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Individual Contribution of Grain Outer Layers and Their Cell Wall Structure to the Mechanical Properties of Wheat Bran

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The mechanical properties of wheat bran and the contribution of each constitutive tissue on overall bran properties were determined on a hard wheat (cv. Baroudeur) and a soft wheat (cv. Scipion). Manual dissection allowed three different layers to be separated from wheat bran, according to radial and longitudinal grain orientations, which were identified by confocal laser scanning microscopy as outer pericarp, an intermediate strip (comprising inner pericarp, testa, and nucellar tissue), and aleurone layer, respectively. Tissue microstructure and cell wall composition were determined. Submitted to traction tests, whole bran, intermediate, and aleurone layers demonstrated elastoplastic behavior, whereas pericarp exhibited elastic behavior. By longitudinal orientation, pericarp governed 50% bran elasticity (elastic strength and rigidity), whereas, in the opposite orientation, bran elastic properties were mostly influenced by the other tissues. Regardless of test orientation, the linear force required to bran rupture corresponded to the sum of intermediate and aleurone layer strengths. According to radial orientation, the intermediate strip governed bran extensibility, but according to longitudinal orientation, all tissues contributed until bran disruption. Tissues from both wheat cultivars behaved similarly. A structural model of wheat bran layers illustrated the detachment of pericarp from intermediate layer within radial bran strips.

KEYWORDS: Wheat bran; aleurone layer; microstructure; arabinoxylans; ferulic acid dehydrodimers; lignin; mechanical properties

INTRODUCTION

The wheat (*Triticum aestivum* L.) grain is a caryopsis, that is, a fruit where the ovary wall is fused to the single seed. Besides the embryo, from the center to the periphery of the grain, one can distinguish the endosperm (composed of the starchy endosperm and the single cell layered aleurone layer), the seed coats (composed of the nucellar epidermis and the testa), and the pericarp (composed of the tube cells, the cross cells, the hypodermis, and the epidermis) (*1*, *2*). In wheat milling technology, the miller aims at best separating the starchy endosperm, which is recovered as white flour, from the outer layers, which are called bran. Wheat bran is therefore composed of pericarp, seed coats, and aleurone layer with some attached remnants of endosperm. Considerable amounts of wheat bran are produced annually that are mostly used in animal feeding. Due to the presence of the aleurone layer, wheat bran constitutes, however, a potential source of micronutrients (*3*) that could be better valorized in human nutrition. New fractionation technologies would be probably necessary to develop efficient cracking strategies of the bran. In this prospect, it is essential to gain better knowledge of the mechanical properties of the bran, which govern mostly its milling behavior.

Traction tests on single kernel isolated strips have been developed that allow access to the fundamental mechanical properties of wheat bran (*4*, *5*). These tests could be applied also to bran layers hand-dissected from the grain. By using such approaches, recent studies on durum wheat (*Triticum durum* Desf.) have shown that the mechanical properties of the bran and strips isolated from the bran (pericarp, aleurone layer) were strongly influenced by the degree of polymer cross-linking in the cell walls of the tissues (*6*). In this respect, the coupling products of ferulic acid appeared as key components for their role in establishing interchain covalent bridges between arabinoxylans and also possibly between arabinoxylans and lignin.

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The aim of this work was to determine the contribution of the wheat (*T. aestivum*) grain outer layers to the mechanical properties of the bran in relation with their microstructure and cell wall composition. Strips were sampled from the bran layers of a hard wheat and a soft wheat, characterized by fluorescence microscopy and chemical analysis, and submitted to traction tests in different orientations. A tentative model of bran mechanical behavior was established and discussed in connection with structural features of the tissues.

MATERIALS AND METHODS

Wheat Samples. Two varieties of wheat (*T. aestivum* L.) of different kernel hardnesses (a hard wheat, cv. Baroudeur, and a soft wheat, cv. Scipion), harvested in France in 2000 (Gascogne and Verneuil, respectively) were used in this study.

Preparation of Wheat Bran Strips and Strips Isolated from Wheat Bran. Strips were isolated by hand according to radial and longitudinal grain orientations. Germ and brush were cut before grain immersion in distilled water for 12 h. For radial orientation, a crease incision was made and the remaining part was soaked again for 2 h. For longitudinal orientation, a crease incision was carried out, and then two strips were isolated by hand from the two lateral faces of the grain before water immersion. Bran strips were obtained by removal of the endosperm using a scalpel. Three different strips were separated by sliding a needle between the different tissues of soaked bran. Then, strips were dried between two plates under ambient conditions to impose upon them a plane shape and, finally, their moisture content (16.5%) was adjusted by conditioning at constant relative humidity (75%) in a saturated NaCl solution overnight at 25 °C. The strips were thereafter cut to approximately 8 mm long and 2−3 mm wide.

Confocal Laser Scanning Microscopy. The multispectral fluorescence microscopy is a usual method of identifying and differentiating the different tissues within wheat bran (*7*, *8*).

Cross sections of the isolated layers were obtained using a cryotome Microm HM 500 OM and observed with a Zeiss LSM 410 Axiovert microscope. The isolated layers and cross sections were examined with a water-immersed x40/NA objective. The native primary fluorescence was detected using two lasers as excitation sources and appropriate long pass filters (a UV argon ion laser, $\lambda_{exc} = 363.8$ nm and $\lambda_{em} > 397$ nm; and a blue argon ion laser, $\lambda_{exc} = 488$ nm and $\lambda_{em} > 515$ nm). Images were obtained serially by scanning the sections with each laser beam in combination with the appropriate filter. Each image was recorded separately in different channels (RGB). There was an average of eight images in each series. Overlaying of the recorder images permitted simultaneous visualization of structures.

Analysis of Lignins in Bran Samples by Thioacidolysis. All of the subsequent analyses were done in duplicate. Thioacidolysis reagent was prepared by introducing 2.5 mL of BF_3 etherate (Aldrich) and 10 mL of ethanethiol (EtSH, Aldrich) into a 100 mL flask and adjusting the final volume to 100 mL with dioxane (pestipur grade). The colorless reagent was used immediately after preparation. Ground bran tissue samples (10−20 mg) were added to 10 mL of reagent and 1 mL of solution of GC internal standard (docosane, 0.15 $\mu g \cdot mL^{-1}$ in CH_2Cl_2) in a glass tube closed with a Teflon-lined screw cap. Thioacidolysis was performed at 100 °C (oil bath) for 4 h. The cooled reaction mixture was diluted with 30 mL of water, and the pH was adjusted to 3−4 (aqueous 0.4 M $NaHCO_3$). The reaction mixture was extracted with CH_2Cl_2 (3 × 30 mL). Combined organic extracts were dried over Na_2SO_4 and then evaporated under reduced pressure at 40 °C. The final residue was redissolved in ~0.5 mL of CH_2Cl_2 before silylation and GC-MS analyses according to the method of Lapierre et al. (*9*). This quantitative determination was carried out from specific ion chromatograms reconstructed at *m*/*z* 269 for the G monomers and at *m*/*z* 299 for the S monomers after an appropriate calibration relative to the docosane internal standard.

Ester-Linked Phenolic Acid Content. Ground samples (20 mg) were saponified for 2 h in the dark with 2.0 M sodium hydroxide (10 mL) at 35 °C under slow agitation. Internal standard, 3,4,5-trimethoxy-(*E*)-cinnamic acid (TMCA, Sigma Chemical Co., St. Louis, MO) was added, and the solution was adjusted to pH 2.0 with 4 M hydrogen chloride. Phenolic acids were extracted twice with diethyl ether (5 mL). Ether phases were evaporated in the presence of argon. The dry extract was dissolved in aqueous methanol (MeOH/water, 50:50, v/v), filtered (0.45 μm), and injected (20 μL) on RP-HPLC using an Alltima (Alltech, Deerfield, IL) C_{18} column, 5 μm (250 × 4.6 mm). Linear gradient elution was performed by acetonitrile and sodium acetate buffer, 0.05 M, pH 4.6, at 1 $mL \cdot min^{-1}$ at 35 °C, from 15:85 to 35:65 in 24 min, from 35:65 to 60:40 in 0.5 min, from 60:40 to 15:85 in 4.5 min, and maintained at 15:85 for 5 min. UV detection was carried out at 280 and 320 nm using a 996 Waters photodiode array detector (Waters, Milford, MA). Standard dehydrodimers (DHD) were kindly supplied by Dr. J. Ralph from the U.S. Dairy Forage Research Center, USDA-ARS, and Department of Foresty, University of Wisconsin, Madison, WI (*10*). Response factors of ferulic acid dehydrodimers determined by Saulnier et al. (*11*) were used. Products were identified using their UV absorption spectra (*12*).

Carbohydrate Content. Freeze-dried strip samples were ball-milled (Dangoumeau, France) for 4 min in liquid nitrogen before hydrolysis. Samples (10 mg) were treated for 30 min with aqueous 72% H_2SO_4 at room temperature (*13*) and then hydrolyzed with sulfuric acid (1.0 M, 2 h, 100 °C). Sugars were converted into alditol acetates (*14*) and analyzed by GLC on a column of HP 225 (50% CNPrph Me siloxan, 30 m × 0.2 mm × 0.15 μm) at 225 °C, using hydrogen as carrier gas and allose (5 mg) as internal standard. Arabinoxylan was calculated as the sum of anhydroarabinose and anhydroxylose. Cellulosic glucose was determined by difference between values obtained with and without the 72% sulfuric acid prehydrolysis step (*15*).

Mechanical Tests. Mechanical tests were performed using Dynamic Mechanical Thermal Analysis DMTA Mk III (Rheometrics Inc., Piscataway, NJ). Humidity control was achieved according to the principle of water vapor saturation at different temperatures (*16*). The humidity of samples was fixed and controlled with air that was bubbled through water at 26.8 °C and that was flushed on the furnace. This process allowed a strip relative humidity of 16.5% to be obtained, corresponding to the usual tempering condition before the milling of this kind of wheat. Sample equilibration was followed by a time sweep test at imposed strain (0.05%) (total time = 10 min; frequency = 10 $rad \cdot s^{-1}$, furnace temperature = 30 °C). The stability of elastic modulus (*E*) was used as an indicator of the sample equilibration.

Uniaxial tension tests were performed at a rate of 0.05 $mm \cdot s^{-1}$ until disruption of the sample. Traction tests were carried out until rupture of bran coat strips. Mechanical properties of the different bran tissues were determined from standard average curves expressing linear strength according to strain: linear strength to elastic deformation and to rupture (f_{ela}, f_{max}), elastic strain (ϵ_{ela}), maximum tensile strain (ϵ_{max}), tensile modulus (*E*), and rupture energy (W_{max}) (**Tables 3** and **4**). Linear strength corresponded to the strength exerted per unit of tissue width to cause elastic deformation (ϵ_{ela}) then fracture (ϵ_{max}). This parameter allowed comparison of the different constitutive tissues of bran, considering them as a single film even though they exhibited very different thicknesses. Deformation was determined as $\Delta L/L_0$. Linear modulus, *E*, was the initial maximum tangent proportionality strength−strain coefficient and highlighted the stiffness of the sample. Finally, the energy to fracture, W_{max}, corresponded to the tensile strength and was expressed in fracture surface units. Tension tests were performed on at least five strips. The coefficient of variation was ≤30%.

RESULTS

Structure and Histological Origin of Bran Coat Strips. The yields in bran tissues estimated from manual dissection were 14.9 and 13.3% of the mass of Baroudeur and Scipion grains, respectively. For both wheat grains, bran strips were ~70 μm thick and were devoid of adherent starch. Three different strips were isolated from the bran by manual dissection. The most external strip, which was translucent, was easily separated. The two other strips were tightly adhered and thus more difficult to isolate one from another. The intermediate strip was brown. The inner strip was dense and white. The thicknesses of these

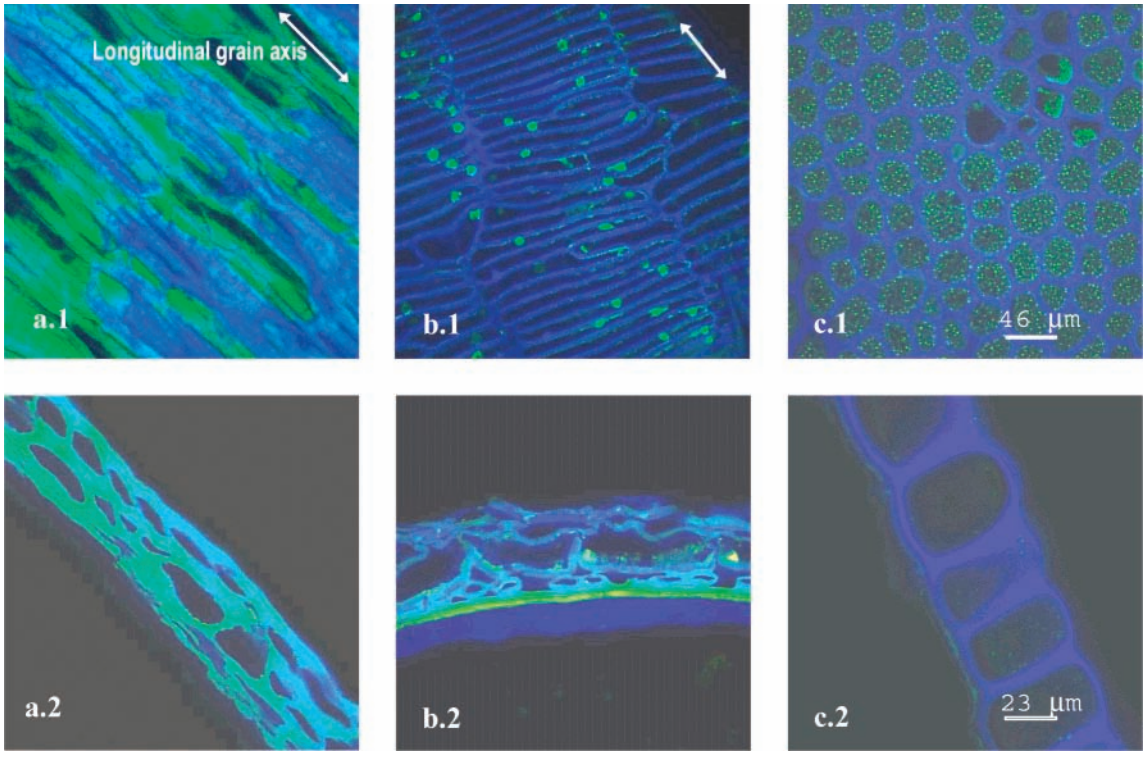

Figure 1. Fluorescence micrographs of pericarp (a), intermediate (b), and aleurone (c) layers of outer faces of tissue strips (a.1 to c.1, same scale) and after cross sections of the same layers (a.2 to c.2, same scale). Images were obtained by superposition of two channels: $\lambda_{exc} = 364$ nm and $\lambda_{em} > 397$ nm for the blue-red emission; $\lambda_{exc} = 488$ nm and $\lambda_{em} > 515$ nm for the green-red emission. Wheat cultivar used was Baroudeur.

strips were 8, 15, and 50 μm from the outer to the inner part of the bran, respectively, and similar for both wheat cultivars. The average percentages of the different strips relative to the bran were 30, 22, and 48% from the outer to the inner part of the bran for cv. Baroudeur and 25, 23, and 52% for cv. Scipion. The histological composition of the strips was assessed by confocal laser scanning microscopy. Because each constitutive tissue of both wheat varieties showed an identical histological composition, only micrographs of Baroudeur are presented (**Figure 1**). The outer strip was composed of longitudinal cells alternately arranged with their principal axis parallel to the principal grain axis (**Figure 1a.1**). It was a layer of three-cell thickness (**Figure 1a.2**) The intermediate strip was a composite of several tissues (**Figure 1b.2**). From the external to the internal face, it contained transversal cells, tubular cells, and two successive homogeneous film-like structures. Finally, the inner strip was composed of polygonal cells forming a regular network with fluorescent granular cell inclusions (**Figures 1c.1** nd **1c.2**).

By confronting these observations with previously published descriptions (*17*), the histological composition of the isolated strips was determined. The outer strip corresponded to the outer pericarp (epidermis and hypodermis), the intermediate strip comprised inner pericarp (transversal and tubular cells), testa, and nucellar tissue, and the inner strip was the aleurone layer.

Cell Wall Composition of the Isolated Strips. *Lignin.* To unambiguously detect the occurrence of lignin structures that could affect the mechanical properties of bran fractions, we subjected the samples to thioacidolysis. Thioacidolysis is an analytical degradation procedure that results in the depolymerization of lignins. The key reaction of thioacidolysis is the cleavage of the labile β-*O*-4 bonds, which are interunit bonds specific for native lignins according to Lapierre et al. (*9*). Guaiacyl (G) and syringyl (S) lignin units only involved in β-*O*-4 bonds respectively give rise to thioethylated G and S monomers (Ar−CHSEt−CHSEt−CH_2SEt, with Ar = G or S ring), with a high reaction yield. In contrast, ferulate esters give partial rise to free ferulates and to the corresponding ethanethiol addition products. On this basis, thioacidolysis may be viewed as a diagnostic test for the presence of lignins in ferulic-containing samples. It must be borne in mind, however, that β-*O*-4 linked G and S units may also occur in the phenolic domain of suberin, which may therefore have a contribution to the recovery of thioacidolysis G and S monomers (*18*).

Table 1. Yield of Lignin-Derived Thioacidolysis G and S Monomers[a] (Micrograms per Milligram) Recovered from Wheat Cv. Baroudeur Bran Coats

tissue	S + G	S/G
aleurone layer	0.38	1.05
intermediate	3.12[b]	1.13
pericarp	1.34	0.75

[a] CV < 10%. [b] CV = 20%.

The results from duplicate thioacidolysis experiments carried out on the Baroudeur tissular fractions are reported in **Table 1**. Although a satisfying reproducibility could be obtained for the S/G molar ratio, the yields were found to be less reproducible, a lower performance that could be related to the difficulty in analyzing such weakly lignified tissular fractions available in low amount. Weak but noticeable amounts of G and S thioethylated monomers were nevertheless released from the bran samples (**Table 1**). Whatever the wheat variety (data not shown for the Scipion samples), the higher monomer level was consistently and significantly released from the intermediate strips, whereas the aleurone layers provided the lowest amounts. The dimethoxylated S monomers were dominant, especially from the aleurone layers. In contrast, *p*-hydroxyphenyl (H) monomers could be observed only as trace components.

Table 2. Phenolic Acid[a] (Micrograms per Milligram) and Polysaccharide[b] (Percent, w/w) Contents of Cv. Baroudeur and Scipion Wheat Bran Coats

wheat cv.	tissue	FA	DHD[c]	cellulose	β-glucans	AX[d]	A/X	X/DHD[e]
Baroudeur	aleurone layer	7.10	0.96	1.5	11.8	21.6	0.38	239
	intermediate	5.01	0.85	13.7	4.2	37.4	0.37	463
	pericarp	3.06	3.90	23.8	2.8	45.6	1.13	79
Scipion	aleurone layer	6.28	0.94	1.1	15.6	20.0	0.39	217
	intermediate	5.00	0.97	11.0	6.3	38.7	0.34	437
	pericarp	4.36	4.34	22.7	9.3	42.6	1.15	67

[a] CV < 10%. [b] CV < 10%; cellulose and β-glucans contents were calculated by the difference between glucose contents obtained with and without 72% sulfuric acid prehydrolysis. [c] DHD: ferulic dehydrodimers as calculated by summing 8,5′-diFA, 8-*O*-4′-diFA, 8,5′-benzo-diFA, and 5,5′-diFA contents. [d] AX: arabinoxylans obtained as the sum of anhydroarabinose (A) and anhydroxylose (X) determined by GLC. [e] Molar ratio.

Thioacidolysis thereby confirmed that the wheat bran fractions were associated with β-*O*-4 structures, the most diagnostic lignin structures.

On the basis of previous thioacidolysis analyses carried out on cereal brans of predetermined lignin content, the thioacidolysis yields observed herein allowed us to evaluate that the lignin content of the outer pericarp and intermediate strips was in the 1−3% range (by weight). In contrast, and not surprisingly, the aleurone layer was found to be less lignified.

Phenolic Acids. Over 95% of the phenolic acids of the bran layers was represented by ferulic acid (FA, *cis* + *trans*) and dehydrodimers (DHD) of ferulic acid (sum of 8,5′-diFA, 8,5′-benzo-diFA, 8-*O*-4′-diFA, and 5,5′-diFA). The wheat brans of the two wheat cultivars Baroudeur and Scipion had similar FA + DHD contents (21−22 μg/mg) (**Table 2**). Of the three strips, the intermediate had the lowest phenolic acid content, which was similar for both wheat cultivars (5.86 and 5.97 μg/mg for Baroudeur and Scipion, respectively). The aleurone layers and the outer pericarp exhibited high FA + DHD contents (7−8 μg/mg). The more phenolic-rich tissue was the aleurone layer for cv. Baroudeur, whereas it was the outer pericarp for cv. Scipion.

An essential difference between the samples lay in the degree of FA dimerization in the different tissues. Pericarp FA was highly dimerized (50−56%), whereas it was only 12−16% in the aleurone layer and in the intermediate strip. The slightly higher relative DHD content of the latter compared to aleurone layer may be due to the presence of inner pericarp. The phenolic acid content of two strips isolated from durum wheat bran, named pericarp and aleurone layer, also were determined (*19*). Values obtained for FA and DHD were in the same range as in the present study for the aleurone layer. However, durum pericarp strip had FA and DHD contents corresponding to the sum of outer pericarp and intermediate strip contents in the present study.

Polysaccharides. The overall polysaccharide content and composition were similar in the corresponding tissues of both wheat cultivars (**Table 2**). The aleurone layers exhibited lower polysaccharide contents because of their cell contents, whereas the other strips were made up essentially with cell wall material. β-Glucans were mostly found in the aleurone layers (33 and 43% of the recovered polysaccharides of Baroudeur and Scipion, respectively), accompanied with minor amounts of cellulose. Cellulose represented 25% (Baroudeur) and 18% (Scipion) of intermediate strip polysaccharides and one-third of the outer pericarp polysaccharides for both cultivars. Arabinoxylans were, however, the major constituents of the cell walls of the bran isolated strips (55−70%). Arabinoxylans of the aleurone layer and intermediate strip of both cultivars had similar arabinose-to-xylose ratios (A/X = 0.38 and 0.37 for Baroudeur aleurone layer and intermediate strip, respectively), suggesting a similar polysaccharide structure. On the other hand, outer pericarp

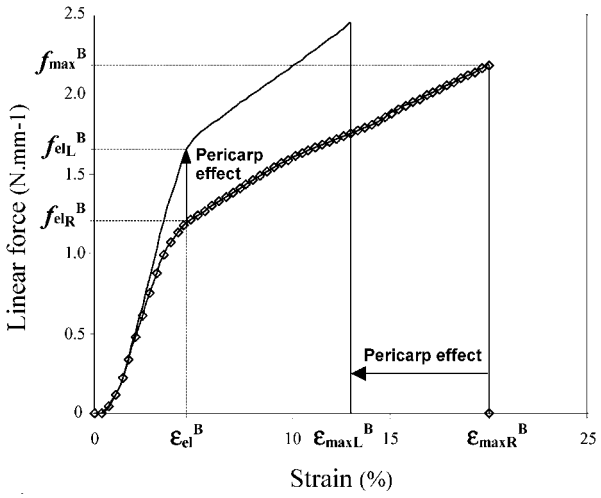

Figure 2. Experimental strain–linear force curves obtained from Scipion bran strips according to radial (⟡) and longitudinal (—) orientations.

arabinoxylans were almost 3 times more substituted (A/X = 1.13 and 1.15 for Baroudeur and Scipion pericarp, respectively). Similar polysaccharide composition and structure were reported for comparable material obtained from other wheat cultivars (*20*).

Owing to the mean structure of cereal arabinoxylans (*21*, *22*), the xylose-to-DHD molar ratio (X/DHD) represents an average of the number of xylose units between two consecutive diferulate bridges linking arabinoxylan chains. This ratio is equivalent to an index of arabinoxylan cross-linking. It appears then that arabinoxylans of the aleurone layer and outer pericarp were, respectively, 2 and 6 times more cross-linked than those of the intermediate strip. It can be noted that Baroudeur tissues were more cross-linked than Scipion tissues.

Mechanical Properties of Wheat Bran. As previously observed on common and durum wheat (*6*), wheat bran issued from Baroudeur and Scipion cultivars exhibited an elastoplastic rheological behavior pointed out by a biphasic strain−linear force curve (**Figure 2**). The first elastic stage corresponded to a reversible material deformation. Beyond the yield point, which occurred at ∼4% deformation, the plastic flow induced a damage of material until its disruption.

Table 3 reports the results of mechanical measurements performed on Baroudeur and Scipion bran strips according to both test orientations. Considering elastic and plastic stages, bran coats showed an anisotropic mechanical behavior, which was similar for both wheat samples.

The elastic stage was characterized by a constant strain threshold whatever the tissue orientation. As a consequence, the anisotropic behavior of bran coats resulted in linear modulus and elastic force ∼1.5 times higher in the longitudinal orientation for both varieties.

Table 3. Mechanical Characteristics of Whole Bran Strips: Resistance to Elastic and Maximum Tensile Strain (f_{ela}; f_{max}), Elastic Strain and Maximum Tensile Strain or Extensibility (ϵ_{ela}; ϵ_{max}), Linear Modulus (E), and Energy Rupture (W_{max}) According to Longitudinal and Radial Traction Test Orientations

wheat cv.		f_{ela} (N·mm^{-1})	f_{max} (N·mm^{-1})	ϵ_{ela} (%)	ϵ_{max} (%)	E (N·mm^{-1})	$W_{max} \times 10^{-2}$ (N·mm^{-1})
Baroudeur	longitudinal	1.9 ± 0.3	2.7 ± 0.3	4.8 ± 0.7	10.3 ± 1.1	39.8 ± 1.5	16.6 ± 3.3
	radial	1.1 ± 0.1	2.4 ± 0.2	4.0 ± 0.3	20.5 ± 4.7	27.9 ± 3.3	29.6 ± 6.3
Scipion	longitudinal	1.5 ± 0.2	2.4 ± 0.2	4.2 ± 0.2	12.0 ± 2.4	35.8 ± 2.5	18.2 ± 5.4
	radial	1.0 ± 0.1	2.1 ± 0.3	3.5 ± 0.5	21.7 ± 4.5	28.5 ± 3.5	27.2 ± 4.6

Table 4. Mechanical Characteristics of Pericarp, Intermediate, and Aleurone Layer Strips: Resistance to Elastic and Maximum Tensile Strain (f_{ela}; f_{max}), Elastic Strain and Maximum Tensile Strain or Extensibility (ϵ_{ela}; ϵ_{max}), Linear Modulus (E), and Energy Rupture (W_{max}) According to Longitudinal and Radial Traction Test Orientations

wheat cv.		tissue	f_{ela} (N·mm^{-1})	f_{max} (N·mm^{-1})	ϵ_{ela} (%)	ϵ_{max} (%)	E (N·mm^{-1})	$W_{max} \times 10^{-2}$ (N·mm^{-1})
Baroudeur	longitudinal	aleurone layer	0.4 ± 0.1	0.7 ± 0.1	2.7 ± 0.0	12.8 ± 2.0	9.3 ± 1.9	6.2 ± 0.9
		intermediate	0.5 ± 0.0	0.9 ± 0.1	3.0 ± 0.6	22.2 ± 1.9	11.7 ± 2.8	13.8 ± 4.6
		pericarp		2.0 ± 0.4		8.7 ± 1.2	20.6 ± 2.8	5.1 ± 2.0
	radial	aleurone layer	0.4 ± 0.1	0.8 ± 0.1	2.5 ± 0.0	14.5 ± 3.0	11.2 ± 1.5	6.0 ± 1.9
		intermediate	0.6 ± 0.1	1.2 ± 0.1	3.2 ± 0.0	21.3 ± 4.0	10.1 ± 2.5	14.8 ± 0.1
		pericarp		0.9 ± 0.1		5.6 ± 0.5	13.5 ± 2.5	2.9 ± 0.5
Scipion	longitudinal	aleurone layer	0.5 ± 0.1	1.0 ± 0.1	2.6 ± 0.1	12.9 ± 2.0	12.0 ± 1.7	8.6 ± 2.2
		intermediate	0.6 ± 0.1	1.2 ± 0.2	3.4 ± 0.0	17.7 ± 1.0	11.7 ± 2.2	11.4 ± 0.8
		pericarp		1.6 ± 0.2		5.6 ± 0.6	28.9 ± 6.7	3.6 ± 0.2
	radial	aleurone layer	0.5 ± 0.1	1.0 ±0.1	2.5 ± 0.1	13.4 ± 3.3	12.7 ± 2.6	9.0 ± 0.3
		intermediate	0.4 ± 0.0	0.8 ± 0.2	3.1 ± 0.0	20.0 ± 0.5	15.9 ± 2.5	16.5 ± 5.0
		pericarp		0.6 ± 0.1		5.6 ± 0.9	10.6 ± 2.0	1.7 ± 0.5

Regarding the plastic stage, bran coats required similar linear forces to fracture in both orientations. In contrast, the rupture strain was the most anisotropic measure, with a value 1.8−2.0 higher according to the radial bran orientation. Finally, in agreement with previous observations made on durum wheat (*6*), the bran coats needed 1.5−1.8 times more energy to rupture by radial orientation proportional to the increase of their extensibility.

Mechanical Study of Individual Bran Layers. Table 4 reports the results of traction tests performed on the aleurone layer, intermediate, and pericarp strips according to the distinct orientations.

The aleurone layer exhibited an elastoplastic rheological behavior. In agreement with its cell structure, it showed an isotropic character. The aleurone layer is one cell thick. Considering the polygonal shape of its constitutive cells, the cell walls form a regular network; therefore, the mechanical properties were unrelated to the traction direction. Accordingly, elastic and plastic characteristics were similar in both orientations. On this point, Scipion aleurone strips exhibited strength 1.4 times higher than that of Baroudeur aleurone strips and needed slightly more energy to rupture ($W = 6 \times 10^{-2}$ and 9×10^{-2} N·mm^{-1} for Baroudeur and Scipion aleurone strips according to radial orientation, respectively).

The intermediate strip showed also an elastoplastic behavior with elastic properties similar to those of the aleurone layer. In the plastic stage, the intermediate strip differed, however, from the aleurone layer by substantially higher extensibility and subsequent higher rupture energy, which was roughly similar for both wheat cultivars. The intermediate strip can be considered as a composite and heterogeneous material comprising distinct adherent tissues. Because of the specific cell structure of each tissue, the strip could form a complex network, which resulted in an isotropic mechanical behavior.

In contrast to the other bran strips, the isolated outer pericarp exhibited a linear elastic rheological behavior characterized by a fragile mode of fracture occurring at ∼6−9% deformation. Its strong anisotropic character was emphasized by strength and linear modulus significantly higher according to the longitudinal test orientation. In agreement with previous data obtained from several distinct plant materials (*23*, *24*), the tissue strength depends on the angle between the force direction and the axis of the pericarp elongated cells. Furthermore, it can be noted that the pericarp strip issued from Baroudeur showed a slightly higher extensibility than the Scipion pericarp, leading to higher rupture energy.

Contribution of Individual Tissue to the Overall Bran Properties. The overall bran mechanical properties depend on both the individual tissue characteristics and their adhesion forces. Bearing in mind the complex structural organization of wheat bran, it was difficult to establish obviously the respective contribution of each layer to bran mechanical properties. However, because the outer pericarp was the single anisotropic tissue, it was plainly responsible for the bran anisotropy. In the elastic strain, the pericarp appeared to be involved in the control of linear modulus of bran according to the longitudinal orientation only (**Figure 2**). With particular respect to Baroudeur results, bran rigidity was indeed roughly equal to the sum of E values of the three layers in the longitudinal orientation, whereas no influence of pericarp E values was noted in the radial orientation:

$$E_R^B = E_R^A + E_R^I$$

$$E_L^B = E_L^A + E_L^I + E_L^P$$

Furthermore, because ϵ_{ela} remained roughly constant for all of the constitutive bran layers, a similar relationship can be observed with the elastic linear force in the limits of bran elastic stage.

$$f_{ela_R}^B = f_{ela_R}^A + f_{ela_R}^I$$

$$f_{ela_L}^B = (f_{ela_L}^A + f_{ela_L}^I + f_{ela_L}^P) \text{ for } \epsilon \in [0; \epsilon_{ela}^B]$$

$$\text{with } f_{ela_L}^B < f_{max_L}^P$$

Regarding the longitudinal orientation, these relationships were evidenced on Baroudeur variety, with 5% difference between bran values and the sum of measures carried out on each constitutive layer (1.8 $N \cdot mm^{-1}$). On the other hand, the relationship was not confirmed with Scipion, with a 32% variation between bran strip and individual tissues values (2.2 $N \cdot mm^{-1}$).

It can be noted that the contribution of pericarp was negligible in the bran elastic strain in the radial orientation but accounted for half of bran work according to the longitudinal orientation.

With regard to the plastic stage of both wheat varieties, linear force of rupture (f_{max}) measured in both bran orientations was equal to the sum of aleurone layer and intermediate strip strengths. This observation is in agreement with the distinct mode of fracture of the different tissues. Considering the fragile behavior of pericarp, the plastic stage of bran was exclusively due to the aleurone layer and intermediate strip works. Consequently, the bran fracture involved these two layers only because it occurred at a strain rate significantly higher than pericarp extensibility.

On the other hand, the bran extensibility appeared to be governed by distinct tissue according to the test orientation. In the radial orientation, $\epsilon_{max_R}{}^B$ was linked to the extensibility of the intermediate strip, which suggested that bran deformation to rupture was governed by the most extensible tissue among layers exhibiting similar strengths.

In the longitudinal orientation, $\epsilon_{max_L}{}^B$ was not related to the pericarp, which acted for the toughest tissue. The longitudinal bran extensibility appeared to be controlled by both tissues because the $\epsilon_{max_L}{}^B$ value was between pericarp and intermediate strip values (**Figure 2**).

Finally, the rupture energy of bran seemed to correspond to the combined work of the individual layers in the limits of the bran extensibility. Therefore, the energy of rupture of radial bran was nearly equal to the sum of energy of the three tissue strips.

$$W_R{}^B = W_R{}^A + W_R{}^I + W_R{}^P$$

As a consequence, the intermediate strip contributed to the largest extent to the energy because it produced 50 and 60% of the bran work for Baroudeur and Scipion, respectively. In contrast, the pericarp involvement appeared to be negligible, with only 10% of bran work.

Conversely, the rupture energy of the longitudinal bran strip was equal to the sum of the energy of the three individual layers until bran rupture. As a consequence, the energy produced by the aleurone layer and the intermediate strip from the rupture strain of bran to their own extensibility threshold (about 30 and 40%, respectively) was lost when the three tissues were coupled in a longitudinal bran strip. The rupture energy of longitudinal bran can be then be expressed by the following relationship:

$$W_L{}^B = (W_L{}^A + W_L{}^I + W_L{}^P) \text{ for } \epsilon \in [0; \epsilon_{max}{}^B]$$

Structural Modeling of Wheat Bran Strips. Through the relationships established above between overall bran mechanical properties and characteristics of the individual tissues, a structural model of wheat bran can be proposed.

The cooperative work of the three layers within longitudinal bran strips led us to compare the bran structure to a composite material composed of adherent tissues which undergo jointly the mechanical stress. In contrast, considering the results obtained according to the radial orientation, the noninvolvement of pericarp in the bran elastic strain prevents comparison with materials composed of three parallel layers simultaneously in tension but suggested a particular organization of the tissues as described in **Figure 3**. In this representation, the bran strip was compared to a trilayered material where the aleurone layer and the intermediate strips were strongly associated. Taking into account the wheat grain morphology and the curvature of peripheral layers according to a grain cross section, the outer pericarp, the most external tissue, showed a length slightly higher than those of the intermediate and aleurone layer strips. This difference in length (δl) would induce a detachment over some length of outer pericarp (l) from the inner pericarp during bran strip drying. Using a parametric modeling of wheat grain (*25*), δl values were evaluated at 3.3 and 3.5% of strip length. As a consequence, on the detached zone (l), pericarp exerted no work during the elastic bran deformation. At the elastic bran yield point ($\epsilon_{ela_R}{}^B$), the length of the bilayered strip (intermediate and aleurone layer) ($l + [l(\epsilon_{ela_R}{}^A + \epsilon_{ela_R}{}^I)]$) was equal to that of the detached pericarp ($l + \delta l$). From this relationship, the minimal length of pericarp detachment can be deduced:

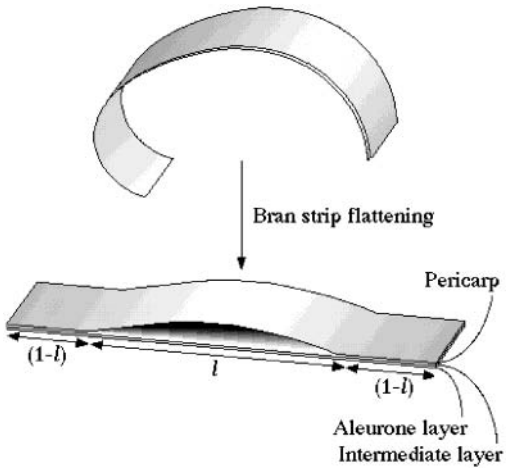

Figure 3. Representation of pericarp strip detachment from the intermediate layer within radial orientation.

$$l = \frac{E_R{}^A + E_R{}^I}{E_R{}^B} \times \frac{\delta l}{\epsilon_{ela_R}{}^B}$$

Using experimental $\epsilon_{ela_R}{}^B$, $E_R{}^A$, $E_R{}^I$, and $E_R{}^B$ values, l could be estimated at 67 and 95% for Baroudeur and Scipion, respectively, suggesting therefore that pericarp was mostly detached from the intermediate strip but remained attached to it at the bran strip extremities only.

From the structural model of bran strip, the mechanical properties of radial bran strips can be defined in the elastic stage by the following expression:

$$\epsilon_{ela_R}{}^B = \left[\frac{l}{(E^A + E^I)} + \frac{(1-l)}{(E^A + E^I + E^P)}\right] \times f_{ela_R}{}^B$$

Considering the high l values, such a mechanical model supported then the experimental observations showing that the elastic force of bran was roughly equal to the sum of the linear forces of the aleurone layer and intermediate strip.

$$f_{ela_R}{}^B = l(f_{ela_R}{}^A + f_{el_R}{}^I) + [(1-l)(f_{ela_R}{}^A + f_{ela_R}{}^I + f_{ela_R}{}^P)] \sim f_{ela_R}{}^A + f_{ela_R}{}^I$$

Although the extent of pericarp detachment would be underestimated for Baroudeur seeing the high coefficient variation

of experimental values, this relationship validated the structural model described above and confirmed the low adherence between outer pericarp and the other bran layers within radial bran strips.

DISCUSSION

Bran coats of wheat grain constitute a complex and heterogeneous material. The intermediate and aleurone layer strips revealed similar elastoplastic behaviors, except plasticity, although their histological structures and cell wall compositions were very different. The plastic strain of the intermediate strip was ~7% higher than for aleurone layer. Therefore, pericarp exhibiting a linear elastic rheological behavior, the plasticity could be considered to be the unique mechanical characteristic that allowed the three strips to be distinguished. Considering the heterogeneous cell wall composition of bran tissues, this mechanical parameter could be connected to a distinct component among the different layers. In the aleurone layer, it proved to be affected by the degree of arabinoxylan cross-linking by ferulic acid dehydrodimers (*26*). Pericarp that showed the most important level of DHD was the least extensible tissue. On the other hand, the intermediate strip contained arabinoxylans twice less cross-linked than aleurone layer (X/DHD = 463 and 239, respectively, for intermediate and aleurone layers of cv. Baroudeur) but was also twice more plastic. Nevertheless, other structural features of the cell walls and cell interfaces may play a role in the mechanical properties of the tissues. For example, lignin or lignin-like structures could be evidenced in the three different strips from their specific thioacidolysis compounds obtained with yields that were similar to DHD. The intermediate strips, which were the more plastic, were also found to be the more lignified. Depending on their content and structure, lignins may act as plasticizers (*27*). In addition, lignin distribution has been reported to affect the position of fracture within various cell wall layers (*28*). Together with ferulate derivatives, the lignification pattern of bran layers could therefore affect the fractionation and mechanical behavior of wheat grain. Finally, besides the influence of its cell structure on its anisotropic character, the pericarp was characterized by a high cellulose content. Therefore, it could be suggested that pericarp cellulose microfibrils take part in the high rigidity of this tissue (*29*).

On the other hand, considering technological applications, pericarp detachment from intermediate layer is interesting. From experimental observations, it resulted probably from hydric history and tissue flattening due to bran strip preparation. Thus, the application of adapted bran hydric conditions to bran could allow an easy separation of the outer pericarp. Conversely, aleurone layer and intermediate strips that appeared to be tightly adheered and extensible would require appropriate conditions that can decrease their adhesive forces and modify specifically their respective plasticity to facilitate their separation. This study defined the fields of investigations to further progress in this way. In particular, it appears now necessary to identify the components responsible for adhesiveness and tissue plasticity. This structural model of wheat bran could help the definition of new rational bran fractionation processes with a view to recovering fractions of interesting nutritional value.

ABBREVIATIONS USED

E, linear modulus; f_{ela}, linear force to elastic deformation; f_{max}, force to rupture; ϵ_{ela}, elastic deformation; ϵ_{max}, rupture strain; W, rupture energy; ^{B}, bran strip; ^{A}, aleurone layer strip; ^{I}, intermediate strip; ^{P}, pericarp strip; $_{R}$, radial strip orientation; $_{L}$, longitudinal strip orientation; l, detachment of radial pericarp strip from inner pericarp; δl, length difference between pericarp and intermediate layer strips due to the curvature of peripheral layers according to a grain cross section.

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