Quality Characteristics of Hard Red Spring and Winter Wheats. I. Differentiation by Reversed-Phase High-Performance Liquid Chromatography and Milling Properties

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ABSTRACT

Reversed-phase high-performance liquid chromatography was used to analyze 15 commercial U.S. hard red spring (HRS) and hard red winter (HRW) wheats. Protein content of these samples ranged from 12.0 to 14.4%. Chromatographic analysis indicated that the HRS and HRW wheats differed in their 70% ethanol-soluble protein (gliadin) chromatograms (absorbance at 210 nm). Total peak areas (ratio to major peak area) for the two late-eluting (more hydrophobic) peaks were larger for HRS than HRW wheats. Milling and analytical properties of these wheats were also compared. HRS wheats showed higher values than HRW wheats for total flour yield and milling score. Ratios of the total amount of break flour to reduction flour were lower for the HRS wheats than for HRW wheats. Somewhat higher damaged starch content and ratio of starch tailings to total isolated starch occurred for HRS wheats. There was little difference in the ratio of free lipid to total lipid content between the two wheat classes.

Wheat characteristics and quality are influenced by both genotype and environment. Because of the importance of quality to millers and bakers, various procedures for differentiating wheat classes are used.

Early efforts to differentiate wheat classes and cultivars were based primarily on grain morphology (Anon 1957, 1984) and kernel hardness (Kosmolak 1978, Simmonds 1974). More recently Zayas et al (1985) used discriminant analysis to differentiate wheat cultivars by image analysis. The differentiation of wheat classes has became more difficult recently due to the crossing of spring and winter wheats.

Since storage proteins are nearly invariable qualitatively within a variety, electrophoresis is routinely used for wheat identification (Wrigley et al 1982). Recently, Bietz et al (1984) and Marchylo et al (1988) showed that reversed-phase high-performance liquid chromatography (RP-HPLC) of gliadins also has good potential for wheat varietal identification.

U.S. hard wheats have long been classified as spring or winter types. For various reasons, many feel that this classification should be maintained. In Japan, for example, hard red spring (HRS) wheats are preferred for specific products. We therefore examined the potential of using RP-HPLC to classify HRS and hard red winter (HRW) wheats and compared the milling and flour analytical properties of these two classes.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals used were of reagent grade or HPLC grade.

Wheat Samples

Fifteen samples each of HRS and HRW wheat were used. All samples were commercial wheats exported from the United States in 1987. Protein content of the HRS and HRW wheats ranged from 12.1 to 14.2% and from 12.0 to 14.4%, respectively (Table I). These samples were selected to represent nearly equal protein content. Moisture, ash, and protein content were analyzed in duplicate according to AACC methods (1979).

Milling

Wheat milling was performed on a Buhler laboratory mill by the procedure specified by the manufacturer. After cleaning the wheat samples with a Carter dockage tester, they were tempered to 15.5% grain moisture for 24 hr. Each 5 kg was then milled at a feed rate of 45 g/min as described by Nagao et al (1976).

TABLE I
Ratio of Peak Areas of Region d to Major Peak Area (peak 1) for Reversed-Phase High-Performance Liquid Chromatograms (A_{210}) of 70% Ethanol-Soluble Proteins (gliadins)

	Peak Area (%)		
Protein (%)[a]	PI[b]	PII[b]	Total[c]
Hard red spring			
12.1	32.3	66.2	98.5
12.3	34.4	64.1	98.5
12.3	25.6	75.5	101.1
12.4	24.5	73.1	97.6
12.5	26.2	76.6	102.8
12.9	34.1	57.9	92.0
13.0	39.1	62.3	101.4
13.1	41.0	55.9	96.9
13.2	30.7	62.2	92.9
13.2	33.1	57.8	90.9
13.9	47.6	43.7	91.3
13.9	48.8	46.7	95.5
14.0	52.8	38.1	90.9
14.0	53.5	37.2	90.7
14.2	45.3	42.8	88.1
Mean	37.9	57.3	95.3
SD	9.80	13.13	4.63
Hard red winter			
12.0	43.9	15.7	59.6
12.2	26.6	12.7	39.3
12.4	43.9	25.0	68.9
12.5	44.0	20.3	64.3
12.5	39.8	19.6	59.4
12.8	41.2	18.0	59.2
12.9	38.0	17.8	55.8
12.9	31.0	11.6	42.6
13.0	38.7	19.0	57.7
13.1	35.4	19.9	55.3
13.8	37.0	10.4	47.4
13.9	41.4	15.9	57.9
14.1	39.2	10.7	49.9
14.3	39.4	16.8	56.2
14.4	34.3	14.2	48.5
Mean	38.3	16.5	54.8
SD	4.89	4.09	7.94

[a] Wheat protein content (N × 5.7) on a 14.0% moisture basis.
[b] Peak I and peak II. Values are expressed as a percentage of major peak area (peak 1).
[c] Peak I + peak II.

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Spacings adjusted for the 2B, 3B, 1M, and 3M rolls were 0.1, 0.08, 0.05, and 0.02 mm, respectively, which fixed the 1B and 2M rolls in positions suitable for our purposes.

Calculation of Milling Score

Milling yield expressed on a percentage basis was calculated on the basis of total recovered product (as is moisture basis). Total flour yield represents a composite of the six flour streams from the Buhler laboratory mill.

Milling score was calculated using flour yield, flour ash, milling time, and patent flour yield as follows:

$$\text{milling score} = 100 - (80 - A) - 50(B - 0.30) - 8.4 - 0.5(65 - C)$$

where A is total flour yield, B is ash content of straight grade flour, and C is patent flour yield (1B + 2B + 1M + 2M). This method is routinely used for the wheat milling quality assessment in Japan.

Damaged Starch

Damaged starch content in the straight grade flour was analyzed according to AACC (1979) method 76-30A. This method is based on the susceptibility of starch granules to hydrolysis by α-amylase.

Preparation of Starch

Starch was isolated from 10 g of straight grade flour by the dough ball process (Pomeranz 1971). The starch suspension obtained by washing the dough ball was centrifuged (5,000 × g, 10 min). After discarding the supernatant, starch tailings (upper starch layer) were separated from the prime starch (lower layer) with a spatula. The ratio of starch tailings to total starch (starch tailings + prime starch) was expressed as a percentage on a dry basis.

Lipid Extraction

Free lipids were extracted from 10-g samples of straight grade flour with n-hexane by shaking overnight. Total lipids were extracted in the same manner except that water-saturated butanol was used (Mecham and Mohammad 1955).

Extraction of 70% Ethanol-Soluble Proteins (Gliadins)

Wheat samples were ground in a coffee grinder to pass through an 80-mesh sieve. Gliadins were extracted from the ground wheats (400 mg) with 70% ethanol (v/v, 8.0 ml) for 30 min with continuous agitation. After centrifugation (30,000 × g, 10 min), a clear supernatant was obtained. The supernatant was filtered through a Millipore filter (0.45 μm) prior to RP-HPLC analysis.

RP-HPLC

RP-HPLC was performed as described by Bietz (1983) and Bietz et al (1984). The RP-HPLC system was composed of a Hitachi 655A-12 liquid chromatograph, a 655A variable wavelength ultraviolet monitor, a 655A-40 autosampler, an L-5000 LC controller, and a D-2000 chromato-integrator. Samples (50 μl) were injected into a 150 × 4.6 mm i.d. Nucleosil 5C 18 RP-P (C18) column. A linear gradient from 80% solvent A (15% acetonitrile [ACN] + 0 05% trifluoroacetic acid [TFA]) + 20% solvent B (80% ACN + 0.05% TFA) to 45% solvent A + 55% solvent B at 1.0 ml/min was used. The column was operated at 40°C. Eluted components were detected at 210 nm.

Peak time reproducibility was within accuracy of the HPLC pump (1%), and the coefficient of variation of peak area analyzed five times for one of the HRS wheat samples (12.1% grain protein) was 1.95%.

Statistical Analysis

Data were statistically analyzed on a personal computer (PC 9801 VM, NEC Co., Japan) by using the commercially available software program developed by Social Survey Research Information Co., Japan.

RESULTS AND DISCUSSION

RP-HPLC

RP-HPLC of storage proteins can identify wheat cultivars (Bietz 1983, Burnouf et al 1983, Bietz et al 1984, Bietz and Cobb 1985, Marchylo et al 1988). Huebner and Bietz (1986) also studied the relationship between baking quality and the amount of a specific gliadin fraction (late-eluting peaks) using RP-HPLC. Because most marketed U.S. wheats are mixtures of cultivars within a particular class, we analyzed gliadin from ground grain rather than a single kernel. In this study, HRS wheats appeared to differ from HRW wheats, while samples in each class appeared homogeneous. Figures 1–5 show gliadin (70% ethanol-soluble) chromatograms (absorbance at 210 nm) for the HRS and HRW wheats studied. If the chromatograms are divided into regions a–d and major peak 1, the HRS wheats differ from HRW wheats in having a larger ratio of region d (peak I + peak II) to peak 1. The difference, however, decreased as wheat protein content increased (beyond 13.8%; Figs. 4 and 5). Because protein contents and their

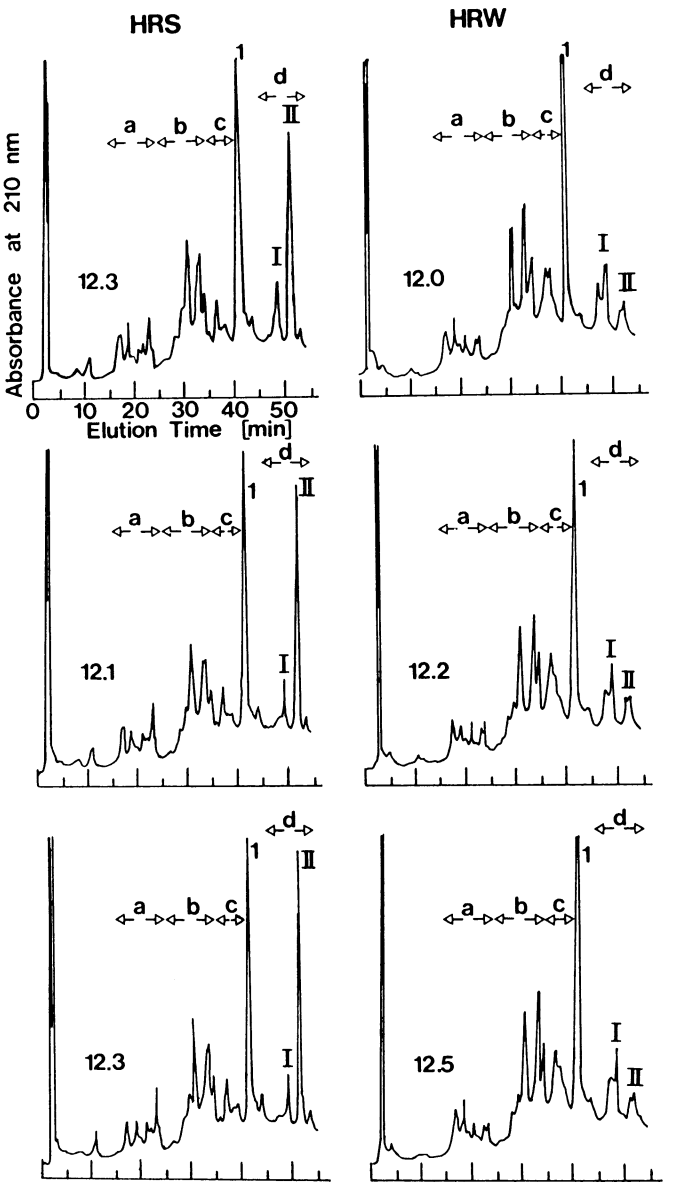

Fig. 1. Comparison of reversed-phase high-performance liquid chromatograms (absorbance at 210 nm) of 70% ethanol-soluble proteins (gliadin) extracted from hard red spring (HRS) and hard red winter (HRW) wheats. **1** = major peak; **I** and **II** = statistically analyzed late-eluting peaks; **a**, **b**, **c**, and **d** = regions defined. Values in figures are wheat protein contents. Wheat protein contents range: 12.1–12.3% (HRS) and 12.0–12.5% (HRW).

solubilities varied among wheat samples, the peak area ratio of peaks I and II was normalized to the relative scale on the basis of peak 1 area. These quantitative differences are summarized in Table I. It was observed that HRS wheats were distinguishable from HRW wheats. Total peak area (peak I + peak II) for HRS wheats ranged from 88.1 to 102.8% (mean = 95.3%, SD = 4.63), whereas HRW wheats showed lower values (mean = 54.8%, SD = 7.94).

Statistical analysis of the chromatograms also provided further information. Highly significant linear correlations were observed for HRS wheats when relative peak areas (%) were plotted against increasing wheat protein contents (%) for peaks I and II (r = 0.87 and −0.92, respectively) (Fig. 6). Correlations for the HRW wheats, however, were much lower (r = −0.09 and −0.42 for peaks I and II, respectively).

Minor differences were also observed in region c. A single peak and a shoulder or broad peak occurred with the HRS wheats, whereas the HRW wheats gave a characteristic sharp single or double peak in this region. Visual analysis of region c for the HRW wheats also provided further information. HRW wheats could be segregated into three groups. The peak pattern for wheats of medium protein content (from 12.5 to 14.1% wheat protein content) was significantly different from those of lower or higher protein wheats. Moreover, the lowest protein HRW wheat (12.0%) had a characteristic double peak that distinguished it from the other wheats. By contrast, HRS wheats were similar at all protein levels in region c. To ascertain if these visual differences could be used for classification purposes further research would be necessary. Bietz and Cobb (1985) noted a significant improvement of resolution at elevated column temperatures. In addition, because various hydrophobic low molecular weight components (pigment, peptides, polar lipids) could also be responsible for absorbance at 210 nm, characterization of each peak in the RP-HPLC chromatograms would need to be studied. A more detailed discussion will be presented in a subsequent paper, using cluster analysis.

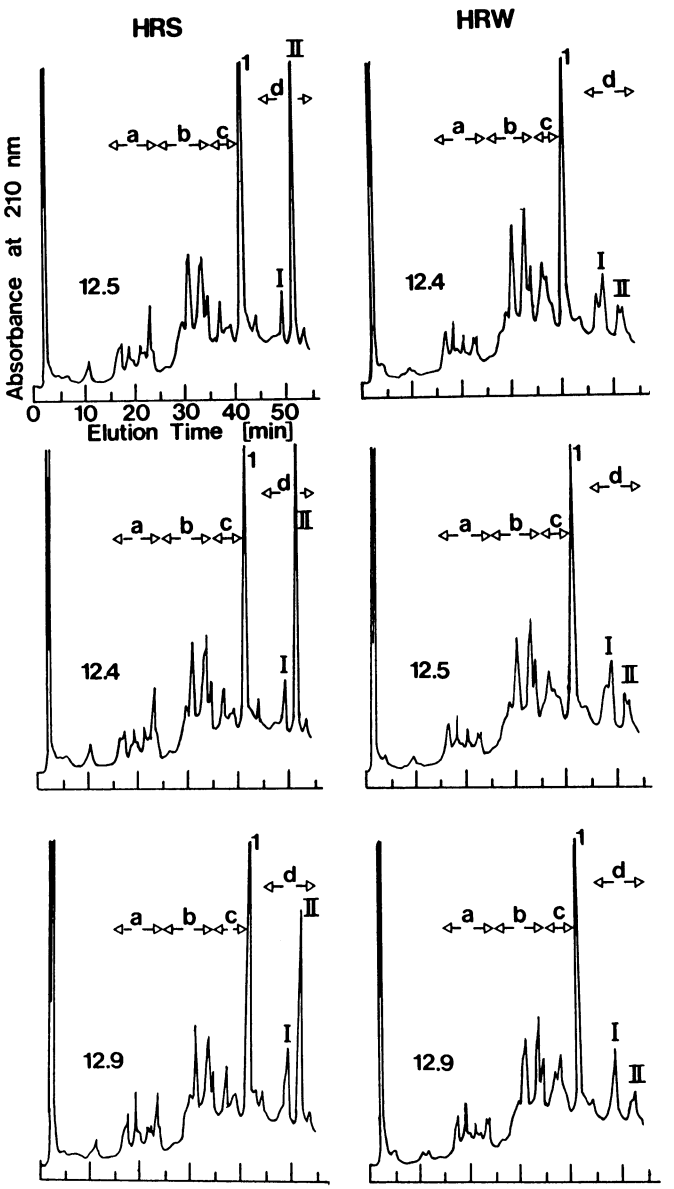

Fig. 2. Comparison of reversed-phase high-performance liquid chromatograms (absorbance at 210 nm) of 70% ethanol-soluble proteins (gliadin) extracted from hard red spring (HRS) and hard red winter (HRW) wheats. **1** = major peak; **I** and **II** = statistically analyzed late-eluting peaks; **a**, **b**, **c**, and **d** = regions defined. Values in figures are wheat protein contents. Wheat protein contents range: 12.4–12.9% (HRS) and 12.4–12.9% (HRW).

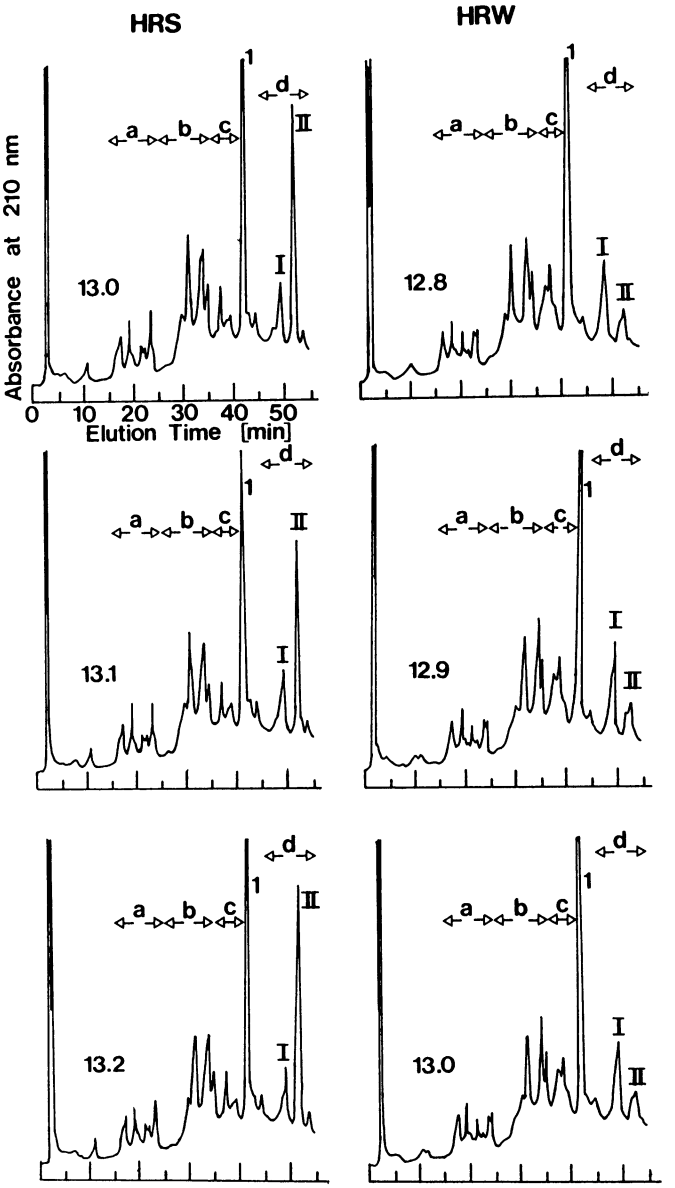

Fig. 3. Comparison of reversed-phase high-performance liquid chromatograms (absorbance at 210 nm) of 70% ethanol-soluble proteins (gliadin) extracted from hard red spring (HRS) and hard red winter (HRW) wheats. **1** = major peak; **I** and **II** = statistically analyzed late-eluting peaks; **a**, **b**, **c**, and **d** = regions defined. Values in figures are wheat protein contents. Wheat protein contents range: 13.0–13.2 (HRS) and 12.8–13.0 (HRW).

TABLE II
Comparison of Flour Analytical and Milling Properties
Between Hard Red Spring (HRS) and Hard Red Winter (HRW) Wheats

Property	Wheat Protein Range (%)					
	12.0–12.9		13.0–13.9		14.0–14.4	
	HRS	HRW	HRS	HRW	HRS	HRW
Wheat protein (%)[a]	12.4 ± 0.27	12.5 ± 0.33	13.4 ± 0.41	13.5 ± 0.47	14.1 ± 0.12	14.3 ± 0.16
Flour[b]						
Protein (%)[a]	11.6 ± 0.33	11.5 ± 0.45	12.3 ± 0.50	12.4 ± 0.39	13.3 ± 0.21	13.4 ± 0.16
Moisture (%)	12.6 ± 0.57	12.7 ± 0.84	12.7 ± 0.61	12.9 ± 0.84	12.1 ± 1.25	12.0 ± 0.62
Ash (%)	0.65 ± 0.04	0.60 ± 0.02	0.58 ± 0.01	0.57 ± 0.02	0.59 ± 0.01	0.62 ± 0.01
FL/TL (%)[c]	40.4 ± 2.85	41.7 ± 3.68	39.6 ± 1.77	39.1 ± 3.48	37.6 ± 0.97	39.4 ± 1.69
Starch tailings (%)[d]	33.8 ± 3.44	28.0 ± 3.42	31.7 ± 1.45	29.6 ± 1.29	22.5 ± 1.25	35.1 ± 5.31
B/M (%)[e]	52.3 ± 3.55	55.4 ± 1.49	49.6 ± 2.74	50.0 ± 6.02	47.8 ± 1.63	53.7 ± 4.26
Total flour yield (%)[f]	75.4 ± 0.95	70.8 ± 1.52	72.9 ± 1.51	69.7 ± 1.28	73.9 ± 0.96	71.3 ± 0.80
Milling score	71.9 ± 1.93	67.9 ± 2.35	72.0 ± 2.37	67.5 ± 2.73	73.0 ± 1.26	68.2 ± 0.64
Damaged starch (%)	6.72 ± 1.41	5.61 ± 0.61	5.88 ± 0.51	5.50 ± 0.69	6.33 ± 0.12	6.09 ± 0.18

[a] N × 5.7.
[b] Straight grade flour.
[c] [Free lipid (*n*-hexane extractable)] × 100/[total lipid (water-saturated butanol extractable)].
[d] (Amount of starch tailings) × 100/(total amount reduction flour).
[e] (Total amount of break flour) × 100/(total amount of reduction flour).
[f] Expressed as a percentage of total flour products recovered.

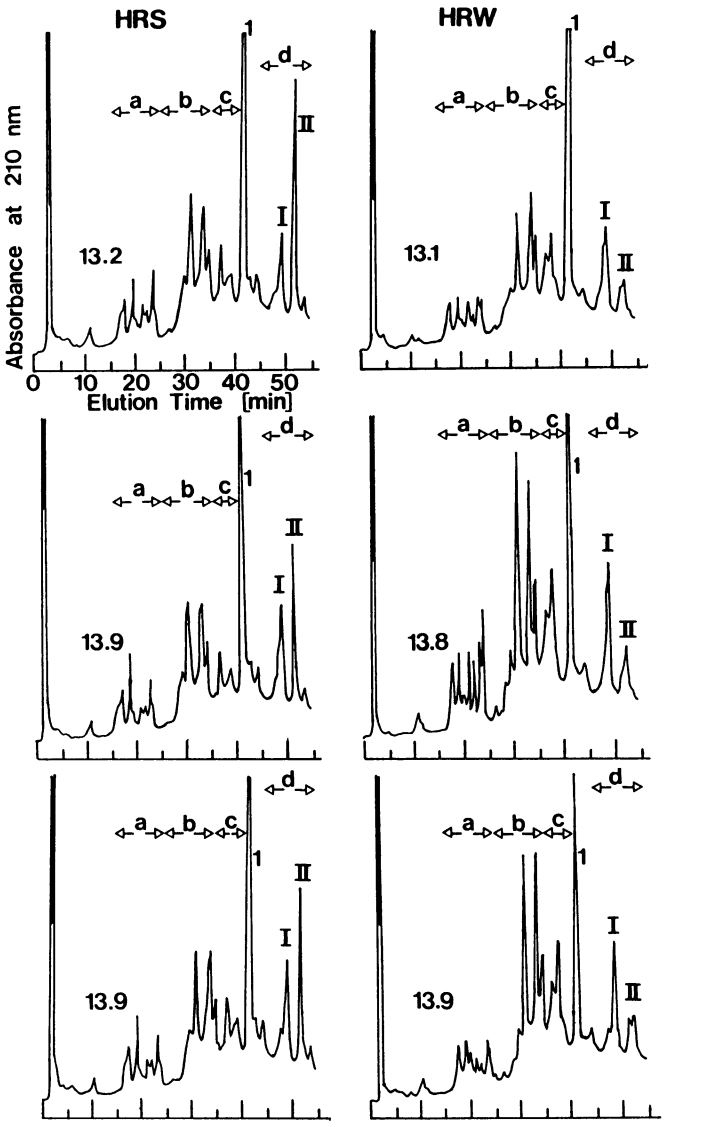

Fig. 4. Comparison of reversed-phase high-performance liquid chromatograms (absorbance at 210 nm) of 70% ethanol-soluble proteins (gliadin) extracted from hard red spring (HRS) and hard red winter (HRW) wheats. **1** = major peak; **I** and **II** = statistically analyzed late-eluting peaks; **a**, **b**, **c**, and **d** = regions defined. Values in figures are wheat protein contents. Wheat protein contents range: 13.2–13.9 (HRS) and 13.1–13.9 (HRW).

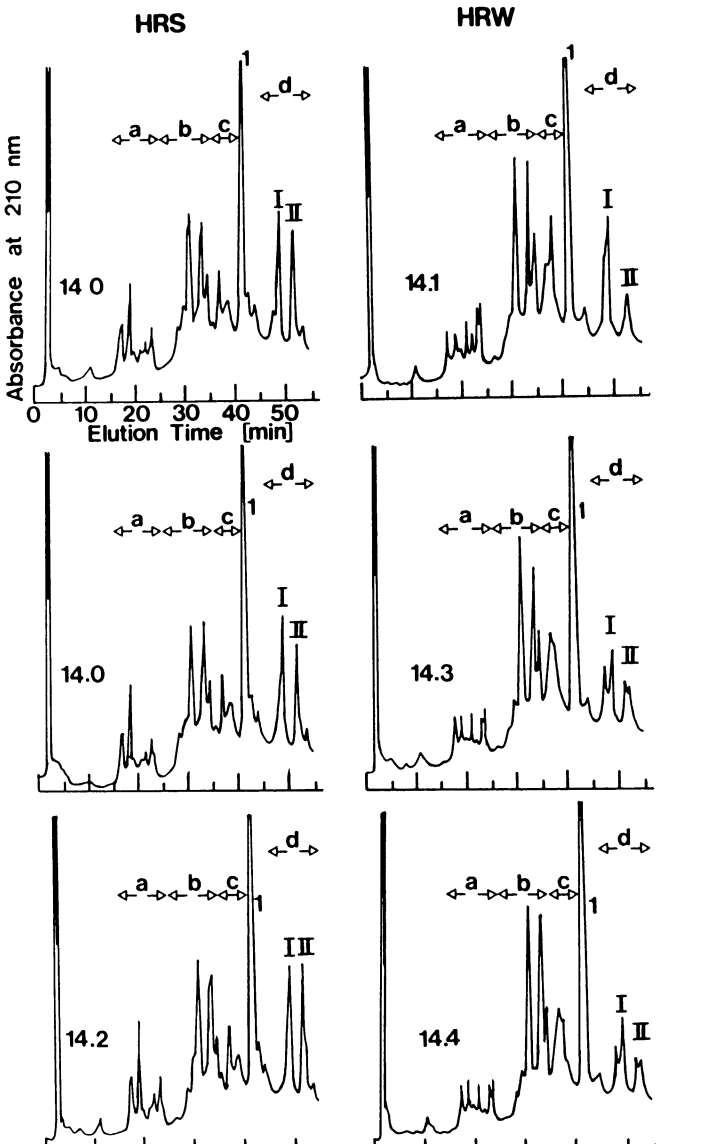

Fig. 5. Comparison of reversed-phase high-performance liquid chromatograms (absorbance at 210 nm) of 70% ethanol-soluble proteins (gliadin) extracted from hard red spring (HRS) and hard red winter (HRW) wheats. **1** = major peak; **I** and **II** = statistically analyzed late-eluting peaks; **a**, **b**, **c**, and **d** = regions defined. Values in figures are wheat protein contents. Wheat protein contents range: 14.0–14.2 (HRS) and 14.1–14.4 (HRW).

TABLE III
Student's Test Between Two Means (for hard red spring and winter wheat) for Analytical and Milling Properties

Property	Wheat Protein Range (%)[a]			
	12.0–12.9	13.0–13.9	14.0–14.4	12.0–14.4
Wheat protein (%)[a]	−0.11	−0.07	−0.20	0.01
Flour moisture (%)	−0.15	−0.28	0.07	−0.13
Flour ash (%)	0.05**[b]	0.01	−0.03	0.02
Flour protein (%)[a]	0.08	−0.04	−0.17	0.10
FL/TL (%)[c]	−1.28	0.49	−1.80	−1.02
Starch tailings (%)[d]	5.78**	2.11*	−12.63*	0.84
Damaged starch (%)	1.11	0.38	0.23	0.63*
B/M (%)[e]	−3.02	−0.41	−5.87	−3.25*
Total flour yield (%)	4.56***	3.22**	1.93*	3.41***
Milling score	4.04**	4.69*	4.83**	4.40***

[a] N × 5.7.
[b] *, **, *** = Significant at the 5, 1, and 0.1% levels, respectively.
[c] [Free lipid (*n*-hexane extractable)] × 100/[total lipid (water-saturated butanol extractable)].
[d] (Amount of starch tailings) × 100/(total starch isolated).
[e] (Total amount of break flour) × 100/(total amount of reduction flour).

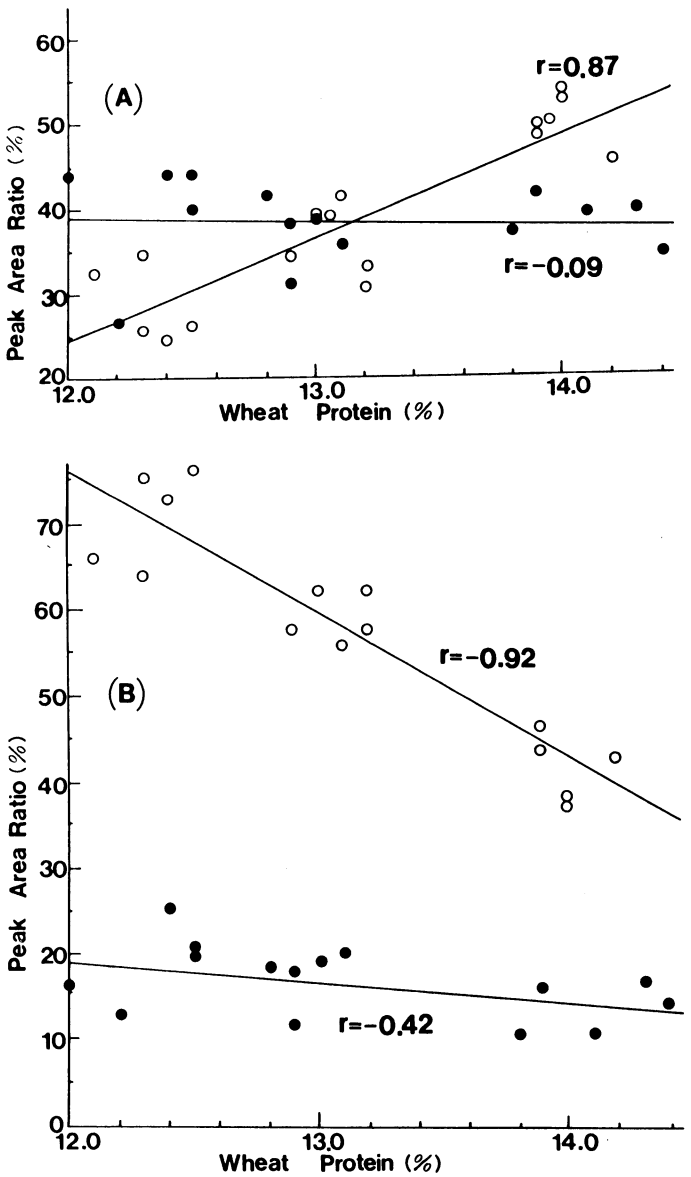

Fig. 6. Scattergrams for peak areas (peak I and peak II) in reversed-phase high-performance liquid chromatograms of 70% ethanol-soluble proteins vs. wheat protein contents. **A,** Peak I vs. wheat protein contents; **B,** = peak II vs. wheat protein contents. ○ = hard red spring, ● = hard red winter. Each peak area was normalized to the relative scale on the basis of major peak area (peak I).

Milling Properties

Tests to compare milling properties of HRS and HRW wheats were conducted at constant moisture conditioning and milling conditions. Results are summarized in Tables II and III. The data were statistically evaluated for each and all wheat protein ranges (12.0–12.9, 13.0–13.9, 14.0–14.4, and 12.0–14.4%, respectively). The data in Table III show that the protein content of the HRS and HRW wheats used in this study were not statistically different.

The two wheat classes differed in break release. The ratio of break flour to reduction flour for the HRS wheats was lower than for the HRW wheats at each wheat protein range (Table II). The difference was significant for samples in the 12.0 to 12.9% wheat protein range and overall in the range 12.0 to 14.4% (Table III). As reported by Pomeranz et al (1988), a highly significant difference was noted for total flour yield and milling score (Tables II and III). HRW wheats showed lower values than the HRS wheats for each and all wheat protein ranges. These results suggest that HRS and HRW wheats also differ in the amounts of wheat farina produced and endosperm mellowness or softness. The difference in wheat hardness might also affect milling properties. Pomeranz et al (1988) observed that HRS wheats were harder than HRW wheats.

The effect of wheat protein content on damaged starch during milling was small, although in general HRS wheats showed higher values than HRW wheats (Table II). The difference was not significant for each protein range but was significant when compared for the entire protein range (Table III).

Analytical Properties

Little difference in moisture, ash, and protein content in the straight grade flours between HRS and HRW wheats was noted (Tables II and III). Although HRS wheats showed higher ash content than HRW wheats, the difference was not significant for the overall protein range (but was significant for samples between 12.0 and 12.9% protein) (Table III).

Starch and Lipid Content

Little difference was observed in the ratio of free lipid to total lipid content (Table II). HRS wheats showed a gradual decrease in the amount of starch tailings as wheat protein level increased, whereas HRW wheats showed a gradual increase (Table II). The difference was significant at each protein range but was not significant for the overall protein range (Table III).

CONCLUSIONS

Our results suggest that RP-HPLC of 70% ethanol-solubles (absorbance at 210 nm) can distinguish HRS from HRW wheats. Additional samples, including representative varieties of their

class, must be investigated to firmly establish the observed relationships. RP-HPLC may differentiate wheat classes. The potential of statistical methods to facilitate such differentiation will be discussed in a subsequent paper.

Comparative studies at optimal moisture level and temper time also will be needed to precisely evaluate milling qualities of the two wheat classes. Based on experience, flour extraction rate decreases when the moisture content of tempered wheat increases beyond an optimal level. Critical moisture content also varies between wheat types. The proportion of vitreous kernels has a major effect on moisture content and milling of wheat (Bradbury et al 1960). Because penetration of water mellows or softens wheat endosperm, the effect of temper time on yield of break flour was also considered to be important. Our results suggested that HRS and HRW wheats require different tempering methods (for example, tempering time).

Since HRS and HRW wheats may differ in both milling and baking properties, wheat identification and classification is essential to ensure optimal end use.

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