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Application of Gel-protein Analysis Compared to Conventional Quality Tests in Characterisation of Iranian Wheat Cultivars

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Application of glutenin macro-polymer (GMP) gel analysis compared to conventional wheat quality indicators such as total protein content, Zeleny, and SDS sedimentation values was evaluated in quality classification of 13 Iranian wheat cultivars. The results showed no significant correlation between total protein content and breadmaking characteristics. Zeleny, SDS sedimentation and GMP tests showed significant correlation with loaf volume and bread height. GMP wet weight and small-strain deformation rheological characteristic of GMP-gel were correlated with large-strain deformation rheological properties of dough measured in Farinograph and bread quality (loaf volume and height). Significant ($\alpha < 0.01$) correlation was found between rheological properties of the GMP gel and Farinograph characteristics of dough. Although GMP wet weight is regarded as a predictive measure for breadmaking quality of wheat, in the light of the results of this rather small sample set we did not find significant correlation between small-strain rheological properties of GMP-gel (storage modulus and tan δ) and breadmaking characteristics.

Keywords: wheat, quality, glutenin macro-polymer (GMP), rheology

Introduction

Proteins are recognized as the most important components governing breadmaking quality of wheat (Payne et al. 1987; Weegels et al. 1996). An important fraction of the glutenins can be isolated from wheat flour as a gel layer named glutenin macro polymer (GMP) (Graveland et al. 1982). The importance of this polymeric glutenin or GMP fraction and its composition in assessing wheat quality and predicting the properties of the final product has been discussed in many recent studies (Moonen et al. 1986; Pritchard 1993; Sapirstein and Suchy 1999; Hamer and van Vliet 2000; Don et al. 2003; Peighambardoust et al. 2005). Not surprisingly, the amount of gel protein is closely related to sodium dodecyl sulphate (SDS) sedimentation volumes of ground wheat flour (r =

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0.95). The sedimentation test, introduced by Axford et al. (1978), and its predecessor, the Zeleny sedimentation test (Zeleny 1947) have been widely used as small-scale tests of breadmaking quality in wheat breeding programs. Sedimentation tests, although ostensibly easy to perform, are complicated by the need for two or more reagent solutions, and seemingly arbitrary time schedules for shaking the suspension, inverting, and resting the measurement cylinders. The SDS protein gel test, in contrast, is relatively straightforward. It requires only one solvent (SDS solution), one mixing step to disperse the flour, and ultracentrifugation to separate the glutenin-enriched gel. A direct relation between the amount and the composition of GMP with breadmaking performance of wheat has been shown (Preston et al. 1982; Payne et al. 1987). Bekkers et al. (2000) also found a positive significant correlation between the elastic modulus of GMP with dough development time in mixing process. Available information in literature mostly deals with relation between the amount and composition of GMP with dough and bread characteristics. To our knowledge, there is limited information about the small-strain rheological characterisation of GMP-gel and its relationship with dough rheology and breadmaking quality of wheat. Thus, the aim of this study was to apply GMP gel analysis (wet weight and rheological characteristics) in evaluating quality characteristics and breadmaking quality of certain Iranian wheat cultivars. A comparison was made between the GMP test and other conventional tests in evaluating the quality of wheat cultivars with different breadmaking qualities.

Materials and Methods

Wheat flour samples

Flour samples of 13 Iranian wheat cultivars with different breadmaking qualities were kindly supplied by Agricultural-Jihad Ministry, Seed and Plant Research Institute, Karaj, Iran. The selected varieties were representative of many wheat samples. Despite to limited sample size, they correspond to three quality classes as weak, medium and strong. The glutenin allelic composition, general quality parameters, agronomy and genetic information of the samples were already reported by Hosseinian-Khoshroo et al. (2010) and Bahraie (2003) are shown in Table 1.

Total protein analysis

The total protein content of the flours was measured by NIR method according to method provided by Williams and Sobering (1993). Triplicate measurements were carried out. The NIR apparatus was first calibrated using the conventional Kejeldal method.

SDS sedimentation and Zeleny tests

SDS sedimentation was measured according to method described by Carter et al. (1999). Zeleny number was measured according to AACC approved methods (AACC 54-11). The experiments were performed in triplicates.

Table 1. Environmental, agronomy and genetic background (specified alleles) of wheat samples[a]

Wheat cultivars		Pedigree	Origin[b]	Region	GH[c]	HW[d] (kg)	TKW[e] (g)	*Glu-A3*	*Glu-B3*	*Glu-D3*
Weak	Sardari	Landrace Kordestan	Kordestan-Iran	4	W	77.9	39	d	h	d
	Alamoot	Kavz/Ti71/3/Maya"s"//Bb/Inia/4/Kj2/5/Anza/ 3/Pi/Ndr//Hys	Karaj-Iran	4	W	77.8	36	e	c	b
	Shiroodi	Attila (CM85836-4Y-OM-OY-8M-OY-OPZ)	CIMMYT-Mexico	1	S	76.5	38	b	b	b
	Dez	Kauze*2/Opata//Kauze CRG-737-1Y-O10M-OY	CIMMYT-Mexico	1	S	76.4	38	ns	ns	ns
Moderate	Hamoon	Falat/Roshan	Zabol-Iran	2	S	79.4	39	c	e	a
	Azar 2	Kvz/Tr71/3/Maya"s"//Bb//Inia/4/Sefid	Azerbaidjan-Iran	4	W	79.7	39	ns	ns	ns
	Marvdasht	HD2172/Bloudan//Azadi	Zarghan-Iran	3	S	79.4	36	D	d	d
	Darab 2	Maya"s"/Nac	CIMMYT-Mexico	2	S	79.8	38	D	h	b
Strong	Zarrin	PK15841	CIMMYT-Mexico ICARDA-Turkey	4	F	80.3	39	B	b	c
	Bezostaya	Introduction cultivar	Russia	4	W	79.9	43	ns	ns	ns
	Innia	LR64/SN64	Mexico	1	F	79.2	39	d	d	d
	Pishtaz	Alvand//Aldan "S"/Ias58	Karaj-Iran	3	S	80.2	45	e	a	b
	Tajan	Bow"s"/Nkt"s" (CM67428-GM-LR-5M-3R-LB-Y)	CIMMYT-Mexico	1	S	82.4	39	a	d	b

[a] Data from Bahraie 2003; Hosseinian-Khoshroo et al. 2010
[b] Climate zone in Iran: 1: Caspian sea shore region, 2: Southern region, 3: Moderate region, 4: Cold region
[c] Growth habit: S: Spring, W: Winter, F: Facultative
[d] Hectolitre weight
[e] Thousand-kernel Weight
ns: not specified

GMP wet weight measurement

GMP wet weight of flour samples was measured according to Graveland (1980). The measurements were carried out at least in triplicate.

Small-strain rheological analysis of GMP-gel

Storage module and tan delta of GMP-gels were measured according to the method provided by Don et al. (2003). Duplicate measurements were carried out.

Farinograph test

The Farinograph characteristics of the flours used were determined using the AACC Approved Method 54-21.

Baking and bread analysis

A mini-baking procedure was performed to bake breads from the flours. Tap water was added according to Farinograph water absorption of the flours (Table 3). NaCl in concentration of 2% (w/w based on flour weight) and a commercial bread improver (Ika-Plus, Turkey) in a concentration of 0.3% (w/w flour basis) were added to the flour. The dough was mixed in a 2 kg spiral kitchen mixer. An initial proofing time of 30 min (at 30ºC, RH=75%) was applied for the mixed dough before dividing, sheeting, rolling and moulding processes. A final proofing time of 60 min (at 30ºC, RH = 80%) on dough pieces (20 g) inside small baking tins (30 × 30 × 40 mm) was performed before baking. Baking was carried out at a temperature of 170 ± 5ºC for 30 min, until the crust is formed. Baking trials were performed in duplicates. Rapeseed displacement method was used to measure the volume of the baked loaves. The height of bread from the middle cut of the loaves was also measured. All measurements were carried out in triplicates.

Results

Physicochemical characteristics of wheat cultivars

Total protein content

Results of total protein measurement are shown in Table 2. Variation in total protein content between wheat cultivars with different quality did not follow any distinct trend. According to the supplier, wheat cultivars were in three quality classes as weak, moderate and strong wheat. However, total protein results did not show such quality classification.

Zeleny and SDS sedimentation tests

Zeleny and SDS sedimentation values of wheat samples are shown in Table 2. Alamoot cultivar showed the lowest and Innia and Bezostaya showed the highest Zeleny and sedimentation values compared to other cultivars. According to the supplier, Alamoot was a weak and both Innia and Bezostaya were strong wheat cultivars. However, as can be seen in Table 2, there are weak cultivars (i.e. Dez) which showed higher sedimentation values.

Table 2. Total protein, SDS sedimentation, Zeleny, GMP contents of wheat samples

Wheat cultivars		Total protein (%)	Zeleny number (mL)	SDS-sediment (mL)	GMP (g/100 g DM flour)
Weak	Sardari	12.1 ± 0.1*[de]**	24.0 ± 0.9[cd]	1.39 ± 0.1[g]	146.2 ± 11.2[de]
	Alamoot	11.4 ± 0.1[g]	12.9 ± 0.1[g]	0.83 ± 0.1[j]	128.3 ± 4.6[e]
	Shiroodi	12.2 ± 0.1[cd]	23.2 ± 1.7[cde]	1.74 ± 0.1[e]	163.0 ± 6.7[cde]
	Dez	12.1 ± 0.1[cde]	25.1 ± 0.6[c]	2.22 ± 0.1[c]	168.4 ± 11.9[cd]
Moderate	Hamoon	11.6 ± 0.1[fg]	23.3 ± 0.5[cd]	1.59 ± 0.1[f]	131.6 ± 24.9[de]
	Azar 2	12.4 ± 0.1[ab]	19.5 ± 1.1[f]	1.31 ± 0.1[h]	141.2 ± 17.4[de]
	Marvdasht	12.3 ± 0.1[bc]	19.3 ± 0.1[f]	1.06 ± 0.1[i]	156.6 ± 3.5[cde]
	Darab 2	12.5 ± 0.1[a]	22.5 ± 1.4[ed]	2.07 ± 0.1[d]	233.8 ± 5.7[a]
Strong	Zarrin	12.0 ± 0.1[e]	22.2 ± 0.4[ed]	2.18 ± 0.1[c]	211.6 ± 9.5[ab]
	Bezostaya	12.5 ± 0.1[ab]	31.4 ± 0.5[a]	2.76 ± 0.1[a]	218.6 ± 13.2[ab]
	Innia	12.5 ± 0.1[a]	28.4 ± 0.5[b]	2.59 ± 0.1[b]	231.7 ± 20.1[a]
	Pishtaz	11.6 ± 0.1[fg]	20.8 ± 0.3[ef]	1.55 ± 0.1[f]	164.0 ± 9.2[cde]
	Tajan	11.7 ± 0.1[f]	22.2 ± 1.0[ed]	1.74 ± 0.1[e]	185.9 ± 13.0[bc]

* Standard deviations
** Similar letters within columns represent significant ($\alpha < 0.05$) difference

Similarly, strong cultivars such as Pishtaz and Tajan exhibited lower sedimentation values. This contradiction likely indicates that, similar to total protein content, Zeleny and SDS sedimentation tests are not able to absolutely determine the quality of wheat.

GMP analysis

GMP wet weight

As can be seen in Table 2, strong cultivars such as Innia, Bezostaya and Zarrin showed the highest GMP wet weights, whereas for weak cultivars (i.e. Alamoot and Sardari) lower amounts of GMP gels were obtained. Compared to other quality test, GMP method is accurate, needing very low amount of flour sample (most suitable for breeding studies).

GMP rheology

In this study, rheological parameters storage modulus (G') and tan delta were measured for GMP gels extracted from wheat cultivars. Higher G' values for most of strong wheats and lower tan delta values for most of weak cultivars were obtained. There also were strong cultivars such as Zarrin and Innia that showed lower G' values (data not shown). Figure 1 demonstrates variations of G' and tan delta for Bezostaya, Hamoon and Alamoot cultivars. Bezostaya, as a strong cultivar, showed a higher G' and lower tan delta values than two others. Alamoot, as a weak cultivar, exhibited lower G' and higher tan delta values. Moderate cultivar Hamoon stayed in between two above-mentioned varieties.

Farinograph results and loaf characteristics

Table 3 shows Farinograph results and loaf volume and height for the wheat cultivars studied. There were variations in Farinograph results in certain samples belonging to different quality classes as provided by the supplier. Nevertheless, in overall, strong cultivars exhibited better rheological properties (i.e. longer stability) and improved loaf characteristics (higher loaf volume) than those recorded for moderate samples, followed by weak cultivars.

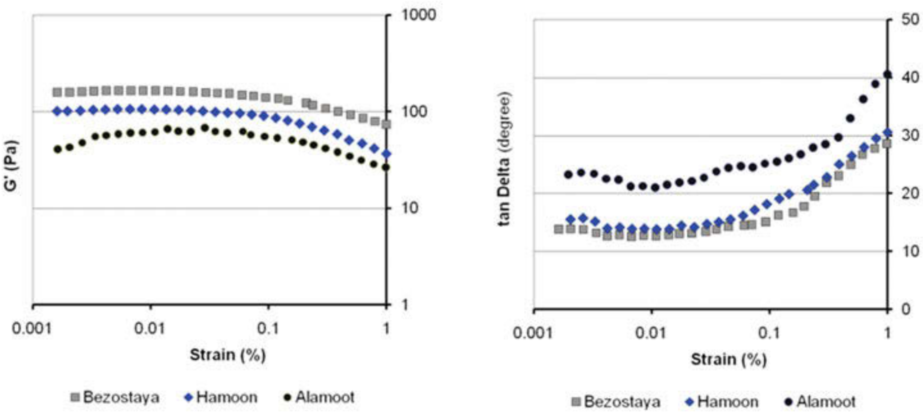

Figure 1. Variations of G' and tan δ for Bezostaya, Hamoon and Alamoot, representing strong, moderate and weak cultivars, respectively

Figure 2 shows Frarinograms of Bezostaya, Hamoon and Alamoot cultivars, representing strong, moderate and weak varieties, respectively. strong cultivars such as Bezostaya showed higher water absorption, dough development time, stability and Farinograph quality number than those of weak clutivars (i.e. Alamoot). Loaf volume and height showed the same trend.

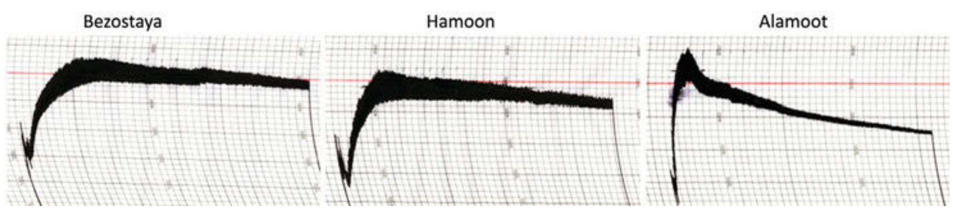

Figure 2. Farinograms of wheat flours from Bezostaya, Hamoon and Alamoot, representing strong, moderate and weak cultivars, respectively

Discussion

Physicochemical characteristics of wheat cultivars

The variation in total protein content of wheat cultivars did not follow a specific trend, as one expects from the supplier quality classification presented in Table 2. As can be seen in this table, there are good quality cultivars that show low total protein content and vice

Table 3. Farinograph analysis and bread characteristics of wheat samples

Wheat cultivars		Water absorption (%)	Dough development time (min)	Stability (min)	Farinograph quality number	Loaf volume (mL)	Loaf height (cm)
Weak	Sardari	63.0 ± 2.0*	3.6 ± 0.4	2.5 ± 0.1	52.0 ± 11.0	57.5 ± 0.5	3.8 ± 0.1
	Alamoot	63.1 ± 1.5	2.0 ± 0.1	1.3 ± 0.2	23.5 ± 0.5	55.0 ± 0.1	3.6 ± 0.1
	Shiroodi	63.5 ± 1.4	3.0 ± 0.4	3.0 ± 1.5	43.3 ± 9.2	73.7 ± 0.6	4.2 ± 0.1
	Dez	65.8 ± 1.9	3.0 ± 0.1	3.3 ± 0.7	46.5 ± 10.5	60.0 ± 1.0	3.8 ± 0.1
Moderate	Hamoon	67.4 ± 2.4	4.0 ± 0.3	4.0 ± 1.6	85.5 ± 28.5	50.0 ± 1.0	3.5 ± 0.1
	Azar 2	63.6 ± 0.2	2.3 ± 0.1	2.3 ± 0.5	85.5 ± 28.5	67.7 ± 1.2	3.7 ± 0.1
	Marvdasht	66.4 ± 1.1	3.0 ± 0.1	3.5 ± 1.1	44.3 ± 4.2	56.0 ± 1.0	3.8 ± 0.1
	Darab 2	67.9 ± 1.5	5.0 ± 0.1	6.5 ± 0.6	72.0 ± 9.0	64.3 ± 0.6	4.4 ± 0.3
Strong	Zarrin	66.7 ± 1.2	4.8 ± 0.3	5.5 ± 1.0	70.0 ± 4.0	79.7 ± 1.2	4.6 ± 0.1
	Bezostaya	68.9 ± 1.2	6.0 ± 0.6	10.0 ± 3.6	124.0 ± 30.0	90.7 ± 1.2	5.5 ± 0.1
	Innia	61.4 ± 0.6	5.3 ± 0.3	7.0 ± 0.5	71.7 ± 1.5	90.7 ± 4.0	5.5 ± 0.1
	Pishtaz	68.4 ± 1.2	5.5 ± 0.1	8.3 ± 3.2	91.5 ± 7.5	66.7 ± 0.6	4.3 ± 0.1
	Tajan	61.7 ± 1.3	5.3 ± 0.3	10.5 ± 1.1	105.5 ± 11.7	77.5 ± 0.5	4.7 ± 0.1

* Standard deviations

versa. In general, total protein content could not represent quality difference between wheat cultivars, as also reported by other researchers (Sapirstein and Suchy 1999). The measurement of wheat quality by SDS sedimentation and Zeleny tests (Table 2) demonstrated quality differences among wheat cultivars. Different group of researchers have used Zeleny and SDS sedimentation tests to evaluate wheat protein quality (Axford et al. 1979; Dexter et al. 1980; Graveland 1980; Preston et al. 1982; Ayoub et al. 1983). Also, these tests are routinely used in cereal laboratories for evaluating overall wheat protein quality, they could not be exclusively and solely used to determine quality in wheat breeding programmes (Blackman and Gill 1979). Therefore, other specific tests such as determination of the amount and quality of high molecular weight fraction (glutenin macropolymer) of wheat protein, which could be carried out with small amounts (ca. 1 g) of flour samples are recently used in cereal laboratories. As shown in Table 2, compared to classical protein quality tests, GMP wet weight gave a better differentiation between quality classes in the wheat cultivars studied. This is in accordance with findings of other researchers (Jeanjean and Feillet 1978, 1980; Moonen et al. 1986; Pritchard 1993; Pritchard and Brock 1994).

GMP analysis

Small-strain rheological analysis of GMP gels (Fig. 1) showed higher storage modulus (elastic behaviour) for strong wheat cultivar. This paralleled Farinogram results for the given cultivars (Fig. 2). Bekkers et al. (2000) demonstrated that gel rigidity (G') is largely determined by the size and interactions of HMW glutenin subunits. Strong cultivars showed higher GMP wet weight values, possibly indicating the presence of HMW glutenins in the gels of strong wheat cultivars. Peighambardoust et al. (2005) reported that the higher the amount of GMP wet weight, the larger the glutenin particles size in the gel.

Table 4. Correlation coefficients between quality tests and loaf properties

	Zeleny number	SDS sediment	Protein content	GMP content	Loaf volume
SDS sediment	0.867**				
Protein content	0.334*	0.519**			
GMP content	0.600**	0.815**	0.403*		
Loaf volume	0.622**	0.758**	0.154	0.716**	
Loaf height	0.703**	0.807**	0.193	0.810**	0.936**

* Significant ($\alpha < 0.05$)
** Significant ($\alpha < 0.01$)

Table 5. Correlation coefficients between GMP-gel rheological properties, Farinogram data and loaf characteristics

	G'	tan δ	DDT	Stability	FQN	Loaf volume
tan δ	–0.530**					
DDT	0.396*	–0.511**				
Stability	0.441**	–0.405*	0.820**			
FQN	0.440**	–0.534*	0.706**	0.835**		
Loaf volume	–0.043	–0.210	0.587**	0.507**	0.482**	
Loaf height	0.034	–0.320	0.750**	0.624**	0.512**	0.936**

* Significant ($\alpha < 0.05$)
** Significant ($\alpha < 0.01$)

This could explain that strong cultivars, which showed higher GMP contents, have larger glutenins leading to more rigid gels with higher G' values, as demonstrated in Figure 1. However, by increasing the strain gels start to deteriorate as evident from a decrease in G' values. The ratio of viscous to elastic behaviour is reflected in tan δ. When tan δ is lower than 45 degrees, elastic behaviour predominates. At values higher than 45 degrees, gel shows more viscous behaviour. In Figure 1, strong cultivar showed lower average tan δ, indicating elastic behaviour for rigid gels. In contrast, weak variety showed higher tan δ values, indicating viscous behaviour for soft gels. Moderate quality cultivar stayed in between. This paralleled Farinogram data shown for three cultivars in Figure 2.

Correlation between different quality tests and bread characteristics

Table 4 shows correlation coefficient between quality tests (including total protein content) and bread characteristics. There was no significant (α<0.01) correlation between the total protein content and bread height and volume. This is in accordance with other studies (Sapirstein and Suchy 1999), indicating that the protein content cannot be regarded as a quality criterion for wheat classification. Despite to the total protein content, other wheat quality tests showed significant correlation with bread characteristics. GMP wet weight and SDS sedimentation value gave higher correlation coefficients compared to conventional Zeleny number. In both methods, SDS solvent is used for extraction or insolubilisation of proteins. Since macro-polymeric fraction of gluten is involved in both SDS and GMP tests, it can explain the better correlation of these tests with loaf characteristics. This is in accordance with other researchers who stated that the polymeric fraction of wheat gluten (GMP) is the most important element of dough gluten network and thus could predict the final product characteristics (Payne et al. 1987; Singh et al. 1990; Popineau et al. 1994; Sapirstein and Suchy 1999).

Correlation between GMP-gel rheoloy, Farinogram data and loaf characteristics

Although, GMP wet weight showed significant correlation with loaf characteristics (Table 4), rheological properties of GMP gel, namely storage modulus (G') and tan δ did not show significant correlation with loaf volume and height (Table 5). Nevertheless, rheological parameters of the GMP gel showed significant correlation (α<0.01) with Farinograph parameters such as farinograph quality number (FQN) and stability. The results indicate that despite to GMP wet weight, small-strain rheological properties of GMP gel could not strongly predict the breadmaking quality of wheat. This is due to the fact that small-strain deformations involved in oscillatory are not similar to those involved in breadmaking processes, i.e. proofing and baking. This is explained by Safari-Ardi and Phan-Thien (1998), who stated that small deformation rheological measurements (dynamic oscillatory testing and creep recovery) cannot be used to describe rheological properties relevant to breadmaking process, where the rheological behaviour of dough is highly non-linear. It has been also reported that small-scale deformation rheology is a suitable method to discriminate between structural components in gluten network structure such as chemical cross links, physical interactions (hydrogen bonds, hydrophobic or electrostatic interactions) versus physical entanglements of polymeric chains (Edwards et al. 2001;

Uthayakumaran et al. 2002). Numerous studies have confirmed that the rheological behaviour of wheat flour dough at large deformation is dominated by the gluten fraction (Dobraszczyk and Morgenstern 2003). Therefore, they are largely responsible for characterisation wheat for end-use quality.

In the light of the results of this rather small sample set with a certain interval of covered dough properties we showed that total protein content cannot be addressed as a wheat quality indicator. Whereas, conventional quality tests such as Zeleny and SDS sedimentation could successively evaluate wheat breadmaking quality. Positive significant correlation was seen between the amount of GMP wet weight and breadmaking properties. Storage modulus and tan δ of GMP gels did correlate well with the loaf volume and height. Although GMP wet weight is considered as a predictive measure of wheat breadmaking quality, GMP rheology could not satisfactorily be used as wheat quality measure.

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