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MICROSTRUCTURE OF STARCH GRANULE RELATED TO KERNEL HARDNESS IN CORN

MICROESTRUCTURA DEL GRÁNULO DE ALMIDÓN RELACIONADA CON LA DUREZA DEL GRANO DE MAÍZ

M. Gaytán-Martínez[1*], J. D. Figueroa-Cárdenas[1], M. L. Reyes-Vega[2], F. Rincón-Sánchez[3] y E. Morales-Sánchez [4]

[1]Centro de Investigación y de Estudios Avanzados, Instituto Politécnico Nacional. Libramiento Norponiente No. 2000. 76230, Juriquilla, Querétaro, Qro. México. Tel: 01 (442) 441-4900, Fax: 01 (442) 441-4900. [2]Food Research Department, Universidad Autónoma de Coahuila. Blvd. V. Carranza s/n. 25280, Saltillo, Coah. México. [3]Universidad Autónoma Agraria Antonio Narro. Buenavista. 25315, Saltillo, Coah. [4]Universidad Autónoma del Estado de Hidalgo. Km 4.5 Carr. Pachuca-Tulancingo. Ciudad Universitaria. 42090, Pachuca, Hgo, México.
* *Autor para correspondencia* (marcelagaytanm@yahoo.com.mx)

SUMMARY

The kernel hardness of 21 corn (*Zea mays* L.) cultivars was studied in relation to texture, flotation index, size and arrangement of the starch granules within the endosperm, using scanning electron microscope. Hardness (7.6 – 16.9 kg-f) was related to the size and the arrangement of the starch granules within the endosperm. Hard corn had large and small polygonal granules in a compact protein matrix. Data obtained from scanning electron microscopy showed a positive correlation of hardness with density and starch granule size, and a negative correlation with flotation index and crystallinity.

Index words: *Zea mays* L., microstructure, starch granule, kernel hardness.

RESUMEN

La dureza del grano de 21 genotipos de maíz (Zea mays L.) fue investigada mediante la textura, índice de flotación, tamaño y arreglo de los gránulos de almidón dentro del endospermo, por medio de un microscopio electrónico de barrido. La dureza (7.6 – 16.9 kg-f) mostró una relación con el tamaño y el arreglo del gránulo de almidón dentro del endospermo. Los maíces duros mostraron gránulos poligonales grandes y pequeños dentro de una matriz proteica compacta. Los datos obtenidos del microscopio electrónico de barrido explicaron la correlación positiva de la dureza con densidad y el tamaño del gránulo de almidón, y la correlación negativa con el índice de flotación y la cristalinidad.

Palabras clave: *Zea mays* L., microestructura, gránulo de almidón, dureza del grano.

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INTRODUCTION

Kernel hardness in corn (*Zea mays* L.) is the main physical parameter for selecting its end-use. There are products, such as "atoles" (porridges), which need low grain hardness, while others products, such as corn flour (dry milling) are favored by a high grain hardness.

The quality of corn is evaluated by physical traits such as kernel size and color, percentages of pericarp, endosperm and germ, flotation index, density, moisture, and percentage of harmful kernels (Norma Oficial Mexicana, 2002). The chemical composition or microstructure characteristics of the grain are not considered. Starch is the main component of corn grain, and the starch granule is a complex hierarchical structure consisting of polysaccharide macromolecules partially arranged in ordered conformations (double and single helixes) and entangled to form supra- and sub-molecular structures. The relationship between the structure and thermodynamic properties of starches can be found at different levels of structural organization. The textural properties of a food, on the other hand, are the group of physical characteristics arising from the structural elements of the food and are sensed primarily by touch. They are related to deformation, disintegration, and flow of the food when acted upon by a force, and are measured objectively by functions of mass, time, and distance (Bourne, 2002). Kernel hardness is one of these textural properties, so it must be related to structural elements of the corn grain.

The main objective of this work was to establish the relationship between the microstructural characteristics of corn kernels and some physical variables that influence the grain hardness.

MATERIALS AND METHODS

Materials. The genetic material studied consisted of 21 cultivars selected with an ample range of hardness (7.6-16.9 kg-f). Seed of these cultivars was harvested in Spring 2003, at the Universidad Autónoma Agraria Antonio Narro, Experimental Station located in Tepalcingo, Morelos, México.

Pericarp, endosperm and germ. The parts of the kernel were separated by hand. They were weighed and the percentage of each part was calculated (10 replicates).

Density. Corn kernels were placed in a 1 L beaker and the empty space was filled with water. The weight of the kernels and the volume of water were determined previously. The moisture content of the kernels was evaluated by using an infrared Ohaus oven, before and after the addition of water, to calculate water absorption. The density of the kernel was calculated as weight divided by volume.

Flotation index. One hundred kernels were placed in a beaker containing 300 mL of $NaNO_3$ solution (1.25 g mL^{-1}). They were stirred to separate the kernels and let stand for 1 min. The number of floating kernels indicated the flotation index (Norma Oficial Mexicana, 2002). The test was performed in duplicate.

Hardness. A TA-XT2 Texture Analyzer (Texture Technologies Corporation, Stable Micro Systems; Surrey, England) was used with a 30° conical probe. Each one of five kernels was punctured at 0.5 mm s^{-1} to a depth of 2 mm. The hard endosperm of the kernels was punctured. The test was performed in duplicate in each sample.

Scanning electron microscopy. Longitudinal sections of a fractured corn kernel were observed using an environmental electron-scanning microscope (ESEM; Phillips Model XL30) with a 20 kV beam (50 μA), GSE detector and spot size of 4.6. The images were taken at 500 to 1500X, to observe the packed cell and starch granule size within the hard and floury (soft) corn endosperm matrix.

Crystallinity. A Rigaku X-ray diffractometer DMAX-2100, which operates at 30 kV and 16 mA with a CuKα radiation of $\lambda = 1.54$. The samples were standardized at 75 % relative humidity. They were placed on a glass surface and scanned from 5 to 35° on the 2 Θ scale.

Statistical analysis. The traits relationship was explored by the Pearson correlation coefficient and by the Path analysis methodology obtained by the PROC CLAIS of SAS (SAS, 1999).

RESULTS AND DISCUSSION

Corn kernel hardness has been studied intensely because of its importance to industrial processes, and a fair amount of data can be found on the subject in the scientific literature, some of it contradictory. Researchers have established relationships between hardness and flotation index (Norma Oficial Mexicana, 2002), amylose and percentage of total starch (Dombrink-Kurtzman and Knutson, 1997), and between type and amount of zein (Lopes and Larkins, 1991; Dombrink-Kurtzman and Bietz, 1993; Moro *et al.*, 1995). However, Robutti *et al.* (1999) reported that protein content of Argentine maize races estimated by Kjeldahl nitrogen determinations, varied between 8.8 and 11.9 % and found no statistically significant correlation between protein content and kernel hardness or endosperm type.

By taking physical factors into account, hardness may be related not only to the chemical characteristics of the grain, but also to its physical characteristics. Both the chemical makeup of the corn kernel as well as the way, in which the molecules and particles are assembled, should be considered.

Positive correlations were found in this study between hardness-density, hardness-starch granule size, and hardness-endosperm. On the other hand, a negative correlation was observed between hardness-flotation index, hardness-crystallinity, hardness-pericarp, and hardness-germ (Table 1). Endosperm is the main component of corn kernels and apparently contributes importantly to kernel hardness, as can be seen by observing the positive correlation between these factors and the negative correlations of hardness with pericarp and germ. The chemical composition of each part of the kernel is different. However, physical traits, such as density, starch granule size and crystallinity are as important as the chemical ones.

The characteristics described above can give an idea of the microstructure of this cereal endosperm. Cells have a variable number of starch granules and granule sizes and shapes, as well as differences in the intrinsic structure of each granule, depending on the amount of amylose, which are reflected as a big or small loosely-packed central region and as variations in the width of the compact and crystalline peripheral region.

Table 1. Pearson product-moment correlations between corn kernel characteristics.

	V6	V7	V8	V9	V26	V27	V28	V29
Min V	7.00	7.60	1.03	7.88	7.27	80.79	3.43	8.00
Max V	93.00	16.90	1.35	14.11	11.21	87.17	7.55	12.87
V6	1							
V7	-0.735*	1						
V8	-0.845*	0.688*	1					
V9	-0.488*	0.587*	0.500*	1				
V26	0.753*	-0.440*	-0.530*	-0.330*	1			
V27	-0.571*	0.486*	0.716*	0.444*	-0.082	1		
V28	0.413*	-0.309*	-0.681*	-0.178	0.144	-0.647*	1	
V29	0.661*	-0.425*	-0.574*	-0.333*	0.429*	-0.636*	0.332*	1

V6 = Flotation index; V7 = Hardness (kg-f); V8 = Density (g/mL); V9 = Starch granule size (μm); V26 = Crystallinity; V27 = Endosperm (% of kernel); V28 = Pericarp (%); V29 = Germ (%); *Significant at 0.05 probability level.

The structural arrangement of the starch inside the kernel is an important factor affecting hardness, more than the presence of a thick pericarp, the amount of fiber between the endosperm cells, or the protein matrix between the starch granules. The scanning electron microscopy revealed that hard corn kernels, had a different starch granule pattern than did soft corn (Figure 1). The starch granules of soft corn are mostly spherical and loosely packed within a protein matrix, as opposed to hard corn whose starch granules are mostly polygonal and densely packed. These results agree with those of Lisle *et al.* (2000) and Ji *et al.* (1998) for rice (*Oryza sativa* L.) kernels who reported that shape, size, and packing of cell in chalky rice grains differed from those in translucent hard grains.

These data indicates a significant ($P < 0.05$) positive correlation between hardness and density, as well as a significant ($P < 0.05$) negative correlation between hardness and flotation index. A densely packed structure is expected to show a high density and a low flotation index, as well as a higher hardness value than a loosely packed structure. Hardness and starch granule size show a significant ($P < 0.05$) positive correlation. It should be taken into consideration that A-type granules (larger than B-type) contain a greater amount of amylose (Seib, 1994) and that granule-bound proteins are more abundant in granules with a higher amylose content (Li *et al.,* 2003), giving the granule a hard structure. Furthermore, B-type granules contain more lipids than do A-type granules (Raeker *et al.,* 1998). High lipid content is not expected to enhance hardness.

The X-ray diffraction patterns from this study showed the typical A-type cereal starches. Hard corn showed less intense diffraction peaks as compared to soft corn, resulting in lower percentage crystallinity values for hard corn starch. Patindol and Wang (2003) reported similar data for rice, and they also found a significant direct relationship between crystallinity and amylopectin, and an inverse one between crystallinity and amylose. Apparently, the intensity of the X-ray diffraction patterns was determined by the amylopectin content, and not by amylopectine structures because of a lack of relationship between crystallinity and amylopectine chain length. In a study of native wheat (*Triticum aestivum* L.) starch granules, Bocharnikova *et al.* (2003) found that starch granule morphology depends mostly on the amylopectin arrangement; it does not change significantly with changes in the amylose content or the thickness of crystalline lamellae. These results agree with our scanning electron microscopy results: larger starch granules that are richer in amylose (data not shown) are more abundant in hard corn than in soft corn.

Hardness, as a texture characteristic of corn kernels, must be considered to be the result of the contribution of several variables, which include individual chemical compounds and the complexes resulting from their interactions, as well as the arrangement of molecules and particles inside the starch granule and the corn kernel.

The path model shown in Figure 2 was proposed to explain the causality of corn hardness. Path analysis was performed, obtaining the following equations:

$$V7 = 0.2365xV8 - 0.5448xV6 + E1$$

$$V8 = 0.0602xV9 - 0.4568xV26 + 0.6514xV27 + E2$$

Where V7 = hardness; V6 = flotation index; V8 = density; V9 = starch granule size; V26 = crystallinity; V27 = percentage of endosperm; E1 = error 1; and E2 = error 2.

The adjustment of the model was estimated by Bentler's Comparative Fit Index (SAS, 1999), and had a value of 0.91. The direct and indirect effects of each variable over hardness are shown in Table 2. It can be seen that the direct effect of density is about one half of that of the flotation index. However, the indirect effects of starch granule size, crystallinity and percentage of endosperm contribute to duplicate the direct effect.

Table 2. Direct and indirect effects on corn kernel hardness[1].

	Direct	Indirect			Total
Density	0.2365				**0.5263**
		P-Granule size	0.0602	0.0142	
		C-Crystallinity	-0.330	-0.0047	
		C-Endosperm	0.440	0.0063	
				0.1436	
		P-Crystallinity	-0.4568	-0.1080	
		C-Granule size	-0.330	0.0356	
		C-Endosperm	-0.082	0.0088	
				-0.0636	
		P-Endosperm	0.6514	0.1540	
		C-Crystallinity	-0.082	-0.0126	
		C-Granule size	0.444	0.0684	
				0.2098	
Flotation index	-0.5448				**-0.5448**

[1]Significant at 0.05 probability level.

According to these results, hardness is influenced mostly by traits related to the starch structure within the grain. Some other traits, such as percentage of pericarp and germ contribute to a minor degree. The amount of some other chemical compounds, such as proteins and lipids (data not shown), did not influence kernel hardness in a significant way.

CONCLUSION

Substantial differences in some physical (flotation index, density, endosperm), and microstructural (starch granule size, crystallinity, cell packing) characteristics were found between hard and soft corn kernels. A positive

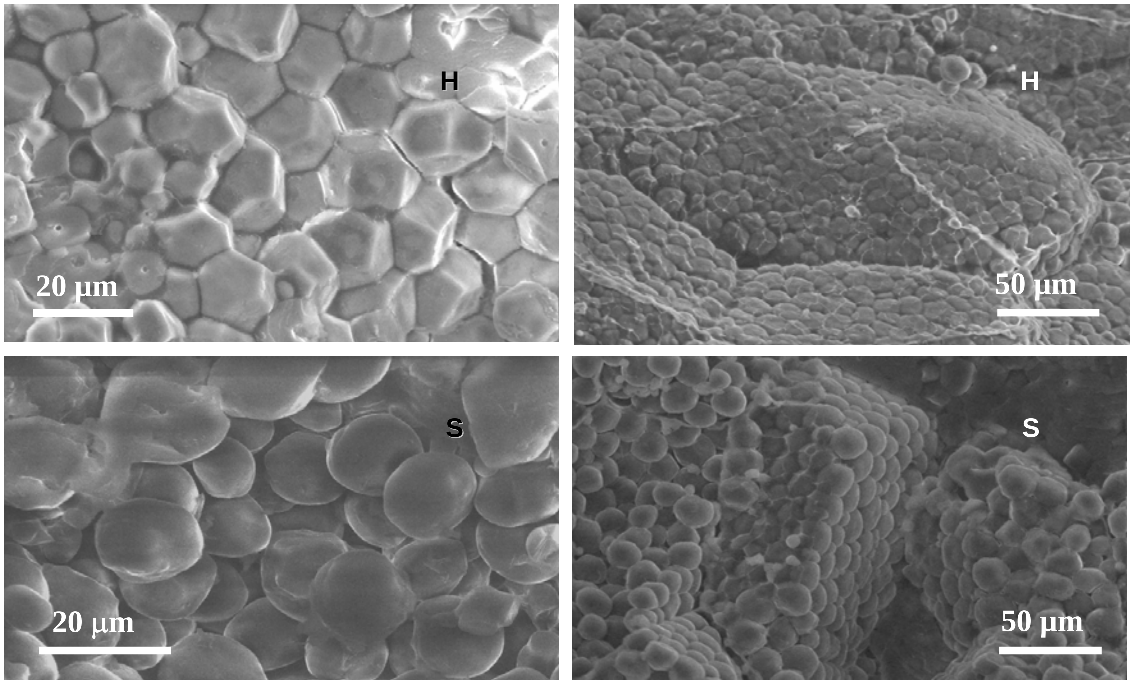

Figure 1. Microstructure of hard (top) and soft (bottom) corn kernels. H represents hard corn and S soft corn.

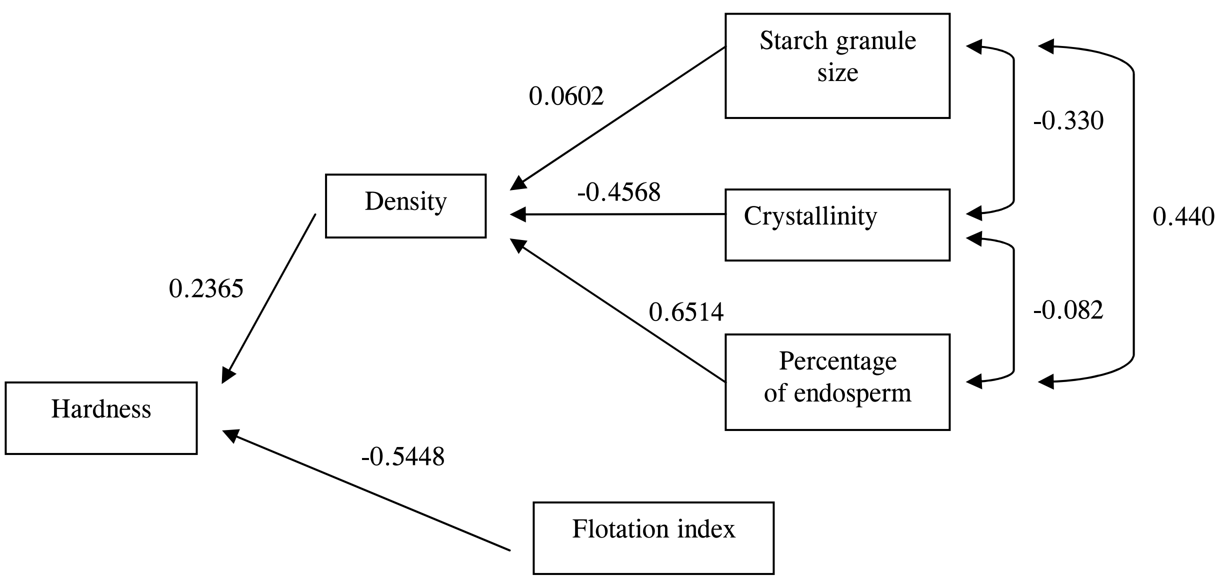

Figure 2. Path diagram of characteristics influencing corn kernel hardness.

correlation was observed between hardness and density as well as starch granule size and endosperm, while hardness showed a negative correlation with flotation index, crystallinity, pericarp and germ. Kernel hardness in soft corn differs from that of hard corn in cellular structure, and in packing of starch granule and may affect end-use among corn genotypes.

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