

ANALYSIS OF WATER-SOLUBLE PROTEINS FROM BARLEY BY ION-EXCHANGE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Received 8 September 1994.

The water-soluble proteins (WSP) from 13 varieties of barley were analysed by ion-exchange high performance liquid chromatography (IE-HPLC) on a non-porous anion-exchange column. The method was rapid, allowing detection of 5 or more major protein peaks in less than 10 minutes. The combination of retention times and peak areas of the five main peaks could be used to identify barley varieties. The WSP content increased with increasing total nitrogen content. However, the amount of WSP in different barley varieties varied greatly. Malting and feed barleys did not show consistent differences in total WSP but the shape of the profile was significantly different for malting and feed varieties. Analysis of water soluble proteins could have value in research associated with crop improvement programmes and in industry variety assessment. The method is quicker and simpler than those used for the analysis of alcohol-soluble proteins.

Key Words: *Barley, HPLC, ion exchange, nitrogen, water-soluble proteins.*

INTRODUCTION

Identification of barley varieties by polyacrylamide gel electrophoresis (PAGE) or high performance liquid chromatography (HPLC) of proteins, has become a routine laboratory test in grain segregation and marketing authorities, in maltings and in barley breeding programmes. The presence of specific proteins detected by electrophoresis or HPLC may also be related to malting and brewing quality^{19,26}. Reverse-phase (RP)-HPLC has been used for both varietal confirmation and examination of the relationship between nitrogen components and grain quality^{1,2,8,12,19-22,24-26,28}. In these studies the proteins were analysed from the alcohol-soluble storage protein (hordein) fraction. The alcohol-soluble proteins make up about half of the total grain protein content. This fraction contains heterogeneous polypeptides and so, in most cases, allows for easy distinction between varieties. However, PAGE and HPLC methods involve extraction and separation taking several hours. The water-soluble protein (WSP) fraction includes non-storage proteins and is abundant in barley. The analysis of WSP may represent a simpler and more rapid extraction (with water) and separation (ion-exchange (IE)-HPLC) technique for identification of barley varieties than the techniques employed for the alcohol-soluble group.

For malting barleys the level of protein is important for several reasons. Firstly, high grain protein content means a reduced level of available starch. Secondly, proteolysis (protease hydrolysis producing amino acids and peptides from hordeins) during malting and mashing is necessary for yeast metabolism²³. Finally, soluble proteins are important in beer head retention and stability¹⁰. The relationship between increasing hordein protein levels and total grain protein content has been examined previously, with both variety and environment having a large affect on this relationship²². The total protein level influences malting quality³ and therefore the interaction of hordeins and malting quality²⁵. Albumins and globulins belong to the water soluble protein fraction which makes up 10-20% of the total barley grain protein content²⁷.

The WSP level varies between varieties and within varieties⁷ depending upon the grain protein content. For varieties at the same protein level the WSP fraction may vary up to 20%⁶. Although the exact role the WSP fraction, in relation to malting is not clearly defined, it is known that important enzymes, eg. β -amylase, are present in this fraction¹⁴.

Several studies have investigated albumin and globulin fractions of barleys using immunological techniques^{13,16-18}. However, analysis of the WSP by HPLC has not been used for barley variety identification or quality analysis. The water-soluble fraction from wheat has been used for variety identification⁸. Battershell and Henry⁴ used ion-exchange (IE)-HPLC to identify and study a barley α -amylase subtilisin inhibitor. This method has been adapted for the analysis of the WSP fraction from barley.

This study reports a simple and rapid procedure for the extraction and separation of WSP from barley using IE-HPLC. The relationship between water-soluble and grain protein levels was also investigated in relation to barley variety and end-use type.

MATERIALS AND METHODS

Thirteen barley varieties (six malting and seven feed) were used for varietal identification by ion exchange high performance liquid chromatography (IE-HPLC). The samples, listed in Table 1, were obtained from the Queensland barley breeding program. All varieties are 2-row barleys except Malebo which is a 6-row variety. Samples were tested at two nitrogen contents. Nitrogen content was determined by an in-house near infrared reflectance calibration.

Extraction of barley water soluble proteins

This method developed by Battershell and Henry⁴ used for protein extraction and separation. Samples were ground in a laboratory mill (Falling Number 3100) with a 0.8 mm screen. Proteins were extracted by mixing 0.5 g of barley flour with distilled water (5 mL) for 30 min, stirring on a vortex mixer every 10 min. The samples were centrifuged at 3000 G for 5 min and the supernatant was passed through a Bio Gel P6 (Bio Rad) desalting column. An aliquot (1 mL) of the

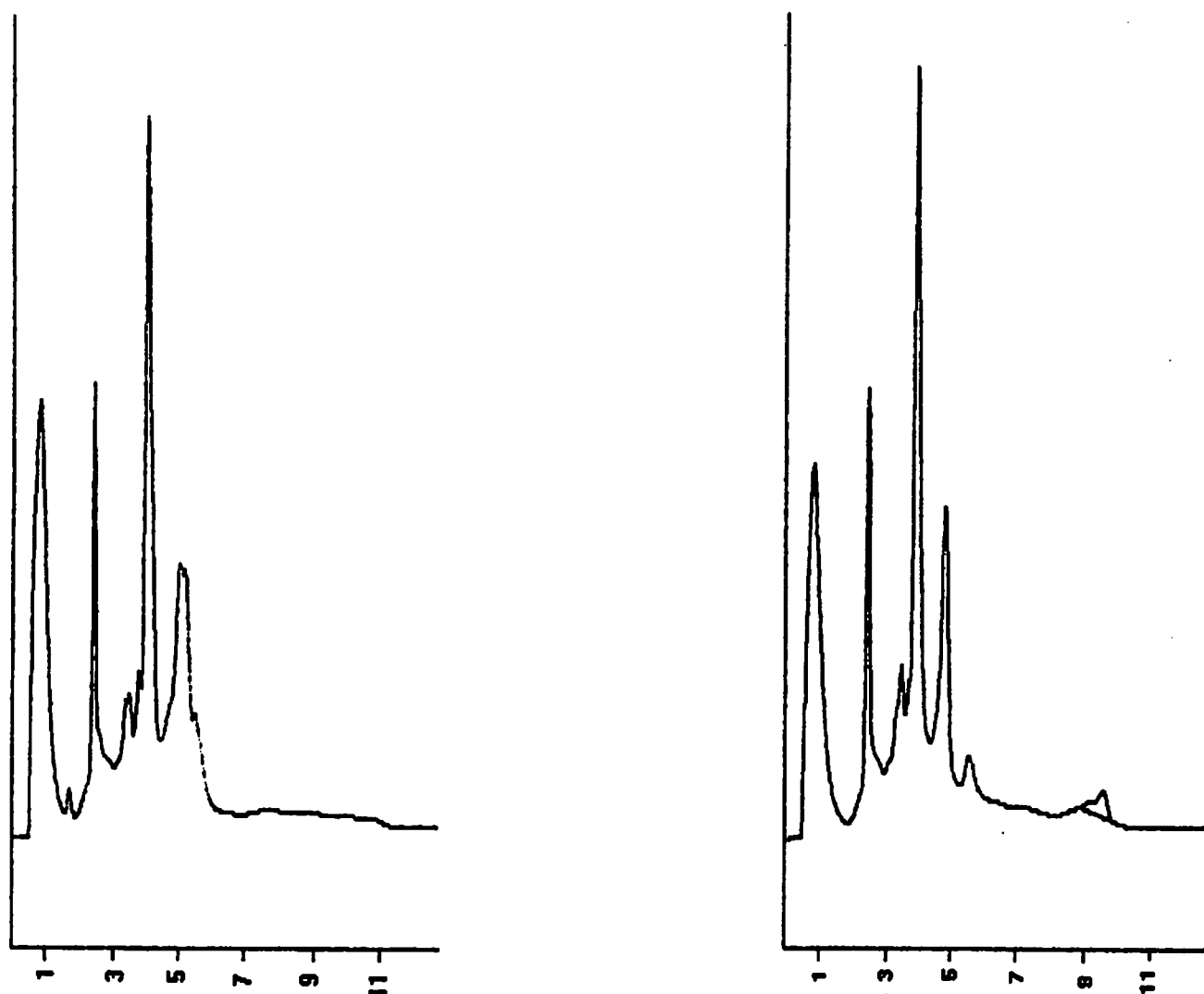


FIG. 1. Comparison of ion exchange HPLC chromatographs for barley varieties Franklin and Corvette.

TABLE 1 Australian barley varieties analysed by IE-HPLC.

Malting varieties	Feed varieties
Grimmett	Malebo
Tallon	O'Connor
Triumph ¹	Skiff ²
Schooner	Corvette
Franklin	Ulandra
Stirling	Gilbert ³
	Galleon

¹Triumph is a European variety although it has been grown commercially in Tasmania.

²Skiff has been used for malt production.

³Gilbert is a feed variety reselected from Koru.

desalted sample was loaded onto the IE-HPLC column at a flow rate of 1.5 mL/min.

Separation of barley water soluble proteins

Water-soluble barley proteins were separated by IE-HPLC. A Bio Rad gradient dual pump module, with sample mixer, sample injector and UV detector (280 nm), was used. The column was a Bio Rad MA7P non-porous column, used for high resolution and fast separation of proteins. Column temperature was at 20°C. The column (30 mm × 4.6 mm) was pre-packed with a polymer matrix support (particle size 7 μm) in which the functional group was polyethyleneimine.

Two buffers were used for the gradient system, Buffer A (5 mM Tris, pH 8.6, degassed) and Buffer B (5 mM Tris, 1 mM CaCl₂, 0.3 M NaCl, pH 8.6, degassed). The gradient from A to B required only 10 min per sample and the solvent flow rate was 1 mL/min.

Statistical Analysis

Peak areas (PA) and retention times (RT) were analysed by analysis of variance (ANOVA). Regressions for nitrogen levels and barley types as well as significance of difference in PA and RT for individual peaks, at different nitrogen levels (high and low) and for different barley types (malting and feed) was also tested.

RESULTS

Identification of varieties

The water-soluble protein fraction from thirteen varieties was separated by ion-exchange HPLC. Each variety gave a distinctive profile. The chromatographs for a malting (Franklin) and a feed (Corvette) variety are presented in Figure 1. For most varieties there were five main peaks. The differences in the retention times and peak heights for the main peaks alone gave a unique identification for each variety. Use of the additional minor peaks would only improve this discrimination.

TABLE 2 Barley varieties with total peak areas and nitrogen contents

Variety	Nitrogen content % oven dry	HPLC peak area (arbitrary units)
<i>Malting</i>		
Grimmett	1.72	4933
	1.67	3804
Franklin	2.01	4632
	1.82	4002
Schooner	1.87	5217
	1.53	4368
Tallon	1.67	4095
	1.42	3762
Triumph	1.82	4942
	1.53	4208
Stirling	2.07	5924
	1.41	4123
<i>Feed</i>		
Corvette	1.80	5398
	1.53	3741
Skiff	2.02	3872
	1.53	3084
O'Connor	1.79	4775
	1.75	3920
Koru	1.99	5229
	1.64	4625
Galleon	1.87	6039
	1.41	5630
Ulandra	1.80	6767
Malebo	1.75	5106

The total peak areas for the varieties analysed were similar (Table 2). The average area for six malting varieties and seven feed varieties was 4504 and 5004 respectively (data not shown). If Skiff (of marginal malting quality) was included in the malting varieties, than the average peak area for those barley classifications was 4357 and 5259.

The peak areas and retention times for the five main peaks plus a sixth peak in some varieties for malting and feed varieties are presented in Table 3a and Table 3b respectively. All varieties had the first peak, in some varieties the largest peak, eluting at approximately 0.85 min. This peak (Peak 1) was significantly different ($P=0.05$) in peak area for high (1677) and low (1071) protein levels (Table 4). There was no

relationship for type of barleys (malting or feed) but there was a significant ($P=0.05$) relationship for type and variety interaction for peak area and retention time. When Skiff was included as a malting variety peak 1 had a significant difference ($P=0.05$) for barley type as well as nitrogen level (data not shown).

A second peak was eluted at around 2.40 min. There was no significant difference in peak areas at different protein levels for this peak. However, there was a significant difference in retention time for barley type ($P=0.05$) (Table 4). Malting varieties eluted at an average of 2.50 min while feed varieties (including Skiff) eluted at an average of 2.36 minutes.

All varieties showed another small peak at about 3.50 min. This peak had a significant difference in area ($P=0.05$) between malting and feed varieties and for protein level (Table 4). The type of barley had a significant effect on retention time ($P=0.05$). Malting varieties average at 3.34 min with feed varieties at 3.47 min. This peak had a significant interaction for barley type and variety for both peak area and retention time (Table 4).

The second main peak was eluted at about 4.00 min for most varieties. Grimmett had a small peak at 3.77 min with its second large peak at 4.39 min. When analysed with the small peak included as peak 4 there was a significant difference ($P=0.05$) between barley types. When the second large peak for Grimmett was analysed as peak five there was no interaction for peak area or retention time with either protein or barley type. No relationship was apparent between the size of the first eluted large peak and this latter large peak at either protein level. For some varieties, the first peak was larger than the second large peak, for others, the reverse was the case. Peaks that eluted after the second main peak (peaks 6 and 7) were inconsistent within a variety and between varieties.

Effect of protein content

The grain protein level of the different varieties influenced the peak areas in the chromatograph patterns. In most cases the higher protein content had a higher total peak area. Similar protein contents between varieties did not mean similar total peak areas. For example, two malting varieties, Franklin and Triumph, had the same protein levels. But the separation profiles were very different (Figures not shown) and Franklin was 10% higher in the total peak area (Table 2).

TABLE 3a Peak areas and retention times of IE-HPLC peaks for malting barleys

Variety	Grain nitrogen (%)	Peak 1		Peak 2		Peak 3		Peak 4		Peak 5		Peak 6	
		Area* and retention time (min)	Area* and retention time (min)	Area* and retention time (min)	Area* and retention time (min)	Area* and retention time (min)	Area* and retention time (min)	Area* and retention time (min)	Area* and retention time (min)	Area* and retention time (min)	Area* and retention time (min)		
Grimmett	1.77	1536	0.83	623	2.67	64	3.40	252	3.75	1797	4.35	589	6.03
	1.67	1462	0.82	329	2.72	3	3.43	86	3.78	1493	4.42	—	—
Franklin	2.01	1332	0.83	626	2.50	407	3.50	1374	3.95	729	4.85	112	5.58
	1.82	834	0.82	238	2.50	404	3.48	1462	3.85	685	4.95	101	5.55
Schooner	1.87	2006	0.83	248	2.55	527	3.00	254	3.95	1746	4.90	—	—
	1.53	1336	0.87	528	2.38	279	3.53	1666	3.95	559	5.18	—	—
Tallon	1.67	1449	0.85	305	2.45	315	3.38	1431	3.98	314	5.18	—	—
	1.42	487	0.73	417	2.38	208	3.27	1884	3.73	764	4.90	144	5.55
Triumph	1.82	1891	0.93	126	2.80	123	3.13	1345	3.82	545	5.02	279	5.45
	1.53	920	0.85	592	2.43	—	—	2369	3.82	778	4.98	—	—
Stirling	2.07	1496	0.87	684	2.27	487	3.38	1848	3.98	196	5.05	—	—
	1.42	825	0.85	549	2.40	340	3.40	1715	4.03	695	4.98	—	—
Mean		1298	0.84	439	2.50	264	3.35	1307	3.88	858	4.90	313	5.69

*arbitrary units

TABLE 3b Peak areas and retention times for feed varieties

Variety	Grain nitrogen (%)	Peak 1 Area* and retention time (min)		Peak 2 Area* and retention time (min)		Peak 3 Area* and retention time (min)		Peak 4 Area* and retention time (min)		Peak 5 Area* and retention time (min)		Peak 6 Area* and retention time (min)	
Skiff	2.02	1798	0.88	55	2.27	161	3.52	1347	4.00	510	5.23	—	—
	1.53	557	0.75	137	2.45	25	3.58	1505	3.85	860	5.20	—	—
Corvette	1.80	1523	0.83	728	2.47	397	3.55	1429	4.07	1260	5.05	—	—
	1.53	1025	0.85	446	2.45	138	3.57	1338	4.05	794	5.13	—	—
O'Connor	1.79	1282	0.85	572	2.32	328	3.42	1733	4.00	860	5.13	—	—
	1.75	1309	0.87	492	2.42	218	3.38	1396	4.00	469	4.65	—	—
Koru	1.99	1527	0.85	660	2.35	409	3.50	1455	4.02	850	5.12	323	5.48
	1.64	936	0.85	573	2.47	388	3.52	1642	3.98	699	5.15	331	5.52
Galleon	1.87	3031	0.85	150	2.20	166	3.50	1745	3.97	758	4.75	120	5.68
	—	—	—	—	—	—	—	—	—	—	—	—	—
Ulandra	1.80	1610	0.85	862	2.32	697	3.37	1660	4.03	1595	4.75	192	5.77
Malebo	1.60	1319	0.87	565	2.28	531	3.35	1552	3.97	941	4.80	90	5.75
Mean	—	1439	0.84	467	2.36	318	3.50	1550	3.98	885	4.93	186	5.69

*arbitrary units

TABLE 4 Levels of probability for differences in peak areas and retention times

Peak	Protein	Type (malting or feed)	protein x type	type x variety
1	<0.001**	0.254	0.785	0.006**
2	0.570	0.723	0.546	0.148
3	0.002**	0.078	0.738	<0.001**
4	0.226	0.142	0.155	0.080
5	0.466	0.865	0.736	0.178
6	0.451	0.142	0.525	0.204

Retention Time	Protein	Type (malting or feed)	protein x type	type x variety
1	0.156	0.036**	0.107	0.006**
2	0.675	0.138	0.303	0.48
3	0.384	0.003**	0.811	<0.001**
4	0.109	0.161	0.143	0.084
5	0.438	0.775	0.283	0.159
6	0.617	0.142	0.524	0.204

The protein content and the total peak area generally showed a positive relationship within each variety. However, there was no significant relationship between protein content and total peak area when the data for malting and feed varieties were combined ($R^2=0.26$, $P=0.05$) but there was a significant relationship for malting varieties ($R^2=0.53$, $P=0.05$).

DISCUSSION

The studies described here demonstrate that it is possible to identify barley varieties using their water soluble protein fraction. The method gave good repeatable chromatographic profiles for replicate extractions and separations. There was some variation in the chromatographs for the same varieties at different nitrogen levels, which would be expected. The value of the small peaks at the end of each chromatograph was inconclusive. Some small peaks may have arisen due to proteinase activity degrading and large peaks. However, a larger number of samples over a range of protein levels,

grown at several environments, would confirm the usefulness of some of the minor peaks of water-soluble protein.

A relationship between total grain protein and total peak area was demonstrated in this study. There was a difference in the relationship for malting and feed varieties. Other studies, including that by Bhatti⁷ showed that for some samples with differences in grain total protein level there was only a slight difference in the water-soluble protein level. On the other hand Baxter and Wainwright⁶ presented data that suggested that for different varieties at the same protein level, there could be a difference of up to 20% in the water-soluble fraction in barley.

In this study, the level of water-soluble protein did not increase linearly with total protein content. However, the difference between grain protein content and water-soluble protein, separated by IE-HPLC, may be useful in selecting potential new malting varieties for a barley breeding program. The method proved discriminating enough to use for varietal identification. Further work with the WSP group and HPLC may also lead to a better understanding of some of the barley quality traits associated with the water-soluble protein fraction.

Acknowledgements. This research was supported by funds from the Grains Research and Development Corporation. Mr D Butler is thanked for performing the statistical analysis and Dr P A. Inkerman for critically reviewing this manuscript.

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