African Crop Science Journal, Vol. 22, No. 1, pp. 31 - 43
ISSN 1021-9730/2014 $4.00

PREDICTION OF BREAD-MAKING QUALITY USING SIZE EXCLUSION HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

M.E. MOROJELE and M.T. LABUSCHAGNE[1]
Small Grain Institute, Private Bag X29, Bethlehem, 9700, South Africa
[1]Department of Plant Science, University of Free State, P. O. Box 339, Bloemfontein 9300, South Africa
Correspondence author: morojelee@arc.agric.za

(Received 31 July, 2013; accepted 7 Februay, 2014)

ABSTRACT

Variation in the distribution of protein molecular weight in wheat (*Triticum aestivum*), influences breadmaking quality of wheat cultivars, resulting in either poor or good bread. The objective of this study was to predict breadmaking quality of wheat cultivars using size exclusion high performance liquid chromatography. Seeds from twenty F_1 and F_2 progeny, and their parents were used. A procedure was followed involving extraction by Sodium Dodecyl Sulphate (SDS), followed by sonication to remove remaining proteins. A computer software Chromsword developed by Shimadzu Corporation was used to estimate different protein classes, after which data were generated from chromatogram. The highest Glutenin/Gliadin (GG) ratio in SDS-soluble and insoluble protein fractions in F_1 progeny, were obtained from Wanda x Sceptre and Nata x SST 124 cultivars, respectively. In F_2 progeny, the cultivar with the highest G/G ratio in SDS soluble protein fractions was Kariega x SST124. Cultivars with high G/G ratios in SDS-insoluble protein fraction were Sceptre x Nata and Kariega x Sceptre. The ratios of high molecular weight/low molecular weight glutenin sub-units (HMW/LMW-GS) in both SDS-soluble and insoluble protein fractions were comparable in F_1 progeny with little variation among cultivars. In F_2 progeny, Kariega x SST 124 led in high HMW-GS/LMW-GS ratio. The highest ratio of polymeric/monomeric protein in both F_1 and F_2 progeny was obtained from Nata x Sceptre in both generations. Therefore, most wheat cultivars revealed high breadmaking quality.

Key Words: Protein fractions, *Triticum aestivum*

RÉSUMÉ

La variation dans la distribution du poids moléculaire de protéines dans le blé (*Triticum aestivum*), influence la qualité panifiable des cultivars du blé, résultant en un pain pauvre ou bon. L'objectif de cette etude était de prédire la qualité panifiable des cultivars de blé en utilisant un liquide chromatographique de performance élevée d'exclusion de taille. Les grains de vingt progénies F_1 et F_2 et leurs parents étaient utilisés. La procédure consistait en une extraction par le Sulphate Dodecyl de Sodium (SDS), suivi par sonication pour enlever les protéines restantes. Un ordinateur de logiciel Chromsword développé par l'Entreprise Shimadzu était utilisé pour estimer différentes classes de protéines, après lesquelles les données étaient générées du chromatogramme. Le rapport le plus élevé Glutenin/Gliadin (GG) dans les SDS-soluble et les fractions de protéines insolubles dans la progénie F_1 était obtenu des cultivars Wanda x Sceptre et Nata x SST, respectivement. Dans la progénie F_2, le cultivar avec le rapport le plus élevé G/dans les fractions de protéines SDS soluble était Kariega x SST124. Les cultivars avec des rapports élevés G/G dans la fraction de protéine insoluble SDS étaient Sceptre x Nata et Kariega x Sceptre. Les rapports du poids moléculaire élevé sur le poids moléculaire bas des sous unités de la glutenine (HMW/LMW-GS) dans le SDS-Soluble et les fractions de protéines insolubles étaient comparables dans la progénie F1 avec une petite variation parmi les cultivars. Dans F_2, Kariega x SST 124 donné un rapport HMW-GS/LMW-GS élevé. Le rapport le plus élevé des protéines polymerique/monomerique dans les progénies F_1 et F_2 était obtenu de Nata x Sceptre dans toutes les deux generations. Par conséquent, laplupart des cultivars de blé ont révélé une qualité panifiable élevée.

Mots Clés: Fractions de protéine, *Triticum aestivum*

INTRODUCTION

Breadmaking quality of wheat flour is determined by its protein composition (Singh *et al.*,1990). Hence, substantial research has been conducted to explain which protein constituents account for the differences in quality (Bietz,1990). On the basis of their solubility, proteins are classified into globulin, which is soluble in alkaline; albumin, soluble in water; gliadin soluble in alcohol; and glutenin soluble in acid solution (Hubbard *et al*., 1997).

Other researchers classified protein constituents according to their molecular size; larger than 100 kilodaltons (Kda) as glutenin, between 100 and 25 kda as gliadin, and smaller than 25 kda as classified into either albumin or globulin (Erickson, 2009). The origins, structures, properties and relationships of the properties of proteins are well documented (Bietz, 1990).

Many cereal proteins interact non-covalently with endosperm constituents such as lipids and carbohydrates, and associate either with non-covalently through hydrogen or hydrophobic bonds or covalently through disulphides, with each other to form high molecular weight complex (Beitz,1990).

In a study by Fleurent (1986) variations in glutenin-gliadin ratio were highly correlated with wheat quality. By varying the proportions of these fractions, while maintaining the total protein level constant, it was found possible to get 20-fold variation in dough resistance and 2.5-fold in extensibility of dough (Kim *et al*., 1988). This showed that the properties of dough are determined by the relative proportions of these fractions. Furthermore, it was observed that failure to correlate glutenin-gliadin ratio with breadmaking quality of cultivars, is attributed to inconsistent solubility of protein from different wheat cultivars (Lookhart *et al*.,1986). Similarly, baking studies employing classical flour reconstitution techniques, have ascertained that glutenin/gliadin proteins are major factors governing wheat quality (Uthayakumaran, 1999).

Glutenin comprises of various types of protein sub-units that are linked to each other by disulphide bonds. Subsequently, these form polymeric and monomeric sub-units with molecular weight in millions (Kasarda, 1989; Wrigley, 1996). Variations in the type and amount of sub-units correlate with quality among cultivars of wheat, most probably influencing the molecular weight distribution of the glutenin polymers (Gupta *et al*.,1993). These sub-units are classified as high and low molecular weight glutenin sub-units. The ratios of polymeric/ monomeric and high/low molecular weight glutenin sub-units are used to predict the quality of wheat for breadmaking purpose.

It is, therefore, imperative that a quick, accurate and affordable method for this purpose is sought. Recently, high performance liquid chromatography was found to be the most successful for this because of its speed, automation, quantitative ability and small sample required. The objective of this study was to predict breadmaking quality of wheat cultivars using size exclusion high performance liquid chromatography.

MATERIALS AND METHODS

A study was performed at Bloemfontein, located in South Africa at1351 m above sea level, 26°18' East and 29° 06' South. The site experiences temperatures of 31 and -4 °C in summer and winter, respectively. Total annual solar radiation is 3315.6 hr, ranging from 249 to 319 hr in summer. The average annual rainfall is 700 mm, occurring between October and April. Soil is 10% montmorillonite clay. The depth of top- and sub-soil is 600-1200 and 400-900 mm, respectively.

Parent materials used in the experiment were commercial wheat cultivars obtained from South Africa germplasm,with good, medium and poor breadmaking quality. These were Kariega and SST 124 (good), Wanda (medium), Nata and Sceptre (poor). Parents were grown in pots under environmentally controlled greenhouse conditions, whereby they were crossed in all possible combinations. Seed materials obtained from the crosses (F_1) were planted and harvested. Seeds harvested from F_1 were planted to obtain F_2 following the same agronomic practices. The plots were laid-out in randomised complete block design, with three replications. Each plot measured 2 m x 1.8 m, with inter-row and intra-row spacings of 45 and 10 cm, respectively.

Proteins in wheat kernels were extracted following a two-step extaction procedure developed by Gupta *et al*. (1993). The first step extracts proteins soluble in dilute SDS; while the second step extracts proteins soluble only with sonication. The procedure in the first step involved suspension of 1.07 mg white flour in 1.5 ml of 0.5% (w/v) SDS phosphate buffer (pH 6.9). Thereafter, the stuff is vortexed for 5 min at 2000 rpm and centrifuged for 30 min at 2000xg to obtain the supernatant protein.

The procedure in the second step involved resuspension of pellets from the first step, in 1.5 ml SDS phosphate buffer again, shake for 5 min with a mechanical shaker and sonicated in an ultrasonic desintegrator (Soniprep 150, Tamro, Molndal, Sweedam) amplitude 5 and fitted with 3 mm exponential microtip for 30 sec. The samples were centrifuged as above to get supernatant proteins. The supernatants were filtered through 0.45 µl filters (Millipore, Durepore membrane filters) before running on HPLC.

Size exclusion HPLC analysis was carried out on a varian HPLC system using a BIOSEP SECC-4000 column (Phenomenex). Separation was done in 30 min by loading 20 µl of sample into an eluant of 50% (v/v) acetonitrile and water containing 0.1% (v/v) trifluvoacetic acid (TFA) at a flow rate of 0.2 ml min^{-1}. 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 and the percentage of large unextractable polymeric protein were calculated according to Gupta *et al.* (1993).

The data used in the analysis were generated from chromatogram in Figures 1 and 2 for SDS-soluble and insoluble protein fractions, respectively.

The data were subjected to analysis of variance (ANOVA) using Agrobase Generation 11 software (2000). Mean separation was done by Least Significant Difference at 5% level of significance. The means for protein fractions such as LPP, SPP, LMP and SMP in both SDS-soluble and insoluble proteins were used to work out the ratios as follows:

$$\text{(a) Glutenin/Gliadin ratio} = \frac{\text{(HMW-GS + LMW-GS)}}{\text{Gliadin}}$$

Where: HMW = High Molecular Weight, GS = Glutenin Sub-units, LMW = Low Molecular Weight

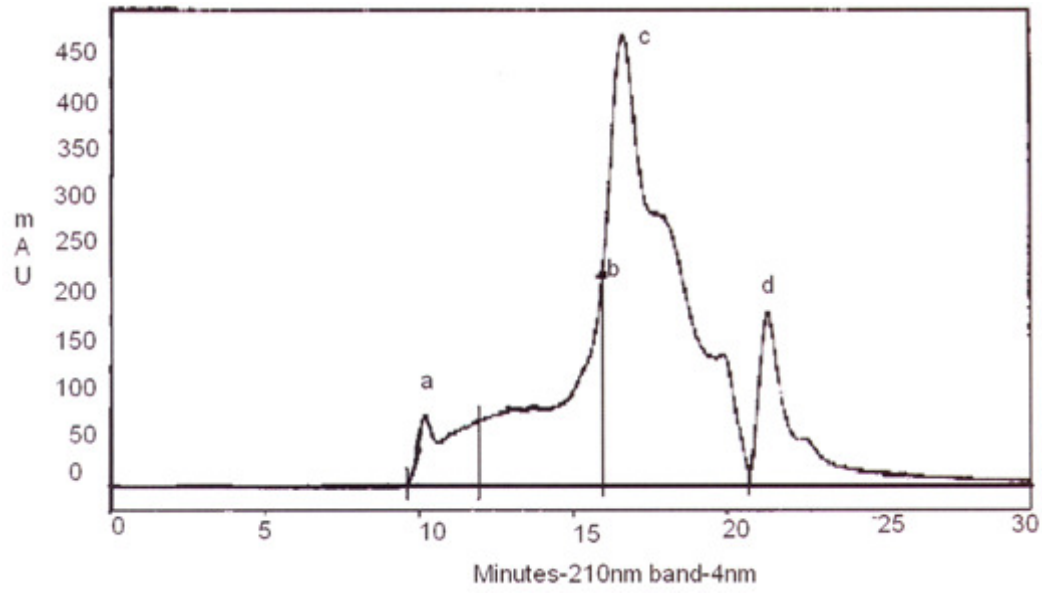

Figure 1. SDS-soluble proteins as separated with SE-HPLC where a = large polymeric proteins (LPP), b = small polymeric proteins, c = large monomeric proteins, d = small monomeric protein, mAU = milli-Amp Unit.

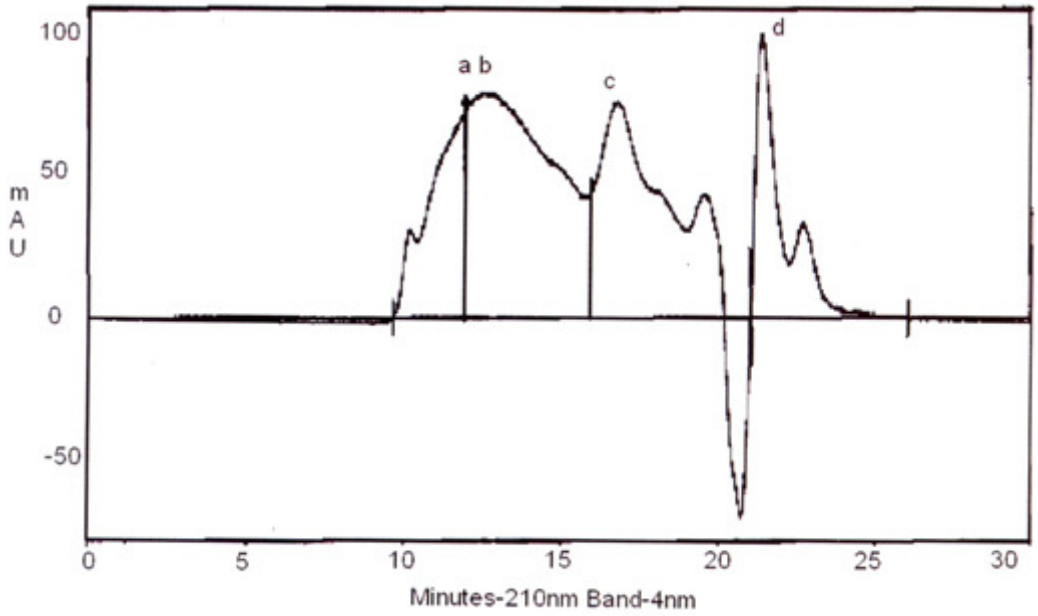

Figure 2. SDS-insoluble proteins as separated with SE-HPLC a = large polymeric proteins (LPP), b = small polymeric proteins, c = large monomeric proteins, d = small monomeric proteins. mAU = milli-Amp Unit.

(b) HMW/LMW ratio = $\frac{\text{HMW-GS}}{\text{LMW-GS}}$

(c) Polymeric/Monomeric ratio = $\frac{\text{Total polymeric proteins}}{\text{Total monomeric proteins}}$

RESULTS

Glutenin/Gliadin ratio. There was a wide range of glutenin/gliadin ratio in both parents and progeny under SDS-soluble and insoluble protein fractions (Table 1). The highest ratio in SDS-soluble protein fraction in F_1 progeny was obtained from Wanda x Sceptre, followed by Nata x SST 124; while the least ratio was in Sceptre and Wanda. Most cultivars obtained ratios around 4:1, followed by ones with ratios above 5:1. Very few cultivars showed ratios below 4:1.

In F_1 progeny, the highest ratio of SDS-insoluble protein fraction was achieved by Nata x SST124 with 6.79:1, followed by Kariega x Wanda with 6.29:1.

The least ratio was exhibited by Kariega x SST 124 with 2.71:1. Similarly, most of the cultivars reached a glutenin/gliadin ratio of around 4:1 with very few above it. Those that had ratio below 4:1 were many, particularly with 3:1. All parents showed a ratio of around 5:1, with insignificant differences among them for SDS-soluble protein fractions. As for SDS-insoluble protein fraction, wider variation in glutten/gliadin ratio was found among parents ranging from as low as 3.09:1 in Sceptre to a high of 6.97:1 in Kariega.

F_2 progeny exhibited high glutenin/gliadin ratios in SDS-soluble protein fractions, with 9 progenies obtaining around 6:1 and another 9 around 5:1. Two progenies (Sceptre x Wanda and Wanda x SST 124) reached the lowest ratios of 3.26:1 and 4.89:1, respectively. In SDS-insoluble protein fractions, glutenin/gliadin ratio was low with a little variation of as high as 4.28:1 to as low as 3.05:1. The highest ratio was obtained in Sceptre x Nata, followed by Kariega x Sceptre; while the least was achieved by Nata X Sceptre. There was very little variation among the parents in SDS-soluble protein fraction, even though their ratio was high. Similarly, parents exhibited a low variation in glutenin/gliadin ratio.

HMW-GS/LMW-GS ratio. The ratios of HMW-GS/LMW-GS in both SDS-soluble and insoluble protein fractions were consistent in F_1 progeny, with little variation among the cultivars. In SDS-

TABLE 1. Ratios of protein fractions used to determine breadmaking quality of wheat

Genotypes	Parents and F_1 progeny					Parents and F_2 Progeny				
	SDS-soluble protein fractions		SDS-insolubleprotein fraction			SDS-soluble protein fractions		SDS-insoluble protein fractions		
	Glutenin/ gliadin ratio	HMW-GS/ LMW-GS	Glutenin/ gliadin ratio	HMW-GS/ LMW-GS	PP/MP ratio	Glutenin/ gliadin ratio	HMW-GS/ LMW-GS	Glutenin/ gliadin ratio	HMW-GS/ LMW-GS	PP/MP ratio
1	4.84:1	4.10:1	4.94:1	3.98:1	3.00:1	5.24:1	1.97:1	3.54:1	3.51:1	2.93:1
2	7.00:1	3.21:1	3.37:1	2.86:1	2.25:1	5.39:1	1.87:1	4.18:1	5.08:1	2.09:
3	6.23:1	3.37:1	4.30:1	3.99:1	2.75:1	6.34:1	1.91:1	3.92:1	3.82:1	2.74:1
4	3.84:1	3.17:1	3.83:1	3.49:1	2.31:1	6.49:1	1.87:1	4.28:1	3.21:1	2.25:1
5	4.07:1	3.04:1	3.70:1	3.12:1	2.11:1	6.46:1	2.71:1	3.57:1	3.11:1	2.07:1
6	4.79:1	3.48:1	4.22:1	2.96:1	2.24:1	4.89:1	2.53:1	3.92:1	3.60:1	2.23:1
7	2.66:1	3.22:1	4.10:1	2.8:1	2.07:1	3.26:1	2.65:1	3.36:1	3.26:1	2.04:1
8	4.14:1	2.15:1	4.30:1	2.53:1	1.73:1	5.96:1	2.17:1	3.30:1	3.77:1	1.71:1
9	6.58:1	1.00:1	6.79:1	3.17:1	1.58:1	5.73:1	2.24:1	3.29:1	3.95:1	1.60:1
0	3.24:1	3.63:1	6.29:1	3.56:1	2.57:1	6.33:1	2.12:1	3.66:1	3.33:1	2.63:1
11	4.86:1	4.16:1	3.89:1	3.09:1	2.68:1	6.63:1	2.90:1	3.21:1	3.53:1	2.61:1
12	4.94:1	4.41:1	3.43:1	2.43:1	3.14:1	6.58:1	2.45:1	3.25:1	3.13:1	2.62:1
13	4.74:1	4.67:1	2.71:1	3.06:1	3.15:1	6.67:1	1.97:1	3.27:1	2.45:1	3.05:1
14	4.84:1	4.44:1	3.01:1	3.26:1	3.02:1	6.57:1	2.35:1	4.27:1	3.69:1	2.93:1
15	4.99:1	4.30:1	3.88:1	3.76:1	3.37:1	5.76:1	2.80:1	3.75:1	4.00:1	3.32:1
16	5.62:1	3.92:1	5.62:1	3.76:1	3.53:1	5.59:1	2.44:1	3.05:1	4.02:1	3.53:1
17	5.23:1	4.52:1	4.54:1	3.46:1	3.31:	6.07:1	3.55:1	3.93:1	4.79:1	2.27:1
18	6.42:1	3.97:1	4.78:1	3.75:1	3.40:1	5.34:1	2.88:1	3.79:1	4.17:1	3.44:1
19	6.38:1	3.80:1	6.09:1	4.06:1	3.41:1	5.30:1	3.08:1	3.23:1	4.14:1	3.51:1
20	6.24:1	3.80:1	4.37:1	3.46:1	3.12:1	5.71:1	2.66:1	3.24:1	4.79:1	3.14:1
21	5.95:1	3.86:1	4.05:1	3.53:1	2.63:1	5.79:1	2.66:1	3.18:1	4.87:1	2.69:1
22	5.92:1	3.62:1	6.72:1	3.62:1	3.02:1	6.20:1	2.92:1	2.81:1	5.01:1	3.13:1
23	5.85:1	4.07:1	6.97:1	4.52:1	2.95:1	5.76:1	2.94:1	3.56:1	4.71:1	3.00:1
24	5.63:1	4.45:1	3.57:1	2.76:1	2.56:1	6.03:1	2.86:1	4.25:1	4.45:1	2.51:1
25	5.55:1	4.22:1	3.09:1	2.17:1	2.46:1	4.93:1	2.98:1	3.81:1	3.86:1	2.45:1
LSD (5%)	1.42	0.98	1.56	0.87	0.77	1.01	0.82	0.91	1.67	0.80

1 = Nata x Wanda, 2 x Wanda x Sceptre, 3 = Sceptre x Kariega, 4 = Sceptre x Nata, 5 = Nata x Kariega, 6 = Wanda x SST124, 7 = scepter x Wanda, 8 = Sceptre x Kariega, 9 = Nata x SST 124, 10 = Kariega x Wanda, 11 = Wanda x Kariega, 12 = Kariega x Nata, 13 = Kariega x SST 124, 14 = Kariega x Sceptre, 15 = SST 124 x Sceptre, 16 = Nata x Sceptre, 17 = Wanda x Nata, 18 = SST 124 x Nata, 19 = SST 124 x Kariega, 20 = SST 124 x Wanda, 21 = Nata, 22 = Wanda, 23 = Kariega, 24 = SST 124, 25 = Sceptre. HMW-GS = High Molecular Weight – Glutenin Subunits, LMW-GS = Low molecular weight-Glutenin Subunits, PP = Polymeric Protein, MP = Monomeric Protein, SDS = Sodium dodycel sulphate

soluble protein fractions, there were more cultivars with ratios of 4:1, followed by 3:1; whereas in SDS-insoluble protein fraction, the ratio of 3:1 was more frequent, followed by 2:1 ratios. In F_2 progeny, high molecular weight/low molecular weight ratio was high in SDS-soluble protein fractions with value between 6.67: and 3.26:1. The most frequent ratios were around 5:1, followed by 6:1. Kariega x SST 124 was leading with high ratio, followed by Kariega x Sceptre. The least ratio was obtained by Sceptre x Nata with a value of 3.26:1, regarded as an outlier (Table 1).

Polymeric proteins/monomeric proteins. There was a difference between PP/MP ratios in F_1 and

F_2 progeny (Table 1). PP/MP ratio in F_1 progeny was more frequent with 3:1 values; whereas a ratio of 2:1 appeared more in F_2 progeny. The highest ratio in both F_1 and F_2 progeny was 3.53:1, obtained from Nata x Sceptre in these two generations. Nonetheless, a difference was observed in the lowest ratio between the two progenies. Nata x SST124 revealed a ratio of 1.58:1 in F_1 progeny; while the same cross recorded 1.60:1. Similarly, parents expressed no difference among them for PP/MP ratio.

Soluble protein fractions. A significant difference ($P<0.05$)was obtained among 20 progeny for larger polymeric proteins (Table 2). Sceptre x Wanda, Kariega x SST 124, Kariega x Sceptre, Sceptre x Kariega, Kariega x Nata and SST124 outperformed the others. Sceptre x Kariega, Wanda x Sceptre and SST124 x Kariega exhibited very low values (9.33, 9.44 and 10.09, respectively). Three parents (Kariega, SST 124 and Sceptre) performed significantly better than the progeny, but not significantly different. Nata performed poorly compared to the other parents.

In terms of smaller polymeric proteins, SST124 x Nata, Kariega x Sceptre, Kariega x SST 124 and Wanda x Nata performed significantly higher than the other progeny; while Sceptre x Nata, Nata x Kariega, Sceptre x Wanda and Kariega x Wanda expressed very low smaller polymeric protein fractions (27.96, 30.12, 32.87 and 34.34, respectively). All the parents performed like their best progeny, but not higher than the highest progeny.

Nata x SST 124 exhibited a high monomeric protein, followed by Sceptre x Kariega and Wanda x Sceptre. Kariega x Wanda revealed a low monomeric protein value. No significant difference ($P>0.05$) was obtained among the parents. However, highly significant differences were obtained between some progeny and their parents. A wide range of smaller monomeric/ polymeric values were obtained among the progeny. Four of the progeny, namely, Sceptre x Nata, Kariega x Wanda, Sceptre x Kariega and Sceptre x Wanda, had the highest values. Parents formed two groups with a large difference between the groups.

Insoluble protein fractions. No significant difference ($P>0.05$) was expressed among the progeny and among the parents for larger polymeric protein (Table 2). Among the parents, Kariega performed dismally. Kariega x Wanda, Nata x SST 124, Sceptre x Kariega and Nata x Sceptre had significantly higher values than the other progeny for smaller polymeric protein.

Among the parents, Wanda performed significantly better than other parents, but slightly lower than the best progeny. Kariega x SST 124 exhibited a lower smaller polymeric protein value than the other progeny. Similarly, Sceptre, one of the parents showed a lower smaller polymeric protein value than the progeny.

There was a wide variation among the progeny for larger monomeric protein; with the highest progeny obtaining a value of 15.70 and a lowest value of 7.95. Nata x SST 124 showed the highest performance, followed by Nata x Sceptre. Kariega x SST 124 perfomed lower than the other progeny. Wanda exhibited higher values than other parents, but it was lower than the best progeny. Nata showed a very low value compared to other parents.

Wanda x Sceptre and Sceptre x Kariega performed significantly better than the other progeny in terms of smaller monomeric protein and their performance was similar to one of their parents (Table 2). Nata x Sceptre, Kariega x Wanda and Nata x SST 124 were significantly inferior to the other progeny, but higher than the lowest parent. Kariega x Wanda, Nata x SST 124 and Sceptre x Kariega obtained the highest total unextractable polymeric protein values and though without significant differences between them. Kariega x SST 124 and Kariega x Nata showed low performance compared to other progeny. The best parent performed significantly lower than the best progeny.

A significant difference was obtained for larger unextractable polymeric protein among progenies, with Wanda x Sceptre, Sceptre x Wanda and Sceptre x Kariega showing the highest values (Table 2). Two parents, SST 124 and Sceptre, revealed the lowest larger unextractable polymeric protein values. Sceptre x Kariega and Wanda x Sceptre performed better

TABLE 2. SDS-soluble and insoluble protein fraction from parents and F_1 progeny of wheat

	SDS-Soluble protein fractions				SDS-Insoluble protein fractions				Unextractable		Total soluble and insoluble PP				TPP	TMP
	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP 2	TUPP	LUPP	LPP	LMP	SPP	SMP	PP	MP
1	12.16	47.34	11.56	5.60	12.97	51.20	12.88	11.19	51.89	51.61	25.13	24.44	98.54	16.79	123.67	41.23
2	9.44	50.40	15.68	5.50	11.29	28.21	9.85	13.03	39.76	54.46	20.73	25.53	78.61	18.53	99.34	44.06
3	9.33	44.83	13.30	6.21	11.20	38.50	9.64	8.58	47.85	54.55	20.53	22.94	83.33	14.79	103.86	37.73
4	11.77	34.34	10.82	11.22	11.19	33.27	9.54	7.60	49.09	48.74	22.96	20.36	67.61	18.82	90.57	39.18
5	10.73	32.87	10.82	10.67	12.02	33.72	10.80	10.11	51.20	52.84	22.75	21.62	66.59	20.78	89.34	42.40
6	11.56	43.01	12.35	10.00	10.72	33.81	11.43	10.02	44.93	48.11	22.28	23.78	75.82	20.62	98.10	43.80
7	13.80	27.96	8.68	10.65	11.05	33.42	11.92	10.32	51.57	44.47	24.85	20.60	61.38	20.97	86.23	41.57
8	13.06	36.86	17.18	10.99	11.54	36.19	14.33	13.87	54.46	46.91	24.60	31.51	73.05	24.86	97.65	56.37
9	11.34	37.29	37.29	9.49	9.63	49.71	15.70	5.73	55.00	45.92	20.97	52.99	87.00	15.22	107.97	68.21
10	11.85	30.12	8.29	11.17	10.25	50.32	14.14	5.35	59.07	46.38	22.10	22.43	80.44	16.52	102.54	39.95
11	12.98	50.88	12.24	5.26	10.98	32.16	10.42	11.99	40.32	45.83	23.96	26.22	83.04	17.25	107.00	39.91
12	13.06	52.57	11.92	4.54	10.51	25.55	10.51	12.14	35.99	44.59	23.57	22.43	78.12	16.68	122.71	39.11
13	13.76	53.72	11.50	4.57	11.88	24.29	7.95	8.87	34.86	46.88	25.64	19.45	78.01	13.44	103.65	32.89
14	13.70	54.07	12.22	5.27	12.28	28.26	8.66	9.74	37.43	47.27	25.98	20.88	82.33	15.01	108.31	35.89
15	13.05	52.77	12.28	4.57	10.93	33.49	8.91	6.97	40.29	45.58	23.98	21.19	86.26	11.54	110.24	32.73
16	11.74	52.57	13.41	4.28	12.61	55.97	14.90	5.01	51.61	51.79	24.35	28.31	108.54	9.29	132.89	37.60
17	12.60	53.93	11.92	3.99	11.26	39.69	11.46	8.09	43.37	47.15	23.86	23.38	93.62	12.08	117.48	35.46
18	10.63	54.50	13.72	2.85	11.11	41.97	11.18	7.00	44.90	51.10	21.74	24.90	96.47	9.85	118.21	34.75
19	10.09	50.65	13.70	5.01	10.54	51.49	12.69	4.68	50.53	51.09	20.63	25.39	102.14	9.69	122.77	36.08
20	10.52	52.10	13.60	4.97	12.28	41.60	12.03	6.72	46.25	53.86	22.80	25.63	93.70	11.69	116.50	37.32
21	10.31	48.70	12.62	7.22	10.79	34.05	9.64	10.01	43.18	51.14	21.10	22.26	82.75	17.23	103.85	39.49
22	9.73	45.14	12.47	6.63	9.89	52.04	14.38	5.25	53.02	50.41	19.62	26.85	97.18	11.88	116.78	38.73
23	11.05	51.94	12.75	4.97	9.10	51.91	11.49	12.77	49.20	45.16	20.15	24.24	103.85	17.74	124.00	41.98
24	11.59	53.29	11.98	4.98	10.67	27.99	10.13	13.38	37.34	47.93	22.26	22.11	81.28	18.36	103.54	40.47
25	11.71	52.55	12.46	4.48	10.36	21.88	10.09	12.21	33.41	46.94	22.07	22.55	74.43	16.69	96.50	39.24
LSD (5%)	2.250	11.983	2.625	6.601	2.408	4.851	1.425	2.069	7.657	9.765	7.006	5.879	3.977	12.765	5.879	2.390

1 = Nata x Wanda, 2 = Wanda x Sceptre, 3 = Sceptre x Kariega, 4 = Sceptre x Nata, 5 = Nata x Kariega, 6 = Wanda x SST124, 7 = scepter x Wanda, 8 = Sceptre x Kariega, 9 = Nata x SST 124, 10 = Kariega x Wanda, 11 = Wanda x Kariega, 12 = Kariega x Nata, 13 = Kariega x SST 124, 14 = Kariega x Sceptre, 15 = SST 124 x Sceptre, 16 = Nata x Sceptre, 17 = Wanda x Nata, 18 = SST 124 x Nata, 19 = SST 124 x Kariega, 20 = SST 124 x Wanda, 21 = Nata, 22 = Wanda, 23 = Kariega, 24 = SST 124, 25 = Sceptre. LPP = Large polymeric protein, SPP = Small polymeric protein, LMP = Large monomeric protein, SMP = Small monomeric protein, TUPP = Total unextractable polymeric protein, LUPP = Large unextractable polymeric protein, PP = Polymeric protein. MP = Monomeric protein.

than the other parents, but lower than the best progeny. Among the parents, no significant difference existed in terms of larger polymeric protein. Sceptre x Kariega, Wanda x Sceptre and Nata X SST124 performed poorly, but better than the least parent. All parents except Wanda, showed similar and low performance compared with the progeny.

Nata x SST 124 exhibited a higher value for larger monomeric protein than all the progeny (Table 2). This was followed by Sceptre x Kariega. The lowest value was expressed by Kariega x SST 124 and Kariega x Sceptre. Similarly, Wanda outperformed the other parents but was lower than the best progeny. Large variation was observed among the progeny, ranging from 61.38 to 108.54, where Nata x Sceptre and SST 124 x Kariega revealed largest values. The progenies were evenly distributed along this range; however, the parents showed low variation among themselves; with the highest being 103.85 and lowest 74.43. Sceptre x Kariega, Sceptre x Wanda, Nata x Kariega and Wanda x SST 124 showed significantly higher values than the other progeny for smaller monomeric protein; while Nata x Sceptre, SST 124 x Kariega and SST 124 x Nata had significantly lower values for smaller monomeric protein. There was no significant difference among the progeny falling between these extremes. All parents, except Wanda exhibited, similar performance which was lower than the best progeny and better than the least progeny.

Soluble protein fractions. Data for soluble protein fractions are presented in Table 2. Highly significant differences were obtained among the progeny for larger polymeric protein, with SST 124 x Kariega ranking first, followed by SST 124 x Nata and SST 124 x Nata. Kariega x SST 124 had a significantly low value for larger polymeric protein. All the parents were not significantly different and their performance was similar to the best progeny. Kariega x Sceptre, Wanda x Nata, SST 124 x Wanda and Kariega x Wanda outperformed the others for short polymeric protein. Most of the progeny obtained values above 50.00 and only Nata x Wanda, Wanda x Sceptre and Wanda x SST 124 exhibited values below 49.00. The performance of the parents exceeded that of the progeny.

Kariega x SST 124, Kariega x Nata, Kariega x Wanda and Sceptre by Nata performed significantly higher than the other progeny for larger monomeric protein; while Wanda and Septre exhibited a significantly low value. All parents had similar values close to 12.00, which was below most of their progeny. Only Wanda x Sceptre, Wanda x SST 124 and Wanda x Nata performed below these parents.

Large variation was observed among the progeny and among the parents for smaller monomeric protein (Table 2). The progeny achieved values ranging from 3.00 to 9.34; while parents ranged from 4.86 to 7.09. Wanda x Sceptre and Nata x Wanda reached the highest values among the progeny, while Nata x Sceptre and Kariega x Nata revealed the lowest values. Sceptre showed high smaller monomeric protein value among the parents while SST 124 exhibited the low smaller monomeric protein value.

Insoluble protein fractions. Two progeny (Wanda x SST 124, Kariega x Nata), out of twenty showed a significant difference from other progeny for larger polymeric protein (Table 2). All other progeny, except Nata x SST 124, obtained values between 10.06 and 12.39, which was a very narrow range. The parents showed a wider range for larger polymeric protein. However, the progeny with the highest values exceeded the best parents and its performance ranked second to it. The progeny with lowest value was higher than the parent with lowest value for larger polymeric protein.

A significant difference was obtained among progenies for smaller polymeric protein, with Kariga x Sceptre and Kariega x Nata having the highest values. A low value was expressed by from Nata x SST 124. No significant differences were observed among the parents.

Sceptre x Nata and Kariega x Sceptre exhibited significantly higher larger monomeric protein values than other progeny (Table 3). Three progenies among these twenty, exhibited a very low performance. The parents showed low differences among themselves. A large variation was observed among the progeny and

TABLE 3. PSDS-soluble and insoluble protein fraction from parents and F_2 progeny of wheat

	SDS – Soluble protein fraction				SDS – Insoluble protein fraction				Unextractable		Total SDS-Soluble and Insoluble PP				TPP	TMP
	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP 2	TUPP	LUPP	LPP	LMP	SPP	SMP	PP	MP
1	11.00	45.04	12.84	8.12	11.12	26.06	13.24	12.47	39.87	50.18	22.16	24.44	98.54	16.79	120.70	41.23
2	10.17	45.81	9.01	9.34	10.06	27.38	14.67	8.35	40.08	49.73	20.23	28.87	78.61	18.53	98.84	47.40
3	10.03	51.94	13.61	5.87	10.14	26.06	13.68	8.41	36.88	50.27	20.17	22.94	83.33	14.79	103.45	37.73
4	10.43	51.60	16.07	3.98	10.22	28.52	15.25	10.11	38.44	49.49	20.65	20.36	67.61	18.80	88.26	39.16
5	10.17	49.69	15.99	4.81	11.00	28.65	10.59	13.77	39.85	51.96	21.17	21.62	66.59	20.78	87.76	42.40
6	11.09	42.52	11.70	5.95	9.60	26.99	10.66	13.44	40.57	46.40	20.69	23.78	76.82	20.02	97.51	43.80
7	10.55	50.31	15.42	4.38	12.37	30.21	11.40	9.86	41.16	53.97	22.92	20.60	61.38	20.97	84.65	41.57
8	11.13	52.43	13.90	3.70	12.14	26.62	12.25	12.32	37.88	52.17	23.27	31.51	73.05	24.86	96.32	56.37
9	11.27	51.53	13.06	5.13	10.96	24.88	11.12	7.26	36.33	49.30	22.23	52.99	87.00	15.22	109.23	68.21
10	11.00	53.44	16.03	4.31	11.01	28.69	11.63	4.62	38.12	50.02	22.01	22.43	80.44	16.52	102.45	38.95
11	10.05	51.94	14.71	4.61	10.94	26.08	9.01	5.69	37.39	52.22	20.99	22.66	83.04	17.25	104.03	39.91
12	10.12	50.47	16.15	3.32	14.17	32.68	13.33	13.12	43.61	58.34	24.29	22.43	78.12	16.68	102.40	39.11
13	9.95	47.12	19.20	5.47	12.39	26.87	13.62	7.96	40.76	55.46	22.34	19.45	78.01	13.44	100.35	32.89
14	11.05	57.13	15.47	5.94	11.70	35.01	14.92	10.92	40.66	51.43	22.75	20.88	82.33	15.01	105.08	35.89
15	11.60	53.40	13.36	4.00	10.91	30.16	10.77	12.32	38.72	48.47	22.51	21.19	86.26	11.54	108.77	32.73
16	11.89	53.22	13.23	3.00	12.19	26.38	10.83	9.97	37.20	50.62	24.08	28.31	108.54	9.29	132.62	37.60
17	11.23	56.41	11.78	4.61	10.96	33.61	9.48	5.79	39.72	49.39	22.19	23.38	93.62	12.08	115.81	35.46
18	12.30	52.98	12.71	4.75	10.88	30.60	10.63	10.33	38.85	46.94	23.18	24.90	96.47	9.85	119.65	34.75
19	12.41	52.94	12.80	4.85	11.94	29.13	9.45	11.69	38.59	49.03	24.35	26.39	102.14	9.69	126.49	36.08
20	12.26	57.88	12.08	5.29	11.16	26.24	9.86	8.71	34.78	47.65	23.42	25.63	93.70	11.70	117.12	37.33
21	12.26	58.88	12.08	5.29	11.37	26.24	9.86	8.71	34.58	48.12	23.63	22.26	82.75	17.23	106.38	39.49
22	11.96	61.87	12.35	5.42	13.21	27.67	9.48	10.85	35.64	52.48	24.17	26.85	97.18	11.88	121.35	38.73
23	11.99	56.93	12.08	6.12	10.31	25.82	8.79	13.78	34.39	46.23	22.30	24.24	103.85	17.74	126.15	41.98
24	11.11	54.78	12.24	4.86	9.14	28.82	10.07	14.32	36.55	45.14	20.25	22.11	81.28	18.36	101.53	40.47
25	11.94	46.76	12.12	7.09	9.89	28.21	9.47	14.67	39.36	45.31	21.83	22.55	74.43	16.69	96.26	39.24
Mean	11.16	52.28	13.60	5.2	11.19	28.31	11.36	10.38	37.20	50.01	22.31	24.91	84.60	15.83	106.93	40.74
LSD (5%)	12.523	10.050	3.54934	1.248	2.2007	2.5025	4.3592	4.4406	7.992	11.780	5.5632	9.438	7.843	3.231	9.003	3.789

1 = Nata x Wanda, 2 x Wanda x Sceptre, 3 = Sceptre x Kariega, 4 = Sceptre x Nata, 5 = Nata x Kariega, 6 = Wanda x SST124, 7 = scepter x Wanda, 8 = Sceptre x Kariega, 9 = Nata x SST 124, 10 = Kariega x Wanda, 11 = Wanda x Kariega, 12 = Kariega x Nata, 13 = Kariega x SST 124, 14 = Kariega x Sceptre, 15 = SST 124 x Sceptre, 16 = Nata x Sceptre, 17 = Wanda x Nata, 18 = SST 124 x Nata, 19 = SST 124 x Kariega, 20 = SST 124 x Wanda, 21 = Nata, 22 = Wanda, 23 = Kariega, 24 = SST 124, 25 = Sceptre. LPP = Large polymeric protein, SPP = Small polymeric protein, LMP = Large monomeric protein, SMP = Small monomeric protein, TUPP = Total unextractable polymeric protein, LUPP = Large unextractable polymeric protein, PP = Polymeric protein. MP = Monomeric protein.

among the parents for smaller polymeric protein. The performances of the parents were above the progeny and the lowest performing parent was far higher than the least performing progeny.

Most of the progeny achieved low values ranging from 4.62 to 8.71; while least parent realised the value of 8.71. No significant difference was found among the progeny for total unextractable polymeric protein; while a significant difference was obtained among the parents (Table 2). Sceptre outperformed all the parents, followed by SST 124. Kariega was the lowest among the parents for total unextractable polymeric. Kariega x Nata, Kariega x SST 124 and Sceptre x Wanda were found significantly higher than other progeny for larger unextractable polymeric protein. The rest of progeny were significantly different from each other. Among the parents, Wanda had a high value than the other four and no significant difference was obtained among the parents.

Correlation between protein fraction and measured quality characteristics. Table 4

TABLE 4. Correlations between protein fractions and measured quality characteristics of wheat

F_1 progeny			F_2 progeny		
Protein fraction	Characteristics	Correlation coefficient	Protein fractions	Characteristic	Correlation coefficient
SDS-soluble LPP	FPC	-0.49*	SDS-soluble LPP	FPSC	-0.32*
	SKCSW	-0.53*		SKCSW	0.38*
	SKCSD	0.55*		SKCSD	0.52*
	FLY	0.58*		FLY	-0.72**
SDS-soluble SPP	SKCSW	-0.41*	SDS-soluble SPP	SDSS	0.52**
	SKCSH	0.39*		MDT	0.56**
	FLY	0.62**		FPC	-0.32*
SDS-soluble LMP	FPC	0.71**		FLY	-0.41*
SDS-soluble SMP	SKCSW	-0.43*	SDS-soluble SMP	FLY	-0.44*
	SKCSH	-0.54**		FPC	0.39*
SDS-insoluble LPP	SDSS	0.62**	SDS-insoluble LPP	SKCSW	-0.34*
	SKCSW	0.55**		SKCSH	0.31*
	SKCSD	0.53**		FPC	-0.49*
	FLY	-0.39*	LUPP	FPC	-0.51*
SDS-insoluble SPP	SKCSH	0.44*			
	SKCSW	0.48*			
	SKCSD	0.45*			
	FLY	0.35*			
SDS-insoluble LMP	FPC	0.62**			
LUPP	SDSS	0.55**			
TUPP	FPC	0.54**			

P< 0.05, ** P < 0.01. LUPP = Large unextractable polymeric protein, TUPP = Total unextractable polymeric protein, LPP = Large polymeric protein, SPP = Small polymeric protein, LMP = Large monomeric protein, SMP = Small monomeric protein, FPC = Flour protein content, Mdt = Mixogram development time, SKCSW = Seed weight; SKCSD = Seed diameter, SKCSH = Seed hardness, FLY = Flour yield

revealed a significant correlation between protein fractions and measured quality characteristics.

F_1 progeny. SDS-soluble larger polymeric protein showed a positive and significant correlation (r = 0.55) with Kernel diameter and break flour yield; and a negative correlated flour protein content and kernel weight. Kernel hardness and break flour yield were positively and significantly correlated with SDS- soluble; while kernel weight was negatively and significantly correlated with it.

SDS-soluble larger polymeric protein showed a moderate positive significant correlation (r = 0.59) with flour protein content; while SDS-soluble smaller monomeric proteins was negatively correlated with kernel weight and kernel diameter. SDS-insoluble larger polymeric protein was positively and highly correlated (r = 0.62) with SDS sedimentation volume (r = 0.55) , kernel weight, kernel diameter (r = 0.39) and break flour yield (r = 0.55). A positively and significant correlation existed between SDS-insoluble smaller polymeric protein and kernel hardness, kernel weight, kernel diameter and break flour yield, while SDS-insoluble larger monomeric protein was highly and positively correlated with flour protein.

Larger unextractable polymeric protein expressed positive and significant correlation with SDS-sedimentation volume (r = 0.38). Total unextractable polymeric protein was also positively and highly correlated with flour protein. Significant correlation obtained in protein fractions was found in Kernel characteristics, break flour yield, SDS-sedimentation and flour protein. Most of the wheat quality characteristics were not correlated with the protein fractions.

F_2 progeny. SDS-soluble larger polymeric protein was significantly and positively correlated with kernel weight (r = 0.38) and kernel diameter (r = 0.52); but negatively with flour protein content and break flour yield. Similarly, SDS-soluble small polymeric protein was significantly and positively correlated with SDS sedimentation and mixogram development time, but negatively with flour protein content and break flour yield. Break flour yield was negatively correlated with SDS-soluble smaller monomeric protein, yet a significant positive correlation was observed between SDS-soluble smaller monomeric and flour protein content. SDS-insoluble larger polymeric protein was positively correlated with kernel weight, kernel hardness and flour protein. Larger unextractable polymetric protein was negatively and significantly correlated with flour protein. The correlation between the wheat quality characteristics existed with very few protein fractions.

DISCUSSION

Glutenin/Giadin ratio. The high glutenin to gliadin ratio observed in progeny and their parents (Table 1) indicated that they are suitable for bread making. A higher ratio increases mixing time, mixogram peak resistance, maximum resistance to extensibility and leaf loaf (Southan and MacRitchie, 1999; Li *et al*., 2012). It is desirable to have a stable ratio of glutenin to gliadin for the purpose of good breadmaking quality. However, glutenin to gliadin ratio can be change by environment factors such as heat stress, soil fertility (Zhu and Khan, 2001). Meintjies (2004) found that different nitrogen application rate greatly influence protein fractions. It is also dependent on the ability of genotypes to change with environment. An extremely high ratio (2.61) may cause very strong visco-elasticity, while a very low ratio (0.97) may as well cause low visco-elasticity. Gliadin contributes to dough elasticity while glutenin contributes to dough viscosity. The recommended glutenin/gliadin ratio is 1.72 to 2.61 (Bekes, 2012). The variation in dough strength parameters is explained by the amount of soluble and insoluble glutenin. Several researchers used glutenin:gliadin ratio to predict breadmaking quality and found it to be accurate and reliable (Gianibelli *et al*., 2001; Bekes, 2012; Wang *et al*., 2012).

Polymeric/monomeric ratio. Many parents and progeny exhibited high ratios of polymeric to monomeric protein suggesting good breadmaking quality. Increased ratio of polymeric to monomeric improve dough extensibility and viscosity of dough, which in turn affects the expansion of the leaf loaf positively (Wang *et al*.,

2012). Polymeric protein is mostly responsible for elasticity of dough, whereas the monomeric proteins is directly related to dough (Abonyi *et al*., 2010). The ratio of polymeric to monomeric protein is directly related to the balance of dough strength and extensibility. These results were consistent with the findings of other researchers. Naeem (2012) conducted a study on developmental and environmental effects on the assembly of glutenin polymers and the impact on grain quality, and found the polymer/ monomeric ratio highly related to bread quality.

High molecular weight sub-units/ Low molecular weight. The results show a high ratio of HMW-GS to LMW-GS in both F_1 and F_2 progeny. The ratio of HMW-GS to LMW-GS plays an important role in visco-elasticity of wheat. Variation in the type and amount of sub-units relate with quality variation among wheat cultivars probably by affecting the molecular weight distribution of the glutenin polymers (Gupta *et al.*, 1993). HMW-GS is not commonly used to determine quality of breadmaking. However, LMW-GS is used concurrently with other tools to verify their results (Bekes, 2012). SST 124 and Sceptre showed lower ratio compared to other parents.

Size Exclusion High performance liquid Chromatography has been used to predict breadmaking quality by many researchers. Singh and MacRitchie (1989) used Size Exclusion High performance liquid Chromatography to predict breadmaking quality and found it to be more accurate.

Correlations among quality parameters. It is apparent that a positive correlation exists between both monomeric and polymeric protein which can be either large or small. When wheat cultivar is being analysed for flour protein content, polymeric to monomeric ratio can be used and give an accurate prediction of whether the cultivar has high or low flour protein content. Another correlation existed between SDS-insoluble large polymeric protein and SDS-sedimentation volume. Southan and MacRitchie (1999) found similar results when studying molecular weight distribution of wheat protein of 15 cultivars in some of South African wheat cultivars. In F_2 progeny, correlation existed between SDS-soluble large polymeric protein and SKCSD, SDS-soluble small polymeric correlated and mixograph development time. Both SDS-soluble large and small polymeric and monomeric proteins can be used to predict SKCSD and mixograph development time, respectively. The polymeric and monomeric protein ratio of wheat cultivars can be used effectively to predict certain the quality parameters of bread made from different cultivars. These results were consistent with the findings of Ohm *et al*. (2009) who investigated relationships of quality characteristics with size-exclusion high performance liquid chromatography chromatogram of protein extract in 45 soft white winter wheat in United State of America and found correlation between polymeric and monomeric ratios and wheat quality.

ACKNOWLEDGEMENT

The authors express sincere gratiutes to Dr. Hilka Marteens and Ms. Saidy Geldenhys for guidance, administrative and technical support. National Research Foundation is acknowledged for financial support.

REFERENCES

Abonyi, T., Tomoskozi, S., Budai, M., Gergely, S., Scholz, E., Lasztity, D. and Lasztity, R. 2010. Gluten formation from flour of kernels in developing wheat grain. *Cereal Research Communications* 38(1): 90-100.

Bekes, F. 2012. New aspects in quality related wheat research: 1. Challenges and achievements. *Cereal Research Communications* 40(2): 159-184.

Bietz, J.A. 1990. High performance liquid chromatography of cereal proteins. *Advances in Cereal Science and Technology* 111:105-159.

Erikson, H.P. 2009. Size and shape of protein molecules at Nanometer level. *Biological Procedure Online* 11(1): 3-51.

Fleurent, E. 1986. Sur une methode chemique d'appreciation de la valeur boulanger des farines de ble. C.R. *Academy Science of Paris* 123: 755 - 758.

Gianibelli, M.C., Larroque, O.R. and MacRitchie, F. 2001. Biochemical, genetic and molecular

characterization of wheat endosperm proteins. Online review. *American Association of Cereal Chemists.* St Paul, Minnesota, USA. pp. 1-20.

Gupta,R.B., K.Khan and Macrithie, F. 1993. Biological basis of flour properties in bread wheat. 1. Effects of variation in the quantity and size distribution of polymeric protein. *Journal of Cereal Science* 18: 23-41.

Hubbard, T.J., Murzin,A.G., Brenner, S. E. and Clothia, C. 1997. Structural classification of Protein. *Nucleic Acid Research* 25 (1):236 - 239.

Kasarda, D.D. 1989. Glutenin structure in relation to wheat quality. pp. 277-302. In: Wheat is unique. *American Association of Cereal Chemists.* St Paul, MN. Minnesota, USA.

Kim, J.J., Kieffer, R. and Belitz, H.D. 1988. Rheological properties of wheat glutenin containing different amount of prolamin from various cereals. *Zeitschrift fur lebensmitte untersuch Forsch* 186:16-21.

Li, X., Cai, J., Bo, F., Liu, F., Jiang, D., Dai, T. and Cao, W. 2012. Effect of shading from joint to maturity on high molecular weight glutenin subunit accumulation and glutenin macropolymer concentration in grain of winter wheat. https://www.google.co.za/#g=effect+of+shading+from+Joint

Lookhart, G.L., Alberts, L.D. and Bietz, J.A. 1986. Comparison of Polyacrilamide gel electrophoresis and high performance liquid chromatography analyses of gliadin polymorphism in wheat cultivars. *Cereal Chemists* 63: 497-500.

Meintjies, G.D. 2004. The use of high performance liquid chromatography for quality prediction of South African wheat cultivars. MSc. Thesis. University of Free State. Bloemfontein, South Africa.

Naeem, H.A., Paulon, D., Irmark, S. and MacRitchie, F. 2012. Developmental and environmental effects on the assembly of glutenin polymers and the impact on grain quality of wheat. *Journal of Cereal Science* 56(1):51 - 57.

Ohm, J.B., Ross, A.S., Peterson, C.J. and Morris, C.F. 2009. Relationship of quality characteristics with size exclusion HPLC chromatogram of protein extract in soft white winter wheats. *Cereal Chemists* 86(2):197-203.

Singh, N.K., Donovan, R. and MacRitchie, F. 1990. Use of sonication and size-exclusion high performance chromatography in the study of wheat flour proteins 11. Relative quantity of glutenin as a measure of breadmaking quality. *Cereal Chemists* 67(2):163-170.

Singh, N.K. and MacRitchie, F. 1989. Use of sonication and size exclusion high perfomance liquid chromatography in wheat flour proteins. II Relative quality of glutenin as a measure of breadmaking quality. *Cereal Chemists* 67:161-170

Southan, M. and MacRitchie, F. 1999. Molecular weight distribution of wheat proteins. *Cereal Chemists* 76 (6): 827-836.

Uthayakumaran, S., Gras, P.W., Stoddard, F.L. and Bekes, F. 1999. Effects of varying protein content and glutenin to gliadin ratio on the functional properties of wheat dough. *Cereal Chemist* 76(3): 389 - 394.

Wang, S., Shing, X., Ge,P., Subburai, S., Li, X., Zeller, S.L., Hsam, K and Yan, Y. 2012. Molecular characterisation and dynamic expression patterns of two types of y-gliadin genes from Aegilops and Triticum species. *Theoritical and Applied Genetics* 125(7): 1371 1384.

Wrigley, C.W. 1996. Giant protein with flour power. *Nature* 381:738-739.

Zhu, J. and Khan, K. 2001. Effects of genotype and environment on glutenin polymers and breeading quality. *Cereal Chemistry* 78(2): 125 - 130.