Hardness of Moroccan Wheats[1]

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Cereal Chem. 67(3):246–250

ABSTRACT

The kernel hardness of 18 common (hexaploid) wheats from Morocco, determined using particle size dispersion, were compared with 10 U.S. varieties of known hardness and baking properties. In addition, their alkaline water retention capacity, damaged starch values, protein content, and vitreousness values were determined. The Moroccan common wheats exhibited a wide range of particle size dispersion values (35.4–58.4). Based on the U.S. wheat samples, we suggest a classification of the Moroccan common wheats into hard and soft types. This classification was corroborated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins associated with the starch granules.

Grain hardness affects the milling behavior of wheat as well as the suitability of the resulting flour for its end use. Hard wheats generally make the best breads, whereas soft wheats are used for cookies (biscuits). Several techniques have been devised to measure wheat hardness but the particle size procedure is still the most simple and reliable technique (Yamazaki and Donelson 1983).

Physical hardness of the grain results from the adhesion forces between starch granules and the protein matrix. These forces are stronger in hard than in soft wheats (Hoseney and Seib 1973). The material surrounding the starch granules contains water-soluble proteins associated with carbohydrates (Barlow et al 1973, Simmonds et al 1973). Greenwell and Schofield (1986) showed that the adherent proteins have molecular weights ranging from 5 kilodaltons to about 76 kDa, and an unbroken positive association was found between the presence of a 15-kDa protein and endosperm softness. Symes (1965) reported that hardness is controlled by one major single gene. In other sample sets other authors did not find the same result (Beard and Poehlman 1954, Baker and Dyck 1975). The gene coding for the 15-kDa protein is on the same chromosome (5D) as the major gene controlling hardness (Greenwell and Schofield 1986).

The relationship of hardness to vitreousness and/or protein content was not found to be significant (Parish and Halse 1968; Symes 1969; Simmonds 1974; Obuchowski and Bushuk 1980a,b; Miller et al 1982, 1984). However, a very strong relationship was found between damaged starch and hardness (Stenvert 1974, Wade 1987, Williams 1967). Wheat hardness may also be affected by environmental conditions (Stenvert 1974).

Wheats grown in Morocco are predominantly durum (*Triticum durum*) wheats used for couscous and pasta production, termed "hard" wheats, and common (*T. aestivum*) wheats (improperly called "soft" wheats) used for bread, cookies, and pastry. The advent of large-scale cookie manufacturing in Morocco has created a need for stricter measures of wheat quality. The present study was undertaken to classify Moroccan common wheats according to their hardness, to rationalize the interpretation using certain U.S. hard and soft wheats as standards, to look at the 15-kDa starch granule protein in Moroccan wheats, and to study the correlations between wheat hardness and protein content, vitreousness, damaged starch, and alkaline water retention capacity.

TABLE I
Classification of Common Wheats Using (LSD) and Tukey's Highest Significant Difference (HSD) Tests

Variety	Particle Size Dispersion[a]	LSD[b] 0.05 (−0.51)	HSD 0.05 (−0.98)
Teggey 9 (5/70-9)	35.4	A	A
Jouda (1646)	35.8	A	A,B
Nesma (149)	36.5	B	B
1724	37.9	C	C
Siete'-Ceros	38.1	C	C,D
Marchouch 9	38.8	D	C,D,E
1710	39.0	D	D,E
1712	39.3	D,E	E
1725	39.7	E	E
1711	41.0	F	F
Acsod 67	41.9	G	F,G
Marchouch 8	42.4	G	G
Marchouch 10	44.4	H	H
Acsod 59	44.5	H	H
Teggey 32 (5/70-32)	47.6	I	I
Sais (1615)	54.5	J	J
Pinyte (2306)	56.1	K	K
Potam	58.4	L	L

[a] Means of two determinations

[b] A–L are indicators of possible class differences (different letters imply that data statistically differ enough to be separated by the methods used).

[1]Cooperative investigations, U.S. Department of Agriculture, Agricultural Research Service, and the Department of Grain Science and Industry, Kansas State University. Contribution 89-403-J, Department of Grain Science and Industry, Kansas State Agricultural Experiment Station, Manhattan, KS 66506.

Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others not mentioned.

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MATERIALS AND METHODS

Wheat Varieties Analyzed

Eighteen Moroccan common wheats were analyzed for hardness. They were all collected from the same location (Marchouch Agronomic Station), crop year 1986. The possible effect of location was tested using 11 cultivars collected from Marchouch (Rabat surroundings) and Menara (Marrakech) research stations. This effect was also tested with five cultivars grown at the Marchouch, Menara, and Boyet (Fès) stations. In order to help differentiate between Moroccan soft and hard common wheats, certain standard American hard and soft wheat cultivars were obtained from Kansas State University, and their particle size dispersion (PSD) values were determined. The Moroccan wheat samples were supplied by the breeding service of the Research Institute (Rabat, Morocco).

TABLE II
Particle Size Distribution Values[a] of 11 Cultivars Collected From Marchouch and Menara Stations and of Five Cultivars from Doyet Station

Variety	Marchouch	Menara	Doyet
Teggey 9	35.4 ± 0.3	40.9 ± 0.1	...
1724	37.9 ± 0.4	45.1 ± 0.1	45.2 ± 0.3
Marchouch 9	38.8 ± 0.3	36.8 ± 0.3	...
1710	39.0 ± 0.0	43.3 ± 0.1	39.4 ± 0.3
1712	39.3 ± 0.1	38.6 ± 0.0	38.8 ± 0.0
1711	41.0 ± 0.0	38.4 ± 0.3	38.9 ± 0.1
Acsod 67	41.9 ± 0.1	45.0 ± 0.3	...
Marchouch 8	42.4 ± 0.3	43.2 ± 0.3	...
Teggey 32	47.6 ± 0.6	39.4 ± 0.3	...
Sais (1615)	54.5 ± 0.1	57.6 ± 0.3	...
Potam	58.4 ± 0.3	52.7 ± 0.1	60.8 ± 0.3

[a] Data shown are means of two repetitions ± the standard deviation.

Particle Size Dispersion Procedure

Wheat samples were manually cleaned to remove foreign material as well as broken, shrunken, and damaged kernels. The samples were then equilibrated at 20°C and 60% relative humidity for several days. Moisture contents of wheat samples were all 13 ± 0.30%. Wheat samples (70 g) were ground using an A.B. Falling Number grinder (model KT 120) rotating at 2,800 rpm and equipped with a sieve with 1-mm openings, as recommended by Williams (1979). The ground meal was homogenized, and 50 g was sifted with a 10XX nylon sieve (136-μm openings) shaken with a Chopin S.A. shaker using a medium speed of 200 rpm. The 10XX nylon sieve was chosen because its throughs define flour as it is industrially known. Different sieving times were tested, and 10-min sieving was chosen because it resulted in a small standard deviation among PSD values of several replicates of the same wheat sample. The material extracted through the 10XX sieve was weighed and expressed as a percentage of the 50 g sieved (PSD value).

Determination of the Parameters Correlated with Hardness

The throughs of the 10XX sieve obtained in PSD determination were used to conduct alkaline water retention capacity (AWRC) and damaged starch analyses.

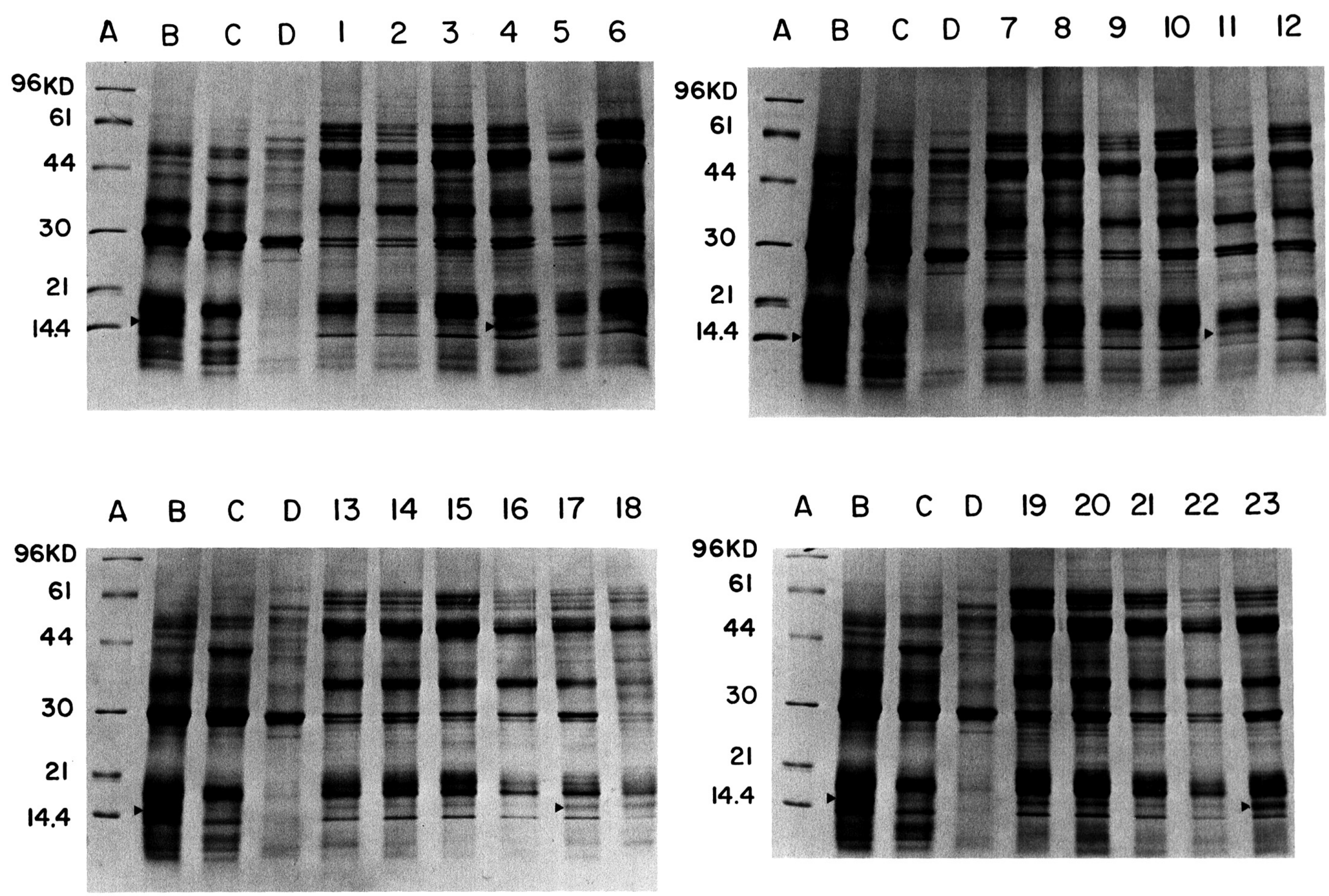

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of starch proteins (Moroccan wheats): **(A)** molecular weight markers (96, 61, 44, 30, 21, and 14.4 kDa); **(B)** soft wheat; **(C)** hard wheat; **(D)** durum wheat; **(1)** 1725; **(2)** 1711; **(3)** 1724; **(4)** 2306; **(5)** 5/70-9; **(6)** 1710; **(7)** 1712; **(8)** Acsad 59; **(9)** Siete Ceros; **(10)** Nesma (149); **(11)** 1615 (Nesma); **(12)** Marchouch 8; **(13)** Marchouch 9; **(14)** Marchouch 10; **(15)** 1646 (Jouda); **(16)** Acsad 67; **(17)** Potam; **(18)** 5/70-32 (Marchouch); **(19)** 5/70-32 (Menara); **(20)** SDS8036; **(21)** 1723; **(22)** Pavon; **(23)** 1618.

AWRC. This test measures the amount of alkaline water held, after centrifugation, by a given amount of flour. Both protein content and the level of damaged starch are important in determining the amount of absorbed water. AWRC is supposed to correlate with hardness. The procedures described by Yamazaki (1953) were used with the exception that the sample size was reduced by one-third.

Damaged starch determination. The analysis was carried out following the AACC method 76-30A (AACC 1983).

Protein determination. The method used was a micro-Kjeldahl procedure in which proteins were quantified by multiplying the nitrogen content by 5.7 (Association Francaise de Normalisation [AFNOR] method NFV03-050).

Vitreousness measurement. This was determined as described by Scotti (1984) by slicing 50 kernels at a time with 12 repetitions using the AFNOR method NF703-70S using a Grobecker Farinator (Tripette et Renaud, Paris, France). The number of vitreous (or mealy) kernels was visually assessed and expressed as a percentage.

TABLE III
Interstation Correlations for Particle Size Distribution (PSD) Values

Correlated PSD Results for Station	No. of Varieties	Sample Correlation Coefficient (*r*)	Level of Significance (%)
Marchouch/Menara	11	0.76	99
Marchouch/Doyet	5	0.92	95
Menara/Doyet	5	0.94	95

TABLE IV
Particle Size Distribution (PSD) Values of Some American Hard and Soft Wheats

Wheat Varieties	PSD (%) Values
Hard red winter	
Newton	45.3
Tam 105	45.8
Mustang	47.3
Hawk	48.5
Probrand	50.0
Soft red winter	
Caldwell	56.3
Hart	57.5
RH9227	59.3
Pike	63.0
Compton	64.8

Electrophoresis of Proteins Surrounding Starch Granules

Flour (5 g) and cold (4°C) water (3 ml) were mixed into dough that was then washed with 200 ml of cold water. Then the gluten and starch were separated, and 10 ml of the starch suspension was centrifuged at 2,000 × *g* for 20 min. To the residue (wet starch), 10 ml of cold water was added followed by centrifugation at 3,000 × *g* for 20 min. The wet starch (residue) was extracted with 1% sodium dodecyl sulfate (SDS) solution at 50°C for 20 min. After extraction, centrifugation was carried out at 2,000 × *g* for 20 min. To the supernatant, 6 ml of acetone was added and centrifuged at 1,000 × *g* for 20 min. The resulting precipitate contains the starch granule protein.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the procedure in the Hoefer Scientific Instruments (San Francisco, CA) catalog. A Hoefer SE600 (18 cm × 16 cm × 1.5 mm) vertical slab gel electrophoresis apparatus was used (4 cm stacking gel and 12 cm separating gel), and a Bio-Rad gradient form (model 385) was used to prepare a linear gradient (7.5–25%) conducted at 12°C and 60 mA constant current. The electrophoresis time was about 5 hr.

RESULTS AND DISCUSSION

PSD values for Moroccan common wheats are reported in Table I. The results show the heterogeneity of Moroccan common wheats in terms of their hardness. The variation coefficient is quite high (16.32%). One-way analysis of variance showed that the varietal effect was highly significant (Fc = 1661.13, $P < 0.01$ [17, 18]). PSD mean values for all cultivars were compared using the least significant difference (LSD) method and Tukey's highest significant difference (HSD) test. From the results obtained (Table I), 12 classes were found with the LSD and HSD tests but with a higher number of overlappings with the latter test. The location effect was also studied (Table II). Considering the five varieties grown at the three locations, two-way analysis of variance showed that a location effect (Fc = 121.66, $P < 0.01$ [2, 15] = 6.36), a genetic effect (Fc = 7779.74, $P < 0.01$ [4, 15] = 4.89), and their interaction effect (Fc = 430.93, $P < 0.01$ [8, 15] = 4.00) were all highly significant ($P < 0.005$). The same conclusion was found with all 11 varieties grown at two locations (Table II). As previously shown by *F* values, the varietal effect was extremely

TABLE V
Characteristics of Common Wheat Cultivars Grown at Marchouch Station (1986 Crop Year)

Wheat Varieties	Particle Size Distribution[a] (%)	Protein[a,b] (%)	Vitreousness (%)	AWRC[c] (dry moisture basis)	% Damaged Starch (dry moisture basis)
Teggey 9 (5/70/9)	35.4	14.65	70	86.2	10.09
Jouda (1646)	35.8	14.58	12	83.9	8.57
Nesma (149)	36.5	16.67	30	83.9	9.62
1724	37.9	13.85	11	82.2	...
Siete'-Ceros	38.1	12.55	12	82.8	8.10
Marchouch 9	38.8	13.99	6	79.3	6.19
1710	39.0	13.24	8	81.0	...
1712	39.3	14.67	8	81.6	8.57
1725	39.7	11.86	4	79.9	5.71
1711	41.0	13.98	3	78.2	...
Acsad 67	41.9	13.22	2	77.6	9.14
Marchouch 8	42.8	15.66	10	80.5	5.34
Acsad 59	44.5	11.79	10	71.8	3.90
Marchouch 10	44.4	14.62	5	78.2	...
Sais (1615)	54.5	14.16	0	65.5	1.43
Pinyte (2306)	56.1	16.58	0	69.0	6.19
Potam	58.4	13.03	0	65.5	1.90

[a] Means of two determinations.
[b] Calculated as 5.7 × %N, dry basis.
[c] Alkaline water retention capacity.

important. This supports what several authors have already reported (Cutler and Brinson 1935, Berg 1947, Beard and Poehlman 1954, Symes 1965, Baker and Dyck 1975). The environmental effect was also important, and this agrees with certain literature reports (Stenvert 1974, Beard and Poehlman 1954), but disagrees with others (Miller et al 1981a and b). However, as shown in Table III, high interstation correlations were obtained as shown before by Taylor et al (1939). This indicates, once again, that differences in hardness come mainly from the varietal criterion. The differences in the sample correlation coefficients show the effect of environment on genetic hardness potential.

In order to suggest a demarcation line between soft and hard Moroccan common wheats, certain standard American hard and soft wheat cultivars were analyzed and their PSDs determined (Table IV). An important gap (6.3% PSD units) occurred between American hard and soft wheats. For Moroccan wheats the major break occurred between Teggey 32 and Sais (1615) varieties. The mean size of that gap was 6.9% units at the Marchouch station (Table II). It was largest (18.2% units) from those varieties at the Menara station. We suggest that Moroccan common wheats may be subdivided into soft and hard wheats. The soft wheats would be Sais (1615), Pinyte (2306), and Potam. The other varieties would all be hard wheats. The environmental conditions under which the wheats are grown do not appear to affect this division.

The division of Moroccan wheat into hard and soft was confirmed by analyzing SDS-PAGE patterns for proteins associated with starch granules. The results are reported in Figure 1 and agree with the findings of Greenwell and Schofield (1986). These data showed that the wheats labeled as soft by the PSD test (Potam, lane 17; 1615, lane 11; and Pinylo 2306, lane 4) have a 15-kDa band that is significantly more intense than those found

TABLE VI
Correlation Results Among the Parameters Determined for Hardness Studies

Correlated Parameters	Simple Correlation Coefficient (*r*)	Level of Significance (%)
	Moroccan Common Wheats	
PSD[a] with:		
Proteins	0.07	NS[d]
Vitreousness	−0.49	95
AWRC[b]	−0.96	99
Damaged starch[c]	−0.77	99
Proteins with:		
Vitreousness	0.24	NS
AWRC	0.11	NS
Damaged starch	0.33	NS
Vitreousness with:		
AWRC	0.56	95
Damaged starch	0.54	95
AWRC with:		
Damaged starch	0.85	99
	Moroccan Durum Wheats	
PSD with:		
Vitreousness	−0.12	NS
Proteins	0.49	NS
Vitreousness with:		
Proteins	0.25	NS
	American Wheats	
PSD with:		
Proteins	−0.70	95
AWRC	−0.91	99
Proteins with:		
AWRC	0.64	95

[a] Particle size distribution.
[b] Alkaline water retention capacity.
[c] All the correlation results involving damaged starch (for Moroccan common wheats) were calculated using 13 samples as reported in Table V.
[d] Not significant at the 95 or 99% levels.

in hard wheats. In Figure 1, the hard cultivars 5/70-32 (lanes 18 and 19) and SD8036 (lane 20) have a relatively intense band near the 15-kDa band, but this is not the softness band because it did not have the same mobility as the 15-kDa band. The "soft" 15-kDa band appears as a broad band or as the faster band of a resolved doublet. Also, the variety 1618 (Fig. 1, lane 23) has an intense 15-kDa band, but this variety was not analyzed for hardness because it had been canceled from the breeding program.

The relationship of wheat hardness to vitreousness, protein content, AWRC, and damaged starch are summarized in Tables V and VI. No significant correlation was found between PSD (hardness) and protein content. Baker and Dyck (1975) suggested that a genetic linkage exists between the genes that control hardness and protein content. Moss (1973) observed that as protein content increased, wheat hardness decreased, and several other authors suggest that the relationship between protein content and hardness is subject to question (Symes 1969; Hoseney and Seib 1973; Simmonds 1974; Obuchowski and Bushuk 1980b; Miller et al 1981b, 1982; Yamazaki and Donelson 1983). On the other hand, correlation studies confirmed the existence of a joint relationship between hardness and vitreousness (significant at the 95% level). The subdivision made earlier on common wheats is somewhat supported by the vitreousness results. In fact, all the presumed soft wheats have no vitreous kernels, and their flours (10XX sieve extracts) were brighter than those of hard wheats. Although some people in grain inspection services rely on grain vitreousness to assess wheat hardness, others do not and stress the fact that this relationship is questionable (Milner and Shellenberger 1953, Simmonds 1974).

A correlation coefficient between AWRC and hardness of −0.96 was found and is highly significant. Because protein content is unrelated to hardness, the strong negative relationship found between PSD and AWRC might be explained by the level of damaged starch. The harder the wheat the higher the level of damaged starch, and the more important the amount of absorbed water will be. It is also interesting to note that presumed soft wheats have the lowest values of AWRC, indicating that they are best-suited for cookie-making compared with the other common cultivars. Damaged starch was correlated with wheat hardness (−0.77, significant at the 99% level). Several authors have found this relationship to exist (Williams 1967, Stenvert 1974, Pomeranz et al 1984, Wade 1987).

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[Received May 5, 1989. Accepted October 23, 1989.]