Determining Endosperm Texture of Developing Hard and Soft Red Winter Wheats Dried by Different Methods Using the Single-Kernel Wheat Characterization System

D. B. BECHTEL,[1,2] J. D. WILSON,[1] and C. R. MARTIN[1]

ABSTRACT

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Field-grown hard (Pioneer 2163, Arkan, Karl, Newton, TAM 107, and TAM 200) and soft (Caldwell and Clark) red winter wheats were harvested at 15, 18, 21, 23, 25, 28, and 35 days after flowering (DAF). Wheat was dried by a variety of methods: air-dried in the spike at 28°C; oven-dried in the spike at 40°C; freeze-dried following both freezing and threshing under liquid nitrogen; field-dried mature grain; and freeze-thawed and air-dried, in which samples were first frozen in liquid nitrogen, thawed at room temperature (23°C), and then air-dried in the spike at 28°C. The U.S. Grain Marketing Laboratory's single kernel wheat characterization system was used to measure various grain parameters including the hardness of individual kernels. Air-dried and oven-dried samples generally had similar hardness indices when compared to that of mature grain. Soft wheats were also softer than hard samples when dried by these two methods. Freeze-dried grain had similar low values of hardness for samples harvested between 15 and 28 DAF, but mature 35 DAF grains had normal hardness indices. Freeze-thawed samples had hardness indices slightly higher than those of either air- or oven-dried grain.

Grain hardness is an important characteristic that plays a significant role in the marketing and processing of wheat both nationally and internationally. A variety of methods exist for the determination of degree of hardness. However, little is known about the mechanisms of hardness. Generally, hardness is thought to result from the strength of starch-protein interactions (Barlow et al 1973). Softness may possibly be related to the presence of the 15 kDa protein called friabilin isolated from starch of soft wheats (Greenwell and Schofield 1986, Schofield and Greenwell 1987). With the presence of friabilin in endosperm residing on the short arm of the chromosome 5D (Jolly et al 1993), friabilin consisting of a family of highly related proteins (Morris et al 1994) and the presence of two classes of starch-bound polar glyco- and phospholipids found in the same pattern as friabilin (Greenblatt et al 1995) suggests that the starch interface must play an important role in determining grain texture in the wheat caryopsis. Little information presently exists as to when endosperm texture (hardness) develops and what effects methods of drying have on hardness. These questions were investigated using the U.S. Grain Marketing Research Laboratory's (USGRL) single kernel characterization system (SKWCS) to measure various grain parameters.

MATERIALS AND METHODS

Six hard red winter (HRW) and two soft red winter (SRW) wheats were grown on the Kansas State University agronomy farm near Manhattan, KS. Spikes of the HRW (TAM 107, TAM 200, Arkan, Newton, Karl, and Pioneer 2163) and SRW (Caldwell and Clark) cultivars were tagged at flowering and harvested at 15, 18, 21, 23, 25, 28, and 35 days after flowering (DAF). Wheat was dried by placing the spikes in ovens with a fan circulating air at either 28°C (air-dried) or 40°C (oven-dried) for approximately one week. Some wheat heads were freeze-dried by first plunging into liquid nitrogen, followed by threshing in liquid nitrogen, and finally by lyophilizing the individual kernels. Two other samples (Karl and Clark) were also frozen in liquid nitrogen, allowed to thaw at room temperature, and then air-dried in the spike at 28°C. Grain harvested at 35 DAF was considered fully mature for this study and was not processed further. Before measurement in the SKWCS, all grain was moisture-equilibrated to the operating range of the instrument (9–14%).

Grain hardness of two replicates of 50 kernels each was measured using the SKWCS. (This prototype was developed at the USGRL and is now commercially available as the Perten 4100, Perten Instruments North America, Reno, NV). A Student's *t*-test showed a *P*-value ($T \leq t$) one-tail for experimental error difference between replicates was 0.47 with a critical *t* of 1.7. Each kernel was hand-placed in the instrument in lieu of using the automated single-kernel feeder because of the great variation in the size of grains. Individual grain weight, grain size, and moisture content were measured for each kernel by the instrument. The entire full-wave crush profile electronically obtained from the load cell of the instrument was saved for each kernel. The instrument's computer software then calculated a hardness index for each sample based on the kernel crush profile, moisture, size, and weight (Martin et al 1993). Data for each 50-kernel replicate was averaged to yield the hardness index and standard deviations for each replicate set were calculated (Table I).

RESULTS

Drying had a profound effect on the hardness index of grain samples. Generally, oven-dried and air-dried wheats had similar hardness indices (Table I). The air-dried samples were used to simulate the field-drying conditions in the laboratory. Lyophilized developing HRW wheats were much softer than any of the other drying methods (Table I) and they maintained their natural pale green color as at the time of harvest. Air-dried, oven-dried, and freeze-thawed and air-dried samples took on the color of mature field-harvested wheats and were typically shriveled when the caryopses were young.

[1]U. S. Grain Marketing Research Laboratory, USDA-ARS, 1515 College Avenue, Manhattan, KS 66502. Mention of company or trade names is for purposes of description only and does not imply endorsement by the U.S. Department of Agriculture.
[2]Corresponding author. E-mail: don@usgmrl.ksu.edu

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Figure 1 shows the relationship of the hardness index among all air-dried wheat samples and development stage. All the hard wheat cultivars had similar curves for the hardness index, with values tending to decrease slightly at the earliest developmental stages. The two soft wheat cultivars had similar shaped curves, except at 15 DAF, where Caldwell grain showed a large increase in apparent hardness. During the earlier developmental stages, the hard wheat cultivars tended to have lower hardness indices, while the soft wheat cultivars exhibited a harder index.

Freeze-drying (lyophilizing) wheat at different developmental stages had the pronounced effect of reducing the overall hardness index of the hard wheats (Figs. 1 and 2, Table I). Mature lyophi-

TABLE I
Single Kernel Hardness Values for Wheats Dried by Different Methods at Various Stages of Development

		Hardness Index[a]				
Cultivar	**Days After Flowering**	**Air-Dried**	**Oven-Dried**	**Mature**	**Lyophilized**	**Freeze-Thawed**
2163	15	71 ± 0.0	79 ± 3.1		38 ± 3.7	
	18	78 ± 2.6	83 ± 4.2		20 ± 18.5	
	21	78 ± 3.2	90 ± 1.7		7 ± 12.3	
	23	84 ± 3.7	92 ± 0.6		17 ± 0.8	
	25	88 ± 3.2	83 ± 4.7		9 ± 8.9	
	28	83 ± 1.5	84 ± 6.3		21 ± 3.5	
	35	84 ± 1.8	75 ± 3.2	75 ± 4.8	74 ± 1.4	
Arkan	15	71 ± 1.0	68 ± 2.5		50 ± 4.1	
	18	82 ± 1.2	89 ± 2.3		16 ± 13.9	
	21	81 ± 0.2	94 ± 0.8		6 ± 13.5	
	23	90 ± 0.8	84 ± 2.7		16 ± 3.7	
	25	92 ± 0.5	90 ± 1.2		8 ± 2.2	
	28	88 ± 4.4	86 ± 0.3		18 ± 6.6	
	35	81 ± 0.2	82 ± 0.8	79 ± 0.5	75 ± 1.8	
Karl	15	80 ± 1.6	90 ± 1.8		35 ± 1.5	
	18	83 ± 7.4	91 ± 0.4		8 ± 10.4	110 ± 4.1
	21	82 ± 3.0	93 ± 0.7		−4 ± 7.0.	99 ± 0.9
	23	89 ± 2.3	88 ± 1.0		14 ± 0.0	100 ± 3.4
	25	90 ± 3.5	93 ± 4.8		−2 ± 8.8	96 ± 5.2
	28	84 ± 4.9	86 ± 5.6		13 ± 0.7	94 ±1.3
	35	81 ± 0.9	82 ± 4.9	75 ± 1.0	79 ± 4.7	77 ± 1.2
Newton	15	71 ± 4.7	62 ± 3.7		57 ± 6.1	
	18	74 ± 1.8	77 ± 5.4		25 ± 17.6	
	21	78 ± 2.2	81 ± 1.4		25 ± 9.1	
	23	86 ± 3.1	99 ± 0.7		49 ± 4.2	
	25	95 ± 5.9	91 ± 1.0		35 ± 5.0	
	28	92 ± 1.3	85 ± 7.1		14 ± 4.8	
	35	91 ± 4.5	84 ± 3.3	81 ± 5.1	89 ± 0.9	
TAM107	15	81± 7.6	73 ± 0.7		46 ± 2.2	
	18	84 ± 3.6	85 ± 4.8		28 ± 12.9	
	21	84 ± 1.1	81 ± 3.6		12 ± 9.6	
	23	87 ± 14.0	94 ± 1.9		36 ± 2.3	
	25	91 ± 5.5	92 ± 2.9		25 ± 2.2	
	28	87 ± 4.4	89 ± 0.9		40 ± 7.7	
	35	87 ± 0.6	80 ± 2.2	84 ± 0.5	83 ± 1.4	
TAM200	15	81 ± 1.2	78 ± 0.1		63 ± 4.2	
	18	75 ± 5.8	84 ± 6.6		33 ± 12.0	
	21	78 ± 3.2	83 ± 10.7		16 ± 11.1	
	23	90 ± 5.9	101 ± 1.0		42 ± 7.6	
	25	85 ± 2.4	93 ± 3.8		26 ± 2.9	
	28	81 ± 6.5	86 ± 5.2		46 ± 3.9	
	35	78 ± 1.0	57 ± 2.8	64 ± 4.8	57 ± 3.6	
Clark	15	33 ± 1.3	55 ± 3.7		32 ± 1.3	
	18	27 ± 7.0	37 ± 0.4		−1 ± 14.8	
	21	23 ± 0.5	25 ± 1.7		−6 ± 14.5	
	23	23 ± 1.9	30 ± 2.6		2 ± 2.4	
	25	27 ± 2.3	28 ± 0.4		−5 ± 4.9	
	28	32 ± 6.9	31 ± 3.8		2 ± 5.4	
	35	24 ± 2.0	23 ± 0.2	20 ± 3.1	21 ± 0.6	
Caldwell	15	65 ± 3.1	56 ± 1.7		45 ± 2.2	
	18	34 ± 4.4	40 ± 6.5		20 ± 11.7	48 ± 1.3
	21	35 ± 5.6	25 ± 0.8		21 ± 15.7	30 ± 5.0
	23	29 ± 2.3	37 ± 1.5		14 ± 1.5	31 ± 0.1
	25	32 ± 1.2	40 ± 6.0		11 ± 6.3	27 ± 5.8
	28	29 ± 8.3	31 ± 8.3		6 ± 3.8	24 ± 9.0
	35	29 ± 1.4	38 ± 2.5	26 ± 4.5	19 ± 0.7	29 ± 0.9

[a] Hardness index is mean of two measurements of 50 kernels each and sample standard deviation.

lized 35 DAF grain had hardness indices similar to those of the field-dried and harvested grain (Table I). The 15 DAF lyophilized grain showed the same phenomenon of increased hardness that was exhibited by the air- and oven-dried soft wheat (Table I). There was also an unexplainable but noticeable peak present at 23 DAF in the lyophilized samples (Fig. 2) in comparison to a slight peak observed between 23 and 25 DAF for air- and oven-dried samples (Fig. 1 and Table I).

The HRW Karl and SRW Caldwell grain was also subjected to a freeze-thaw cycle followed by air-drying. The freeze-thaw cycle caused the Karl grain to have higher hardness indices than any of the other drying processes, whereas freeze-thawing had a less pronounced effect on the hardness indices of Caldwell caryopses (Figs. 3 and 4).

DISCUSSION

The presiding theory regarding endosperm hardness holds that it is the strength of starch-protein interactions that causes endosperm hardness (Barlow et al 1973). That the interaction between starch and protein results in the phenomenon called hardness is supported by the fact that proteins from hard and soft wheats have mechanical properties similar to those of the starches from hard and soft wheats (Barlow et al 1973). Finding the 15 kDa protein friabilin consistently associated with soft wheat starch (Greenwell and Schofield 1986, Schofield and Greenwell 1987), and more recently, differences between bound polar lipids on wheat starch granules (Greenblatt et al 1995) gives further evidence that the starch-protein interactions are possibly mediated by the surface of the starch granule and could play a significant role in determining endosperm hardness. We have previously shown that such starch-protein interactions can influence the size and shape of starch granules that can be visualized with digital image analysis (Bechtel et al 1993, Zayas et al 1994). Consequently, the type or degree to which these interactions occur between starch and protein during growth and development may result in the phenomenon called grain hardness.

The present study was undertaken to determine when hardness (softness) developed in wheat caryopses. The results clearly show that hard wheat grain is hard throughout development and that soft wheat grain is soft during development. The curious trend for HRW wheats to appear softer and for soft wheats to appear harder earlier in development when caryopses are small is a phenomenon that was observed during the development of the SKWCS. The phenomenon is possibly machine-induced as a result of software or instrument design as the instrument was designed to work with fully mature, normal-sized wheat kernels and not the small shriveled caryopses that were processed through it.

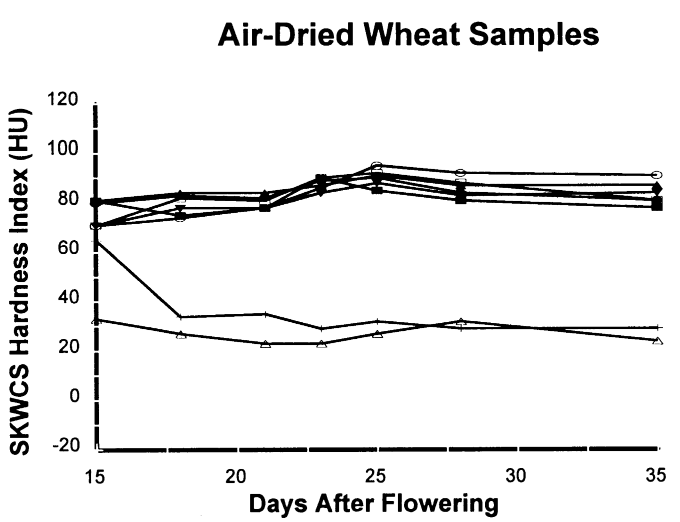

Fig. 1. Hardness index vs. development stage of wheats that were dried in an oven at 28°C. Pioneer 2163 (▼); Arkan (□); Karl (●); Newton (○); TAM107 (▲); TAM200 (■); Clark (∇); Caldwell (+).

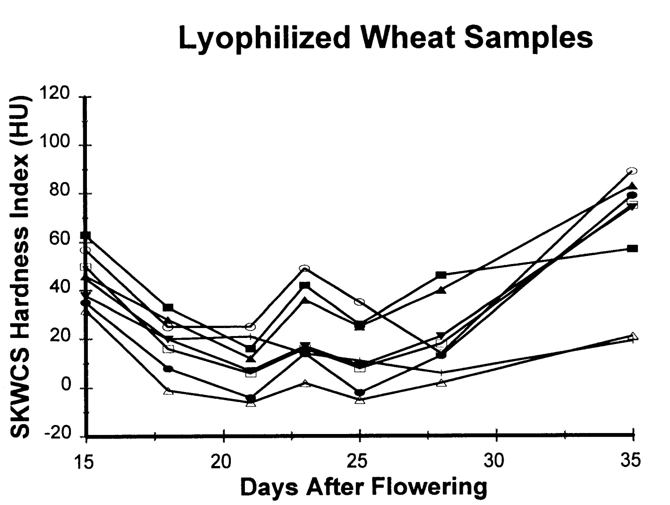

Fig. 2. Hardness index vs. development stage of wheats that were lyophilized. Pioneer 2163 (▼); Arkan (□); Karl (●); Newton (○); TAM107 (▲); TAM200 (■); Clark (∇); Caldwell (+).

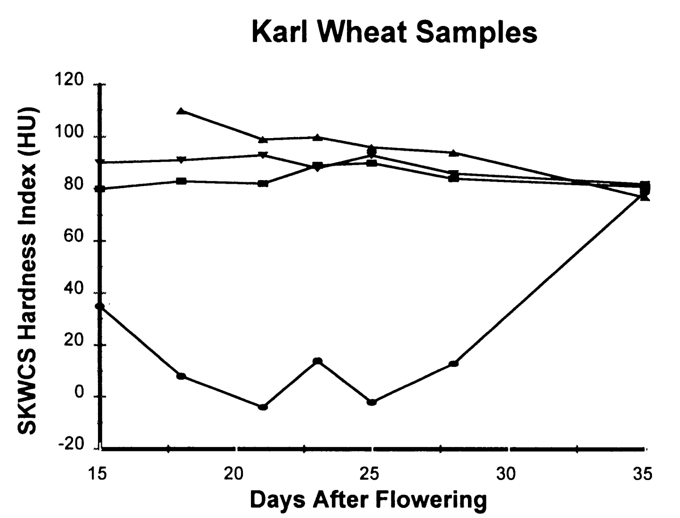

Fig. 3. Hardness index vs. stage of development for Karl wheat. Air-dried (■), oven-dried (▼), lyophilized (●), and freeze-thawed (▲).

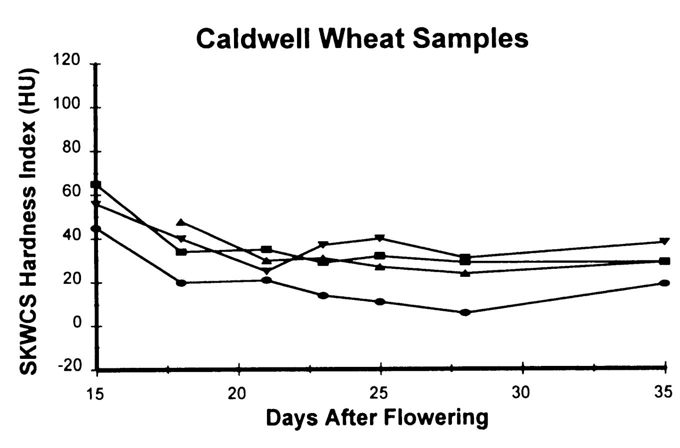

Fig. 4. Hardness index vs. stage of development for Caldwell wheat. Air-dried (■), oven-dried (▼), lyophilized (●), and freeze-thawed (▲).

Air- and oven-drying did not substantially change the hardness index for either soft or hard wheats used in this study. A Student's *t*-test comparing these two drying regimens showed a *P*-value ($T \leq t$) one-tail for experimental error difference between replicates was 0.2 with a critical *t* of 1.7. Freeze-drying had a marked effect on the hard wheat grain by reducing the hardness index to that of soft wheat (Fig. 2). The effect was less pronounced for the soft wheat grains, but the lyophilized developing soft wheat caryopses were still softer than the air-dried ones (Table I). Lyophilization is a way of drying biological samples in a somewhat natural state without a great deal of enzymatic activity during removal of water. The retention of the green color and size of the caryopses tend to support the view that little cellular degradation and alteration occurred during lyophilization. The other drying methods all yielded wheat grains that were shriveled and brown. Our previous experiences with the preservation of wheat endosperm tissue by freezing (Bechtel and Barnett 1986a,b) suggests that samples that were freeze-thawed and then dried had extensive predrying structural damage inflicted on the endosperm tissue.

We contend that the natural mixing of cellular components during the final stages of drying of the wheat caryopsis after physiological maturity is reached results in the phenomenon called wheat hardness by allowing certain cellular components to bind to the starch granules. This model is further substantiated by the fact that the hardness index for hard wheats starts to increase for the lyophilized samples once physiological maturity is reached (≈25 DAF) (Fig. 2). What components are involved in establishing the binding (or lack of binding) needs to be resolved, possibly through microscopy. Possibly the mixing of cellular components allows friabilin and other molecules to bind to starch granules and prevent a tight binding of starch to storage protein in the soft wheats, leading to a soft wheat. One perplexing dilemma is the fact that storage proteins have not been significantly synthesized at 15–18 DAF to allow for much storage protein to be present to bind to the numerous starch granules already present.

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