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Electrophoretic and immunochemical characteristics of wheat protein fractions and their relationship to chapati-making quality

P. Prabhasankar*

Flour Milling, Baking and Confectionery Technology Department, Central Food Technological Research Institute, Mysore 570 013, India

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Abstract

The major portion of wheat produced in India is used in the preparation of traditional foods, such as chapati and its variants. The quality of chapati is mainly influenced by the quality of wheat used. The present study was aimed at predicting the suitability of wheat for chapati-making by assessing the biochemical and immunochemical characteristics of 10 major commercially released Indian wheat cultivars. Wheat proteins and their fractions, such as soluble proteins (albumin and globulin), gliadin and glutenin, of different wheat cultivars, were isolated and quantified. The albumin and globulin ranged from 0.9 to 2.0%, gliadin 4.0–6.4%, soluble glutenin 0.9–1.1%, insoluble glutenin 2.5–4.6%. The variety DWR 240 had the maximum content of gliadin. The variety NIAW 612 had the maximum percent of glutenin. Wheat varieties having higher amounts of gliadin protein resulted in poor quality chapati ($r = -0.68$; $P < 0.05$). Albumin and globulin did not have any role in governing chapati-making quality. The proteins subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that varieties having good chapati-making quality exhibited distinct electrophoretic patterns. For the first time, a dot-blot tecimique was developed, using anti-gliadin antibodies, to differentiate wheat varieties with respect to chapati-making quality. The results indicated that either SDS-PAGE or dot-blot techniques could be conveniently used for identification of wheat varieties suitable for chapati making. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Electrophoresis; Wheat; Dot-blot; Chapati; Gliadin

1. Introduction

Wheat is the basic raw material for the preparation of chapati and its culinary variants (Haridas Rao, Leelavathi, & Shurpalekar, 1986a). The quality of wheat greatly influences the quality of chapati. Normally, the quality of wheat is assessed by chemical, rheological and baking tests. Since the quality of wheat is governed by the interaction of many constituents, it is difficult to judge quality by any single test. Test-baking of the product is cumbersome and is not always possible and it also requires large sample sizes. Therefore, there is a need to develop a rapid and sensitive and single test to assess the quality of different Indian wheat varieties and their suitability for chapati-making.

Electrophoretic and immunologically-based methods have been reported elsewhere for assessing bread baking quality of wheat varieties (Andrew, Blundell, & Skerritt, 1993; Brett, Mills, Tatham, Fido, Shewry, & Morgan, 1993; Hill, Giersch, Loh, & Skerritt, 1999; Lukow, Payne, & Tkauchuk, 1989; Payne, Nightingale, Krattiger, & Holt, 1987). It is noteworthy that Indian wheat varieties differ strikingly in their composition and functionality as compared to wheat grown elsewhere (Shurpalekar, Kumar, Rao, Ranga Rao, Vatsala, & Rahim, 1976). Considerable work has also been reported on the influence of quality and quantity of protein fractions on chapati characteristics (Austin & Ram, 1971; Haridas Rao et al., 1986a; Ram & Nigam, 1981; Rao, Leelavathi, Rao, & Shurpalekar, 1986; Saxena, Prasada Rao, & Haridas Rao, 1997; Sharma & Bains 1976; Shurpalekar & Prabhavathi, 1976; Shurpalekar et al., 1976). Predominant factors which affect chapati-making quality of

* Fax: +91-821-517233.
E-mail address: prabhasankar@lycos.com

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wheat varieties are damaged starch content and water absorption of flour (Haridas Rao et al., 1986a; Rao et al., 1986) However, there are few studies on the electrophoretic and immunochemical characterisation of wheat protein fractions of Indian wheat varieties. These detection methods could provide vital tools in assessing different wheat varieties with respect to their functionality and suitability for chapati-making. Hence, the present study is aimed at assessing the biochemical and immunochemical characterisation of Indian wheat varieties and their relation to chapati-making quality.

2. Materials and methods

2.1. Collection of wheat varieties

Commercially-released aestivum wheat varieties (DWR 162, DWR 195, DWR 225, DWR 240, DWR 241, NIAW 421, NIAW 474, NIAW 514, NIAW 579, NIAW 612), obtained from Mahatma Phule Krishi Vidyapeeth Agricultural Research and Wheat Breeding Station, Niphad, Nasik, India, were used in this study. After collection, wheat grains were fumigated and stored at 4 °C. Whole wheat flour was obtained by milling the wheat in a plate mill using similar conditions of clearance between plates and their revolutions per minute for all samples to keep the grinding conditions similar.

2.2. Quality of wheat

2.2.1. Physicochemical characterisation

SDS-sedimentation values and protein contents of whole wheat flour samples were determined by standard methods (AACC, 2000).

2.2.2. Protein fractionation and determination

Proteins from the 10 flour samples were fractionated, using a modified sequential extraction by standard methods (Curioni, Pogna, Pasini, Spettoli, Voltarel, & Dal Belin Peruffo, 2000; Weiss, Vogelmeier, & Gorg, 1993). Samples (5 g) of the ground wheat were extracted with 20 ml of Tris–HC1 buffer (50 mM, pH 8.8) for 1 h at 4 °C with vortexing at 15 min intervals and centrifuged (20,000 ***g***, 20 mm). The supernatant, containing the albumin and globulin protein, was carefully removed and stored at −20 °C. The pellet was then extracted with Tris–HC1 buffer and the supernatants of these extraction steps were combined, representing the albumin/globulin (salt-soluble) protein fraction. Following a water wash to remove buffer ions, the pellet was then extracted, as above, but with 75% aqueous ethyl alcohol (v/v), thus obtaining the gliadin (ethanol-soluble). Following a water wash, the pellet was extracted with 20 ml of SDS/DTT buffer for 2 h at room temperature with occasional vortexing. After centrifugation, the supernatant, which corresponded to soluble glutenin, and residual protein, were also stored at −20 °C.

The protein content in each fraction was determined by the Kjeldhal method ($N \times 5.7$), following the AACC procedure (AACC, 2000).

2.3. Electrophoresis

All electrophoretic separations (SDS-PAGE) were performed on a horizontal electrophoresis system (Broviga, India). SDS-PAGE analysis was carried out according to Laemmli (1970) and as modified by Singh and Shepherd (1985). The SDS-gels contained a 4% polyacrylamide stacking gel and a resolving gel of a 10% polyacrylamide. The buffer system of Laemmli (1970) was employed. Samples [30 µl aliquots from whole wheat flour (5 mg) extracted with 250 µl of sample buffer; in the case of gliadin, a 30 µl aliquot from 1 ml 70% ethanol extract of whole wheat flour (1 g)] were applied into precast application slots (size $7 \times 2 \times 0.25$ mm^3). Upon completion of electrophoresis, the proteins were fixed in methanol/acetic acid/water (40/10/50) for 30 mm and then with Coomassie brilliant blue (CBB). However, in the case of gliadin, it was stained with silver stain as per standard procedure (Weiss et al., 1993).

2.4. Production of polyclonal antibodies

Immunization of rabbits with gliadin (Sigma, USA) was carried out according to the method of Prabhasankar, Ragupathi, Sundaravadivel, Annapoorani, and Damodaran (1993) with slight modifications. New Zealand white rabbits were injected with gliadin. The initial immunization in thrice-concentrated Freund's complete adjuvant (250 µg gliadin in 500 µl of 16 mM acetic acid per rabbit) was followed by two further immunizations, consisting of 150 µg gliadin per rabbit in Freund's incomplete adjuvant, 2 or 4 weeks later. Doses were divided with half being given subcutaneously and half intradermally. Rabbits were bled to enable the serum response to be monitored and then animals were rested for 2 months. Good responders (high serum titres) were given booster injections of 100 µg of gliadin intradermally. One week after booster injections, test bleeds were obtained; serum was separated and stored at −20 °C until further use. Antibodies were purified from antisera by using an ammonium sulphate precipitation method (Mc Kinney & Parkinson, 1987) and purified antibodies were stored in small aliquots along with 10% BSA (Bovine Serum Albumin) and 0.01% sodium azide.

2.5. Dot-blot analysis

Dot-blot assay was carried out using wheat flour as per the protocol of Skerritt, Diment, and Wrigley

Table 1
Physicochemical characterisation of different wheat varieties

Varieties	Moisture (%)	SDS-sedimentation value (ml)[a]	Protein (%)[a]	Specific sedimentation value[a]	Dry gluten (%)[a]
DWR 162	9.6	43.5	12.5	3.5	11.5
DWR 195	8.9	51.5	11.0	4.7	10.0
DWR 225	9.8	55	11.5	4.8	10.5
DWR 240	9.9	71	14.5	4.9	13.2
DWR 241	9.0	73.5	15.0	4.9	13.9
NIAW 421	9.1	65.5	12.0	5.5	10.9
NIAW 474	8.9	72.5	14.0	5.2	12.9
NIAW 514	9.1	72.0	13.5	5.3	12.5
NIAW 579	9.2	65.0	12.0	5.4	10.9
NIAW 612	10.0	70.0	12.9	5.4	11.9

[a] Values based on 14% moisture basis.

(1985). Wheat grains were powdered, using a mortar and pestle; 2.5 mg of ground wheat grains were extracted with 1 ml of 70% ethanol and 4 μl of extract (10 μg) was spotted on NCP (Pall Gelman Laboratory, USA), using a microlitre syringe, followed by blocking with 2% gelatine in PBS-T (phosphate buffered saline, pH 7.4, containing 0.05% Tween 20). Immunochemical reactions were carried out by a standard protocol (Curioni et al., 2000) with slight modifications. Initially the blot was blocked with 2% gelatin in PBS-T, followed by treating with anti-gliadin antibodies (Sigma, USA). Then the blot was washed with PBS-T and then treated with anti-rabbit IgG-ALP conjugate (Bangalore Genei, India). Finally, the blot was treated with BCIP/ NBT (5-bromo-4-chloro-3-indoyl phosphate/nitro blue tetrazolium) substrate.

2.6. Chapati-making quality

Chapatis were prepared and evaluated according to the method of Haridas Rao, Leelavathi, and Shurpalekar (1986b). Chapatis were evaluated for the following quality parameters: appearance, tearing strength, colour, pliability, aroma and eating quality, by assigning maximum scores for each parameter (0–10) except eating quality (0–20). The chapatis having higher scores were considered to have better quality. The shear value of chapatis was determined by measuring the force required to shear a piece of chapati 2.5×8.0 cm using a Texture analyser (Model TA-Hdi, Stable Microsystems, UK) under the following conditions. Load cell 5 kg; plunger speed 100 mm min^{-1} Warner–Bratlzer shear attachment. Data presented are averages of quadruplicate determinations.

2.7. Statistical analysis

Statistical analysis was carried out by standard methods using Excel'97 software.

3. Results and discussion

3.1. Wheat quality

The protein content (Table 1) in different wheat varieties ranged from 11 to 15% and it was highest in variety DWR 241, followed by DWR 240 and NIAW 474, while it was minimum in the variety DWR 195. The gluten followed a similar pattern to the protein and it ranged from 10 to 13.9%. The SDS-sedimentation value also showed a similar trend to protein, in general, and it ranged from 43.5 to 73.5 ml (Table 1). The specific sedimentation, which reflects the quality of protein, showed that NIAW varieties had superior quality, with values ranging from 5.2 to 5.5 while DWR 162 had inferior quality.

It is well known that variation in the quality of wheat and its suitability for different products is attributed to the variation in the composition of gluten protein (Creesey, Campbell, Wrigley, & Griffin, 1987; Kolster & Vereijken, 1993; Ram & Nigam, 1981). In the 10 varieties tested for chapati quality, albumin and globulin ranged from 1.1 to 2.7%, gliadin ranged from 4.0 to 6.4% and glutenin from 4.0 to 7.2%. The variety DWR 240 had the maximum content of gliadin, followed by NIAW 612. The varieties DWR 162 and NIAW 421 had lowest content of gliadin. The variety NIAW 612 had the maximum contents of glutenin whereas DWR 240 had a minimum value. The varieties NIAW 421 and DWR 241 had the lowest amounts of albumin and globulin (Fig. 1).

3.2. Electrophoresis

SDS-PAGE analyses of whole wheat protein and the gliadin fraction from 10 varieties were carried out in a 10% gel, as depicted in Figs. 2 and 3. In the case of whole wheat protein, the high molecular weight glutenin region had a polymorphic pattern (MacRitchie,

Fig. 1. Comparison of wheat protein contents with chapati-making quality (CMQ) of Indian wheat varieties. A, albumin + globulin; B, gliadin; C, glutenin.

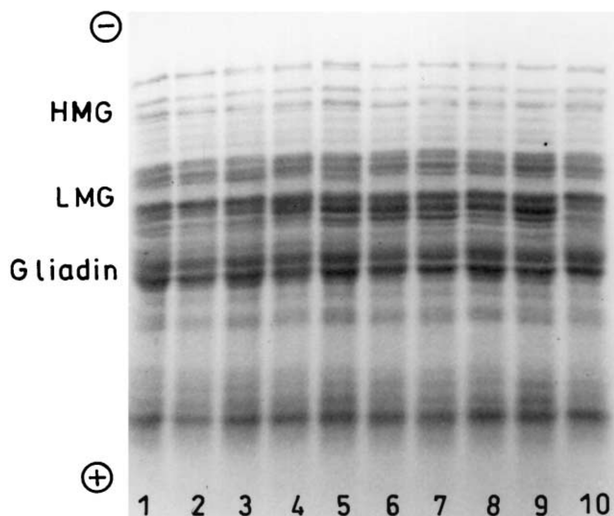

Fig. 2. SDS-PAGE of whole wheat protein—stained with Coomassie brilliant blue. Lanes: 1. DWR 162; 2. DWR 195; 3. DWR 225; 4. DWR 240; 5. DWR 241; 6. NIAW 579; 7. NIAW 474; 8. NIAW 514; 9. NIAW 612; 10. NIAW 421.

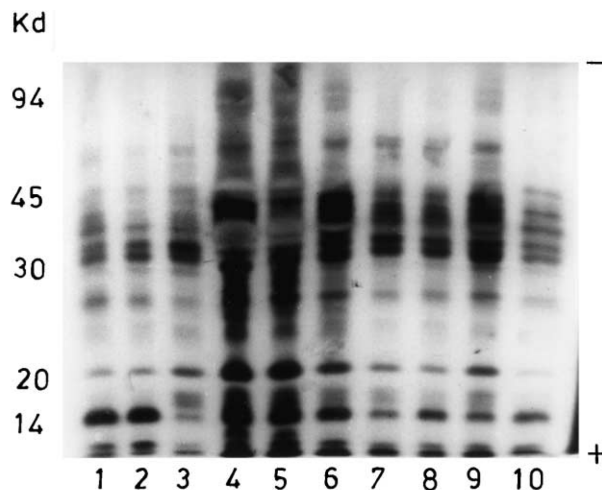

Fig. 3. SDS-PAGE of gliadin—stained with silver staining protocol. Lanes: 1. DWR 162; 2. DWR 195; 3. DWR 225; 4. DWR 240; 5. DWR 241; 6. NIAW 579; 7. NIAW 474; 8. NIAW 514; 9. NIAW 612; 10. NIAW 421.

de Cross, & Wrigley, 1990). This was in line with earlier reports. Numerous studies from different laboratories have shown a correlation between the presence of particular HMW subunits of glutenin and baking performance of wheat flour (Koister & Vereijken, 1993; MacRitchie et al., 1990; Payne, 1987). The electrophoretic pattern of whole wheat protein, from different wheat varieties, exhibited polymorphism in the HMG and LMG regions. However, in the present study, the electrophoretic pattern of whole wheat flour did not show any distinct pattern. In the case of the gliadin gel electrophoretic pattern (Fig. 3), each variety exhibited a distinct pattern, but with common bands at 40 kDa and the intensity of this band varied with variety. The varieties DWR 240, DWR 241 and NIAW 579 had more intense bands and these varieties also had more gliadin content. In the case of NIAW 421, intensity of the band was very low and it also had less gliadin content.

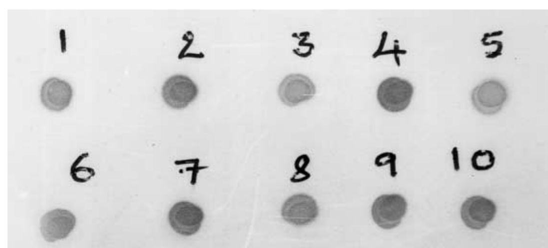

Fig. 4. Dot-blot analysis of wheat varieties. 1. DWR 162; 2. DWR 195; 3. NIAW 421; 4. DWR 240; 5. NIAW 514; 6. DWR 225; 7. DWR 241; 8. NIAW 612; 9. NIAW 474; 10. NIAW 579.

3.3. Dot-blot analysis

In order to simplify the electrophoresis, a dot-blot system was developed using anti-gliadin antibodies. This assay reflects the qualitative variation in the gliadin content of wheat varieties. In the present study, DWR 240 and DWR 241 gave darker spots and also had high gliadin contents. Variety NIAW 421 gave a lighter spot and also had a lower percentage of gliadin (Fig. 4). All other varieties gave moderately intense spots.

3.4. Chapati-making quality

Table 2 shows that the qualities of chapati made from different wheat varieties differed considerably. The chapati water absorption values ranged from 71 to 76%; puffing height ranged from 5.0 to 5.7 cm and shear values ranged from 939 to 1562. The variety NIAW 421 had the highest chapati water absorption, whereas DWR 240 had the lowest water absorption. DWR 240 had lowest chapati puffing height and the variety NIAW 421 had highest value. Most of the NIAW chapatis had the lowest shear values whereas DWR 240 had the highest value. The chapatis made from varieties NIAW 421, DWR 162 and NIAW 514 had higher scores for pliability, aroma and eating quality as well as high overall quality scores. These varieties also had lower

Table 2
Chapati-making characteristics of Indian wheat varieties

S. no.	Sample code	Water absorption (%)	Chapati puffing height (cm)	Shear value (g/kg)
1	DWR 162	73	5.4	968
2	DWR 195	73	5.2	999
3	DWR 225	74	5.2	1032
4	DWR 240	71	5.0	1562
5	DWR 241	72	5.3	1264
6	NIAW 421	76	5.7	939
7	NIAW 474	74	5.3	991
8	NIAW 514	74	5.2	939
9	NIAW 579	73	5.1	998
10	NIAW 612	74	5.4	979

Table 3
Sensory evaluation of chapatis made from different Indian wheat varieties[a]

S. no.	Sample code	Appearance[b] (10)	Tearing strength[b] (10)	Pliability[b] (10)	Aroma[b] (10)	Eating quality[b] (20)	Overall score[b] (60)
1	DWR 162	8.5b,c	9.0c	9.0b	8.5b	18.0d	53.0c,d
2	DWR 195	8.5b,c	9.0c	8.5a	8.5b	17.0c	51.5c
3	DWR 225	9.0d	8.5b	8.5a	8.5b	16.5b	51.0a
4	DWR 240	6.0a	8.0a	9.0b	8.0a	16.0a	47.0a
5	DWR 241	7.5b	8.0a	9.0b	8.5b	17.0c	50.0b
6	NIAW 421	9.0d	9.5d	9.0b	9.0c	18.0d	54.5e
7	NIAW 474	8.0b,c	8.5b	8.5a	8.0a	16.5a	49.5b
8	NIAW 514	8.0b,c	9.0c	9.0b	9.0c	18.0d	53.0b,c
9	NIAW 579	8.0b,c	8.5b	8.5a	8.5a	16.0a	49.5a,b
10	NIAW 612	8.5c	9.0c	9.0b	8.5b	17.5d	52.5d
	S.E.M.[c] (df = 39)	±0.07	±0.06	±0.06	±0.03	±0.04	±0.21

[a] Values in parentheses are maximum score for individual sensory parameters.
[b] Near values for a particular column with a different letter differ significantly ($P < 0.05$).
[c] Standard error of means at 39 df.

Table 4
Correlation matrix for different protein fractions and chapati-making characteristics of wheat varieties

	Puffing height	Shear value	Water absorption	Appearance score	Tearing strength	Pliability	Aroma	Eating quality	Total quality score
Soluble protein	−0.55	−0.16	+0.03	+0.19	−0.04	−0.69	−0.24	−0.32	−0.19
Gliadin	−0.48	+0.72*	−0.38	−0.72*	−0.49	+0.01	−0.47	−0.57	−0.68*
Glutenine	+0.43	−0.66*	+0.43	+0.80**	+0.69*	−0.12	+0.53	+0.61	+0.76**

*$P<0.05$; **$P<0.01$.

shear values confirming the softer texture. The variety DWR 240 had the lowest value for overall quality score (47/60) which was judged by the sensory panel as the least suitable variety for chapati-making (Table 3).

3.5. Relationship between sensory score, protein contents and electrophoretic and dot-blot pattern

A correlation study was carried out between various protein fractions and chapati-making characteristics, such as chapati puffing height, shear value and chapati water absorption. In the present study, soluble protein was not correlated with any of these parameters. Gliadin was positively correlated with shear value ($r=+0.72$ at $P<0.01$) whereas glutenin was negatively correlated with shear value ($r=-0.66$ at $P<0.05$) (Table 4). Among the chapati sensory parameters, pliability score was negatively correlated with soluble protein content ($r=-0.69$ at $P<0.05$). Gliadin content was negatively correlated with appearance score ($r=-0.72$ at $P<0.05$) and total quality score ($r=-0.68$ at $P<0.05$). The glutenin content was correlated with appearance score ($r=+0.80$ at $P<0.01$), tearing strength ($r=+0.69$ at $P<0.05$) and total quality ($r=+0.76$, $P<0.01$) (Table 4 and Fig. 1). Ram and Nigam (1981) made similar observations earlier. Electrophoretic and dot-blot assays also support the above correlation studies. Electrophoretic patterns of gliadin fractions from different varieties showed distinct patterns for each variety. But, each variety had a predominant band at 40 kDa and its intensity varied from variety to variety. For example, varieties DWR 240, DWR 241 and NIAW 579 gave a more intense band in that particular region and also had a high percentage of gliadin. Similarly, the variety NIAW 421 gave a less intense band and had a low gliadin content. In other words, the varieties which gave the darker bands in the 40 kDa region have poor chapati-making quality (Fig. 3). Using this electrophoretic pattern, varieties could be easily evaluated for chapati-making quality. The presently-developed SDS-PAGE electrophoretic method could be an alternative tool to the existing method for evaluation of chapati-making quality of different wheat varieties. In order to simplify the electrophoresis, dot analysis was developed using the anti-gliadin antibodies. The dot-blot assay reflects the qualitative variation in gliadin content present in the wheat varieties. The intensity of the spot was directly proportional to the gliadin content. In the present study, the varieties DWR 240, DWR 241 and NIAW 579 gave darker spots and also had poor chapati-making quality. The variety NIAW 421 gave a lighter spot and also had good chapati-making quality (Fig. 4). The above electrophoretic and dot-blot patterns also confirmed that the varieties with low gliadin content are more suitable for chapati. These methods are more advantageous than the existing methods. Particularly, the dot-blot requires less sample size, less time-consumption and allows high sample throughput. Thus, the methods described in this paper could be conveniently used to differentiate the wheat varieties with respect to chapati-making quality.

4. Conclusion

For the first time, SDS-PAGE and dot-blot were used for predicting the chapati-making quality of wheat. The wheat varieties having good chapati-making quality exhibited distinct pattern in SDS-PAGE and less intense spots in dot-blot assays.

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