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In-Depth Study of Durum Wheat Grain Tissue Distribution at Milling

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The starchy endosperm proportion in durum wheat grain and its ability to be isolated from the peripheral tissues appear as main intrinsic characteristics potentially related to the milling value but still difficult to assess. In this study, several durum wheat samples displaying distinct grading characteristics were analyzed and processed through a pilot mill. The histological composition of grains and milling fractions was monitored by using identified biochemical markers of each wheat grain tissue. Contrasted milling yields of semolina and flour were observed between samples, despite displaying a similar starchy endosperm proportion determined by hand dissection. These yields were related both to differences in the starchy endosperm extraction and to the presence of the aleurone layer, particularly its cellular content. Furthermore, two distinct types of fractionation behavior of the aleurone layer were distinguished depending on the wheat grain sample. Extraction of the envelopes and embryonic axis into semolina and flours were found negligible in comparison with the other tissues.

Durum wheat grain (*Triticum durum* Desf.) is traditionally used for the production of pasta, couscous, and several other products (Abecassis et al. 2012). They are mainly made of semolina, produced from milling. Grain milling indeed has as an aim to isolate the starchy endosperm (SE) into fine products (semolina and flours) apart from the other grain parts recovered into bran and short (SH) fractions, after a succession of grinding, sifting, and purifying steps. The milling quality of each wheat sample is determined through the respective fraction yield and the efficiency of separation between products from the SE and the peripheral tissues (Abecassis 1993). Several wheat grain characteristics that influence either the extraction yield or tissue separation have already been identified.

The SE texture was the most studied characteristic; it dramatically impacts the ratio between semolina and flour (Matveef 1963; Dexter and Matsuo 1981) and appears to depend both on nitrogen fertilization (Grahmann et al. 2014) and genetic background (Bnejdi and El Gazzah 2008). It was found to be related to the grain protein content, which mainly impacts semolina production below a threshold of around 10% (Dexter et al. 1989; Samson et al. 2005).

A high recovery of the total semolina (TS) and total flours (TF) obviously depends on the initial proportion of SE, that is, the ratio of SE to other tissues, a ratio whose absolute determination is difficult to obtain (Matveef 1965). Therefore, hand dissection of the wheat grain tissues remains the most employed method for directly assessing the SE proportion (Barron et al. 2007). Rapid evaluation of this characteristic was suggested by using the thousand kernel weight (TKW) measurement, assuming that the larger the grain size, the greater the SE-to-bran ratio (Matsuo and Dexter 1980; Dexter et al. 1987; Lempereur et al. 1997). However, low correlations

were found between TKW values and the semolina and clear flour (Matsuo and Dexter 1980) or bran yields (Abecassis and Chaurand 1996) with $R = 0.48$ and $R = 0.66$, respectively. Moreover, different semolina yields were observed with grains selected at similar grain size from different cultivars (Lempereur et al. 1997). Abecassis and Chaurand (1996) thus concluded that, above a TKW threshold of 30 g, not only the SE and bran proportions but also additional factors were involved in the respective yields.

The ability to efficiently isolate the SE from the other grain tissues during the milling process is also one of the main characteristics involved in durum wheat quality. It could play a role in the milling yield variability observed above a specific TKW threshold, as reported by Abecassis and Chaurand (1996), but this aspect was rather less explored. It influences either the loss of the SE particles in bran fractions or the presence of peripheral tissues in TS+TF. The presence of peripheral tissues in TS+TF is currently assessed by using the ash content measurement, which stands as the most important quality factor for commercialization of the milling products in several European countries (Troccoli et al. 2000; Dexter and Marchylo 2001; Gruber and Sarkar 2012). The validity of this marker to monitor the peripheral tissues has already been questioned, because ash content was shown to be proportional to the initial grain ash content (Abecassis and Feillet 1985; Cubadda 1988; Chaurand et al. 1999; Sissons et al. 2012). Experiments with debranning devices also revealed the existence of a mineral gradient in grains that increases from the inside to the outside but does not allow clear monitoring of the grain outer layers in the milling fractions (Dexter et al. 1994; Fares et al. 1996).

Efforts have been focused on the identification of better markers to monitor the presence of peripheral tissues in milling fractions such as the phenolic acids (Lempereur et al. 1998; Peyron et al. 2002) or the alkylresorcinols (Chen et al. 2004). Furthermore, a few biochemical markers were identified in hand-isolated common wheat grain tissues and successfully used to determine the histological composition of corresponding whole grains and fractions (Antoine et al. 2004; Hemery et al. 2009; Barron et al. 2011). Measurement of the starch content was used to specifically monitor the SE, because it is only found in this tissue embedded in a protein matrix, whereas the aleurone layer cellular content (ACC) was quantified through the measurement of phytic acid contained in the cytoplasmic globoids (Evers and Bechtel 1988; Pomeranz 1988). ACC was distinguished from the quantification of the aleurone cell walls (ACW) by using *p*-coumaric acid measurement and gravimetric determination (Hemery et al. 2009). Hyaline, testa, and inner pericarp were quantified as a single layer, defined as the intermediate layer by Barron et al. (2007), through the measurement of alkylresorcinol content in accordance with their specific location in wheat grains (Landberg et al. 2008). The outer pericarp and embryonic axis were monitored with the 4-*O*-8',5'-5"-dehydrotriferulic acid and wheat germ agglutinin measurements, respectively, taking into account their specific grain tissue locations (Miller and Bowles 1982; Antoine et al. 2004). Application of these biochemical markers was previously used successfully to monitor debranning and estimate the proportion of peripheral tissues in durum wheat grains (Ríos et al. 2009a; Barron et al. 2011). However, their potential to monitor each tissue behavior at milling was never validated.

In this study, the biochemical markers defined in common wheat to monitor the tissue distribution in milling fractions were found to be useful to study the behavior during processing of eight durum wheat samples with contrasting grading characteristics. Furthermore, the study explored specifically how the separation between the SE and the grain peripheral tissues affected

the TS+TF yield, and it revealed two possible fractionation mechanisms of the aleurone layer (AL).

MATERIALS AND METHODS

Durum Wheat Grain Samples and Characterization.

Eight durum wheat samples (S1–S8) from pure cultivars grown and harvested between 2007 and 2012 in different crop sites were collected and cleaned to remove impurities, broken kernels, and shriveled grains. All samples were characterized by using international standardized grading factors. TKW was determined by weighing a 30 g sample and counting the grains with an electronic seed counter. Results were reported as mass (g) of a thousand kernels (ISO International Standard 520:2010). Test weight was determined with a chondrometer (ISO International Standard 7971-1:2009) and expressed in kg/hL. At least three determinations were performed for each sample. Protein content was measured with the Kjeldahl method (AACC International Approved Method 46-12.01) using a nitrogen-to-protein ratio of 5.7, with at least two measurements performed for each sample.

Determination of the SE Proportion in Wheat Grain.

Twenty grains were randomly chosen, weighed, and hand dissected. First, the embryonic axis was removed from whole grains with a needle after removal of the surrounding outer pericarp. Then the grains were soaked in water for 16 h at room temperature. An incision was made along the crease to open the grain, and the scutellum was removed with a scalpel. The SE was gently scratched away from the AL inner surface and was kept apart with all of the washing waters. The embryonic axis, scutellum, and whole peripheral layers were dried over phosphorus pentoxide at 40°C, and the SE present in the washing waters was freeze-dried. After drying to constant mass, all of the tissues were weighed and proportions of each tissue expressed relative to the sum of all of the weighed tissues. The weight loss according to the initial grain weight was found to be lower than 3%. Four determinations were performed for each sample.

Milling Process.

Durum wheat samples were milled on a pilot scale (150 kg/hL) as described by Ríos et al. (2009b) after tempering grains to 17% water content. The milling process included four break steps, four sizing steps, and six purifying steps. Milling yields were calculated for each fraction gathered when the mill was fully charged and expressed on a dry basis after measurement of the moisture content (AACCI Approved Method 44-15.02). A total of 18 milling fractions were recovered, including four break flours, four reduction flours, six semolina fractions, and four bran fractions: SH, sized fine bran (SFB), purified fine bran (PFB), and coarse bran (CB) from a total dry mass of 50 kg. A TS sample was gathered from a bypass immediately before the sampling. A TF fraction was later created by mixing all of the obtained flours in their respective proportions. Milling fractions and grains were stored at 4°C before analysis.

Determination of Grain Tissue Distribution Using Biochemical Markers.

Biochemical markers previously identified to monitor each grain tissue in common wheat were measured as described by Hemery et al. (2009) with slight modifications. Quantification of the biochemical markers in hand-isolated outer pericarp, intermediate layer, and embryonic axis

from each wheat grain sample served as reference values for the corresponding tissues, but starch content in TS was used as a reference value for the SE, because this milling fraction recovers the overall variability of the biochemical composition found in endosperm cells (Evers and Bechtel 1988). The relative mass proportion of ACW in AL was determined by gravimetric quantification as described by Brillouet et al. (1988) and modified by Hemery et al. (2009) and Chen et al. (2013) to allow proper determination of ACW and ACC with the corresponding markers, *p*-coumaric acid and phytic acid, determined on hand-dissected AL. In brief, an AL sample (100 mg) ground under cryogenic conditions with a SPEX CertiPrep 6750 grinder (SPEX CertiPrep, Metuchen, NJ, U.S.A.) and dried on phosphorous pentoxide was weighed and submitted to two delipidation steps in hexane, followed by a centrifugation step at $3,000 \times g$ for 15 min. The dried pellet was suspended in HPLC-grade water with 1.5% SDS and 5mM sodium metabisulfite. A pronase (type XIV pronase from *Streptomyces griseus*, Sigma-Aldrich, Saint Louis, MO, U.S.A.) solution in water at 1 mg/mL was added, and it was kept at 40°C for 2 h with regular mixing. Chemicals, enzymes, and soluble compounds were then washed twice with water, and the pellet was cleaned and dried in a graded series of ethanol, acetone, and diethyl ether. The final pellet corresponding to purified ACW in pure AL was dried on phosphorous pentoxide, weighed until constant mass, and was expressed in proportion to the original mass of ground AL.

Each grain tissue proportion was deduced from the ratio of the marker amount in the analyzed sample to the marker amount in the pure hand-isolated tissue. Each biochemical measurement was performed twice on the analyzed sample and the pure tissue; therefore, a total of four values for tissue proportion were obtained for each sample. Results were expressed as mean and standard deviation. The outer pericarp and intermediate layer were quantified separately, but because the intermediate layer contains layers from the grain inner pericarp, results were expressed as a whole tissue corresponding to the “envelopes.”

Based on tissue proportions, different calculations were carried out. Assessment of the respective parts of the TS+TF yield, brought by the SE and the different peripheral layers, were calculated as follows:

$$Y(\text{tissue})_{\text{TS+TF}} = \%T_{\text{TS}} \times Y_{\text{TS}} + \%T_{\text{TF}} \times Y_{\text{TF}} \quad (1)$$

where Y indicates yield and %T indicates % tissue. The relative proportion of each tissue extracted in the TS+TF was calculated with the following equation:

$$TE_{\text{TS+TF}} = \frac{(\%T_{\text{TS}} \times Y_{\text{TS}} + \%T_{\text{TF}} \times Y_{\text{TF}})}{(\%T_{\text{FractionSum}})} \times 100 \quad (2)$$

where TE indicates tissue extraction and FractionSum indicates the sum of a single tissue proportion in all of the milling fractions corrected by their respective yields.

The SE extraction was deduced from the endosperm loss in bran fractions in order to reduce the analytical error by using the following formula:

$$\text{SE extraction} = (1 - \%SE_{\text{CB}} \times Y_{\text{CB}} - \%SE_{\text{PFB}} \times Y_{\text{PFB}} - \%SE_{\text{SFB}} \times Y_{\text{SFB}} - \%SE_{\text{SH}} \times Y_{\text{SH}}) / \%SE_{\text{FractionSum}} \quad (3)$$

Statistical Analysis.

All statistical analyses were performed with XLSTAT software (Addinsoft, Paris, France). Tukey's honest significant difference test for multiple sample comparison was chosen over the Fischer's LSD with regard to its more conservative nature. The retained significance level was 0.05.

RESULTS AND DISCUSSION

Durum Wheat Sample Characterization, Endosperm Proportion, and Milling Yields.

Characterization of the cleaned grain samples was performed according to three international grading factors (TKW, test weight, and protein content). Proportion of the SE was determined by weighing after hand dissection. All the results are reported in Table I.

TKW and test weight values spanned between 39.9 and 60.2 g, and between 82.4 and 87.5 kg/hL, respectively, and were consistent with the observed current range for French durum wheat cultivars (Raggiri et al. 2014). TKW values therefore set the samples above the threshold defined by Abecassis and Chaurand (1996), in which other factors than the SE-to-bran ratio were suggested to be involved in the milling yield. The protein content varied largely between 12.9 and 17.2% but was found to be above the threshold level of 10%, below which the SE texture is affected, as pointed out by Samson et al. (2005). No significant differences in the SE proportion (spanning between 82.5 and 83.2% db) were measured between the samples despite displaying contrasting TKW and test weight values. Samples were milled at a pilot scale and TS, TF, and bran (CB + PFB + SFB + SH) yields determined and expressed on a dry basis (Table II). TS yields were between 73.1 and 75.0%, and TF yields were between 8.6 and 11.2%, leading to TS+TF yields between 82.4 and 84.5%. These results were within the range of values generally obtained by using the same pilot mill, as previously described (Abecassis and Chaurand 1996; Chaurand et al. 1999; Peyron et al. 2002) for French durum wheat cultivars.

Low relationships between TS+TF milling yields and TKW ($R = 0.57$) and test weight ($R = 0.58$) were found in accordance with the results of Matsuo and Dexter (1980). However, differences in TS+TF milling yields appeared to be owing either to changes in the SE extraction or the presence of peripheral tissues in these milling fractions, because no significant differences in SE proportion between the analyzed wheat grain samples were observed. Nevertheless, ash contents of TS+TF fractions were lower than the generally accepted threshold of 0.9% (Troccoli et al. 2000; Gruber and Sarkar 2012).

Analysis of Grain Tissue Distribution in the Milling Fractions.

Histological Characterization of Wheat Grains and Milling Fractions.

Although the endosperm and outer layer proportions can be determined in wheat grain by hand dissection, this method is not feasible for milling fractions. Therefore, the use of biochemical markers as described by Hemery et al. (2009) was considered for the characterization of durum wheat milling fractions. Indeed, use of these biochemical markers to identify different grain tissues in debranning fractions (Ríos et al. 2009a) and grains (Barron et al. 2011) was already shown to be efficient. To be able to study in detail the behavior of each durum wheat tissue at milling, two distinct validations of these biochemical markers have to be done. First, it is necessary to check that the sum of the tissues determined independently in each analyzed fraction reaches 100%, assuming that the lack of quantification of the scutellum is

negligible considering its low amount in grain (Pomeranz 1988; Barron et al. 2007). In a second step, the sum of each grain tissue determined in the obtained milling fractions (TS, TF, SH, SFB, PFB, and CB) needs to be equal to the corresponding tissue proportion determined directly for each wheat grain sample.

Analysis of each grain tissue proportion in each milling fraction and grain is reported for one of the eight wheat samples (S2) as an example in Table III.

Results showed that all of the grain tissues were present whatever the observed milling fractions, but their relative amounts appeared to depend on the fraction nature. Therefore, the milling process occurred well at a histological scale but could not achieve tissue purification, as already suggested by Peyron et al. (2002). As shown in Table III for sample S2, the average sum of the determined grain tissue proportions for each milling fraction was around 100%. This average sum was also found between 92.3 and 104.3 % for the overall analyzed sample set ($n=8$, data not shown) and if TS fractions were slightly overestimated, with a tissue sum amounting to between 103.4 and 105.6%, the SH fractions were the most underestimated, with a tissue sum of between 87.3 and 96.4%. However, taking into account the cumulative analytical error, results were considered reliable toward the relative error of 10%.

Moreover, the sum of each grain tissue proportion determined in the distinct milling fractions was compared with the tissue proportion determined directly on the grains. As shown in Table III and checked for all of the durum wheat samples, quantification of each grain tissue was similar between grains and the sum of milling fractions. A satisfactory recovery of the total wheat grain sample was also observed, because the total sum of grain tissues equaled an average of 102.1% and the total sum of tissues in milling fractions reached a mean of 103.1% for the overall wheat sample set.

Therefore, the use of the biochemical markers defined in common wheat (Hemery et al. 2009) could be successfully applied to monitor the fate of each durum wheat grain tissue during milling.

To allow comparison between grain samples, the tissue composition of the different fractions was standardized to 100%. Values were processed through principal component analysis (PCA), and the obtained pattern of similarity is shown in Figure 1A.

Similar histological composition for each milling fraction was obtained whatever the durum wheat sample. The first axis of the PCA biplot, which accounted for 74.3% of the data variability, was mainly correlated with the proportion of envelopes ($r = 0.96$), ACC ($r = 0.96$), and ACW ($r = 0.87$) and inversely correlated with SE proportion ($r = -0.99$). This relationship can be related to the site in the grain where tissue separation occurred at milling. Along this axis, milling fractions can be clearly separated into three groups: the first one being formed with TS and TF, the second group with SH, and the last group with the other bran fractions (SFB, PFB, and CB). The second axis of the biplot accounted for 18.9% of the data variability and appeared mainly related to the embryonic axis proportion ($r = 0.94$). Only SH fractions were distinguished from the other milling fractions according to this parameter.

Based on the mean composition of each type of milling fraction for the overall set of durum wheat grain samples reported in Figure 1B, TS appeared mainly composed of the SE (95.9%) with a noticeable amount of ACC (2.4%) and ACW (0.7%). Low proportions of envelopes (0.5%) and embryonic axis (0.4%) were also found in TS. In comparison with TS, TF contained

less SE (89.0%) and more peripheral tissues, with 6.9% ACC, 2.0% ACW, 1.6% envelopes, and a comparable amount of embryonic axis (0.5%).

SH contained more SE than the other bran fractions (57.4%) but also more peripheral tissues than TS and TF (15.5% envelopes, 15.4% ACC, and 8.2% ACW). As illustrated with the second PCA axis of Figure 1A, this product displayed the highest composition in embryonic axis (3.5%); thus, it probably contained interesting compounds for nutritional value as part of the grain B vitamins, minerals, α -tocopherols (Pomeranz 1988), and sterols (Nyström et al. 2007) present in the germ. Other bran fractions contained higher proportions of envelopes (41.5–43.7%, along with comparable amounts of the other tissues except the SE, which was found in a higher proportion in fine bran fractions (SFB and PFB).

Our results confirmed the previous work of Peyron et al. (2002), who roughly determined the relative proportion of main grain tissues in the different milling fractions but provided a more detailed knowledge of the wheat grain sample behavior, because a larger number of grain tissues were determined as well as the respective proportion of ACC and ACW.

Contribution of the SE and Peripheral Tissues to TS+TF Yields.

From the histological composition, the respective contributions of each durum wheat grain tissue to TS+TF yields were calculated and reported in Figure 2.

As expected, a major part of the TS+TF yields corresponded to the SE, with values between 78.5 and 80.3%. A significant increase of these yields (between 2.0 and 3.0%) was owing to the presence of ACC. Indeed, for durum wheat samples S7 and S3, which showed low to intermediate contribution of the SE extraction to TS+TF yield (78.5 and 79.4%, respectively), extraction of the ACC particularly contributed to increase the milling performance (+3.0%). The presence of the ACW contributed to a lesser extent to the milling yield increase (between 0.5 and 0.9%), at around the same order as the envelopes (between 0.3 and 0.9%) and the embryonic axis (between 0.2 and 0.6%). The highest increases in the milling yields owing to the presence of peripheral tissues were observed for samples S3 (+4.8%) and S7 (+4.9%), whereas the lowest was observed for sample S4 (+3.4%).

Therefore, TS+TF yield variability was mainly owing to differences in the SE and ACC proportion, with minimal impact from the presence of the other peripheral tissues.

Correlations between TS+TF ash content and both percentage of the envelopes and the ACC contribution to TS+TF yield ($R = 0.65$ and 0.61 , respectively) were found. Thus, extraction of the peripheral tissues was undeniably linked to an ash content increase of the fractions. However, depending on the respective contribution of ACC or the envelopes, the corresponding ash increase did not reflect similar biochemical composition of the fractions. Although food products containing AL have been clearly associated with beneficial effects on consumer health (Brouns et al. 2012; Price et al. 2012), the envelopes contain the major part of potentially contaminating mycotoxins and heavy metals (Ríos et al. 2009a, 2009b; Sovrani et al. 2012), pesticides (Fleurat-Lessard et al. 2007), and microorganisms (Laca et al. 2006).

Extraction Efficiency Between the SE and the Other Grain Tissues.

Extraction of the Durum Wheat Grain Tissues in TS and TF.

Previous results suggested an impact of the SE and bran separation on the obtained TS+TF yields. To better explore this separation, proportions of the initial grain SE, ACC, ACW, envelopes, and embryonic axis recovered in TS+TF were quantified and compared between the different durum wheat grain samples. Results are presented in Table IV. As an example, 94.53% of the initial grain SE from sample S1 was extracted in TS+TF, the remaining 5.47% being lost in SH and bran fractions.

Differentiation of five significantly distinct groups between the different wheat samples was possible according to the proportion of initial grain SE recovered in TS+TF fractions, with measured values spanning from 94.53 to 95.96%. These values are on the same order as previously described by Peyron et al. (2002), with SE extraction of between 94.63 and 96.38%.

Significant differences were measured between the samples for both the extraction of ACC (six distinct groups) and ACW (four groups). Sample S4 displayed the lowest ACC extraction (36.20%), whereas samples S3 and S7 displayed the highest (45.29 and 48.22% respectively), in accordance with the previous observations concerning the main grain tissue contributions to TS+TF yield. Between 40 and 50% of the initial grain ACC was therefore recovered in TS+TF, whereas only 19–30% of the ACW was extracted in the same fractions, suggesting an incomplete aleurone cell breakage with slight fragmentation of the ACW but a release of the ACC in the mill streams. However, the ratio between extracted ACC versus ACW appeared to depend on the wheat samples. Moreover, these differences of AL extraction in TS+TF appeared strongly correlated with those in TS alone. Close examination of head-end and tail-end semolina fractions showed that differences of ACW extraction between samples appeared at the first milling steps, whereas differences of ACC extraction occurred mainly at later steps (data not shown). Indeed, the determination coefficient between ACW extractions in TS+TF and in head-end semolina was equal to 0.78. The determination coefficient between ACC extractions in TS+TF and in tail-end semolina was equal to 0.73. Such differences in ACC and ACW fractionation behavior were also reported by Greffeuille et al. (2005) for hard-type common wheat.

The percentage of the envelope extraction displayed a smaller variability (only three distinct groups) to that observed for the SE and ACC or ACW. Their presence was also significantly lower and did not vary proportionally with the other tissues. No significant differences in embryonic axis extraction were observed, mainly owing to the difficulty of increasing its measurement accuracy. However, considering its low mass in grains, its presence remains negligible.

Taking into account the observed differences of extraction between ACC and the ACW, further study of the AL fractionation at milling was undertaken.

Comparison Between AL Composition Before (in Grain Tissues) and After Milling (in CB).

To closer examine the AL fragmentation, ACW proportions in hand-dissected undamaged whole outer layers isolated with the crease were compared with those in CB recovered after milling, where the AL can be damaged owing to the process. Results are reported in Figure 3.

Significant differences between values obtained for hand-isolated undamaged tissue and potentially damaged AL in CB were observed for samples S1 and S2. Indeed, for these samples, the $ACW/(ACC+ACW)$ ratio increased significantly in CB in comparison with the hand-isolated tissue, whereas it remained almost stable or decreased among the other samples. Two hypotheses could be suggested to explain the specific behavior of these two samples (S1 and S2): a higher

resistance of the ACW, which led to a lower level of fragmentation in comparison with the other durum wheat samples, or a higher SE compression against the AL at milling, which led to a larger ACC release without complete recovery of the AL tissue and thus an observed concomitant apparent increase of the ACW proportion in CB. Results from Table IV do not particularly reveal a higher percentage of ACC in TS+TF from S1 and S2 wheat grains in comparison with other samples, but the percentages of ACW extraction were found to be significantly lower. These results indicated that, for these specific samples, the AL was emptied of its cellular content with low fragmentation of its cell walls. By contrast, for the other samples the ACW proportion did not increase drastically, as shown in Figure 3, and appeared to be more related to a removal of the entire cells from the AL.

CONCLUSIONS

In this study, durum wheat samples displaying a large range of grading characteristics were milled and their tissue distribution monitored in recovered fractions by using biochemical markers. The obtained results demonstrated the reliability of this method to monitor durum wheat grain tissues and characterized the histological composition of the different fractions. The use of these specific biochemical marker measurements, in comparison with ash, allowed distinguishing between the presence of AL or other peripheral tissues in fractions that will be used for food production. Because the presence of these tissues does not have the same consequences on consumers' health, it is particularly useful for milling fraction characterization.

It was also found that despite displaying a similar SE proportion in grains, the different durum wheat samples presented distinct TS+TF yields. This result was found to be mainly because of distinct SE and ACC extractions, the other grain peripheral tissues being extracted with less variability and to a lesser extent in these milling fractions (<2%). Because ACC was shown to be the main durum wheat grain tissue present in TS+TF after the SE, a closer study of AL rupture behavior was undertaken. This investigation revealed two potentially distinct fractionation mechanisms: one corresponding to a cell emptying mechanism with low damage of the ACW, whereas the other corresponded more to an overall cell removal. Therefore, further examination of the AL mechanical resistance in the different samples to better understand the origin of these different types of tissue rupture appears interesting to undertake.

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Fig. 1. A, Principal component analysis similarity map corresponding to all of the milling fractions from the different durum wheat grain samples according to their tissue composition. **B**, Average histological composition of each milling fraction. Each value is the mean of eight values corresponding to the different grain samples, with standard deviation in parentheses. SE = starchy endosperm; ACC = aleurone layer cellular content; ACW = aleurone cell walls; TS = total semolina; TF = total flour; SH = shorts; SFB = sized fine bran; PFB = purified fine bran; and CB = coarse bran.

Fig. 2. A, Contribution of the different durum wheat grain tissue to the TS+TF yield (percentage of dry mass): starchy endosperm (SE), aleurone layer cellular content (ACC), aleurone cell walls (ACW), envelopes, and embryonic axis. **B**, Corresponding values (mean of four determinations). Different superscripts in each column mark significant differences ($P \leq 0.05$). TS = total semolina, and TF = total flour.

Fig. 3. Aleurone cell wall (ACW) proportion (evaluated with biochemical markers) in hand-dissected undamaged whole outer layers isolated with the crease and in coarse bran recovered after milling, where the aleurone layer can be damaged because of the process. Significant differences between values obtained for hand-isolated undamaged tissue and potentially damaged aleurone layer in coarse bran are indicated with an asterisk. ACC = aleurone layer cellular content.

TABLE I
Characterization of the Wheat Grain Samples^z

Sample	TKW (g)	TW (kg/hL)	Protein Content (% db)	SE (% db)
S1	39.9a	82.4a	13.9c	82.6a
S2	53.3d	83.6bc	12.9a	82.8a
S3	54.4d	84.0c	15.1e	83.1a
S4	49.2c	85.4d	13.8c	82.5a
S5	60.2e	87.5e	13.6b	82.9a
S6	50.5c	83.4b	13.5b	82.5a
S7	47.0b	83.1b	14.4d	82.5a
S8	47.4b	85.6d	17.2f	83.2a
LSD	1.0	0.3	0.1	1.0

^z Means with different letters in each column were significantly different based on Tukey's honest significant difference test ($P \leq 0.05$). TKW = thousand-kernel weight; TW = test weight; and SE = starchy endosperm.

TABLE II
Milling Yield of Total Semolina (TS), Total Flour (TF), Their Calculated Sum, and Bran Fractions (CB + PFB + SFB + SH), and the Purity of the TS+TF Products Assessed with Their Cumulative Ash Content^z

Sample	TS (%)	TF (%)	TS+TF (%)	Bran (%)	Ash Content (%)
S1	73.1	9.2	82.4	17.6	0.79
S2	75.0	8.6	83.6	16.4	0.74
S3	74.0	10.2	84.2	15.8	0.89
S4	73.5	9.8	83.3	16.7	0.76
S5	74.5	10.0	84.5	15.5	0.84
S6	71.5	11.2	82.7	17.3	0.89
S7	73.6	9.8	83.4	16.6	0.89
S8	73.5	10.7	84.1	15.9	0.76

^z All values are expressed on a dry basis. CB = coarse bran; PFB = purified fine bran; SFB = sized fine bran; and SH = shorts.

TABLE III
Yield and Tissue Composition of Each Milling Fraction and Their Sum in Comparison with the Wheat Grain Tissue Composition for Sample S2^z

Milling Fraction	Yield (% db)	SE (%)	ACC (%)	ACW (%)	Envelopes (%)	Embryonic Axis (%)	Tissue Sum (%)
TS	75.0	100.0 (± 1.4)	2.3 (± 0.1)	0.5 (± 0.0)	0.6 (± 0.0)	0.3 (± 0.1)	103.7 (± 1.6)
TF	8.6	84.9 (± 1.2)	7.1 (± 0.1)	2.3 (± 0.1)	1.7 (± 0.1)	0.5 (± 0.1)	96.6 (± 1.6)
SH	2.6	57.9 (± 0.6)	12.6 (± 0.4)	6.6 (± 0.3)	12.3 (± 0.6)	2.4 (± 0.6)	91.8 (± 2.3)
SFB	0.4	26.2 (± 0.3)	22.1 (± 0.3)	11.5 (± 0.5)	41.8 (± 0.6)	1.5 (± 0.3)	103.1 (± 1.9)
PFB	7.4	21.1 (± 0.3)	22.4 (± 0.4)	13.6 (± 0.7)	44.4 (± 0.4)	1.1 (± 0.3)	102.7 (± 2.0)
CB	6.0	16.3 (± 0.2)	21.6 (± 0.9)	23.8 (± 0.9)	43.3 (± 0.8)	0.9 (± 0.1)	105.9 (± 2.6)
Sum	100.0	86.5 (± 1.2)	5.7 (± 0.2)	3.3 (± 0.1)	7.0 (± 0.1)	0.5 (± 0.1)	102.9 (± 1.7)
Grain	...	85.6 (± 1.1)	5.9 (± 0.1)	3.4 (± 0.1)	6.5 (± 0.1)	0.9 (± 0.3)	102.2 (± 1.6)

^z Envelope values correspond to the sum of outer pericarp and intermediate layer determination. Each result is the mean of four determinations, and standard deviation is in parentheses. SE = starchy endosperm; ACC = aleurone layer cellular content; ACW = aleurone cell walls; TS = total semolina; TF = total flour; SH = shorts; SFB = sized fine bran; PFB = purified fine bran; and CB = coarse bran.

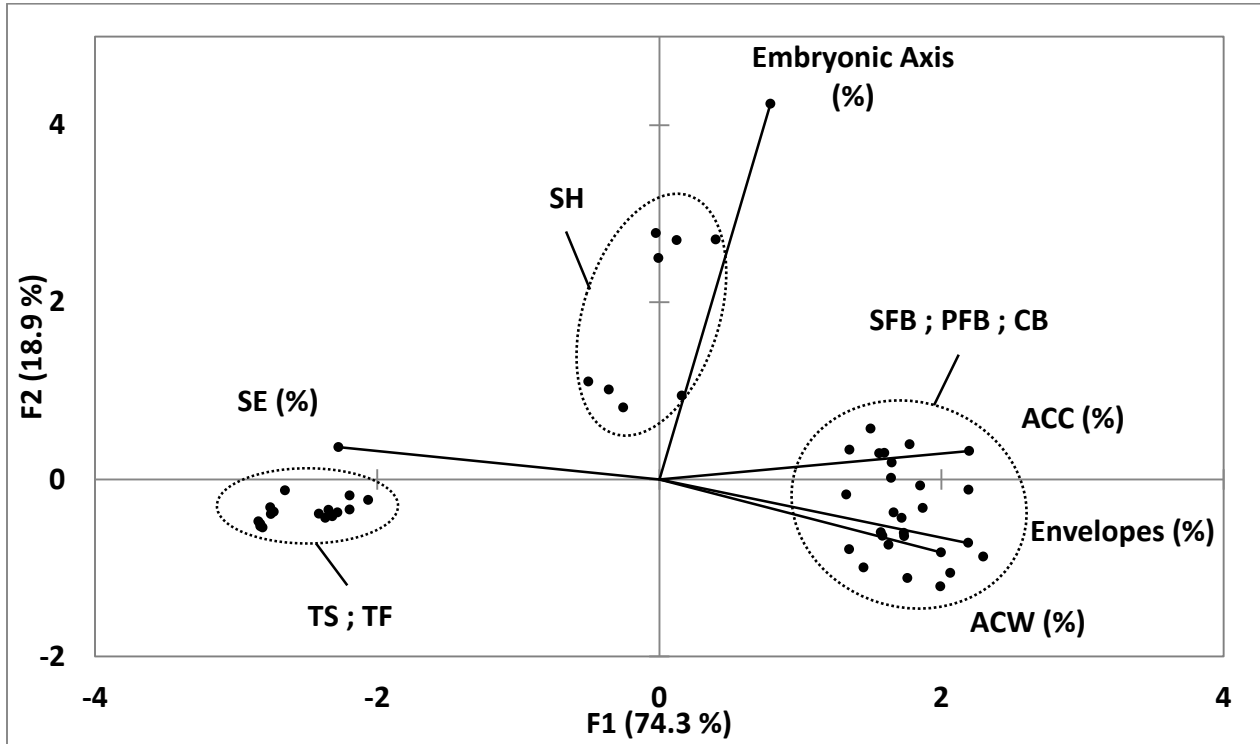
TABLE IV
Percentage of Extraction of Each Tissue Recovered in TS+TF Relative to the Grain^z

Sample	SE (%)	ACC (%)	ACW (%)	Envelopes (%)	Embryonic Axis (%)
S1	94.53a	41.37c	19.05a	8.21bc	57.31a
S2	95.05b	41.16c	19.12a	8.56bc	60.10a
S3	95.46d	45.29e	28.05d	9.50c	62.76a
S4	95.22c	36.20a	25.07b	6.43a	73.97a
S5	95.96e	43.40d	25.62bc	5.65a	60.61a
S6	95.10b	39.41b	30.51e	6.26a	53.49a
S7	95.92e	48.22f	26.54bcd	6.37a	71.89a
S8	95.39d	39.23b	27.35cd	7.94b	48.84a
LSD	0.06	0.86	1.12	0.89	28.64

^z Each result is the mean of four values. Means with different letters in each column were significantly different ($P \leq 0.05$). TS+TF = sum of total semolina plus total flour; SE = starchy endosperm; ACC = aleurone layer cellular content; and ACW = aleurone cell walls.

Figure 1

(a)

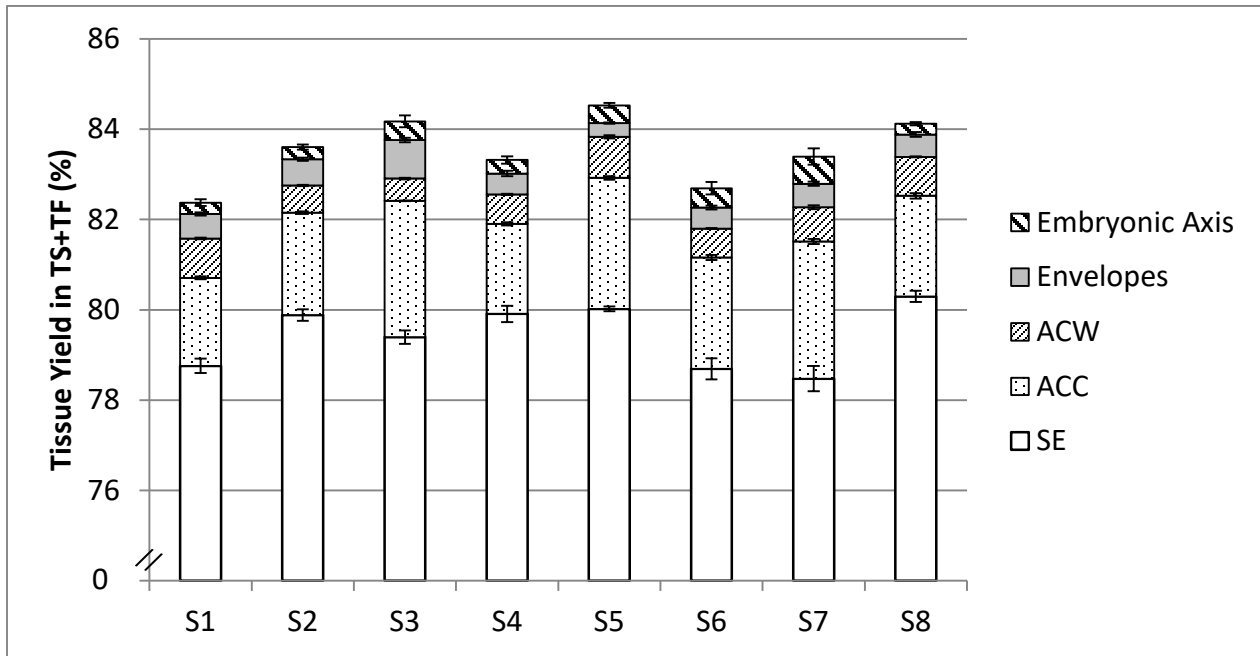


(b)

Fraction	SE (%)	ACC (%)	ACW (%)	Envelopes (%)	Embryonic Axis (%)
TS	95.9 (± 0.7)	2.4 (± 0.5)	0.7 (± 0.2)	0.5 (± 0.2)	0.4 (± 0.2)
TF	89.0 (± 1.4)	6.9 (± 1.0)	2.0 (± 0.4)	1.6 (± 0.3)	0.5 (± 0.1)
SH	57.4 (± 4.1)	15.4 (± 1.5)	8.2 (± 2.0)	15.5 (± 2.9)	3.5 (± 1.2)
SFB	22.7 (± 1.6)	21.1 (± 2.7)	13.1 (± 4.2)	41.5 (± 3.4)	1.7 (± 0.5)
PFB	19.8 (± 1.8)	22.5 (± 2.7)	13.8 (± 3.7)	42.6 (± 3.9)	1.3 (± 0.5)
CB	16.2 (± 1.8)	22.6 (± 3.6)	16.3 (± 6.4)	43.7 (± 4.4)	1.1 (± 0.5)

Figure 2

(a)



(b)

<i>Sample</i>	<i>SE</i> (%)	<i>ACC</i> (%)	<i>ACW</i> (%)	<i>Envelopes</i> (%)	<i>Axis</i> (%)	<i>Total Yield</i> (%)
S1	78.8 ^A	2.0 ^A	0.9 ^D	0.5 ^{B,C}	0.3 ^A	82.4
S2	79.9 ^C	2.3 ^B	0.6 ^B	0.6 ^C	0.3 ^A	83.6
S3	79.4 ^B	3.0 ^E	0.5 ^A	0.9 ^D	0.4 ^{A,B}	84.2
S4	79.9 ^{C,D}	2.0 ^A	0.6 ^B	0.5 ^B	0.3 ^A	83.3
S5	80.0 ^{C,D}	2.9 ^D	0.9 ^D	0.3 ^A	0.4 ^{A,B}	84.5
S6	78.7 ^A	2.5 ^C	0.6 ^B	0.5 ^B	0.4 ^{A,B}	82.7
S7	78.5 ^A	3.0 ^E	0.8 ^C	0.5 ^{B,C}	0.6 ^B	83.4
S8	80.3 ^D	2.2 ^B	0.9 ^D	0.5 ^{B,C}	0.2 ^A	84.1
<i>LSD</i>	0.3	0.1	0.0	0.1	0.2	-

Figure 3

