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COMPARISON OF ENDOSPERM MICROSTRUCTURE OF WHEAT AND DURUM WHEAT USING DIGITAL IMAGE ANALYSIS*

J. Fornal[1], *T. Jeliński*[1], *J. Sadowska*[1], *E. Quattrucci*[2]

[1]Division of Food Sciences, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences
Tuwima 10, 10-718 Olsztyn, Poland
[2]National Institute of Nutrition, via Ardeatina 546, 00178 Rome, Italy

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A b s t r a c t. Six wheat varieties were analysed to quantify the relationship between the microstructure and hardness of endosperm. The microstructure of endosperms was characterized using scanning electron microscopy (SEM). Digital image analysis was used to transform the scanned microphotographs and to characterize the image objects. With the DIA algorithms employed for the microstructure quantifications, the grains of different hardness could be differentiated within the variety by analysing the endosperm. The results encourage further studies on wheat grains with improved methods for DIA image acquisition and transformation.

K e y w o r d s: digital image analysis, hardness, fracture resistance, endosperm microstructure, wheat

INTRODUCTION

Wheat is considered of the greatest value among cereals because of it's processing characteristics and chemical composition. Basically, wheats are classified as hard, soft and durum. The processability of these wheats differs due to the differences in the content and types of proteins. It may be assumed that friabilin, which has been identified among the proteins of soft wheats [7], is responsible for wheat softness, not the all protein of matrix [2, 14]. Distinct differences in the mechanical properties (grinding, crushing, abrasion and indentation resistance) between hard and soft wheats benefit the routine methods of classification [1]. The characteristic differences are also observed in the kernel microstructure of hard and soft wheats. As a close relationship between fracture resistance and microstructure of wheat grains was previously proved, [6,12], the microstructure features may be expected to be a sensitive and precise classification factor.

The digital image analysis is used in cereal studies:
- to discriminate between cereal types and wheat cultivars by characterizing either the kernel geometry [9,15-17] or characteristic features of isolated starch granules [3,18],
- to identify impurities both weed seeds and other cereal grain [19] ,
- to identify mechanically damaged or microbiologically attacked grains [13].

Because of the encouraging results of these studies, the use of digital image analysis techniques in the evaluation of various microscopic pictures seemed purposeful, the more that it enables the instrumental objectivity of microscopic measurements, unattainable till now [8].

The purpose of the present work was to develop a procedure to discriminate between the wheat of various hardness on the basis of

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differences in the endosperm-cell structure, as evaluated by scanning electron microscopy.

MATERIAL AND METHODS

Material

Six Italian wheat varieties: Centauro, Gemini, and Mec (*Triticum aestivum*) and Appulo, Creso, and Durango (*Triticum durum*) were used.

Methods

Kernel resistance for compression

Resistance to compression was measured with an Instron 1011 compression device, (Instron Ltd, England) at the crosshead speed of 10 mm/min. Kernels were compressed uniaxially, perpendicularly to the kernel crease (Fig. 1). Fracture force (F_{kernel} [N]) and relative force (force value corrected for different thickness of kernels, F_{thick} [Nmm^{-1}]) were assumed to be the characteristic parameters of kernel resistance for fracture. The determinations were made in 30 repetitions.

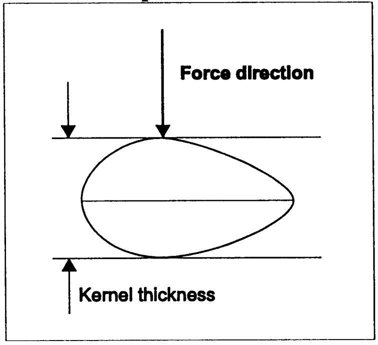

Fig. 1. Position of kernel during compression.

Scanning electrone microscopy (SEM)

The specimens of cross-sectioned wheat kernels (1.5 mm thick) dried in critical point and coated with carbon and gold were examined in a JSM 5200 microscope at 15 kV. The microphotographs (magnification x1000) were done with a Nikon camera.

Digital image analysis (DIA)

Hewlett Packard IIp (Hewlett Packard, Japan) scanner connected by an SCSI interface to a PC-AT 486 computer, equipped with a Falcon PCI (Univision Techn. Inc.) vision processor board, were used for the image acquisition from the SEM microphotographs. Digital image analysis was made with Image Pro Plus (IPP) v.1.2. (Media Cybernetics) software.

DIA algorithm for SEM picture of endosperm

The endosperm images (magnification 1000x) sized 592×395 pixels were stored as TIFF files with 256 grey levels. The area of interest (AOI) was limited to the size of 588×391 pixels (smaller by 2 pixels on both sides) to eliminate the outlines produced by the filter. The pictures were scaled based on a reference bar in the right bottom corner of each picture. To avoid mistakes and to speed up the work, a "macro" program was used that included the following activities:

a) to select the best LUT values for each picture,
b) to convert the picture into negative,
c) to filter with Sobel filter.

Sobel filter extracts and enhances edges and contours in an image by expressing intensity differences (gradients) between neighboring pixels as an intensity value. This is done by combining the difference between top and bottom rows in a neighborhood, with the difference between left and right columns using the following formula:

$$E = (X^2+Y^2)^{1/2}$$

where

$$X = (C+2F+I) - (A+2D+G)$$

$$Y = (A+2B+C) - (G+2H+I)$$

and the neighbourhood is arranged as:

A	B	C
D	E	F
G	H	I

d) to threshold at 135 grey level,
e) to measure automatically the following features of bright objects: area, A [μm^2];

perimeter, P [µm]; diameter, D_m [µm]; compactness, C [-], and number of objects, N [-] while rejecting those with surface area lower than 0.5 µm^2.

Twenty particular images was evaluated for kernels of every wheat variety.

Statistical analysis of the results was carried out with a Statistica ver.5 (StatSoft, USA) program [5].

RESULTS

The methods of wheat hardness classification used most frequently are based on testing the grinding resistance of bulk grain, e.g., Stenvert, Brabender, PSI and PRI tests [11], and NIR test [4]. The determination of mechanical resistance of a single kernel [10] allows both correct classification of the wheat grains (hard and soft) and accurate examination of the varietal variability in the mechanical resistance. The force required to deform the grain, the manner in which fracture occurs, fragment size and sifting behaviour depend upon kernel hardness [1]. Then, the fracture resistance of kernels can be used for general discrimination between soft and hard wheats.

The fracture resistance of wheats (Centauro, Gemini, Mec) as measured by the fracture force was by about half as small as the fracture resistance of durum wheats (Appulo, Creso, Durango) (Table 1). Despite a big dispersion of values in the variety groups (high standard deviation), variance analysis showed that the difference in hardness between wheat groups was statistically significant at P=0.05. It is known that apart from the rheological behaviour, the kernel size also affects the fracture force F_{kernel}. Because the kernels were compressed perpendicularly to the kernel crease, thickness was assumed as the characteristic dimension of kernel size and the values F_{thick} correcting and leveling the kernel-size variations, were calculated (Table 1). The variance analysis of F_{thick} values confirmed the statistically significant differences between the varieties and also between the wheat and durum wheat groups and, consequently, it proved different rheological properties of kernels from these groups, irrespective of the wheat variety.

There are distinct differences in the microstructure of wheats and durum wheats (Figs 2A and 2B). The endosperm of wheats has a loose structure with big spherical and elliptic starch granules between the which there are empty spaces. This results in a low fracture resistance of kernels. The pictures of wheats having a higher fracture resistance usually show more small starch granules that, together with protein, fill the spaces between the large starch granules.

Table 1. Fracture resistance of wheat kernels

Variety	Force at first fracture	
	F_{kernel} (N)	F_{thick} (Nmm^{-1})
Centauro	122.6±30.66	37.3±10.58
Gemini	112.7±18.26	32.7±5.62
Mec	101.4±24.88	30.0±6.97
Appulo	213.4±44.84	66.7±13.35
Creso	219.9±45.91	71.5±15.53
Durango	176.2±50.55	50.3±14.97

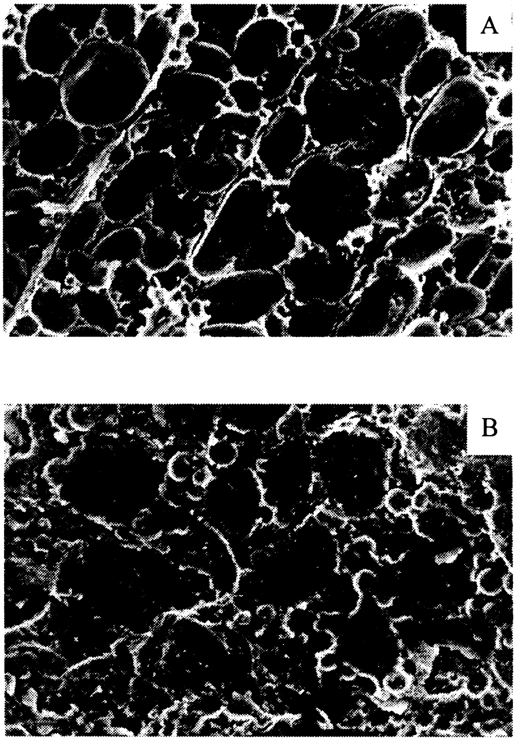

Fig. 2. SEM endosperm micrograph of soft (A) and durum (B) wheats.

In the case of durum wheats, the starch granules are firmly packed in the protein matrix and covered by a protein layer adhered to their surface.

DIA transformation of SEM microphotographs gave distinctly different computer images (Figs 3A and 3B). The images of wheats showed mostly large objects, while the durum wheat images presented a higher number of small objects. Thus, the values of area, diameter, perimeter, and compactness were higher for soft wheats and the number of objects was higher for durum wheats (Table 2).

The variance analysis showed statistically significant (at P = 0.05) varietal differences for all values of the measured features of the images. The analysis of correlation between the studied values led to the following conclusions:

- High correlation coefficient between two measures of kernel fracture resistance allows using them interchangeably when evaluating kernel hardness (Table 3);

- Near maximum correlation coefficients for relationships between area, perimeter and

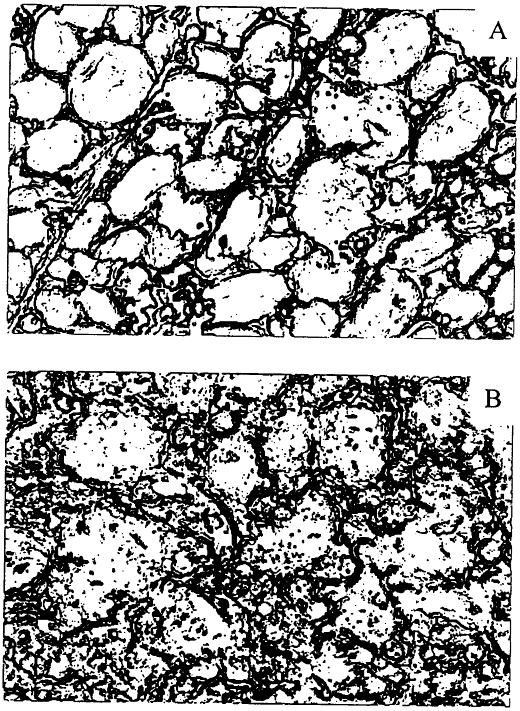

Fig. 3. DIA- transformed image of endosperm of soft (A) and durum (B) wheats.

Table 2. Geometrical features of objects of wheat endosperm images

Variety	Area [μm^2]	Diameter [μm]	Perimeter [μm]	Compactness [-]	No of objects [-]
Centauro	20.12±2.93	3.00±0.43	77.51±14.49	24.20±5.69	123.4±19.0
Gemini	16.16±1.93	3.13±0.46	63.61±9.49	20.34±3.61	161.0±17.6
Mec	19.86±5.77	2.85±0.31	75.38±23.00	23.15±7.32	123.6±33.7
Appulo	8.09±0.94	2.53±0.14	32.44±3.47	10.67±1.03	282.6±20.8
Creso	11.88±2.15	2.54±0.23	45.67±8.50	14.33±2.75	215.4±23.2
Durango	12.57±7.18	2.33±0.43	49.96±26.38	16.21±7.72	210.1±63.1

Table 3. Correlation coefficients for the relationships between fracture resistance and geometrical features of image objects

Parameter	Features of image objects					F_{kernel}
	Area	Diameter	Perimeter	Compactness	Number	
Area	x					
Diameter	0.725	x				
Perimeter	0.999*	0.733	x			
Compactness	0.995	0.741	0.998*	x		
Number	-0.990*	-0.720	-0.990*	-0.990*	x	
F_{kernel}	-	-0.791	-	-0.921*	0.893*	x
F_{thick}	-	-0.733	-	-0.900*	0.864*	0.992*

*significant at P = 0.05.

compactness, which are in a close mathematical relationship, confirmed a good accuracy of the DIA algorithm, allowing at the same time to assume only one feature, i.e., value C (compactness) for further calculations (Table 3);

- High correlation coefficients between the independent features of picture objects (diameter, compactness, and number of objects) and fracture resistance of kernels prove that there is a close relationship between the microstucture elements and wheat hardness (Table 3).

When accurately defining the discrimination between wheats and durum wheats using Fisher linear discriminant analysis (at Wilks' coefficient M = 0.0088, which is a very low value, confirmed high statistical significance of the discriminatory power of the current model) the following classifying functions were calculated:

$$\Phi_1 = - 2592.39 + 766.90\, D_m + 100.52\, R + 5.85\, N - 1.57\, F_{kernel} \quad (1)$$

for wheats and

$$\Phi_2 = - 1894.89 + 519.14\, D_m + 91.17\, R + 5.63\, N - 0.36\, F_{kernel} \quad (2)$$

for durum wheats.

Significant differences in coefficients of these classification functions quantified very well observed differenes in microstructure and hardness (expressed by kernel fracture force) for wheats and durum wheats.

Discriminant analysis also allowed us to determine the share of studied parameters in the overall discrimination. The partial Wilks' coefficients for diameter, kernel fracture force, compactness, and number of objects, which are 0.0991, 0.3588, 0.8835, and 0.9859,

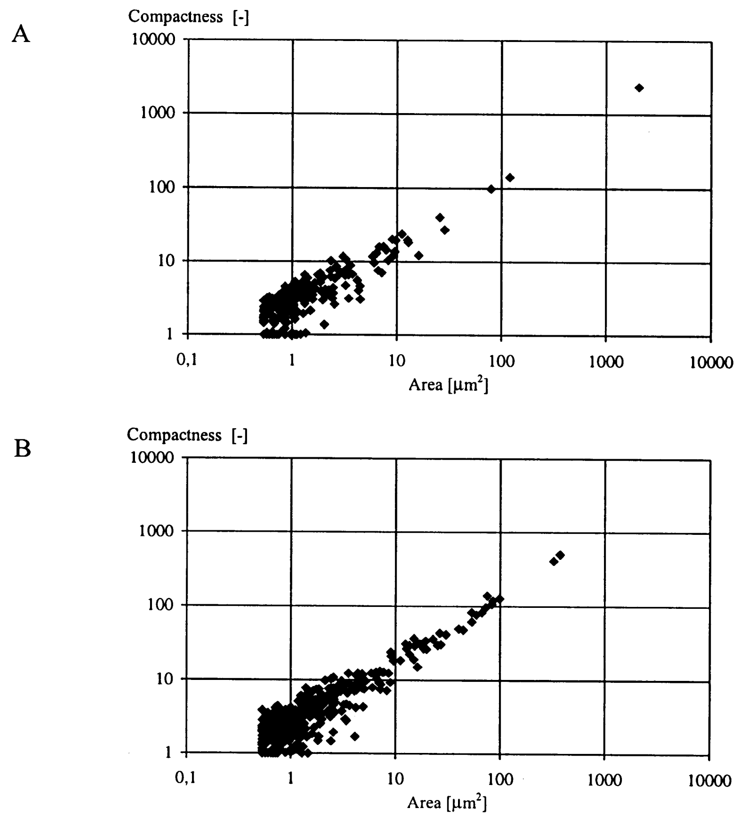

Fig. 4. Graphical presentation of the image structure differences for wheat (A) and durum wheat (B). Note lower number of image objects on (A).

respectively, indicates that diameter contributes most to overall discrimination kernel fracture force, compactness, and number of objects.

Studying the relationship between the measured features of image objects it was found that the graphical presentation of the correlation compactness/number of object obviously illustrate very well the differences between soft and durum wheats being determined by DIA. Figures 4A and 4B show the characteristic arrangement of DIA-measured points for wheat and durum wheat. Total to the number of points (responding number of DIA objects) is higher for durum wheat than for wheat, but the points representing very large DIA objects (above 1000 μm^2) occured only in wheats.

CONCLUSIONS

All of measured features of image objects were found to closely correlated with the fracture resistance of kernels and discriminated very well between durum and soft wheat microstructure.

The share of the studied parameters in the overall discrimination, according to partial Wilks' coefficients is: image object diameter, kernel fracture force, compactness, and number of image objects.

Further studies are necessary to verify whether the cell structure features found herein are regular for a much greater number of varieties.

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