly affect the semolina yield and purity, confirming previous conclusions drawn from common wheat (Larkin et al 1951).

The extraction rate of semolina was negatively correlated to starch content of bran fractions ( $r = -0.55$ ). The amount of adherent endosperm to the bran particles is a determining technological aspect and the tissue adhesion is therefore a component of the semolina milling value. However, this exercise revealed a negligible influence of the structural irregularity of the aleurone layer-endosperm interface ( $\phi$  and  $R$ ), indicating the involvement of other predominant physicochemical parameters.

The projection of the wheat samples on the principal plan (Fig. 4B) mainly showed a distribution of wheat samples along an axis defined by the semolina-to-feeds ratio except for Primadur, Lloyd S1 and Lloyd S2. This classifies the various cultivars according to their milling value. Ardente and Lloyd S3, which exhibited both high vitreousness and TGW, showed the highest extraction rate in semolina whereas Lloyd S1 and S2 supplied the lowest. These two samples were mostly characterized by low values of kernel vitreousness, and consequently by high yields in flour. In contrast, Primadur differed from the other samples by a low TGW explaining its isolated location on the plan.

On the other hand, the projection on the plan of Nefer N+, which showed the highest starch content in the bran fraction, allowed assessment of the relevance of this factor on the wheat milling behavior. If the loss of endosperm in bran appeared to influence the semolina milling value, grain physical characteristics such as vitreousness and endosperm-to-bran coat ratio still appeared predominant on the wheat quality.

## DISCUSSION

Evidence of the influence of genetic and agricultural factors on the semolina milling value and the relevance of intrinsic grain characteristics such as endosperm mechanical resistance or endosperm-to-

bran coat ratio are now well established (Chaurand et al., 1999). Nevertheless, the significant differences of milling behavior between cultivars exhibiting similar kernel vitreousness and grain morphology led to the consideration of the structure of the aleurone layer as a potential factor explaining this variability.

The investigation on structural characteristics of the aleurone layer required both a representative grain selection and an accurate measurement location within each selected grain to obtain significant results from a large set of wheat samples. The present approach found the respective influence of several factors on the layer characteristics. As a result, the thickness and the irregularity of the aleurone layer were subjected to large variations within grains and between grains from distinct wheat samples. Wheat cultivars and crop site have significantly influenced both thickness and irregularity of the tissue. Conversely, grain mass and morphology, which were affected by nitrogen addition (Simmons and Meredith 1979) showed no effect on these parameters.

Furthermore, the present study provided an answer to a question raised for many years about the possible involvement of the structure of the aleurone layer in the wheat milling properties. First, evidence was established that the tissue thickness did not affect significantly wheat milling behavior. In spite of significant variations in aleurone thickness, the TWG and endosperm-to-bran coat ratio appeared most specifically dependent on the starchy endosperm mass (Lempereur et al 1997). As a consequence, the thickness of the aleurone layer did not significantly affect the respective extraction rate of feeds and semolina during the process.

Additionally, if the amount of starchy endosperm lost in bran fractions was subjected to a relevant variation according to the wheat sample, this variability can not be explained by the structural heterogeneity of the aleurone-endosperm interface characterized by the two distinct variables ( $R$  and  $\phi$ ). This result is not in agreement with a previous study on common wheat many years ago (Crewe and Jones 1951). In the durum process, the structure of the aleurone layer might not be a determining factor of the tissue dissociation generated by the milling.

Therefore, it is now necessary to investigate further the mechanism involved in the tissue adhesion. Butcher and Stenvert (1973) pointed out the relevant role of the moisture penetration across the grain during tempering and suggested that the moisture level of the aleurone and subaleurone interface is the most critical factor determining the efficiency of the milling operation. On the other hand, investigations on the components involved in the mechanical strength of this interface could be an interesting step toward understanding tissue adhesion. The cell walls of the aleurone layer and of the starchy endosperm are characterized by a high content of feruloylated arabinoxylans (Bacic and Stone 1981). Recently, several studies reported an implication of ferulic acid dehydromers in the cell-cell adhesion (Fry 1986; Waldron et al 1997). It can be hypothesized that the concentration and the distribution of these components could partly control tissue adhesion and may constitute new markers of the wheat milling quality. Further work requiring a shift in the scale analysis will be necessary to get more insight into the molecular basis of adhesion within tissues.

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