Quality Characteristics of Hard Red Spring and Winter Wheats. II. Statistical Evaluations of Reversed-Phase High-Performance Liquid Chromatography and Milling Data

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ABSTRACT

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Reversed-phase high-performance liquid chromatography (RP-HPLC) and milling results used to compare qualitative and quantitative differences between hard red spring (HRS) and hard red winter (HRW) wheats were statistically evaluated by cluster analysis. Absolute RP-HPLC peak areas and elution times were normalized to main chromatographic peak. Classification was performed using common peaks (peaks with the same elution times). Cluster analysis of normalized chromatographic data successfully separated 15 HRS and 15 HRW wheats by class. HRS wheats formed a more compact cluster and HRW wheats more individual clusters. When milling properties of the wheats were compared by this technique, cluster analysis could differentiate the two classes.

Reversed-phase high-performance liquid chromatography (RP-HPLC) of storage proteins can identify cereal cultivars (Burnouf et al 1983, Bietz et al 1984, Bietz and Cobb 1985). Because RP-HPLC is fast, accurate, reproducible, and relatively easy to perform, it should also be useful during breeding to optimize wheat quality and utilization (Huebner and Bietz 1987). RP-HPLC data can also readily be analyzed; computer analysis may identify quality-related proteins, thus enhancing the value of RP-HPLC (Huebner and Bietz 1987).

Several approaches have been used for data analysis, although further research is needed to make it fully automated. For example, Cohen et al (1987) described the use of pattern recognition for qualitative analysis of chromatograms. Huebner and Bietz (1987) studied quality-related proteins using computer analysis of RP-HPLC results. Marchylo et al (1988) studied the applicability of computer analysis for predicting the concentration of wheat cultivars in an admixture and concluded that further improvement, especially in normalization of chromatograms, is needed for precise determination. These efforts were mainly based on liner regression analysis.

Another technique of computerized multivariate analysis has recently become available. This method, cluster analysis, differentiates various items by analyzing data similarities. The technique is based on several methods: a single linkage method, complete linkage method, median method, centroid method, and group-average or Ward's method. Its results are shown by dendrograms.

This study attempts to differentiate wheat classes by applying cluster analysis to RP-HPLC chromatograms and also compares milling properties of hard red spring (HRS) and hard red winter (HRW) wheats.

MATERIALS AND METHODS

Data Used for Statistical Analysis

Wheat samples used are listed in the previous paper (Endo et al 1990). Each set of 15 HRS and HRW wheats was representative of commercial wheats exported from the United States in 1987.

RP-HPLC data for gliadin proteins and milling properties were summarized previously. RP-HPLC was performed as described by Bietz (1983) and Bietz et al (1984). For milling, wheats were tempered to 15.5% moisture for 24 hr. Tempered grains were milled on a Buhler laboratory mill using standard milling conditions (Nagao et al 1976).

Normalization of Chromatographic Data

Although RP-HPLC chromatograms were automatically integrated with a D-2000 Hitachi chromato-integrator, it was necessary to normalize peak elution times and areas to precisely compare different runs.

Because protein levels varied among samples, Marchylo et al (1988) compared chromatograms by scaling the largest peaks to equal heights. In addition, it has been shown that a decrease in hydrophobic bonding ability leads to a decrease in retention times (Noyes 1983). Bietz and Cobb (1985) reported an increase in elution time with column use. Sapirstein and Bushuk (1985) normalized electrophoregrams using a reference band system.

Elution times of each peak and the area under each peak were normalized to a relative scale based on elution time of the largest peak (main peak), which always eluted at 41 min (Table I). By this means, the elution times were reported as the values subtracted from the main peak time. Peak areas were normalized to the ratio of the main peak area. Comparisons were facilitated by specifying exact elution times through this normalization.

Computerized Statistical Analysis

Data were statistically analyzed by cluster analysis using multivariate analysis on a personal computer (PC 9801 VM, NEC Co., Japan). The commercially available software (Multivariate Analysis) was developed by Social Survey Research Information Co., Japan. With this analysis, various items are grouped or classified in a cluster by their similarities of data (Watari and Kishi 1981). Clustered results are shown by figures designated as dendrograms (branching structures). The linkage in a dendrogram

TABLE I
Specification of Absolute (main peak = P9) and Normalized Elution Times for 11 Peaks Used for Cluster Analysis

Peak	Mean (min)[a]	Deviation
P1	−21.7	0.20
P2	−19.8	0.11
P3	−19.0	0.12
P4	−17.5	0.30
P5	−11.8	0.12
P6	−10.0	0.20
P7	−8.2	0.14
P8	−4.3	0.41
P9[b]	41.2[b]	0.19[b]
P10	7.2	0.14
P11	9.9	0.20

[a] Difference of each elution time from that of main peak.
[b] Main peak. Data were expressed as absolute values.

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shows the order of similarities in an ascending scale (designated as the distance index). Relations between items are shown by linked lines.

Parameters for RP-HPLC analysis were normalized peak area ratios as specified by elution time. The differences in milling properties were evaluated using amount of flour stream, milling score, and damaged starch content as parameters.

RESULTS AND DISCUSSION

Normalization of RP-HPLC Data

RP-HPLC chromatograms of gliadin proteins from HRS and HRW wheats consisted of as many as 22 separate peak areas and elution times; consequently, 44 parameters could have been used for computerized analysis. For the initial cluster analysis, however, only peak areas were used. Because an increase in elution time with column use was reported by Bietz and Cobb (1985), Simpson et al (1985), and Marchylo et al (1988), chromatograms were normalized to correct the peak elution time variability from different runs.

The largest peak eluted at 41 min in all chromatograms (Table I), so all results were normalized to this peak. Figure 1 shows an example of normalization. As previously reported, HRS and HRW wheats could be differentiated using only one or two chromatographic peaks. Using cluster analysis, 11 peaks common (same elution times) to HRS and HRW wheats were selected to examine the relations between and within these wheat classes. Information for these 11 peaks is summarized in Table I, and the numbered peaks are shown in Fig. 1. Little difference was observed for the elution times of main peaks between HRS (mean = 41.3, SD = 0.15) and HRW (mean = 41.2, SD = 0.22) wheats. Peak 11 was identical to peak II in the previous paper (Endo et al 1990). Deviations of elution times were within 0.20 min, except for peaks 4 and 8. To obtain better peak time reproducibilities, further research including normalization techniques is

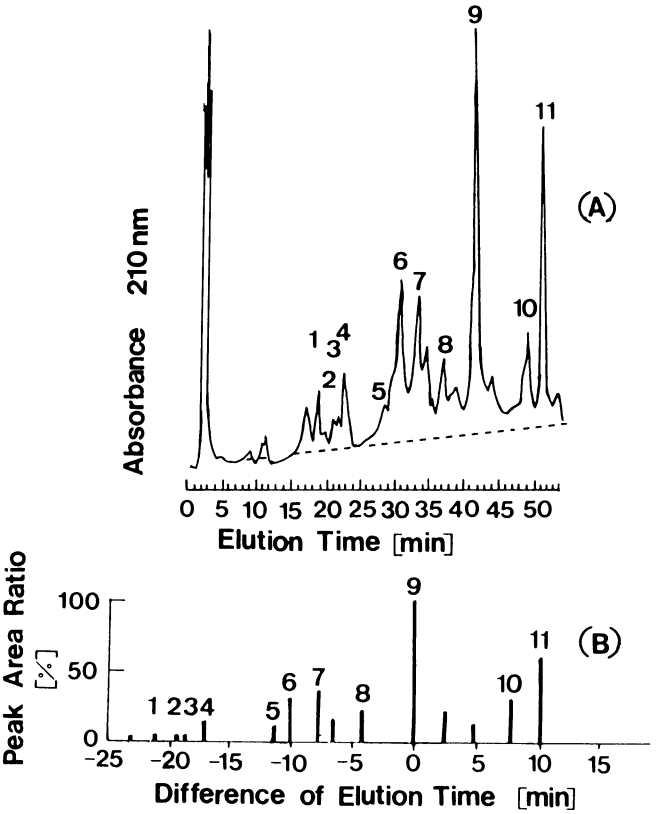

Fig. 1. A, Reversed-phase high-performance liquid chromatography of gliadin extracted with 70% ethanol from hard red spring wheat (12.3% protein). Baseline used for analyzing peak area (- - -). **B,** Typical normalized chromatogram. Eleven peaks common to hard red spring and winter wheats (specified by elution times) were used for cluster analysis.

TABLE II
Normalized Peak Area Ratio for Hard Red Spring (HRS) and Winter (HRW) Wheats

Wheat Protein (%)[a]	Ratio of Peak Area (%)										
	P1	P2	P3	P4	P5	P6	P7	P8	P9[b]	P10	P11
HRS											
12.1	2	2	2	10	13	28	40	22	100	24	66
12.3	2	3	2	14	10	35	43	21	100	35	64
12.3	3	2	2	10	11	30	45	18	100	29	76
12.4	2	2	3	11	11	31	42	24	100	31	73
12.5	2	2	2	10	12	31	43	18	100	31	77
12.9	4	1	2	10	10	29	38	20	100	38	58
13.0	4	2	2	11	10	31	44	20	100	39	62
13.1	6	2	3	13	11	31	43	20	100	41	56
13.2	4	2	2	10	11	30	42	21	100	38	62
13.2	4	2	2	14	10	30	41	21	100	37	58
13.9	5	1	2	11	13	30	47	16	100	43	44
13.9	5	1	2	11	14	30	47	17	100	43	47
14.0	5	1	2	10	11	28	33	17	100	35	38
14.0	5	1	1	10	10	28	37	16	100	35	37
14.2	6	1	2	11	10	23	34	13	100	34	43
HRW											
12.0	3	2	1	3	16	25	25	56	100	23	16
12.2	3	2	1	5	13	24	23	55	100	21	13
12.4	3	2	1	6	18	27	23	55	100	22	25
12.5	3	1	1	3	12	23	24	26	100	22	20
12.5	3	1	1	3	17	23	21	51	100	25	20
12.8	4	2	2	9	18	29	26	64	100	44	18
12.9	3	2	1	7	18	25	21	50	100	35	18
12.9	3	2	2	7	17	25	21	50	100	35	12
13.0	3	2	1	6	15	23	20	50	100	36	19
13.1	3	2	2	7	14	24	20	35	100	37	20
13.8	3	3	2	9	15	34	27	72	100	48	10
13.9	4	2	1	6	15	33	28	60	100	40	16
14.1	3	3	2	10	17	35	26	69	100	48	11
14.3	3	2	1	8	17	36	29	44	100	26	17
14.4	3	2	1	8	18	37	30	73	100	28	14

[a] Values are expressed on a 14.0% moisture basis (N × 5.7).
[b] Main peak.

necessary. Noyes (1983) recommended periodic adjustments in the gradient program to compensate for elution time variability. In addition, since samples used in this study were mixtures of varieties, to ensure identities for each peak between chromatograms, some other method including electrophoretic analysis is necessary. Lookhart and Albers (1988) analyzed each RP-HPLC peak by gel electrophoresis.

Normalized peak areas for HRS and HRW wheats are summarized in Table II.

Cluster Analysis of RP-HPLC Data

Cluster analysis was carried out using the normalized peak area ratios as parameters. It was evident that HRS and HRW wheats analyzed in this study could be differentiated by this computerized statistical analysis technique (Fig. 2).

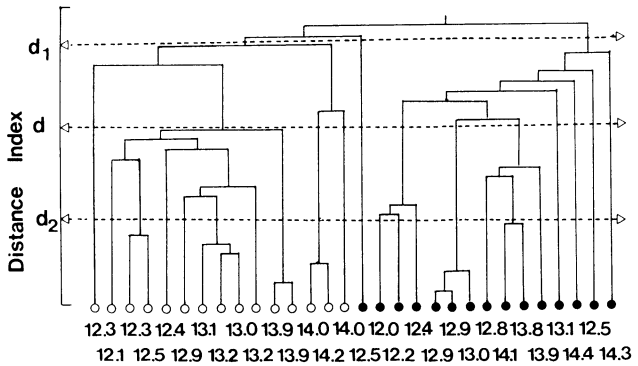

Fig. 2. Dendrogram of cluster analysis for reversed-phase high-performance liquid chromatograms of 70% ethanol-soluble protein (gliadin). Normalized peak area ratios for 11 peaks common to hard red spring and winter (HRS and HRW) wheats (same elution times) were used as parameters. Figures under the cluster showed the protein contents of wheat grains on a 14.0% moisture basis (N × 5.7). HRS wheats (○), HRW wheats (●). Lines d, d_1, and d_2 are applied distance indexes.

Quantitative analysis of the dendrogram further grouped wheats within classes. The Y axis of the dendrogram shows the distance index (values were not printed out by the software program), which indicates similarities between items in a cluster. Items linked at lower values indicate higher similarities between them. In other words, various items could be classified into some groups by properly applying a distance index.

The dendrogram in Figure 2, for example, could be classified into 3, 12, or 22 groups by arbitrarily applying the distance indexes of d_1, d, and d_2, respectively. Thus, at the highest distance index, d_1, HRS and HRW wheats were linked. HRS wheats were classified into four groups by distance index d. Group A comprised a wheat sample with protein content of 12.3%. Group B samples had protein contents of 12.1, 12.3, 12.5, 12.4, 12.9, 13.1, 13.2, 13.0, 13.2, 13.9, and 13.9%; group C samples were 14.0 and 14.2%, and group D samples 14.0% protein. HRW wheats could be classified into seven groups: 12.5 (group 1); 12.0, 12.2, 12.4 (group 2); 12.9, 12.9, 13.0, 12.8, 14.1, 13.8, 13.9 (group 3); 13.1 (group 4); 14.4 (group 5); 12.5 (group 6); and 14.3% protein (group 7). Applying distance index d_2 would distinguish 10 and 12 groups for HRS and HRW wheats, respectively.

RP-HPLC characteristics of HRS wheat gliadins are closely related to protein contents (Fig. 2). Most HRS wheats could be classified into more compact clusters than HRW wheats. Because RP-HPLC of gliadins can identify cultivars on the basis of genetic differences, HRW wheats, which were classified into more groups, may be more heterogeneous mixtures.

This type of computer analysis of RP-HPLC data might effectively classify unknown new varieties if a dendrogram for all wheat were available.

Cluster Analysis of Milling Properties

Figure 3 shows the dendrogram based on results of milling

TABLE III
Wheat Milling Data for Hard Red Spring (HRS) and Winter (HRW) Wheats

Wheat Protein (%)[a]	Flour Stream (%)[b]								Milling Score	Damaged Starch (%)
	1B	2B	3B	1M	2M	3M	Bran	Shorts		
HRS										
12.1	11.6	10.5	2.6	32.8	15.0	2.4	15.8	9.3	69.45	8.69
12.3	14.4	10.1	2.4	33.7	13.7	1.9	14.5	9.3	73.25	6.46
12.3	12.0	10.2	2.7	36.4	13.4	1.8	15.5	8.0	70.80	5.99
12.4	14.1	10.4	2.6	33.9	12.9	1.9	15.1	9.1	73.05	5.99
12.5	12.7	11.1	3.0	31.1	14.5	2.2	16.1	9.3	74.80	5.48
12.9	12.3	9.8	2.5	32.9	14.6	2.0	16.3	9.6	70.50	6.11
13.0	11.6	10.5	2.6	33.9	14.6	1.9	15.8	9.1	75.50	5.99
13.1	10.2	10.0	2.9	30.8	15.3	2.3	18.0	10.5	70.25	5.58
13.2	10.1	11.4	3.3	29.2	14.9	2.3	18.7	10.1	69.10	4.59
13.2	12.5	10.3	2.4	32.0	14.8	2.1	15.9	10.0	73.00	6.36
13.9	9.6	10.1	3.0	32.4	15.2	2.1	18.2	9.4	70.65	5.58
13.9	10.7	10.9	2.8	31.3	15.1	2.3	16.3	10.6	73.20	6.19
14.0	11.2	10.0	2.6	32.3	16.3	2.4	15.9	9.3	73.80	6.11
14.0	11.1	10.1	2.5	31.1	16.8	2.4	15.8	10.2	73.65	6.23
14.2	11.9	9.7	2.6	30.7	15.8	2.2	16.5	10.6	71.55	6.49
HRW										
12.0	11.1	11.0	3.1	31.2	14.3	2.1	17.8	9.4	70.20	7.18
12.2	12.8	10.7	2.7	30.5	14.1	2.1	16.8	10.3	71.05	5.21
12.4	10.5	11.1	3.4	28.2	14.0	2.4	20.0	10.4	67.50	5.63
12.5	11.9	10.2	2.8	27.2	14.6	2.3	20.0	11.0	65.05	5.67
12.5	12.0	10.0	3.2	29.3	14.3	2.0	19.4	9.8	69.70	5.08
12.8	11.2	11.7	3.0	31.3	12.8	1.7	19.0	9.3	68.30	4.89
12.9	10.8	11.1	2.9	28.8	13.4	2.2	20.2	10.6	66.36	4.79
12.9	8.8	12.1	3.5	31.3	12.6	2.0	20.4	9.3	64.80	4.92
13.0	9.5	12.6	3.5	29.1	12.7	1.7	20.6	10.3	65.65	5.00
13.1	9.0	10.0	3.1	31.4	13.5	1.9	21.3	9.8	67.95	4.39
13.8	8.6	10.7	3.1	30.6	13.9	2.2	20.5	10.4	65.10	5.08
13.9	10.4	9.5	2.6	31.8	15.1	2.2	18.1	10.3	71.10	6.60
14.1	10.8	12.2	2.9	31.0	12.9	1.9	17.5	10.8	67.75	5.08
14.3	11.6	10.7	3.1	28.3	14.9	2.4	18.0	11.0	67.85	6.07
14.4	10.5	10.2	3.0	31.6	14.7	2.3	17.2	10.5	68.90	6.11

[a] Protein content of wheat (N × 5.7) on a 14.0% moisture basis.
[b] Yield was expressed as a percentage of total recovered products.

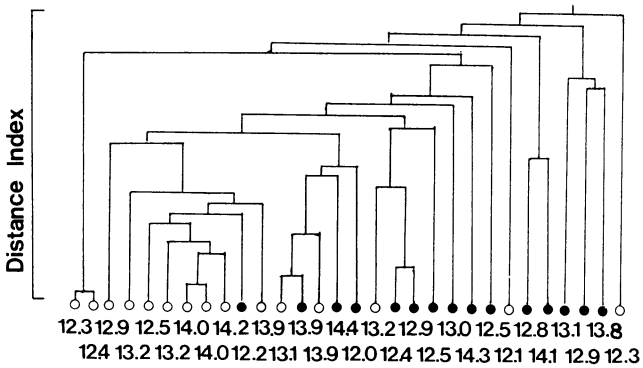

Fig. 3. Dendrogram of cluster analysis for milling properties. Amounts of flour stream, milling score, and contents of damaged starch for straight grade flours were used as parameters. Wheats were milled under standard conditions on a Buhler laboratory mill. Figures under clusters show grain protein contents on a 14.0% moisture basis (N × 5.7). HRS wheats (○), HRW wheats (●).

properties. The parameters measured are summarized in Table III. In most cases, the two wheat classes differed in milling properties. HRW wheats showed a much wider range of clusters or individual clusters than HRS wheats (Fig. 3). These results may relate to RP-HPLC results.

As reported by Pomeranz et al (1988), it would also be necessary to mill wheat classes at optimum temper level for precise comparison. However, the dendrogram (Fig. 3) indicates that HRS and HRW wheats differ in milling properties even when compared at an equivalent protein level. There is little relationship between HRS and HRW wheats.

Furthermore, the milling data may better classify spring and winter wheats than protein content. If protein is the major factor affecting milling properties, the cluster would be linked at each protein level. For example, a cluster of some HRS and some HRW wheats would be obtained (HRS, 12.3 and 12.4% protein; HRW, 12.2 and 12.4% protein). Figure 3 indicates that milling properties mainly depend on wheat class and are almost independent of protein content.

These results suggest that classification of wheats as spring and winter is superior to that based on protein level. The current classification of HRS and HRW wheats serves the needs of both millers and bakers.

CONCLUSIONS

Computerized cluster analysis is effective and useful in analyzing large amounts of RP-HPLC data. Still, we may have only compared mixtures of numerous varieties of each class. Nevertheless, we used this technique successfully to separate HRS and HRW wheats into two groups. To firmly establish this method, however, it would be necessary to compare representative varieties. Peak area ratio was the parameter used, but elution times should also be further examined.

The value of cluster analysis could also be enhanced through combined analysis including RP-HPLC, milling properties, analytical properties, wheat hardness, and so on. In the future, cluster analysis should be able to classify new varieties more easily and automatically even for those wheats developed from crosses between various classes (spring and winter, soft and hard).

Based on this study, we demonstrated that HRS and HRW wheats differed in milling properties and that wheat classification using the these designations has a greater advantage for flour millers and bakers than a protein level type classification.

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