Milling and Cookie Baking Quality of Near-Isogenic Lines of Wheat Differing in Kernel Hardness[1]

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ABSTRACT

Two sets of near-isogenic lines of wheat were milled on a modified Brabender Quadrumat Senior experimental mill and were identified as being either hard or soft. Those identifications were made during milling. The resulting flours were tested for starch damage, presence of the 15-kDa starch granule protein, and sugar-snap cookie spread. The 19 lines derived from Falcon, 10 hard and nine soft, had acid-polyacrylamide gel electrophoresis (A-PAGE) patterns of gliadins identical to each other and to those of Falcon. Likewise, the 11 lines derived from Heron, six hard and five soft, had A-PAGE patterns identical to each other and to those of Heron. As expected, the A-PAGE patterns were genotypic and not related to the hardness or softness characteristic. Milling and baking parameters correlated highly with classification of the flours as being hard or soft, rather than with classification according to the flour's gliadin (A-PAGE) pattern.

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Hardness is an important quality characteristic of wheat. One method often used to determine hardness is particle size index (PSI) as given in method 55-30 of the American Association of Cereal Chemists (AACC 1983), which is based on sieving and weighing ground or abraded material (Symes 1965, Miller et al 1981). In general, hard wheats require more conditioning (tempering to a higher moisture content) than do soft wheats. Hard wheats produce coarser particles that flow more readily than fine ones (Neel and Hoseney 1984). This leads to better bran cleanup and higher extraction rates for hard wheats at any given flour color or ash content. Additionally, hard wheat flours contain a significant amount of mechanically damaged starch granules (Moss 1978, Evers and Stevens 1985). Typically, soft wheat flours are preferred for pastry, cake, or cookie processes, whereas hard wheat flours are preferred for bread processes.

Wheat endosperm hardness is controlled by one major gene (Symes 1965, 1969), which is modified by other genes (Symes 1965, Yamazaki and Donelson 1983, Law and Krattiger 1987) and, to a lesser extent, by environment (Miller et al 1984, Pomeranz et al 1985). Several explanations have been offered for the mechanism(s) involved (Symes 1969, Barlow et al 1973, Simmonds et al 1973, Stenvert and Kingwood 1977, Greenwell and Schofield 1986, Hoseney 1987).

Proteins extracted from isolated starch have been separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Greenwell and Schofield 1986). Consistent positive correlations have been found between the presence of an intense 15-kDa protein band and endosperm softness (Greenwell and Schofield 1986, Bakhella et al 1990, Malouf et al 1992). This protein may reduce adhesion between starch granules and endosperm proteins or may be a fortuitous marker for the absence of other (as yet unknown) components that act as the binding forces between starch and protein.

Malouf et al (1992) demonstrated that tablets made from soft wheat starch had a smaller tensile strength compared to those made from hard wheat starch. However, when the 15-kDa protein was removed from the soft wheat starch, the resulting tablets had a tensile strength similar to that of the tablet made from hard wheat starch. This strongly suggests that the 15-kDa protein

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was responsible for the difference in hardness.

Crossing hard and soft wheats leads to progeny that are either hard or soft. Both hard and soft lines can be backcrossed to either parent, resulting in lines that are near-isogenic except for the hard or soft trait (Symes 1965). Symes (1969) used such near-isogenic lines of soft wheats and hard wheats to study the effect of wheat hardness on bread-baking quality. Hard wheat flours typically result in sugar-snap cookies with smaller diameters than those of soft wheat flours (Sollars 1956, Yamazaki et al 1977, Abboud et al 1985). The objective of this study was to use cookie baking to quantify end-use product quality of the near-isogenic lines. Milling characteristics, starch damage, gliadin protein patterns, and the presence or absence of the 15-kDa starch granule protein were determined and compared with the cookie diameter for each line.

MATERIALS AND METHODS

Wheat Samples

Two sets of near-isogenic lines, including reciprocals, for soft wheat kernels and hard wheat kernels were used in this study. The lines were developed by Symes (1969), with hardness classifications based on particle size index (PSI). Each line had seven backcrosses to the recurrent parent. Both recurrent parents, Heron, a soft wheat, and Falcon, a hard wheat, were used as controls. The 30 backcross lines of Heron/7*Falcon and Falcon/7*Heron contained both hard and soft samples. The seeds were generously supplied by Michael Mackay, curator of the Australian Winter Cereals Collection (Tamworth, NSW).

All the lines in this study were grown in a 49-m^2 soilbed in a glasshouse. Nutrients and moisture were applied to avoid any plant stress, and temperatures were maintained at 18–20°C throughout the growing period.

Pike, a U.S. soft wheat, and Mustang, a U.S. hard wheat, were obtained from the USDA-ARS, U.S. Grain Marketing Research Laboratory (Manhattan, KS). Marquis, a Canadian spring wheat, was obtained from Robert Busch, USDA-ARS (St. Paul, MN).

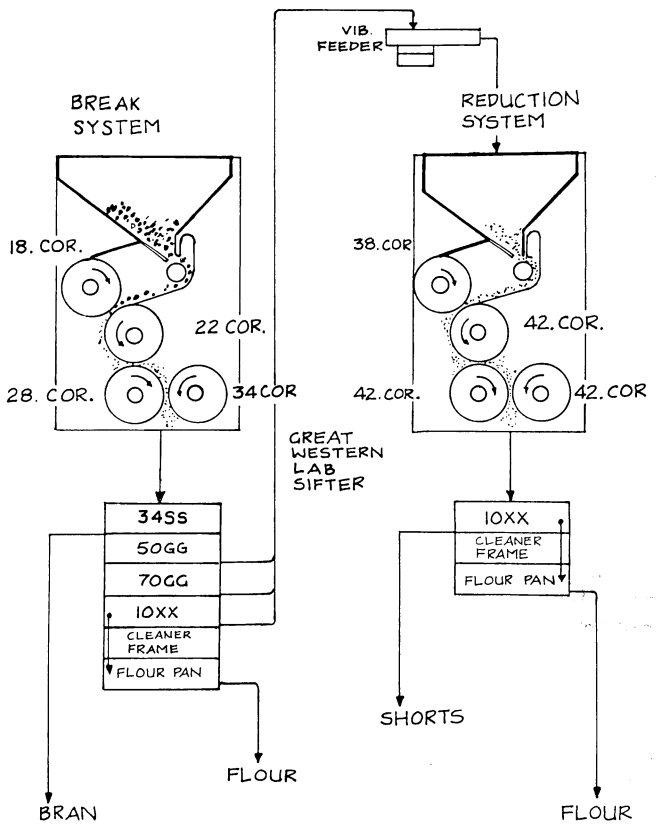

Fig. 1. Flow of the modified Brabender Quadrumat Senior experimental mill used for milling all wheats.

Milling

The coded samples were conditioned before milling by adding sufficient water to bring the moisture content of each sample to 15% and allowing them to temper for approximately 18 hr. Milling was performed using the modified micromilling flow shown in Figure 1. Grinding was accomplished with the break and reduction heads from a Brabender Quadrumat Senior experimental mill (C. W. Brabender Instruments, South Hackensack, NJ). A vibratory feeder was used to control the feed rate of middlings stock to the reduction head at 25 g/min.

All sifting was performed on 12-in^2 sieves rotated at 180 rpm by a Great Western laboratory sifter (Great Western Sifters, Leavenworth, KS) with a 4-in. throw. A cleaner frame, equipped with two cotton belt cleaners, was placed between a 130-μm sieve and the flour pan to keep the flour cloth clean. Sifting time was set at 2 min. Particle size distributions were calculated from weights of materials remaining on each of the sieves. All material passing through a 520-μm sieve and remaining over a 130-μm sieve was sent to the reduction head from the break sifter.

One kilogram of a hard red winter wheat was used to bring grinding heads up to operating temperature, and the coded samples were then milled in random order. Characterization of the samples, according to whether they milled as soft wheats or as hard wheats, was performed by an experienced miller as the samples were being milled.

Moisture (method 44-15A), ash (method 08-01), and protein (method 46-11A) were determined in duplicate for each flour (AACC 1983). Statistical analysis was performed using PC-SAS software (version 6.03, SAS Institute, Cary, NC).

Damaged Starch

Flour starch damage was determined enzymatically according to AACC method 76-30A (AACC 1983). Each flour was analyzed in duplicate.

Gliadin Analysis

Flour was extracted with 70% (v/v) ethanol for 1 hr (Lookhart et al 1982). Five drops (approximately 0.5 ml) of glycerol and one drop (approximately 0.05 ml) of methyl green dye solution were added to the supernatant. Electrophoresis was performed with a 6% polyacrylamide gel at pH 3.1 (Lookhart et al 1986).

TABLE I
Summary of Milling Data for Near-Isogenic Wheats

Sample	Hardness[a]	No. in Set	Break Flour (%)[b]	Combined Bran (%)[b]	Total Flour (%)[b]	Miller's Evaluation
Heron	Soft	1	29 a	25 a	72 a	Mellow
Falcon/7*Heron	Soft	5	29 a	26 a	72 a	Mellow, floury stock
Falcon/7*Heron	Hard	6	24 b	21 b	76 b	Very sharp midds and sizings
Falcon	Hard	2	23 b	22 b	77 b	Very sharp midds and sizings
Heron/7*Falcon	Hard	10	23 b	23 b	77 b	Very sharp midds and sizings
Heron/7*Falcon	Soft	9	28 a	25 a	73 a	Mellow sizings, some midds slightly sharp

[a]Classified according to Australian Winter Cereals Collection notations.
[b]Mean values followed by same letter within a column are not significantly different at $P < 0.05$.

TABLE II
Summary of Analytical Data for Near-Isogenic Wheats

Sample	Hardness[a]	Protein (%)[b]	Ash (%)[b]	Damaged Starch (%)[b]	Cookie Diameter (cm)[b]
Heron	Soft	11.3 a,b	0.51 a,b	4.4 a,b	16.7 a
Falcon/7*Heron	Soft	10.8 a	0.49 a	4.0 a	16.7 a
Falcon/7*Heron	Hard	11.9 b,c	0.49 a,b	7.6 c	15.6 c
Falcon	Hard	12.5 d	0.53 b	7.8 c	15.4 c
Heron/7*Falcon	Hard	12.0 c,d	0.53 b	7.9 c	15.3 c
Heron/7*Falcon	Soft	11.8 b,c	0.50 a,b	4.7 b	16.3 b

[a]Classified according to Australian Winter Cereals Collection notations.
[b]Mean values followed by same letters within a column are not significantly different at $P < 0.05$.

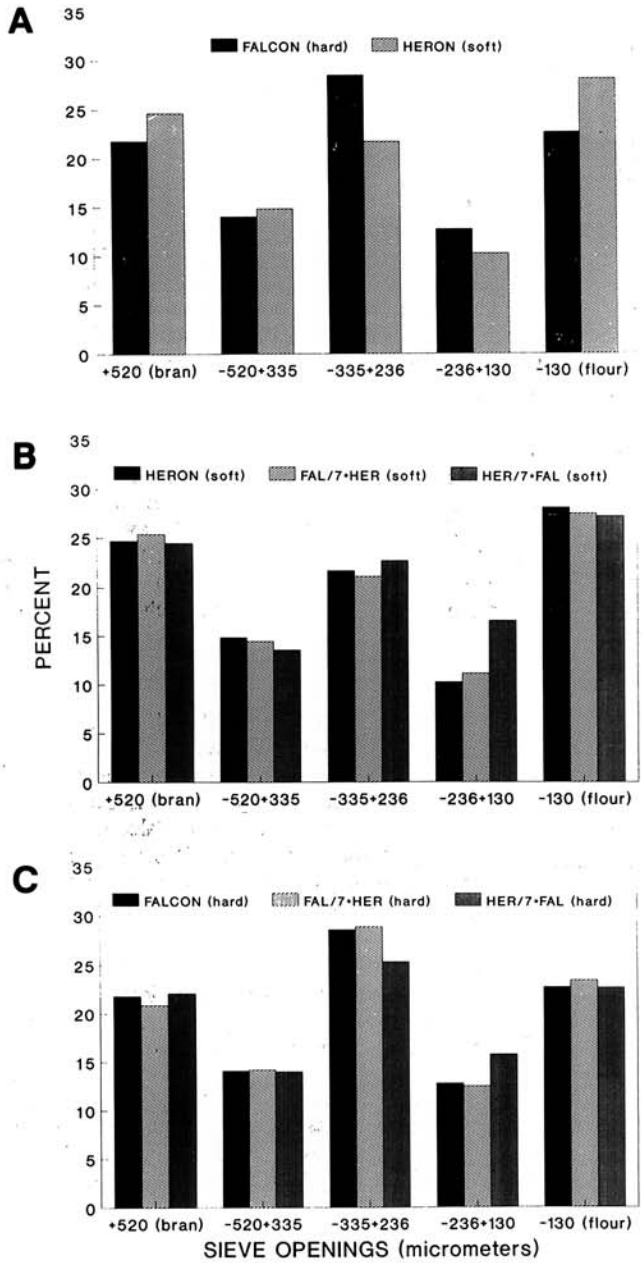

Fig. 2. Particle size distribution of material released from the break rolls. **A,** Parent wheats Falcon (hard) and Heron (soft). **B,** Soft wheats. **C,** Hard wheats. +520 = overs of screen with 520-μm openings; −520+335 = throughs of screen with 520-μm openings and overs of a screen with 335-μm openings; −335+236 = throughs of screen with 335-μm openings and overs of a screen with 236-μm openings; −236+130 = throughs of screen with 236-μm openings and overs of a screen with 130-μm openings; −130 = throughs of screen with 130-μm openings.

Temperature was kept constant (20°C). Electrophoresis voltage (500 V) and running time (2 hr) optimized band resolution. Staining and destaining procedures were based on those described by Lookhart et al (1982). The cultivars Marquis and Falcon were used as electrophoresis standard samples.

Starch Granule Protein Analysis

Five grams of flour and 3.1 ml of water were mixed into a dough, then allowed to relax for 15 min. The dough was washed with 200 ml of cold water, and the gluten and starch were separated. The starch suspension was centrifuged at 2,000 × g for 20 min, resuspended, and centrifuged again. Starch granule proteins were extracted at 50°C with 2 ml of 1% (w/v) SDS for 20 min and centrifuged at 2,000 × g for 20 min. Six milliliters of acetone was added to the supernatant to precipitate the proteins. Proteins were separated by SDS-PAGE with a linear gradient of acrylamide from 7.5 to 25% (m/v). Run time was approximately 5 hr (Malouf et al 1992).

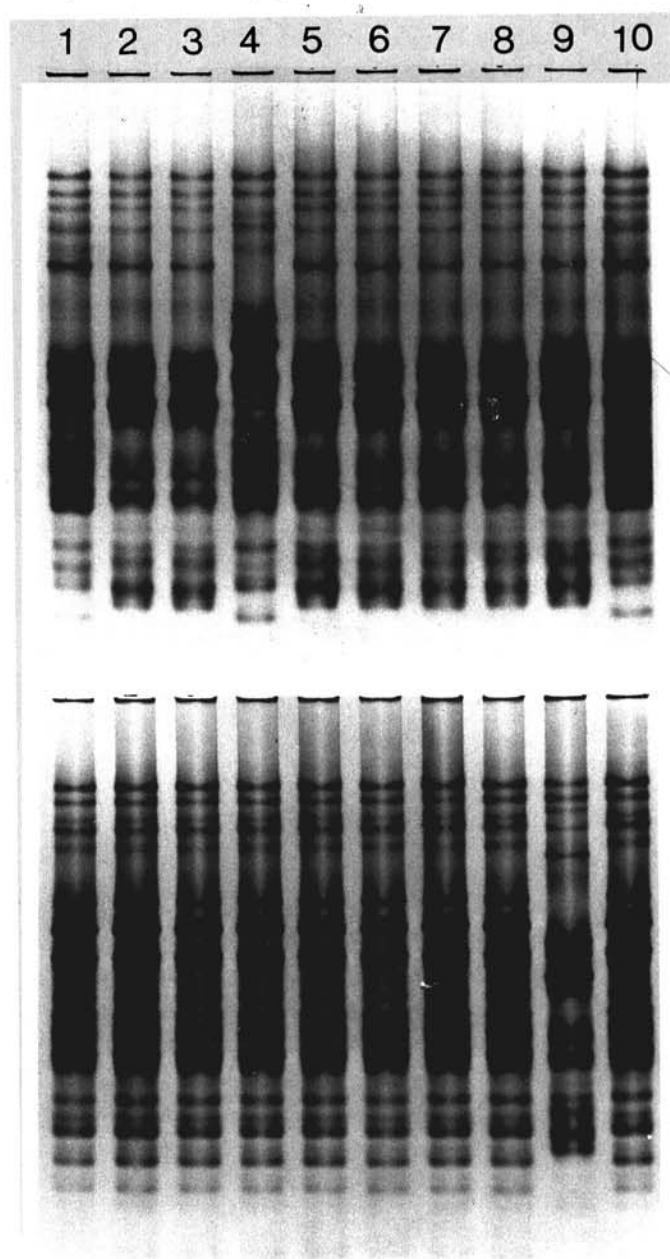

Fig. 3. Acid-polyacrylamide gel electrophoresis mapping of gliadin proteins showing the patterns of both hard and soft samples. **Top,** Lanes 1 and 10, Marquis; Lanes 2 and 3, Heron/7*Falcon (soft); Lane 4, Heron (soft); Lane 5, Falcon (hard); and Lanes 6–9, Heron/7*Falcon (hard). **Bottom,** Lanes 1–7, Falcon/7*Heron (hard); Lane 8, Heron (soft); Lane 9, Falcon (hard); and Lane 10, Falcon/7*Heron (soft).

Cookie Baking

Cookies were baked using a modification of the procedure of Finney et al (1950). Modifications included using a constant baking absorption of 25% flour basis (Abboud et al 1985) and the addition of 0.23% distilled monoglycerides (Myverol, Eastman Chemicals Division, Kingsport, TN) to the shortening cream. Two cookies were baked in each test, and tests were run in triplicate. Cookies were evaluated on the basis of diameter (larger spread denoting superior flour quality).

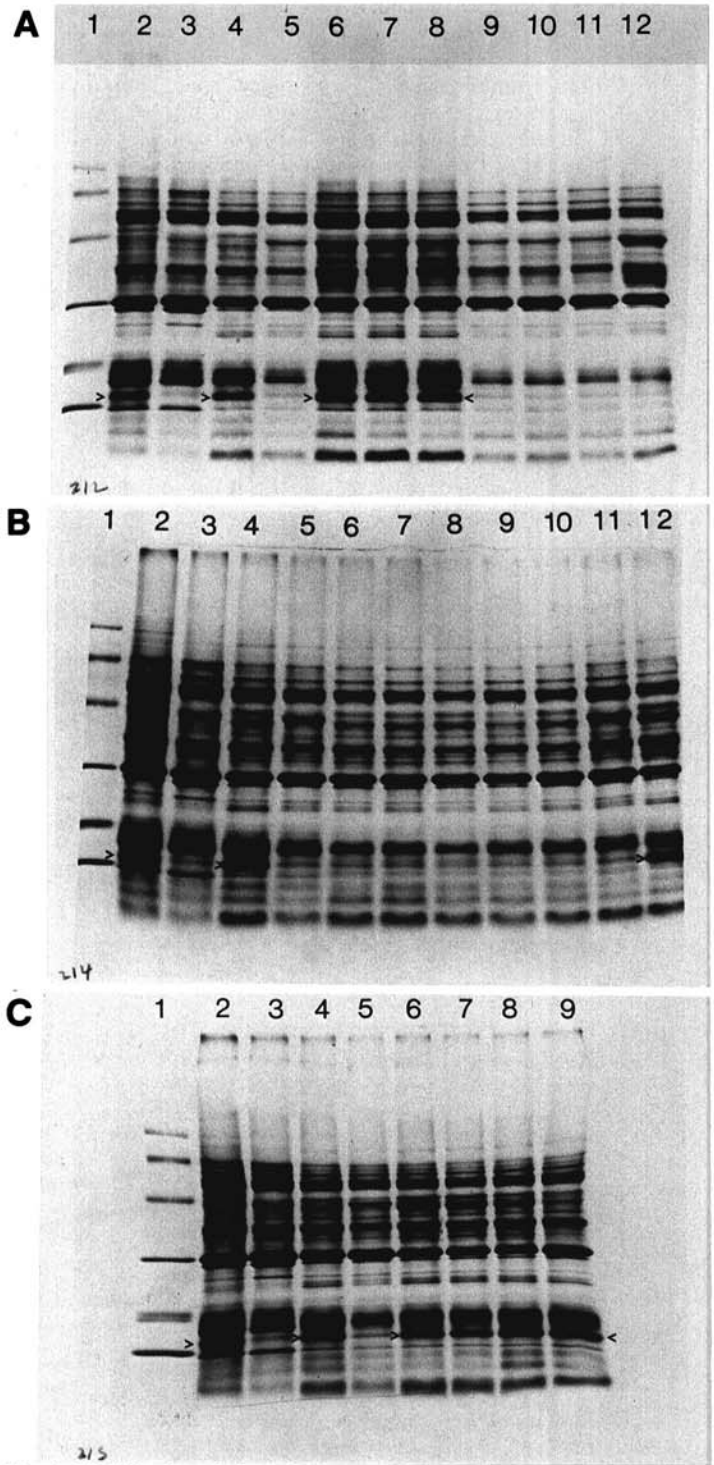

Fig. 4. Classification of wheat hardness, using the 15-kDa starch granule protein as an index. For all gels (**A–C**): Lane 1, molecular weight markers; Lanes 2 and 3, proteins extracted from U.S. soft (Pike) and hard (Mustang) wheats; Lane 4, Heron (soft); Lane 5, Falcon (hard). **A,** Lanes 6 and 7, Heron/7*Falcon (soft); Lane 8, Heron (soft); Lane 9, Falcon (hard); and Lanes 10–12, Heron/7*Falcon (hard). **B,** Lanes 6–11, Falcon/7*Heron (hard); Lane 12, Heron (soft). **C,** Lanes 6–9, Falcon/7*Heron (soft). The 15-kDa protein in soft wheat starch is indicated by arrow on the left side of the gel.

RESULTS AND DISCUSSION

Milling

Coded samples, tempered under constant conditions, were judged for hard or soft characteristics during milling and then compared with the characterization supplied by the Australian Winter Cereals Collection. The hard or soft milling characterization from this study was in complete agreement with the Australian Winter Cereals Collection classification. The soft wheats produced higher percentages of break flour and bran than the hard wheat lines did (Table I). Ash values of the straight-grade flours of each near-isogenic line (Table II) were found to be similar to that of its recurrent parent. Flour protein values of the soft wheats (isolines and parents) were not significantly different than those of the hard wheats (isolines and parents).

Milling characteristics of the near-isogenic lines did not follow the milling characteristics of their respective backcross parents but, rather, followed the PSI criteria on which they had been selected. The hardness of the 16 hard lines was independent of the backcross parent, as was the hardness of the 14 soft lines.

The particle size distribution of the ground material from the break rolls for the Falcon (hard) and Heron (soft) parents is shown in Figure 2A. More break flour was produced from Heron than was produced from Falcon. Soft wheats typically give flours of finer particle size. Figures 2B and 2C compare the means of the particle size distributions of the near-isogenic lines with those of their respective parents. Means of the stock quantities for either the soft or the hard samples were nearly identical, independent of their parentage.

Starch Damage

As might be expected, the wheats that had been classified as hard had greater amounts of starch damage after milling than did the soft wheats (Table II). In both cases, the average values of starch damage for the soft wheat progenies and the soft parent (Heron) were not significantly different. The wheats that were classified as hard progenies did not differ in starch damage from the hard recurrent parent, Falcon.

Gliadin Proteins

Gliadin proteins were mapped by acid-PAGE to determine whether they were truly near-isogenic. The patterns of both hard and soft samples of the Heron/7*Falcon lines matched those of the recurrent hard parent (Falcon) (Fig. 3A). Likewise, the patterns of the hard and soft samples of the Falcon/7*Heron lines all matched those of the soft parent (Heron) (Fig 3B). Therefore, genetic fingerprinting of the gliadin proteins did not differentiate end-use quality characteristics of those flours.

Starch Granule Proteins

Starch granule proteins extracted from a typical soft wheat (Pike) and a typical hard wheat (Mustang) were used for comparison in this investigation. The results were consistent with previous studies (Greenwell and Schofield 1986, Bakhella et al 1990) in that the 15-kDa starch granule protein was more abundant in soft wheats than in hard wheats (Fig. 4). The presence of the softness marker (15-kDa band) follows the physical softness-hardness parameters and not the parentage of the wheat.

Cookie Baking

The flours from each of the near-isogenic lines gave cookies with a wide range of diameters. Flours from soft milling wheats resulted in larger diameter cookies than did flours from hard milling wheats (Table II). Flours from soft milling wheats resulting from backcrosses with the hard wheat as the recurrent parent (Heron/7*Falcon) gave cookies with significantly smaller diameter than the other soft wheat flours did. No significant differences were seen among flours of the hard wheat lines.

CONCLUSIONS

Abboud et al (1985) reported that protein content has only a minor effect on cookie diameter, in contrast to an unidentified genetic factor that strongly affects cookie spread. In the present study, where flour protein quantity varied, the factors associated with hardness had a major influence on cookie diameter, milling characteristics, and starch damage. Gliadin proteins, as fingerprinted by A-PAGE patterns, were not useful in differentiating end-use characteristics. The softness factor, marked by high levels of the 15-kDa starch protein, consistently predicted suitability for cookie baking. It is not clear from this study whether the 15-kDa protein is responsible for the difference in baking quality or is just a useful marker.

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