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Evaluation of Tissue Dissociation of Durum Wheat Grain (*Triticum durum* Desf.) Generated by the Milling Process

S. Peyron*, A. Surget, F. Mabille, J. C. Autran, X. Rouau and J. Abecassis

Unité de Technologie des Céréales et des Agropolymères. ENSAM – INRA Montpellier 2, place P. Viala 34 060 Montpellier cedex 1

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ABSTRACT

The three major botanical components (starchy endosperm, aleurone layer and pericarp) of eight durum wheat samples exhibited significantly different compositions and concentrations in phenolic acids. The starchy endosperm, the aleurone layer and the pericarp were respectively characterised by a low content in ferulic acid (FA), a high content in *trans*-sinapic acid (*t*-SA), and a high content in ferulic acid dehydrodimers (DHD). These three chemical markers can be exploited to differentiate the three grain botanical parts within milling fractions and to evaluate the milling efficiency, particularly the separation between bran and endosperm. The histological dissociation of the wheat grain generated by the milling process can be investigated further into details using the three phenolic acids markers. A separability index (S*i*) was proposed in order to quantify the ease of dissociation of endosperm from bran. Differences in S*i* values between wheat varieties grown under various agricultural conditions demonstrated the relevant variability of this character. The structural and molecular factors implied in the control of tissue dissociation are discussed in details.

Keywords: durum wheat, phenolics, botanical components, milling, tissue dissociation.

INTRODUCTION

The milling process of durum wheat (*Triticum durum* Desf.) aims at isolating the starchy endosperm without contamination by peripheral layers of the grain (i.e. aleurone layer and pericarp). According to Chaurand *et al.*[1], the semolina yield resulting from the milling depends on three groups of factors: (i) external factors related to harvesting conditions as represented by the weight of impurities; (ii) internal factors like the endosperm/bran ratio (semolina + flour/total feeds) and the mechanical resistance or friability of endosperm (semolina/flour ratio); and (iii) the ease of separating the endosperm from the hulls, which depends on wheat characteristics. The last factor, i.e. the dissociation between botanical parts of the grain, appears as the most critical for the durum wheat milling industry. A wrong separation between starchy endosperm and bran layers can induces (i) the lost of starchy endosperm in the bran fraction which decreases the semolina yield and (ii) the presence of bran particles in semolina fractions which affects the semolina purity.

The assessment of the separation of botanical parts during milling involves a quantitative analysis of the relevant constituents within the different milling fractions. The purity of semolina fractions is usually assessed by their ash content[3]. However, the occurrence of an ash gradient within the kernel prevents the exact determination of the semolina contamination[2]. The counting specks is an another standard practical method used in mill industry. If this procedure is usable for assessing pericarp in

* *Corresponding author. Tel: (33) 4 99 61 28 90; Fax: (33) 4 67 52 20 94; E-mail: peyron@ensam.inra.fr*

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semolina, the relevant variability in tissue pigmentation prevent its use for a comparative study of wheats[4]. Alternative methods by image analysis have been developed in the past. The most used takes advantage from the fluorescence characteristics of the wheat seed coats[5,6]. This method appears useful for the measurement of the degree of common wheat flour refinement[7,8]. However, this procedure was unsuitable for semolina and bran fractions of durum wheat[9] because of the particle-size heterogeneity and the crystalline aspect of starchy endosperm. The quantification of botanical parts in the bran fraction implies the use of specific indicators for each layer. It is well established that vitamin and minerals are concentrated in the aleurone layer[10,11] whereas lignin and cellulose occur specifically in pericarp[12]. Therefore, their quantification coupled to the measure of starch as marker of contaminating endosperm would allow to determine the proportion of each layer in the bran. However, this approach requires the implementation of several analyses and relatively large amounts of biological material.

The external parts of wheat grain exhibit a large range of phenolic acids[13,14]. Until now, these compounds have been especially studied for their antioxidant properties and, except for ferulic acid, their use as histological markers have never been investigated[15]. Detailed phenolic acid composition could however reveal differences within grain layers and constitutes an alternative way to study, by a single analysis, the dissociation of the different botanical parts of grain during milling.

The aim of this work was to study the relevance of phenolic acids as markers of the different botanical part of the grain. The phenolic acids were identified and quantified within hand-isolated pericarp, aleurone layer, and endosperm, and their specificity was employed to determine the distribution of each tissue in the fractions obtained from conventional durum milling process. By this way, a 'separability index' was defined in order to estimate the wheat ability to endosperm-bran dissociation and evaluate the milling efficiency on various durum wheat samples.

MATERIAL AND METHODS

Wheat samples

The study was performed on eight durum wheat samples. Four varieties ('Ardente', 'Primadur', 'Nefer', 'Lloyd') with distinct morphological grain characteristics were grown in 1999 at Station d'Amélioration des Plantes (INRA, Maugio, France). The influence of crop site was investigated on Lloyd variety which was grown in parallel at three locations: Maugio (S1: southeast of France); Crambade (S2: southwest of France) and Ouzouer (S3: Northern France). The effect of nitrogen addition was studied on Nefer variety at Crambade crop site: Nefer N− received 50 kg/ha nitrogen over the cropping period whereas Nefer N+ was fertilised with 100 kg/ha of nitrogen.

Milling fractions

Milling fractions were obtained from a conventional milling process performed in a semi-industrial semolina mill (150 kg/h)[1]. 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% wet basis (w.b.) for 15 h, and then to 17% for 3 h prior to the 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).

Isolation of pericarp and aleurone layer

Wheat grains were immersed in distilled water for 12 h. Grain ends were cut and eliminated. The remaining part was soaked again for 2 h in water. An incision was made in the crease and the endosperm was eliminated using a scalpel. Aleurone layer and pericarp were isolated by insertion of a razor blade between the two layers. After rinsing, tissue fragments were dried at 25 °C in order to reach 15% w.b.

Isolation of starchy endosperm

The operation was performed on dried wheat grain (≈12% w.b.). The extremity carrying the embryo was cut and then eliminated. The external surface of the grain was abraded using a miniaturised sander in order to eliminate the totality of bran layers. The grain was then opened at the level of the crease and the bran layers located in the crease fold were also eliminated by abrasion.

Analysis of esterified phenolic acids

Wheat bran fractions (coarse bran, sizing fine bran, purifying fine bran and short), starchy endosperm,

aleurone layer and pericarp isolated by hand were ground (Freeze-mill 6570, Avantec, France) and freeze-dried before phenolic acids extraction. Ground samples (80 mg) were de-esterified for 2 h in the dark with 2 M NaOH (10 mL) at 35 °C. The internal standard, 2·3·5 trimethoxy-*trans*-cinnamic acid (TMCA), was added (50 µg) and the solution was adjusted to pH 2 with 4 N HCl. Phenolic acids were extracted twice with diethyl ether (5 mL). Ether phase was evaporated at 30 °C under argon. The dried extract was dissolved in methanol/water (50/50:v/v), filtered (0·45 µm) and injected (20 µL) on RP-HPLC using an Alltima (Alltech, Deerfield, USA) C_{18} column 5 µm (250 × 4·6 mm). UV detection was carried out using a 996 Water photodiode array detector (Waters, Milford, MA, USA) at 320 nm. Linear elution gradient was performed by acetonitrile and sodium acetate buffer 0·05 M (pH 4·0) at 1 mL/min at 35 °C, from 15/85 to 35/65 in 30 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. Response factors of ferulic acid dehydrodimers determined by Saulnier *et al.*[16] were used. Two quantifications of phenolics content were performed on each sample (Isolated tissue and milling fraction).

Moisture and ash contents

Moisture content was determined according the ISO 711-1978 method and ash content was determined according to the ISO 2171-1980 method.

RESULTS

Phenolic acid composition in the different botanical parts of wheat grains

A large array of phenolic acids occurred in the three purified botanical parts of the different wheat grain samples (Table I). As previously reported[17], their concentrations and relative proportions were variable according to the histological layers resulting in

Table I Mean values of phenolic acid contents[a] (µg/100 mg D.M.) in various botanical parts of grain (starchy endosperm, aleurone layer and pericarp) for the eight durum wheat varieties

	t-FA	*c*-FA	*t*-SA	5-5′	8-5′ diFA	8-5′ benzo	8-O-4	Total phenolics
Starchy endosperm								
Ardente	0·62	0·26	0·25	0·03	–	0·08	0·08	1·32
Primadur	0·76	0·30	0·21	0·03	–	0·07	0·07	1·44
Nefer	0·58	0·18	0·26	0·03	–	0·06	0·06	1·17
Nefer N+	0·65	0·22	0·26	0·03	–	0·06	0·07	1·29
Nefer N−	0·65	0·23	0·26	0·02	–	0·06	0·06	1·28
Lloyd S1	0·46	0·20	0·17	0·02	–	0·04	0·04	0·93
Lloyd S2	0·75	0·25	0·22	0·02	–	0·05	0·06	1·35
Lloyd S3	0·83	0·31	0·24	0·03	–	0·06	0·07	1·54
Aleurone layer								
Ardente	20·98	9·82	13·28	1·40	1·69	3·56	4·38	55·11
Primadur	28·75	15·71	14·37	1·69	1·79	3·27	4·67	70·25
Nefer	25·67	11·19	18·66	1·32	1·44	2·63	3·46	64·37
Nefer N+	23·19	10·40	13·45	1·22	1·43	2·64	3·51	55·84
Nefer N−	24·73	10·55	14·25	1·11	1·40	2·50	3·17	57·71
Lloyd S1	29·36	12·81	16·07	1·69	1·73	3·04	4·25	68·95
Lloyd S2	24·41	12·38	13·09	1·95	1·89	3·79	5·48	62·99
Lloyd S3	23·67	10·70	12·80	2·25	2·63	5·14	6·53	63·72
Peicarp								
Ardente	30·97	9·56	3·19	5·55	4·81	10·22	12·86	77·16
Primadur	44·28	13·69	4·56	6·62	6·21	11·48	14·51	101·35
Nefer	34·72	9·63	2·59	5·00	5·14	9·40	12·02	78·50
Nefer N+	30·21	7·17	1·92	5·38	4·92	5·13	11·69	66·42
Nefer N−	33·21	7·66	2·03	5·11	5·35	10·13	11·62	75·11
Lloyd S1	45·89	14·02	6·57	5·50	4·80	9·34	12·74	98·86
Lloyd S2	39·21	12·66	3·07	6·01	4·70	8·78	12·75	87·18
Lloyd S3	45·41	9·80	2·36	6·47	4·14	7·14	13·41	88·73

– Less than 10^{-2} µg/100 mg.
[a] Coefficient of variation $\leq 5{\cdot}0\%$.

an increasing concentration gradient from the centre to the periphery of the grain. Phenolic acids occurring mostly in the cell walls, their concentration measured on whole tissue depends both on the cell wall phenolics content and on the mass proportion of the cell wall in each layer.

In the starchy endosperm, the low phenolic acid content can be then explained both by the thinness of the cell walls and the low degree of feruloylation of arabinoxylans. *Trans*-ferulic acid (*t*-FA) was the major phenolic compound and accounted for 43% to 61% of the total content in phenolic acids, depending on samples. Lower quantities of *Trans*-sinapic acid (*t*-SA) and *cis*-ferulic acid (*c*-FA) were extracted and three FA dehydrodimers (5-5′, 8-5′ benzofuran and 8-O-4 DHD) were detected in low amounts (6% of the total extracted phenolic acids, according to the wheat sample).

The same phenolic compounds were identified in the aleurone layer but another form of DHD (8-5′ diFA) and other monomers (caffeic acid, vanillic acid and *p*-coumaric acid (*p*-CA)) were also detected in low amounts. The concentration in phenolic acids was more than 40 times higher in the aleurone layer than in the starchy endosperm, but despite this large difference, the proportion of each compound was roughly similar in the two botanical parts. Pericarp exhibited the highest phenolic acid content of the three grain tissues. FA occurred in similar amount than in the aleurone layer but the DHD content was 3 to 4 times higher. In addition, the *t*-SA content in pericarp was about 4 times lower than in the aleurone layer.

Considering the result set obtained from all samples, the content in phenolic acids in the different botanical parts appeared highly dependent on the wheat variety and the crop site. The values were also affected to a low extent by nitrogen addition. However, the genetic and agronomic variabilities were insignificant compared to the differences measured between the three botanical parts suggesting that the phenolic acid composition can be used as a marker of the botanical part of grain, enabling their detection in the milling fractions.

Determination of the proportions of the three botanical parts in durum wheat milling fractions

All major phenolic acids (*t*-FA, *c*-FA, *t*-SA, DHD) detected in the isolated tissues occurred in each milling fraction (Table II). To determine the percentage of the three botanical parts (starchy endosperm (%*e*), aleurone layer (%*a*) and pericarp (%*p*)) in the milling fractions, phenolic acid contents measured in each layer and fraction can be combined in the following equation system:

$$\begin{aligned} &\%e + \%a + \%p = 100\\ &(\%e)\mathrm{C}_1e + (\%a)\mathrm{C}_1a + (\%p)\mathrm{C}_1p = \mathrm{C}_1F\\ &(\%e)\mathrm{C}_2e + (\%a)\mathrm{C}_2a + (\%p)\mathrm{C}_2p = \mathrm{C}_2F \end{aligned}$$

With C_1F and C_2F = concentrations of two distinct phenolic acids measured in the milling fraction.

C_1e, $\mathrm{C}_2e/\mathrm{C}_1a$, $\mathrm{C}_2a/\mathrm{C}_1p$, C_2p = concentrations of the same phenolic acids measured in starchy endosperm (*e*)/aleurone layer (*a*)/pericarp (*p*).

The system resolving gives the expressions:

$$\%a = \frac{(\mathrm{C}_2F - \mathrm{C}_2e)(\mathrm{C}_1p - \mathrm{C}_1e) - (\mathrm{C}_2p - \mathrm{C}_2e)(\mathrm{C}_1F - \mathrm{C}_1e)}{(\mathrm{C}_2a - \mathrm{C}_2e)(\mathrm{C}_1p - \mathrm{C}_1e) - (\mathrm{C}_1a - \mathrm{C}_1e)(\mathrm{C}_2p - \mathrm{C}_2e)} \times 100$$

$$\%p = \frac{\mathrm{C}_1F - \mathrm{C}_1e - (\%a)(\mathrm{C}_1a - \mathrm{C}_1e)}{\mathrm{C}_1p - \mathrm{C}_1e} \times 100$$

$$\%e = [1 - (\%a + \%p)] \times 100.$$

The values of phenolic acid concentration used in the equation system (C_1 and C_2) are selected according to their specific distribution within the three botanical parts. As previously showed, *t*-SA was more particularly concentrated in the aleurone layer while DHD were mainly located in the pericarp. In addition, FA was 40 times more concentrated in the aleurone layer and pericarp than in the starchy endosperm. These compounds constituted adequate markers of the individual layers and were therefore used (two by two) in the equation system. The use of FA, *t*-SA and the four form of DHD led to a combination of 15 results for each milling fraction. The proportions determined by this method corresponded then to an average of these 15 results.

Table III shows the botanical composition of the various milling fractions obtained from the eight wheat samples. The extraction of aleurone layer in flour and semolina, and the occurrence of starchy endosperm in bran observed on each sample emphasised the limits of the milling process in demonstrating the imperfect separation between these two botanical parts. However, the distribution of the three botanical parts was variable according to the wheat sample which demonstrated a distinct milling efficiency. For example, difference of 40% was noted between Nefer N− and Lloyd S3 for the semolina contamination by bran layers. Concurrently, the loss of starchy endosperm in bran fractions was also

Table II Mean values of phenolic acid contents[a] (μg/100 mg D.M.) in milling fractions (total semolina, total flour and feeds) for the eight durum wheat cultivars

	t-FA	*c*-FA	*t*-SA	5-5′	8-5′ diFA	8-5′ benzo	8-O-4	Total phenolics
Total semolina								
Ardente	1·19	0·12	0·40	0·05	0·05	0·14	0·12	2·07
Primadur	1·44	0·13	0·41	0·05	0·05	0·12	0·12	2·32
Nefer	1·05	0·13	0·47	0·06	0·05	0·10	0·12	1·98
Nefer+	1·22	0·14	0·46	0·05	0·04	0·10	0·10	2·11
Nefer N−	1·26	0·17	0·45	0·05	0·04	0·10	0·11	2·18
Lloyd S1	1·02	0·12	0·36	0·04	0·02	0·08	0·08	1·72
Lloyd S2	1·34	0·14	0·36	0·04	0·03	0·08	0·08	2·07
Lloyd S3	1·42	0·15	0·36	0·05	0·03	0·10	0·10	2·21
Total flour								
Ardente	2·59	0·23	1·74	0·16	0·12	0·35	0·36	5·55
Primadur	2·96	0·00	1·59	0·16	0·12	0·33	0·36	5·52
Nefer	2·40	0·24	1·70	0·13	0·10	0·27	0·30	5·14
Nefer N+	2·76	0·22	1·81	0·14	0·12	0·27	0·36	5·68
Nefer N−	2·70	0·24	1·70	0·13	0·10	0·25	0·33	5·45
Lloyd S1	2·11	0·18	1·27	0·10	0·06	0·18	0·22	4·12
Lloyd S2	2·26	0·28	1·30	0·11	0·08	0·20	0·26	4·49
Lloyd S3	2·90	0·31	1·51	0·13	0·09	0·23	0·32	5·49
Shorts								
Ardente	14·52	1·54	4·23	1·02	0·87	2·06	2·29	26·53
Primadur	14·73	1·09	4·20	1·10	0·84	1·84	2·32	26·12
Nefer	12·16	1·15	3·53	0·78	0·62	1·40	1·82	21·46
Nefer N+	11·34	0·83	2·95	0·75	0·60	1·25	1·67	19·39
Nefer N−	10·67	0·77	2·75	0·68	0·61	1·17	1·58	18·23
Lloyd S1	12·59	1·21	4·61	0·72	0·58	1·24	1·62	22·57
Lloyd S2	12·51	1·13	3·59	0·77	0·62	1·22	1·62	21·46
Lloyd S3	14·55	1·09	3·66	0·92	0·53	1·31	1·98	24·04
Sizing fine bran								
Ardente	23·19	3·13	4·90	2·28	1·82	4·30	4·79	44·41
Primadur	30·71	3·26	6·17	2·93	2·29	4·86	6·02	56·24
Nefer	19·77	2·26	4·67	1·47	1·19	2·59	3·41	35·36
Nefer N+	24·04	2·54	4·73	2·62	1·86	4·02	5·08	44·89
Nefer N−	26·97	2·99	5·56	2·62	1·40	2·78	3·76	46·08
Lloyd S1	30·74	3·06	7·95	2·76	1·85	4·01	5·54	55·91
Lloyd S2	28·44	3·23	6·28	2·87	2·09	4·47	5·55	52·93
Lloyd S3	26·69	3·04	4·75	2·53	1·56	3·32	4·94	46·83
Purifying fine bran								
Ardente	24·76	2·83	7·19	1·98	1·63	3·98	4·32	46·69
Primadur	32·28	2·87	6·83	2·52	1·85	4·19	5·34	55·88
Nefer	27·45	3·02	5·87	2·07	1·58	3·72	4·55	48·26
Nefer N+	25·21	2·20	5·11	1·94	1·47	3·30	4·26	43·49
Nefer N−	28·22	2·55	4·91	2·36	1·81	4·25	5·10	49·20
Lloyd S1	31·52	3·43	9·68	2·10	1·61	3·54	4·59	56·47
Lloyd S2	31·74	3·46	7·42	2·18	1·22	2·53	3·38	51·93
Lloyd S3	32·19	2·88	5·60	2·36	1·39	3·11	4·86	52·39
Coarse bran								
Ardente	27·50	3·48	7·10	2·83	2·19	5·14	5·88	54·12
Primadur	33·66	3·24	7·47	3·14	2·32	4·85	6·32	61·00
Nefer	22·96	2·96	5·01	2·26	1·65	3·87	4·80	43·51
Nefer N+	27·94	2·88	5·52	3·04	2·13	4·65	6·08	52·24
Nefer N−	28·91	2·99	5·59	3·09	2·28	5·12	6·07	54·05
Lloyd S1	35·99	4·35	10·48	2·85	1·96	4·23	5·94	65·80
Lloyd S2	35·17	4·04	7·65	3·07	2·08	4·30	5·94	62·25
Lloyd S3	36·54	3·40	6·34	3·18	1·67	3·48	6·17	60·78

[a] Coefficient of variation $\leq 5{\cdot}0\%$.

Table III Histological compositions of the milling fractions obtained from the eight wheat samples

	Total semolina	Total flour	Shorts	PFB	SFB	CB
Ardente						
Endosperm (%*e*)	98·7 ± 0·2	89·0 ± 0·6	59·9 ± 1·6	38·2 ± 3·0	28·7 ± 3·1	19·0 ± 3·4
Aleurone layer (%*a*)	1·1 ± 0·1	11·0 ± 0·2	29·3 ± 0·5	29·6 ± 0·9	49·6 ± 0·9	45·8 ± 1·0
Pericarp (%*p*)	0·2 ± 0·3	0·0 ± 0·8	10·8 ± 0·9	32·2 ± 2·1	21·6 ± 2·2	35·2 ± 2·4
Primadur						
Endosperm (%*e*)	98·6 ± 0·1	90·5 ± 0·7	64·5 ± 0·9	33·7 ± 2·1	34·2 ± 2·6	25·5 ± 2·7
Aleurone layer (%*a*)	1·4 ± 0·1	9·5 ± 0·3	26·9 ± 0·4	33·3 ± 0·9	40·2 ± 1·2	42·9 ± 1·2
Pericarp (%*p*)	0·0 ± 0·2	0·0 ± 1·1	8·7 ± 1·3	33·0 ± 1·2	25·6 ± 1·4	31·5 ± 1·5
Nefer						
Endosperm (%*e*)	98·6 ± 0·2	92·3 ± 0·5	71·4 ± 1·5	54·8 ± 1·6	39·9 ± 3·0	42·4 ± 3·1
Aleurone layer (%*a*)	1·1 ± 0·0	7·7 ± 0·1	17·5 ± 0·4	22·1 ± 0·2	27·5 ± 0·4	22·5 ± 0·6
Pericarp (%*p*)	0·3 ± 0·2	0·0 ± 0·6	11·1 ± 1·9	23·1 ± 1·8	32·5 ± 2·6	35·2 ± 2·5
Nefer N+						
Endosperm (%*e*)	98·4 ± 0·1	88·8 ± 0·9	69·0 ± 2·1	31·1 ± 2·8	33·5 ± 2·8	17·0 ± 3·4
Aleurone layer (%*a*)	1·5 ± 0·0	11·2 ± 0·1	20·2 ± 0·3	29·6 ± 0·6	33·8 ± 0·9	33·7 ± 1·4
Pericarp (%*p*)	0·1 ± 0·1	0·0 ± 1·1	10·7 ± 1·8	39·4 ± 2·2	32·7 ± 1·9	49·3 ± 2·0
Nefer N−						
Endosperm (%*e*)	98·5 ± 0·1	90·1 ± 0·7	72·9 ± 1·2	36·7 ± 3·3	33·5 ± 3·2	21·6 ± 2·7
Aleurone layer (%*a*)	1·3 ± 0·0	9·9 ± 0·1	17·7 ± 0·2	35·5 ± 1·0	29·7 ± 0·8	33·6 ± 0·8
Pericarp (%*p*)	0·2 ± 0·1	0·0 ± 0·8	9·4 ± 1·0	27·8 ± 2·3	36·8 ± 2·4	44·8 ± 1·9
Lloyd S1						
Endosperm (%*e*)	98·8 ± 0·0	93·2 ± 0·3	69·7 ± 0·5	32·3 ± 3·0	28·6 ± 2·9	18·1 ± 3·0
Aleurone layer (%*a*)	1·2 ± 0·0	6·8 ± 0·2	26·4 ± 0·3	36·3 ± 1·0	52·1 ± 1·2	53·4 ± 0·9
Pericarp (%*p*)	0·0 ± 0·1	0·0 ± 0·5	3·9 ± 0·3	31·5 ± 2·0	19·4 ± 1·7	28·5 ± 2·1
Lloyd S2						
Endosperm (%*e*)	98·9 ± 0·2	92·2 ± 0·4	71·3 ± 1·2	29·1 ± 2·7	36·3 ± 2·7	20·2 ± 3·1
Aleurone layer (%*a*)	1·1 ± 0·0	7·8 ± 0·1	25·5 ± 0·3	40·3 ± 0·8	53·8 ± 0·8	51·5 ± 1·2
Pericarp (%*p*)	0·0 ± 0·2	0·0 ± 0·5	3·2 ± 0·8	30·5 ± 1·9	9·9 ± 1·9	28·3 ± 1·9
Lloyd S3						
Endosperm (%*e*)	99·1 ± 0·1	91·1 ± 0·8	71·9 ± 1·7	44·6 ± 3·0	42·3 ± 2·9	31·4 ± 3·1
Aleurone layer (%*a*)	0·9 ± 0·0	8·9 ± 0·2	27·0 ± 0·5	31·9 ± 0·6	39·6 ± 1·4	44·5 ± 1·1
Pericarp (%*p*)	0·0 ± 0·2	0·0 ± 1·0	1·1 ± 1·1	23·5 ± 1·4	18·1 ± 1·5	24·1 ± 2·0

subjected to large variations (e.g. 42% between Nefer and Lloyd S1). Because the milling process was performed at constant settings, these variations of dissociation between the three botanical parts can be attributed to intrinsic characteristics of grains. The two determining factors were the initial tissue composition of grain (i.e. starchy endosperm/bran layers ratio) and the wheat ability to aleurone layer-starchy endosperm separation, which can be pointed out by the expression of a 'separability' index.

Definition of a 'separability' index

A separability index (S*i*) can be deduced from the extraction profile of tissues generated during the milling process and was defined as the difference in relative proportion of extracted bran and endosperm for a fixed total extraction rate:

$$Si = (\%E) - [(\%A) + (\%P)]$$

with: $\%E$ = relative proportion of extracted starchy endosperm;
$\%A$ = relative proportion of extracted aleurone layer;
$\%P$ = relative proportion of extracted pericarp.

From a theoretical point of view, an ideal milling process should lead to a total extraction of the starchy endosperm without bran contamination (Fig. 1). In such a model, S*i* would reach 100% for an extraction rate equal to the endosperm percentage of the grain (ET). A comparative study of wheat

samples, on the basis of their bran-endosperm separation property only, required then to measure S*i* for a total extraction rate equal to their respective ET value.

In practice, the extraction profile of each botanical part was established by incrementing their proportion in each milling fraction in function of the cumulated extraction rate of the fractions (Table IV). Figure 2 shows the curves obtained with the Ardente variety selected as a representative example. From each extraction profile stated on the eight wheat samples, S*i* was determined by picking out the values of relative proportion of extracted starchy endosperm (%*E*), aleurone layer (%*A*) and pericarp (%*P*) for a total extraction rate equal to ET value. ET value was determined at the final step of milling process by a mass balance analysis taking into account the composition and extraction rates of each fractions. The sum of the results revealed the initial grain composition from each wheat sample.

starchy endosperm proportion in the grain
= ET
= $\sum$(%*e* in the fraction × fraction yield;)
aleurone layer proportion in the grain
= AT
= $\sum$(%*a* in each fraction × fraction yield;
pericarp proportion in the grain
= PT
= $\sum$(%*p* in the fraction × fraction yield.)

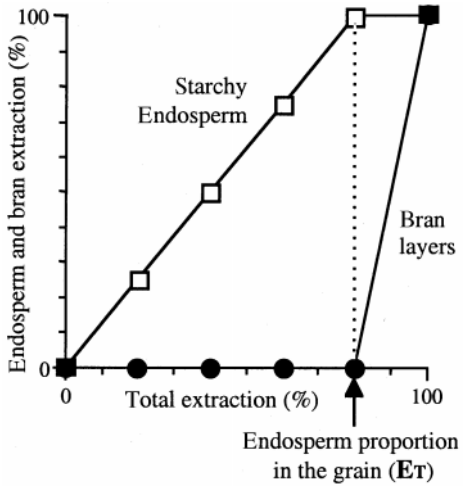

Figure 1 Representation of bran and endosperm extraction profiles in a model of ideal process.

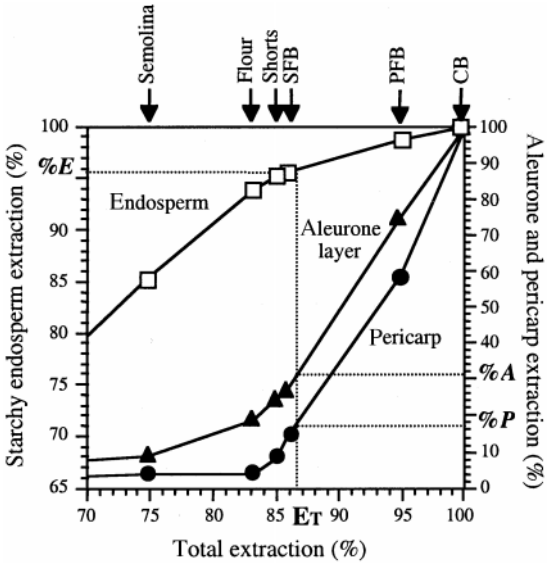

Figure 2 Extractions of starchy endosperm (□), aleurone layer (▲), and pericarp (●) in function of total grain extraction (*Ardente* variety).

Table IV Extraction rate (percentage of dry matter – % D.M.) of the milling fractions (total semolina, total flour and feeds) for the eight wheat samples

	Total semolina	Total flour	Shorts	PFB	SFB	CB
Ardente	74·8	8·3	1·9	9·0	0·8	5·1
Primadur	72·5	7·9	1·5	8·9	0·9	8·2
Nefer	72·9	9·7	2·4	7·0	0·1	8·0
Nefer N+	72·6	8·7	3·2	7·8	0·3	7·2
Nefer N−	73·1	8·5	3·1	7·7	0·4	7·2
Lloyd Sl	72·2	9·5	2·0	7·5	0·8	8·0
Lloyd S2	71·3	10·3	3·5	7·9	0·4	6·6
Lloyd S3	75·4	8·0	2·4	7·5	0·4	6·2

Table V Initial proportion (%) in endosperm and bran (aleurone + pericarp) of grain; relative proportion of bran and endosperm extracted for extraction rate equal to ET and S*i* values

	Initial proportion of endospem[a] (ET)	Initial proportion of bran[a] (1−ET)	Extracted endosperm (%E)[b]	Extracted bran (%A + %P)[b]	S*i*
Ardente	86·61 ± 1·01	13·39 ± 1·01	95·83	49·15	46·68
Primadur	85·44 ± 0·77	14·56 ± 0·77	94·66	58·02	36·64
Nefer	89·06 ± 1·18	10·94 ± 1·18	94·63	77·19	17·44
Nefer N+	85·62 ± 1·95	14·38 ± 1·95	95·65	53·31	42·34
Nefer N−	86·60 ± 1·85	13·40 ± 1·85	95·30	55·91	39·38
Lloyd S1	85·45 ± 0·71	14·55 ± 0·71	96·38	48·21	48·18
Lloyd S2	86·86 ± 1·64	13·14 ± 1·64	95·39	37·36	58·03
Lloyd S3	88·14 ± 1·80	11·86 ± 1·80	95·61	51·74	43.38

[a] Values determined using the phenolics composition.
[b] Values determined for a total extraction rate = ET.

Variability in histological dissociation of the grains

Table V reports the endosperm (ET) and bran layers (AT + PT = 1−ET) proportions of the grains, the relative proportions of extracted tissues (%A, %P and %E) and the S*i* values obtained from the eight wheat samples. This index points out substantial variations between wheat samples and allowed to classify them according to their ability to the bran-endosperm dissociation as unique criterion. Regarding the experimental design and the low number of analysed samples, the respective influences of the wheat variety, the crop site and the nitrogen addition on the bran-endosperm dissociation can not be unequivocally established. However, large differences between the four varieties cropped at Mauguio (S1) were observed. Primadur and Nefer cultivars showed S*i* values, respectively 21% and 63%, lower than Lloyd and Ardente which exhibited similar tissue distributions and identical S*i* values. In detail, Nefer exhibited a particular milling behaviour characterised by an imperfect dissociation between the aleurone layer and the starchy endosperm. It can be noted that this particular milling behaviour was due to both a low bran layer proportion and a poor ability to bran-endosperm dissociation (S*i*).

With regards to the influence of the crop site, the results obtained with Lloyd variety harvested at three distinct locations revealed less variations within botanical part distribution than those observed between the four cultivars. The proportions of each botanical part within the various fractions were then comparable except for a high proportion of starchy endosperm in the bran fraction issued from Ouzouer cultivar (S3) which was related both to a lower S*i* value and a low initial proportion of bran layers.

At last, the influence of nitrogen addition on the botanical dissociation appeared the least significant. The botanical parts in the different milling fractions were in similar amounts and S*i* values were alike in both conditions.

With regards to the result set, S*i* which express the dissociation of the three botanical parts appeared thus dependent on the wheat variety and, to a lower extent, on the crop site but was only slightly affected by nitrogen addition. Like the botanical composition of grain, it proved to be a determining factor of the milling efficiency.

DISCUSSION

The composition in phenolic acids proved to be a useful marker of the three distinct botanical parts of durum wheat grain. In particular, the high contents in *t*-SA and DHD of aleurone layer and pericarp, respectively, allowed the tissue quantification in the different milling fractions. The validity of the results was confirmed by the total amounts of each botanical part recovered from the milling fractions. Depending on wheat samples, the respective proportions of each botanical part after milling were 85·4–88·1% of starchy endosperm, 6·5–8·9% of aleurone layer and 3·8–5·1% of pericarp. However, it must be noted that the aleurone layer proportion was probably overestimated since it included the germ, which represents 2–3% of the grain weight. The analysis of the phenolic acids of purified germ showed the occurrence of a significant amount of *t*-SA. Taking this into account, our results are in

agreement with the values reported in a histological study of the wheat grain.[18]

The phenolic acid concentrations of external layers of durum wheat grain exhibited genetic and agronomic variabilities, which prevent from their use as absolute marker of tissues. Nevertheless, this approach allows to compare the histological dissociation generated by the milling process for different varieties. Since this procedure require a delicate grain hand-dissection, it can not be applied in the commercial environment but must be considered as an investigation tool to assess the milling behaviour of durum wheat and to define the basis of durum wheat milling quality. Besides, our results are in agreement with some previous observations[9,19] concluding that ash content is not a precise indicator of tissue dissociation. The amount of aleurone layer in semolina fraction was weakly correlated to the ash content ($R^2 = 0{\cdot}77$). As previously mentioned, this could be explained by the gradient distribution of minerals within the endosperm, which is variable according to the cultivar, the agro-cultural condition and the crop year.

The distribution of the three botanical parts (starchy endosperm, aleurone layer and pericarp) in the different milling fractions proved to be distinct for the four studied cultivars and appeared influenced to a lower extent by the crop site. The most significant variations involved the starchy endosperm and the aleurone layer while the pericarp appeared more constantly distributed for all wheat samples. The expression of the separability index S*i* emphasised the imperfect dissociation between the aleurone layer and the starchy endosperm and discriminated the wheat samples according to their milling performances. S*i* appeared to influence in a low extent the semolina contamination ($R^2 = 0{\cdot}41$) but was significantly correlated to the loss of endosperm in the bran fractions ($R^2 = 0{\cdot}84$). On this basis, S*i* constitutes a major component of the semolina milling value defined by Abecassis[20] in 1991 as the wheat ability to produce a high yield in semolina of a defined purity.

The distribution of starchy endosperm is a decisive and complex aspect of the semolina milling value, which is related to several factors. Endosperm division between flour and semolina fractions constitutes a first determining factor for the milling performance. This point has been previously reported[21] and it is now well established that the semolina/flour ratio is strongly correlated to the endosperm vitreousness[1]. The second relevant factor is the proportion of residual starchy endosperm in bran fractions, which is also subject to high variability. It could be suggested that this factor is more especially related to the ease of separating the endosperm from the bran. Several years ago, the structural irregularity of the aleurone layer-starchy endosperm interface was considered as a factor determining the easiness for detachment of endosperm from bran during the milling process[22]. However, until now, this structural parameter has never been studied in detail and this hypothesis has never been confirmed.

The aleurone layer distribution appeared also as a determining parameter of the semolina milling value. In particular, our results demonstrated significant variations in the amount of aleurone layer in semolina according to cultivars. This suggested a high variability in the mechanical properties of this tissue. Indeed, the separation between aleurone layer and endosperm during the milling process is based on differences in mechanical resistance between the two tissues. The aim of wheat conditioning is to increase bran plasticity while keeping endosperm hardness. The variations in semolina contamination by aleurone fragments can be then explained by differences in layer strengthening. This parameter was recently studied and several methods have been developed to measure tissue mechanical properties. They allowed to reveal a large variability in bran resistance and to demonstrate the significant influence of moisture content of tissues[23,24]. Consequently, differences in tissues chemical compositions or differential distribution of water during the wheat conditioning could be responsible of variations in bran extensibility and could explain the variability in semolina contamination.

In conclusion, our results allowed to specify the differences in the milling behaviour of wheat samples. More particularly, empirical knowledge of the milling process allowed to identify the endosperm-bran separability as an major component of the wheat milling value. This study demonstrated that this factor can be quantified. It is now essential to characterise the physicochemical bases controlling this separability. Actually, the adhesion forces between aleurone layer and endosperm, the structural irregularity of aleurone layer-endosperm interface and the mechanical properties of the grain peripheral layers constitute the priority fields of research to further progress in the understanding of milling behaviour and to define new selection criterions of wheat varieties.

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