

Use of Size-Exclusion High-Performance Liquid Chromatography for Wheat Quality Prediction in Ethiopia

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ABSTRACT

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Wheat quality testing facilities in Ethiopia are limited. The aim of this study was to determine whether size-exclusion high-performance liquid chromatography (SE-HPLC) could be used in breeding programs for quality testing. Thirteen Ethiopian and two South African wheat cultivars were evaluated in two diverse environments for milling and dough characteristics. SE-HPLC was done on the same samples. Across environments, both SDS-soluble and SDS-insoluble polymeric proteins significantly influenced important quality characteristics such as SDS-sedimentation and mixograph development time. The large monomeric proteins, which are mainly gliadins, had a consistently significantly negative

effect on quality. The increase of polymeric protein as opposed to monomeric protein led to improvement of quality characteristics. The SDS-soluble and SDS-insoluble polymeric proteins were equally important in quality prediction. The amount of polymeric proteins was significantly higher in the high-protein environment. Despite a large environmental effect on most fractions, a large ratio of polymeric proteins to monomeric proteins (both SDS-soluble and SDS-insoluble) can be a good indicator of baking quality. SE-HPLC is therefore an option to use in breeding programs in Ethiopia for quality evaluation.

SE-HPLC is a powerful tool to study native protein aggregates and physicochemical basis of baking strength and has potential for rapid assessment of baking quality of bread wheat genotypes in breeding programs (Dachkevitch and Autran 1989). SE-HPLC accurately separates the three main classes of wheat endosperm proteins into glutenin, gliadins, and albumins-globulins (Larroque et al 1997). The results obtained with this technique have been highly correlated with breadmaking quality (Batey et al 1991). Correlations were found between quantity of polymers and technological parameters linked with mixing (Singh et al 1990, 1991; Gupta et al 1992). The amount of some polymeric proteins was correlated with gluten baking strength and loaf volume (Haddad et al 1995). In Ethiopia, almost no quality testing facilities are available and, up to now, cultivars were selected only on agronomic characteristics and disease resistance. There is an increasing demand for improved quality but the facilities are not available and funding is scarce. Some research stations have HPLC machines or access to such machines currently used for other purposes. If SE-HPLC can be used for quality prediction, it can be used in Ethiopian breeding programs. Procedures like the SDS-sedimentation test would be very effective and make it cheaper to predict breadmaking quality. But if a HPLC machine is available, the breeder can also gain information on the glutenin-to-gliadin ratios in various cultivars and how they are influenced by the environment. This additional information may help the breeder to better structure a selection strategy. Therefore, the aim of this study was to determine whether SE-HPLC could be used for quality prediction in Ethiopian wheat breeding programs.

MATERIALS AND METHODS

Thirteen popularly grown Ethiopia cultivars and two South African cultivars of known quality (Kariega and SST825) were used in this study. The main criterion of selection for the Ethiopian material was agronomic performance. The trials were made at two environments in Ethiopia in 2001: Adet Research Center, in an area with a higher protein potential; and Motta, in an area with low protein potential. A randomized complete block design with

three replicates was used. The plot size was 3 m² at both locations. All recommended wheat management practices were exercised.

After harvesting, the material was transported to South Africa and measurements were made in triplicate at the laboratories of the Small Grains Institute, Bethlehem, South Africa: hectoliter weight (kg/hL), break flour yield (Approved Method 26-21A, AACC 2000), flour yield (AACC Approved Method 26-21A), flour color (Kent Jones, C76), flour protein content (Approved Method 39-11), SDS-sedimentation test (AACC Approved Method 56-70), vitreous kernels (kernels were sliced and counted), single kernel characteristic system (SKCS) hardness index (AACC Approved Method 53-31), SKCS seed diameter (AACC Approved Method 53-31), SKCS seed weight (AACC Approved Method 53-31), mixograph development time (AACC Approved Method 54-40A), farinograph water absorption (AACC Approved Method 54-21). As the material had to be transported to South Africa, not enough seed was available for the alveograph or to determine loaf volume.

Proteins were extracted from the same wheat flour with a two-step extraction procedure developed by Gupta et al (1993). The first step extracts the proteins soluble in dilute SDS, while the second extract contains proteins soluble only after sonication. For the first extraction, 11 mg of white flour was suspended in 1.0 mL of 0.5% (w/v) SDS-phosphate buffer (pH 6.9) and vortexed for 10 sec. Samples were then stirred for 5 min at 2,000 rpm and centrifuged for 30 min at 10,000 × g to obtain the supernatant protein. The pellet was subsequently resuspended in SDS buffer as above and sonicated in an ultrasonic disintegrator (Branson B12 sonifier) for 30 sec, amplitude 5, and fitted with a 3-mm exponential microtip. The samples were centrifuged as above to obtain a supernatant of proteins. The extracts were filtered through 0.45-μm filters (Millipore, Durapore membrane filters) before running on HPLC. Size-exclusion HPLC analyses were performed on a Varian HPLC system using a BIOSEP SEC-4000 Phenomenex column. Separation was achieved in 30 min by loading 20 μL of sample into an eluent of 50% (v/v) acetonitrile and water containing 0.1% (v/v) trifluoroacetic acid at a flow rate of 0.2 mL/min. Proteins were detected by UV absorbance at 210 nm. Areas of the different peaks were calculated.

The percentage of total unextractable polymeric protein in the total polymeric protein ([SDS-insoluble large and smaller protein polymers]/[SDS-soluble and insoluble large and smaller protein polymers]) and the percentage of large unextractable polymeric protein in the total large polymeric protein ([SDS-insoluble large protein polymers]/[SDS-soluble and SDS-insoluble large protein polymers]) was calculated (Gupta et al 1993).

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TABLE I
Measured Quality Characteristics at Two Locations (Adet and Motta) in Ethiopia^a

Sample No.	Name	Loc	SDS	VK	FPC	FLY	MDT	WGHT	DIAM	HI
1	Har457	A	42	72	9.1	60.4	2.3	35.42	2.38	46.85
		M	38	40	7.4	60.2	2.6	36.46	2.48	47.91
2	Har2348	A	37	68	9.3	60.8	2.2	35.09	2.43	9.81
		M	33	52	8.7	58.8	2.2	36.04	2.49	23.33
3	Har2807	A	37	72	10.4	63.7	1.9	30.97	2.05	59.88
		M	29	70	8.4	59.7	1.7	37.02	2.29	59.18
4	Har2096	A	39	68	9.5	61.5	2.4	31.92	2.14	56.46
		M	31	68	8.6	62.7	2.5	30.82	2.19	52.56
5	Har2562	A	48	74	10.5	61.9	3.0	32.85	2.23	60.41
		M	37	72	8.7	59.7	3.2	33.84	2.35	60.42
6	ET13A2	A	54	68	9.8	63.5	2.0	29.87	2.21	24.78
		M	31	50	8.2	64.2	2.0	28.88	2.18	21.22
7	Har1709	A	52	72	9.3	63.7	2.5	30.54	2.15	48.47
		M	35	54	6.7	61.3	3.1	30.38	2.17	41.80
8	Har1685	A	53	60	9.1	58.6	2.9	25.54	1.90	55.47
		M	42	24	6.8	62.8	3.1	33.59	2.33	28.20
9	Har604	A	76	68	8.8	61.5	3.7	31.79	2.15	50.19
		M	43	36	6.7	60.6	4.5	35.23	2.52	44.50
10	Har1522	A	54	72	9.6	56.5	5.0	25.48	2.01	67.39
		M	33	58	6.7	57.5	6.0	24.74	1.93	58.18
11	Har1775	A	42	84	10.3	61.9	4.2	25.63	1.79	62.46
		M	32	58	6.9	59.0	5.0	26.72	2.02	56.75
12	Har1868	A	59	82	9.1	57.9	2.7	27.56	1.99	58.03
		M	43	24	6.3	58.7	6.0	31.01	2.24	31.35
13	Har2505	A	55	48	8.9	62.7	2.7	28.53	2.03	42.71
		M	33	56	7.0	68.8	4.2	28.90	2.15	49.89
14	Kariega	A	80	62	10.2	63.9	2.5	31.28	2.21	38.62
		M	55	20	7.3	63.7	3.3	36.41	2.49	18.12
15	SST825	A	56	84	10.3	61.5	3.8	26.90	2.13	61.87
		M	43	50	7.2	59.4	5.0	34.55	2.63	46.13
	Average	A	52	68	9.6	61.1	2.9	30.58	2.16	48.69
		M	37	54	7.3	60.9	3.8	32.18	2.28	40.61

^a A, Adet; M, Motta; SDS, SDS sedimentation value; VK, vitreous kernels; FPC, flour protein content; FLY, flour yield; MDT, mixograph mixing time; Wght, SKCS single kernel weight; Diam, SKCS single kernel diameter; HI, hardness index.

The measured HPLC fractions were SDS-soluble and SDS-insoluble, with each subdivided into large polymeric proteins (LPP), smaller polymeric proteins (SPP), large monomeric proteins (LMP), mainly gliadins, and smaller monomeric proteins (SMP), mainly albumins and globulins. All statistics were analyzed with Agrobases 2000 software. To determine the real contribution of the protein fractions, and to make sure that correlations were not due to the protein content of the lines, each fraction was divided by the protein content, and then used again to determine correlation values.

RESULTS

Adet had an average flour protein content of 2.3% higher than Motta (Table I). Adet, consequently, had a much higher average SDS-sedimentation. All hardness parameters were higher at Adet.

The ANOVA (Table II) indicated that for all eight measured fractions, there were no significant differences between the replicates at Adet, but at Motta there was a replicate effect for five of the fractions. There was a strong genotype effect at Adet for LPP, LMP, SMP, and SDS-insoluble LPP; at Motta only SPP and LMP had a strong genotype effect. Across the two locations, the genotype effect was significant for all four SDS-soluble fractions. The location effect was highly significant for all eight fractions. The replicate × location effect was significant for five fractions, and location × sample effect was significant for four fractions. It therefore seems that the environment largely influenced the fractions.

For the ratios between the different fractions (Table III) SDS-soluble LPP/SMP and SPP/SMP and SDS-insoluble LPP/SMP were not much different between the two locations. For all other fractions, the ratios were consistently higher at Adet, the high protein environment.

Correlations between specific subunits and quality characteristics were limited for Adet. SDS-insoluble LMP were negatively

correlated with SDS-sedimentation and TUP were positively related with hardness (Table IV).

At the low protein environment Motta, the SDS-insoluble SPP were significantly positively and LMP significantly negatively correlated with SDS-sedimentation and mixograph development time. The reverse was true for flour protein content where SPP had a strong negative and LMP a strong positive relationship with protein content. TUP were significantly correlated with flour protein content (negatively) and mixograph development time.

When the two data sets were combined (Table IV) for the SDS-soluble fractions, LPP were highly significantly correlated with SDS-sedimentation, vitreous kernels, and flour protein content. SPP were significantly correlated with vitreous kernels and flour protein content. The LMP were negatively correlated with SDS-sedimentation, vitreous kernels, and flour protein content. SMP were positively correlated with vitreous kernels and flour protein content.

For the SDS-insoluble protein fractions, the LPP were again highly significantly correlated with SDS-sedimentation, vitreous kernels, and flour protein content. The LMP were significantly negatively correlated with vitreous kernels and flour protein content.

TUP significantly positively correlated with SDS-sedimentation, vitreous kernels, and flour protein content. LUP significantly positively correlated with all the same characteristics with the exception of vitreous kernels.

When the protein component was standardized to make sure that protein content does not cause correlations (Table V), protein content was strongly negatively correlated with all measured protein fractions. Motta SDS-insoluble SPP were significantly positively correlated with mixograph development time and SDS sedimentation and negatively correlated with vitreous kernels. Motta SDS-insoluble LPP, SPP, and LMP were positively correlated with mixograph development time. SDS-insoluble SPP were also correlated with SDS sedimentation (positively) and vitreous kernels

TABLE II
Mean Squares of Protein Fractions at Separate Locations and Across Locations^{a,b}

Source	Loc	SDS Soluble Proteins				SDS Insoluble Proteins			
		LPP	SPP	LMP	SMP	LPP	SPP	LMP	SMP
Rep	A	3.93	1.65	42.43	15.48	5.13	3.31	1.63	5.98
	M	9.53**	40.65**	112.74**	1.40	318.11**	7.40	391.75**	15.41
Sample	A	3.35*	20.10	124.13**	44.18*	13.32*	12.13	12.57	22.06
	M	0.487	4.28**	12.38*	8.06	14.55	12.76	22.29	15.99
	A+M	2.01*	13.57*	83.04**	31.94**	9.45	16.14	24.67	11.64
Loc	A+M	128.76**	678.59**	6,044.88**	1,314.84**	426.32**	607.52**	788.66**	243.28**
Rep × Loc	A+M	6.73**	21.15*	77.58**	8.44	161.62**	5.35	196.69**	10.69
Loc × Sample	A+M	1.82*	10.82	53.48**	20.30	18.41*	8.75	10.19	26.40*

^a A, Adet; M, Motta. LPP, large polymeric proteins; SPP, small polymeric proteins; LMP, large monomeric proteins; SMP, small monomeric proteins.

^b *,** $P = 0.05$ and 0.01 , respectively.

TABLE III
Ratios of Different Protein Fractions at Two Locations^a

Extraction	Ratios	Adet	Motta
SDS soluble	LPP/SPP	0.37	0.35
	LPP/LMP	0.12	0.06
	LPP/SMP	0.30	0.30
	SPP/LMP	0.33	0.15
SDS insoluble	SPP/SMP	0.84	0.87
	LPP/SPP	0.56	0.48
	LPP/LMP	0.63	0.40
	LPP/SMP	2.13	1.17
	SMP/LMP	1.14	0.82
	SMP/SMP	3.84	2.38

^a LPP, large polymeric proteins; SPP, small polymeric proteins; LMP, large monomeric proteins; SMP, small monomeric proteins.

(negatively). For Adet and Motta combined, SDS-soluble LMP significantly negatively influenced SDS sedimentation and vitreous kernels (the same as in Table IV) but were positively correlated with mixograph development time. SDS-insoluble LPP was positively correlated with mixograph development time. This was different from the uncorrected protein correlations where the SDS sedimentation, vitreous kernels, and flour protein content were all positively correlated with this fraction. SDS-insoluble SPP and LMP were positively correlated with mixograph development time and negatively with vitreous kernels. This was very different from the previous table, where SDS-insoluble LMP were negatively correlated with SDS-sedimentation and vitreous kernels, and SDS-insoluble SPP were negatively correlated with SDS-sedimentation. SDS-insoluble SMP were negatively correlated with vitreous kernels and positively correlated with single kernel diameter.

Correlations between the ratios of the protein components and the quality characteristics (Table VI) revealed that the ratio between SDS-soluble LPP and LMP significantly influenced SDS-sedimentation, vitreous kernels, and flour protein content. With the exception of SDS-sedimentation, the ratio between SPP and LMP influenced the same characteristics. For the SDS-insoluble fractions, the ratio between LPP and LMP and between SPP and LMP significantly influenced SDS-sedimentation, vitreous kernels, and flour protein content. The ratio between LPP and SMP and between SPP and SMP had the same effect on all the characteristics with the exception of vitreous kernels for the first ratio and vitreous kernels and flour protein content for the second ratio. The SDS-insoluble LPP/SPP also significantly influenced flour protein content. When the protein corrected fractions were used for the correlations, SDS-soluble LPP/LMP ratio was still positively correlated with flour protein content, but there were no other significant correlations. For SDS-soluble SPP/LMP, the correlations stayed much the same. For SDS-insoluble LPP/SMP, correlations with SDS sedimentation and flour protein was much the same, but there was no correlation with vitreous kernels as previously. For SDS-insoluble LPP/SMP, the correlation with SDS-sedimentation was

TABLE IV
Significant Correlations Between Specific Protein Fractions and Quality Characteristics^{a,b}

Location	Character 1	Character 2	Correlation	
Adet	SDS insol LMP	SDS sedimentation	-0.577*	
	TUP	Hardness	0.562*	
Motta	SDS insol SPP	SDS sedimentation	0.653*	
	SDS insol SPP	Flour protein content	-0.605*	
		Mixograph development time	0.589*	
	SDS insol LMP	SDS sedimentation	-0.559*	
		Flour protein content	0.709**	
		Mixograph development time	-0.566*	
	TUP	Flour protein content	-0.707	
		Mixograph development time	0.642*	
	Motta and Adet	SDS sol LPP	SDS sedimentation	0.446*
			Vitreous kernels	0.513**
		Flour protein content	0.649**	
SDS sol SPP		Vitreous kernels	0.420*	
		Flour protein content	0.620**	
SDS sol LMP		SDS sedimentation	-0.404*	
		Vitreous kernels	-0.502**	
		Flour protein content	-0.702**	
SDS sol SMP		Vitreous kernels	0.546**	
		Flour protein content	0.675**	
SDS insol LPP		SDS sedimentation	0.526**	
		Vitreous kernels	0.432*	
		Flour protein content	0.555**	
SDS insol SPP		SDS sedimentation	0.590**	
SDS insol LMP		SDS sedimentation	-0.742**	
		Vitreous kernels	-0.419*	
		Flour protein content	-0.562**	
SDS insol SMP		SDS sedimentation	-0.383*	
TUP	SDS sedimentation	0.595**		
	Vitreous kernels	0.415*		
	Flour protein content	0.571**		
LUP	SDS sedimentation	0.486**		
	Flour protein content	0.451*		

^a SDS, SDS sedimentation value; LPP, large polymeric proteins; SPP, small polymeric proteins; LMP, large monomeric proteins; SMP, small monomeric proteins; TUP, % total unextractable polymeric proteins; LUP, % large unextractable polymeric proteins.

^b *,** $P = 0.05$ and 0.01 , respectively.

replaced with a correlation with mixograph mixing time, and the correlation with protein content became negative. The other correlations stayed the same as previously discussed.

DISCUSSION AND CONCLUSIONS

In the individual environments, the SE-HPLC fractions had a smaller effect on quality characteristics than the combined environments. Both the soluble and insoluble LPP, which are mainly the HMW glutenins, highly significantly influenced important quality characteristics such as SDS sedimentation and mixograph mixing time. The SPP, which are mainly the LMW glutenins, also positively influenced these characteristics. In other studies where

TABLE V
Significant Correlations Between Specific Protein Fractions Corrected for Protein Content and Quality Characteristics^{a,b}

Location	Character 1	Character 2	Correlation
Adet	SDS insol SPP	Flour protein content	-0.726**
	SDS insol LMP	Flour protein content	-0.626*
Motta	SDS sol LPP	Flour protein content	-0.771**
	SDS sol SPP	Flour protein content	-0.857**
	SDS sol LMP	Flour protein content	-0.966**
		Mixograph development time	0.821**
	SDS insol LPP	Flour protein content	-0.820**
		Mixograph development time	0.648*
	SDS insol SPP	Flour protein content	-0.929**
		Mixograph development time	0.768**
		SDS sedimentation	0.560*
		Vitreous kernels	-0.609*
	SDS insol LMP	Flour protein content	-0.849**
		Mixograph development time	0.623*
Adet and Motta	SDS sol LMP	SDS sedimentation	-0.401*
		Vitreous kernels	-0.710**
		Flour protein content	-0.926**
		Mixograph development time	0.516**
	SDS insol LPP	Flour protein content	-0.378*
		Mixograph development time	0.383*
	SDS insol SPP	Vitreous kernels	-0.606**
		Mixograph development time	0.620**
		Flour protein content	-0.759**
	SDS insol LMP	Vitreous kernels	-0.708**
		Mixograph development time	0.427*
		Flour protein content	-0.933**
SDS insol SMP	Vitreous kernels	-0.525**	
	Flour protein content	-0.727**	
	Single kernel diameter	0.386*	

^a SDS, SDS sedimentation value; LPP, large polymeric proteins; SPP, small polymeric proteins; LMP, large monomeric proteins; SMP, small monomeric proteins.

^b *,** $P = 0.05$ and 0.01 , respectively.

proteins were also extracted using sonication, the first fraction (HMW glutenins) can be used as a measure of breadmaking quality, and the third fraction (gliadins) was negatively correlated with loaf volume (Singh 1990, 1991). The correlation between quality of polymers and technological parameters was confirmed by Gupta et al (1992). In this study, all eight protein fractions were significantly influenced by growing conditions, but for all four SDS-soluble fractions there was also a strong genotype effect. This was also found by Dachkevitch and Autran (1989), where the insoluble polymers were not taken into consideration and positive correlation was found between glutenins and technical parameters. Both SDS-soluble and SDS-insoluble LMP (mainly the gliadins) had a consistently significant negative effect on important quality traits. The SDS-soluble SMP (mainly globulins) positively influenced quality, while the SDS-insoluble globulins had a negative effect on quality. The total unextractable proteins as well as the large unextractable proteins both had a large positive influence on quality, as reported by Jia et al (1996). The importance of the polymeric proteins versus the monomeric proteins was confirmed in the fact that an increase in the polymeric proteins in the ratio, either small or large or SDS-soluble or SDS-insoluble proteins, led to an improvement in important quality characteristics such as SDS-sedimentation and flour protein content. The polymeric proteins almost consistently were much higher in the high protein environment, and the poor protein environment saw an increase in undesirable monomeric proteins. This was also found by Johansson et al (2001). The F1/F2 ratio was a good predictor of quality (Dachkevitch and Autran 1989), but in our study, a high ratio of any polymeric to monomeric proteins led to improved quality. In general, the SDS-soluble and SDS-insoluble proteins were equally important in quality prediction, which is in contrast with other reports (Gupta et al 1993; Bean et al 1998) that found SDS-insoluble proteins to be more important. When the effect of flour

TABLE VI
Significant Correlations Between Ratios of Protein Fractions and Measured Quality Characteristics^a

Ratio	Quality Characteristic	Correlations ^{b,c}
SDS soluble LPP:LMP	SDS sedimentation	0.406*
	Vitreous kernels	0.507**
SDS soluble SPP:LMP	Flour protein content	0.653** (0.578**)
	Vitreous kernels	0.428* (0.379*)
SDS insol LPP:SMP	Flour protein content	0.621** (0.561**)
	SDS sedimentation	0.650** (0.487**)
SDS insol LPP:SMP	Vitreous kernels	0.469*
	Flour protein content	0.598** (0.430**)
	SDS sedimentation	0.487**
SDS insol SPP:LMP	Flour protein content	0.430* (-0.716**)
	Mixograph mixing time	(0.392*)
	SDS sedimentation	0.751** (0.751**)
SDS insol SPP:SMP	Vitreous kernels	0.387* (0.387*)
	Flour protein content	0.505** (0.505**)
SDS insol LPP:SPP	SDS sedimentation	0.526** (0.526**)
	Flour protein content	0.395* (0.395*)

^a SDS sedimentation value; LPP, large polymeric proteins; SPP, small polymeric proteins; LMP, large monomeric proteins; SMP, small monomeric proteins.

^b Correlations for protein corrected ratios are in parentheses.

^c *,** $P = 0.05$ and 0.01 , respectively.

protein content was standardized for all samples, the significant correlations for Motta increased significantly. SDS sedimentation became less pronounced in the correlations for combined data for Adet and Motta, and mixograph development time featured more. SDS-soluble LMP still negatively influenced SDS sedimentation, but was correlated positively with mixograph mixing time. All the monomeric proteins were negatively correlated with vitreous kernels. So even with the effect of the protein content minimized, the protein fractions were still significantly correlated with quality characteristics.

Therefore, in this study, SDS-soluble and SDS-insoluble LPP and SPP and percentage of total and large unextractable proteins were good predictors of quality characteristics. A large polymeric-to-monomeric protein ratio was also related to better quality. If cultivars can be selected for increased polymeric proteins and reduced monomeric proteins, it should lead to an improvement in quality in all environments. Although HPLC was done on flour in this study, equally good results were obtained using crushed kernels (data not shown). Therefore, SE-HPLC can be used in Ethiopian breeding programs to assess breadmaking quality.

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