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Development of Enzyme-Linked Immunosorbent Assay for Evaluation of Chapati-Making Quality of Wheat Varieties

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An indirect enzyme-linked immunosorbent assay (ELISA) has been developed for evaluation of chapati-making quality of wheat varieties. Polyclonal antibodies against gliadin, low molecular weight glutenin (LMG), and high molecular weight glutenin (HMG) were developed and utilized in the ELISA. Twenty-eight Indian wheat varieties were utilized in the ELISA. Out of these antibodies, an antigliadin antibody response was negatively correlated with farinograph water absorption ($r = -0.89$ at $P < 0.01$), chapati dough water absorption ($r = -0.91$ at $P < 0.01$), overall chapati sensory score ($r = -0.95$ at $P < 0.01$), chapati puffed height score ($r = -0.95$ at $P < 0.01$), and positively correlated with shear value of chapati ($r = 0.76$ at $P < 0.01$). Anti-LMG antibody response was not correlated with any of these parameters, whereas anti-HMG response positively correlated with chapati dough water absorption ($r = 0.44$ at $P < 0.05$), farinograph water absorption ($r = 0.45$ at $P < 0.05$), and overall chapati sensory score ($r = 0.44$ at $P < 0.05$), and negatively correlated with shear value ($r = -0.38$ at $P < 0.05$) and chapati puffed height ($r = -0.44$ at $P < 0.05$). The results indicate that wheat varieties with good chapati-making quality were having less antigliadin antibody response.

KEYWORDS: Chapati; wheat; ELISA; rheology; pasting characteristics

INTRODUCTION

Chapati, unleavened baked flat bread, is the staple diet of the majority of the people living in the Indian subcontinent. Typically, chapati dough is rolled thin and is baked in such a way as to give a puffed ball. Wheat is the basic material for chapati and its culinary variants. The quality requirement of wheat flour is specific for different products. In general, the quality of wheat for chapati is assessed by chemical, rheological, and baking tests (*1*). Because the quality of wheat is governed by the interaction of many constituents, it is difficult to judge quality by any single test. Test baking is not always possible, especially for a large number of samples where only small amounts of whole meal are available. Therefore, there is a need to develop a rapid, sensitive, single test to assess the chapati-making quality of Indian wheat varieties.

The application of immunological methods to study and compare cereal proteins from different species actually predates the use of various chemical and rheological tests. Recently, immunological-based methods haven been reported for assessing dough strength wheat flour (*2*). Similarly, Brett et al. (*3*) reported the immunological identification of LMW (low molecular weight) subunit of glutenin associated with bread-making quality of wheat flours. Hill et al. (*4*) also reported a sandwich immunoassay to predict the quality of wheat with respect to the processing condition.

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Indian wheat varieties differ in their composition and functionality (*5*). Immunochemical-based quality-assessing methods could provide a vital tool in assessing different wheat varieties with respect to their functionality and suitability for chapati. Considerable work has also been reported on the interaction of quality and quantity of protein fractions with respect to chapati-making quality (*6*−*10*). Recently, we reported on a dot-blot method for evaluation of chapati-making quality of Indian wheat varieties (*11*). However, the plate ELISA method has not been reported for evaluation of chapati-making quality of Indian wheat varieties. Hence, the aim of the present study is the development of an indirect ELISA for evaluation of chapati-making quality.

MATERIALS AND METHODS

Raw Materials. Commercially grown 28 different aestivum wheat varieties (DWR 162, DWR 195, DWR 225, DWR 235, DWR 240, DWR 241, HD 2189, HD 2501, HD 2735, HI 977, GW 190, LOK-1, MACS 2496, NIAW 34, NIAW 301, NIAW 421, NIAW 474, NIAW 499, NIAW 514, NIAW 579, NIAW 588, NIAW 590, NIAW 606, NIAW 612, NIAW 5439, PBW 343, PBW 373, SONALIKA) obtained from Mahatama Phule Krishi Vidyapeeth Agricultural Research Station, Niphad, Nashik, India were used in this study. Whole-wheat flour was obtained by milling the wheat in a plate mill using similar conditions of clearance between plates and their grinding conditions (*1*).

Physical and Chemical Characteristics of Wheat Varieties. Physical characteristics of wheat varieties, such as hardness and hectoliter weight, were determined as follows. Grain hardness was

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determined using a Grain Hardness Tester (Kiya Kusho Ltd., Tokyo, Japan). Wheat grain was kept on a platform of hardness tester with crease facing toward the platform. Force was applied to crush the grain by turning the knob. The force (in kilograms), shown on the dial when the grain was crushed, was noted. The test was repeated with 10 grains and average values were reported. Hectoliter weight of wheat grains was determined using hectoliter weight apparatus using standard protocol (*12*). Chemical characteristics of wheat varieties such as moisture, ash, protein, SDS-sedimentation value, specific sedimentation value, damaged starch, and falling number were determined by AACC methods (*13*).

Functional Characteristics of Wheat Varieties. Functional characteristics of wheat varieties such as Farinograph and pasting characteristics were according according to AACC (*13*).

Chapati-Making Quality. Chapaties were prepared and evaluated for sensory properties according to the method of Haridas Rao et al. (*1*). Chapati dough color was determined using a Reflectance Spectrophotometer (Shimadu, Japan) as per the manufacturer's protocol. Chapati water absorption was measured by Research Water Absorption Meter (Henry-Simon Ltd., UK) as per the standard method (*6*). Chapaties were scored on a scale ranging from 0 to 10 for the following quality parameters: appearance, tearing strength, pliability, aroma and on a scale ranging from 0 to 20 for eating quality. Higher scores indicate better quality of chapati. The shear values of chapati were determined by measuring the force required to shear a piece of chapati measuring 2.5 × 8.0 cm using a Texture analyzer (model TA-Hdi, Stable miro system, UK) under the following conditions: load cell, 5 kg; plunger speed, 100 mm min^{-1}; blade set with Warner-Bratlzer shear attachment. Data presented are average of quadruplicate determinations.

Protein Fractionation. Proteins from different Indian wheat varieties were fractionated using a modified sequential extraction by standard methods (*14*, *15*). Samples (5 g) of the ground wheat were extracted with 20 mL of Tris-HCl buffer (50 mM, pH 8.8) for 1 h at 4 °C with vortexing at 15-min intervals and centrifuged (20000*g*, 20 min). The supernatant containing the albumin and globulin protein was carefully removed and discarded. The pellet was then extracted with Tris-HCl buffer and the supernatants of these extraction steps were discarded. After 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). After a water wash, the pellet was extracted with 20 mL of SDS/DTT buffer for 2 h at room temperature with occasional vortexing. After centrifuging, the supernatant, which corresponds to LMG, and pellet, which corresponds to HMG, were also stored at −20 °C. These extracts were used for production of antibodies and ELISA measurements.

Production of Polyclonal Antibodies. Immunization of rabbits with gliadin/LMG/HMG was performed according to the method of Prabhasankar et al. (*16*) with slight modifications. New Zealand white rabbits were injected with gliadin/LMG/HMG. The initial immunization in three-times concentrated Freund's complete adjuvant (250 μg gliadin/LMG/HMG in 500 μL of 16 mM acetic acid per rabbit) was followed by two further immunizations of 150 μg gliadin/LMG/HMG per rabbit in Freund's incomplete adjuvant 2 and 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 the animals were rested for 2 months. Good responders (high serum titers) were given a booster of 100 μg gliadin intradermally. One week later, blood was collected; serum was separated and stored at −20 °C until further use. Antibodies were purified from antiserum by using ammonium sulfate precipitation method, and purified antibodies were stored in small aliquots along with 10% BSA and 0.01% sodium azide.

Development of Indirect ELISA for Gliadin/LMG/HMG. Preliminary standardization of indirect ELISA methods for quantification of wheat protein fractions was performed according to the method of Skerritt (*17*) with slight modifications. The optimal combination of the dilutions of the antibody and enzyme-labeled secondary antibody for use in ELISA was ascertained by following method. Indirect ELISA method was developed for quantification of gliadin using antigliadin antibodies and commercially available anti-rabbit IgG-Alkaline Phosphatase Conjugate as enzyme tracer. The optimal dilutions of antigliadin antibody and enzyme conjugate were 1:15 000 and 1:10 000, respectively. Similarly, ELISA methods for quantification of LMG and HMG in wheat varieties were developed. The amount of flour used was also standardized. The optimal concentration was found to be 500 μg of flour per variety for ELISA. The antibody responses for different wheat varieties were recorded. Accordingly, 200 μL of protein (gliadin, LMG, and HMG) extracts from different wheat varieties were diluted to the concentration of 650 ng with carbonate buffer pH 9.6; 50 mmol/L was added to the wells of microtiter plates and incubated for 2 h at 37 °C or overnight at 4 °C. After washing the plates three times with wash buffer (PBS−containing 0.5 g/L tween 20), the empty sites were blocked with a 200-μL solution of gelatin in PBS. The plates were then incubated for 1 h at 37 °C and washed again with wash buffer. This was followed by the addition of antigliadin/LMG/HMG antibodies, incubation for 1 h at 37 °C, followed by addition of anti-rabbit IgG-AKP and incubation of the plates at 37 °C for 1 h.

Table 1. Summary of Analytical Data for Indian Wheat Varieties[a]

characteristics	range	average	standard deviation
hardness (kg)	9.0–11.9	10.4	0.95
hectoliter weight (kg /hL)	71–86	79.9	4.13
moisture (%)	8.7–10.1	9.3	0.83
ash[b] (%)	1.4–2.0	1.6	0.19
SDS-sedimentation value[b] (mL)	50–73	67.0	5.20
protein[b] (%)	11.5–15.9	14.0	1.37
specific sedimentation value	3.7–5.8	4.9	0.95
damaged starch[b] (%)	12–24	19.4	3.57
falling number (s)[b]	347–684	511	92.0

[a] n = 28. [b] Values expressed on 14%.

Statistical Analysis. Statistical analyses such as mean, standard deviation, and correlation were performed by standard methods using Excel'97 software.

RESULTS AND DISCUSSION

Physical and Chemical Characteristics of Wheat Varieties. The values of hardness ranged from 9 to 11.9 kg. The results indicated that most of wheat varieties tested belonged to the medium-strong category. Wheat variety Sonalika had lowest grain hardness. Macs 2496 variety had highest hardness. Hardness indicates the milling and its suitability for bread-making quality. Wheat with lower hardness values has poor milling quality and the flour obtained will not be suitable for bread or chapati. These products require medium-strong to strong varieties. DWR 195, NIAW 42, NIAW 499, and NIAW 579 had lower hardness values whereas DWR 235, HD 2189, HD 2501, HD 2735, NIAW 34, MACS 2496, and NIAW 5439 had higher hardness values. All other varieties had moderate hardness (**Table 1**). The values of hectoliter weight ranged from 71 to 86 kg/hL. The hectoliter weight followed the similar trend of hardness. Sonalika had minimum hectoliter weight. Macs 2496 had maximum hectoliter weight. DWR 162, DWR 225, DWR 235, DWR 240, DWR 241, HD 2189, HD 2501, HD 2735, Macs 2496, NIAW 590, NIAW 612, and NIAW 5439 had hectoliter weight values between 81 and 86. All other varieties' hectoliter weight values varied between 71 and 80 (**Table 1**).

The results indicate that moisture values ranged from 8.7 to 10.1%. Varieties DWR 195 and NIAW 421 had the lowest moisture values. NIAW 606 had the highest moisture value (**Table 1**). Ash content ranged from 1.4 to 2.0%. DWR 225 and DWR 241 had the lowest ash content, whereas varieties DWR 240, NIAW 34, NIAW 421, and NIAW 606 had the highest ash content. SDS-sedimentation values ranged from 50 to 73 mL. The Sonalika had the lowest sedimentation value,

Table 2. Farinograph Characteristics of Different Wheat Varieties

sample code	water absorption (%)	dough development time (min)	dough stability (min)	mixing tolerance index (BU)
DWR 162	73.5	7.0	4.5	45
DWR 195	74.0	5.5	4.5	45
DWR 225	75.0	6.0	6.5	55
DWR 235	74.5	5.5	4.5	45
DWR 240	76.0	5.0	7.0	45
DWR 241	78.0	6.5	5.5	20
HD 2189	80.0	6.0	4.0	60
HD 2501	78.5	5.5	2.5	65
HD 2735	79.0	6.0	5.5	20
HI 977	74.0	6.0	4.5	40
GW 190	78.0	7.5	5.5	20
LOK-1	79.0	6.0	4.0	60
MACS 2496	73.0	7.5	5.5	20
NIAW 34	81.5	5.5	4.0	60
NIAW 301	80.0	5.5	2.5	65
NIAW 421	81.5	7.5	5.5	20
NIAW 474	75.5	7.5	5.5	20
NIAW 499	72.5	5.0	4.0	60
NIAW 514	77.0	5.0	2.5	40
NIAW 579	74.5	6.0	6.0	60
NIAW 588	74.0	5.0	2.5	40
NIAW 590	73.5	6.0	6.0	60
NIAW 606	73.5	6.0	5.5	40
NIAW 612	74.0	9.8	8.0	70
NIAW 5439	76.5	5.0	5.5	20
PBW 343	77.5	5.0	2.5	40
PBW 373	78.5	5.0	2.5	40
SONALIKA	72.5	6.0	3.0	60

Table 3. Chapati-Making Characteristics of Indian Wheat Varieties

sample code	chapati dough color (ΔE)	chapati dough water absorption (%)	chapati-puffing height (cm)	shear value (g)
DWR 162	12.9	73	4.2	1170
DWR 195	13.0	73	4.3	1280
DWR 225	16.3	74	4.5	1275
DWR 235	14.9	74	4.3	1202
DWR 240	14.9	71	4.5	1450
DWR 241	16.2	72	4.6	1375
HD 2189	16.2	79.5	5.2	990
HD 2501	14.3	78.0	5.0	1045
HD 2735	15.5	78.5	5.1	1241
HI 977	14.2	75.5	4.9	1201
GW 190	16.2	76.5	5.3	1025
LOK-1	15.9	80	5.7	910
MACS 2496	14.2	72.5	4.6	1350
NIAW 34	15.7	81.0	5.7	900
NIAW 301	16.2	73.5	5.1	1215
NIAW 421	15.7	76.0	5.6	930
NIAW 474	14.2	72.0	4.3	1179
NIAW 499	14.3	71.0	4.1	1250
NIAW 514	15.2	74.0	4.2	1090
NIAW 579	14.7	75.0	4.3	1090
NIAW 588	14.8	74.5	4.5	1125
NIAW 590	14.9	74.0	4.6	1250
NIAW 606	15.2	72.5	4.4	1300
NIAW 612	14.2	73.0	4.5	1250
NIAW 5439	14.1	79.0	5.2	1210
PBW 343	16.1	77.0	5.5	930
PBW 373	16.4	79.0	5.6	950
SONALIKA	15.5	72.0	4.5	850

whereas Macs 2496 had the highest sedimentation value. Protein content ranged from 11.5 to 15.9% and followed a similar trend of sedimentation values. The variety Macs 2496 had the highest value of protein followed by HD 2501 and DWR 235. All other varieties belong to the medium-strong range. Specific sedimentation value ranged from 3.7 to 5.8. NIAW 612 had the lowest specific sedimentation value. NIAW 34 had the highest specific sedimentation value indicating that the variety had better-quality protein. The variety Sonalika had the lowest protein value. Another important quality parameter was the extent of damaged starch. The strong grains normally yield flours with high levels of damaged starch. The damaged starch values ranged from 12 to 24.1%. Sonalika had the lowest and NIAW 301 had the highest starch damage values, respectively. The falling number values ranged from 453 to 680 s (**Table 1**). DWR 453 had the lowest and NIAW 514 had the highest falling number values, respectively.

Rheological Characteristics of Wheat Varieties. The results indicated that farinograph water absorption ranged from 72.5 to 81.5%; dough development time ranged from 5 to 9.8 min; dough stability ranged from 2.5 to 8.0 min, and mixing tolerance index ranged from 20 to 70 BU. Sonalika and NIAW 499 had minimum farinograph water absorption. NIAW 34 and NIAW 421 had maximum farinograph water absorption. DWR 240, NIAW 514, NIAW 588, NIAW 5439, PBW 343, and PBW 373 had the lowest dough-developing time. NIAW 612 had high dough-developing time. The varieties HD 2501, NIAW 301, NIAW 514, NIAW 588, PBW 343, and PBW 373 had low dough stability. NIAW 612 had high dough stability. DWR 241, HD 2735, Macs 2496, NIAW 421, NIAW 474, GW 190, and NIAW 5439 had a low mixing tolerance index. NIAW 612 had the highest mixing tolerance index (**Table 2**). Farinograph and pasting characteristics also indicate that most of the Indian wheat varieties belong to the medium-strong type. This is in line with earlier reports (*1*).

Chapati-Making Quality. The results indicated (**Table 3**) that chapati dough water absorption ranged from 71 to 81%. Chapati-puffing height ranged from 4.1 to 5.7 cm. DWR 240 and NIAW 499 had minimum chapati dough water absorption. NIAW 34 had maximum chapati dough water absorption. DWR 162 had the lowest dough color value. PBW 373 had the maximum dough color value. NIAW 499 had the lowest chapati-puffing height. Lok-1 and NIAW 34 had the maximum chapati-puffing height. The variety Sonalika had the lowest shear value; the variety DWR 240 had the highest shear value (**Table 3**).

Results indicated that tearing strength score ranged from 7 to 9.0. Appearance score values ranged from 7 to 9.0. Pliability score ranged from 6.5 to 9.0. Aroma score ranged from 7 to 9.0. Eating quality score ranged from 13 to 19.0. Total score ranged from 43 to 55. NIAW 5439 had the lowest tearing strength score, whereas the varieties Lok-1, NIAW 421, and PBW 373 had the highest tearing strength score. NIAW 514 and Sonalika was rated lowest for appearance score and varieties GW 190, NIAW 421, NIAW 34, and PBW 373 were rated highest for appearance score. DWR 195 was rated lowest for pliability scores, whereas varieties HD 2189, LOK-1, and NIAW 421 were scored highest for pliability scores. DWR 225, NIAW 588, NIAW 474, and SONALIKA had the lowest scores for aroma and NIAW 34, HD 2189, LOK-1, NIAW 421, and PBW 343 had the highest scores for aroma. LOK-1, NIAW 421, and NIAW 34 had the highest score for eating quality, whereas MACS 2496, NIAW 606, and NIAW 499 had the lowest score for eating quality. NIAW 421 had the highest overall sensory score, whereas the varieties MACS 2496, NIAW 499, and NIAW 606 had the lowest values for overall sensory score. Based on overall sensory score, seven varieties (NIAW 421, NIAW 34, LOK-1, PBW 343, PBW 373, HD 2189, and GW 190) had excellent chapati-making quality. Six varieties (HD 2501, NIAW 579, NIAW 301, HD 2735, DWR 235, and HI 977) had good chapati-making quality. Four varieties (DWR

Table 4. Sensory Evaluation of Chapati Prepared from Different Indian Wheat Varieties[a]

sample code	tearing strength (10)	appearance score (10)	pliability score (10)	aroma score (10)	eating score (20)	total score (60)
NIAW 421	9.0 ± 0.29	9.0 ± 0.29	9.0 ± 0.26	9.0 ± 0.29	19.0 ± 0.41	55.0 ± 0.41
NIAW 34	8.5 ± 0.26	9.0 ± 0.29	9.0 ± 0.29	9.0 ± 0.26	19.0 ± 0.44	54.5 ± 0.44
LOK-1	9.0 ± 0.29	8.5 ± 0.26	9.0 ± 0.26	9.0 ± 0.29	19.0 ± 0.44	54.5 ± 0.50
PBW 343	8.0 ± 0.29	8.5 ± 0.29	8.5 ± 0.29	9.0 ± 0.29	18.5 ± 0.41	53.5 ± 0.44
PBW 373	9.0 ± 0.29	9.0 ± 0.26	8.5 ± 0.26	8.5 ± 0.29	18.0 ± 0.44	53.0 ± 0.50
HD 2189	8.5 ± 0.26	8.5 ± 0.29	9.0 ± 0.26	9.0 ± 0.29	18.0 ± 0.41	53.0 ± 0.29
GW 190	8.0 ± 0.26	9.0 ± 0.29	8.5 ± 0.29	8.5 ± 0.26	18.5 ± 0.29	52.5 ± 0.44
HD 2501	8.5 ± 0.26	8.5 ± 0.29	8.5 ± 0.29	8.5 ± 0.26	17.0 ± 0.44	51.0 ± 0.50
NIAW 579	8.5 ± 0.29	8.5 ± 0.26	8.5 ± 0.29	8.0 ± 0.26	16.5 ± 0.41	50.0 ± 0.41
NIAW 301	7.5 ± 0.29	8.0 ± 0.26	8.0 ± 0.29	8.0 ± 0.29	17.5 ± 0.44	49.0 ± 0.44
HD 2735	8.5 ± 0.26	8.0 ± 0.29	8.5 ± 0.26	8.0 ± 0.26	16.5 ± 0.41	49.0 ± 0.50
DWR 235	8.5 ± 0.29	8.5 ± 0.26	8.0 ± 0.29	8.0 ± 0.26	15.5 ± 0.44	48.5 ± 0.41
HI 977	8.0 ± 0.29	8.0 ± 0.29	8.0 ± 0.26	8.0 ± 0.29	16.0 ± 0.29	48.0 ± 0.29
DWR 241	8.0 ± 0.29	8.0 ± 0.26	8.0 ± 0.29	8.0 ± 0.29	15.0 ± 0.41	47.0 ± 0.44
NIAW 514	7.5 ± 0.26	7.0 ± 0.29	7.5 ± 0.26	8.0 ± 0.29	17.0 ± 0.44	47.0 ± 0.41
NIAW 5439	7.0 ± 0.26	7.5 ± 0.29	7.5 ± 0.26	7.5 ± 0.26	17.5 ± 0.41	47.0 ± 0.41
DWR 240	8.0 ± 0.29	8.0 ± 0.26	7.5 ± 0.26	8.0 ± 0.29	15.0 ± 0.29	46.5 ± 0.29
DWR 225	8.0 ± 0.29	8.0 ± 0.26	8.0 ± 0.29	7.0 ± 0.26	14.0 ± 0.41	45.0 ± 0.41
NIAW 474	8.0 ± 0.29	7.5 ± 0.26	8.0 ± 0.29	7.0 ± 0.26	14.5 ± 0.29	45.0 ± 0.44
NIAW 590	8.0 ± 0.29	7.5 ± 0.26	8.0 ± 0.29	8.0 ± 0.26	13.5 ± 0.29	45.0 ± 0.41
DWR 162	7.5 ± 0.26	8.0 ± 0.29	7.0 ± 0.26	8.0 ± 0.29	14.0 ± 0.29	44.5 ± 0.29
NIAW 612	7.5 ± 0.26	8.0 ± 0.29	7.5 ± 0.29	8.0 ± 0.26	13.5 ± 0.29	44.5 ± 0.41
SONALIKA	7.5 ± 0.26	7.0 ± 0.29	7.0 ± 0.26	7.0 ± 0.26	16.0 ± 0.41	44.5 ± 0.44
NIAW 588	8.0 ± 0.29	7.5 ± 0.26	8.0 ± 0.29	7.0 ± 0.29	13.5 ± 0.44	44.0 ± 0.41
DWR 195	7.5 ± 0.29	7.5 ± 0.26	6.5 ± 0.26	7.5 ± 0.29	14.5 ± 0.41	43.5 ± 0.44
MACS 2496	7.5 ± 0.29	7.5 ± 0.29	7.5 ± 0.29	7.5 ± 0.29	13.0 ± 0.29	43.0 ± 0.29
NIAW 499	7.5 ± 0.26	7.5 ± 0.26	7.5 ± 0.29	7.5 ± 0.29	13.0 ± 0.29	43.0 ± 0.41
NIAW 606	7.5 ± 0.29	7.5 ± 0.29	7.0 ± 0.29	8.0 ± 0.26	13.0 ± 0.29	43.0 ± 0.29

[a] Values in the parentheses are the maximum score for individual sensory parameters. Scale of total score: 52–60, excellent; 48–51, good; 46–47, fair; below 45, poor.

241, NIAW 514, NIAW 5439, and DWR 240) had fair chapati-making quality, and the remainder had poor chapati-making quality (**Table 4**).

Development of ELISA Methods. The results indicate that response against antigliadin antibodies ranged between 0.345 and 0.750. Variety Lok-1 had the lowest response whereas varieties MACS 2496, DWR 195, NIAW 474, NIAW 579, NIAW 606, and Sonalika had maximum response against the antigliadin antibodies. The responses against the anti-LMG antibodies ranged between 0.307 and 0.703. The varieties PBW 373 had a minimum response whereas the variety HD 2189 had a maximum response. The response against anti-HMG antibodies ranged between 0.303 and 0.710. The variety DWR 162 had the lowest response whereas DWR 240 and NIAW 34 had the maximum response (**Figure 1**).

Correlation Studies between Protein Fractions as Evaluated by ELISA and Rheological Characteristics of Wheat Varieties. Antigliadin antibody responses were negatively correlated with farinograph water absorption ($r = -0.89$ at $P < 0.01$) and chapati dough water absorption ($r = -0.91$ at $P < 0.01$). However, the chapati water absorption ($r = 0.44$ at $P < 0.05$) and farinograph water absorption ($r = 0.45$ at $P < 0.05$) were positively correlated with anti-HMG antibody response. LMG antibody's responses were not correlated with any of these parameters. Andrew and co-workers (*2*) reported a highly significant correlation between absorbance values of ELISA farinograph dough development time and extensograph maximum resistance in Australian wheat varieties. Hill et al. (*4*) showed a correlation between ScFv [single-chain fragment engineered from a monoclonal antibody to high molecular weight gluten subunits (HMW-GS)] binding and R_{max} (maximal resistance) in Australian wheat varieties, which is a major predictor of dough strength. Skerritt (*17*) also developed an immunoassay method to measure dough strength of Australian wheat varieties, and in his study, he showed significant correlation between antiglutenin antibodies and mixograph, farinograph, and extensograph characteristics.

Correlation Studies between Protein Fractions as Evaluated by ELISA and Chapati-Making Characteristics of Wheat Varieties. Among the three antibodies, antigliadin antibody's responses were well correlated with most of the parameters (**Figure 2**). Similarly, Brett et al. (*3*) reported the immunological identification of LMW subunit of glutenin associated with bread-making quality (extensograph characteristics) of Australian wheat flours. The Fc-antibody-based method (*4*) was used to predict the quality of Australian wheat with respect to the processing condition. Andrew et al. (*2*) performed similar kinds of studies for bread-making properties such as farinograph, mixograph, etc., of Australian wheat cultivars. Skerritt (*17*) showed significant correlation between different antibodies against glutenin proteins and loaf volume of bread (from loaves with and without bromate) in Australian wheat varieties. However, the present study mainly focused on the chapati-making characteristics of Indian wheat cultivars. All the sensory parameters, such as appearance score ($r = -0.77$ at $P < 0.01$), tearing strength ($r = -0.69$ at $P < 0.01$), pliability score ($r = -0.75$ at $P < 0.01$), aroma score ($r = -0.81$ at $P < 0.01$), eating quality ($r = -0.91$ at $P < 0.01$), overall quality score ($r = -0.95$ at $P < 0.01$), indicated that correlation was negative with antigliadin antibody response. Shear value was positively correlated ($r = 0.76$ at $P < 0.01$), whereas chapati-puffed height score was negatively correlated ($r = -0.95$ at $P < 0.01$) with antigliadin antibody response. The anti-HMG antibody's responses positively correlated only with eating quality ($r = 0.51$ at $P < 0.05$) and overall quality score ($r = 0.44$ at $P < 0.05$). Shear value ($r = -0.38$ at $P < 0.05$) and chapati-puffed height ($r = -0.44$ at $P < 0.05$) were negatively correlated with anti-HMG antibody's response. The anti-LMG antibody response was not correlated with any of these parameters. This is in line with our earlier report wherein dot-blot was reported for evaluation of chapati-making quality of Indian wheat varieties (*11*). The extraction procedure for the gliadin is also much easier than other fractions. Hence, the present study suggested that the ELISA method developed using the antigliadin antibody could be conveniently used for evaluating the chapati-making quality of Indian wheat varieties.

Conclusion. An antibody-based ELISA method for prediction of chapati-making quality of wheat varieties has been developed. The polyclonal antibodies against wheat storage proteins such as gliadin, HMG, and LMG were developed and used in ELISA. Antigliadin antibody was found to be more suitable in ELISA for evaluation of chapati-making quality of Indian wheat varieties. The results from the ELISA that has been developed using antigliadin antibody showed a strong negative correlation with chapati-making quality. The ELISA is simple and easy to use and is applicable to different wheat varieties. Wheat varieties with good chapati-making quality had less antibody response. Hence, the newly developed ELISA method in the present study could be conveniently used for predicting the chapati-making quality of wheat varieties. Among the 28 Indian wheat varieties tested for chapati-making quality, NIAW 421, NIAW 34, PBW 343, and LOK-1 had excellent chapati-making quality.

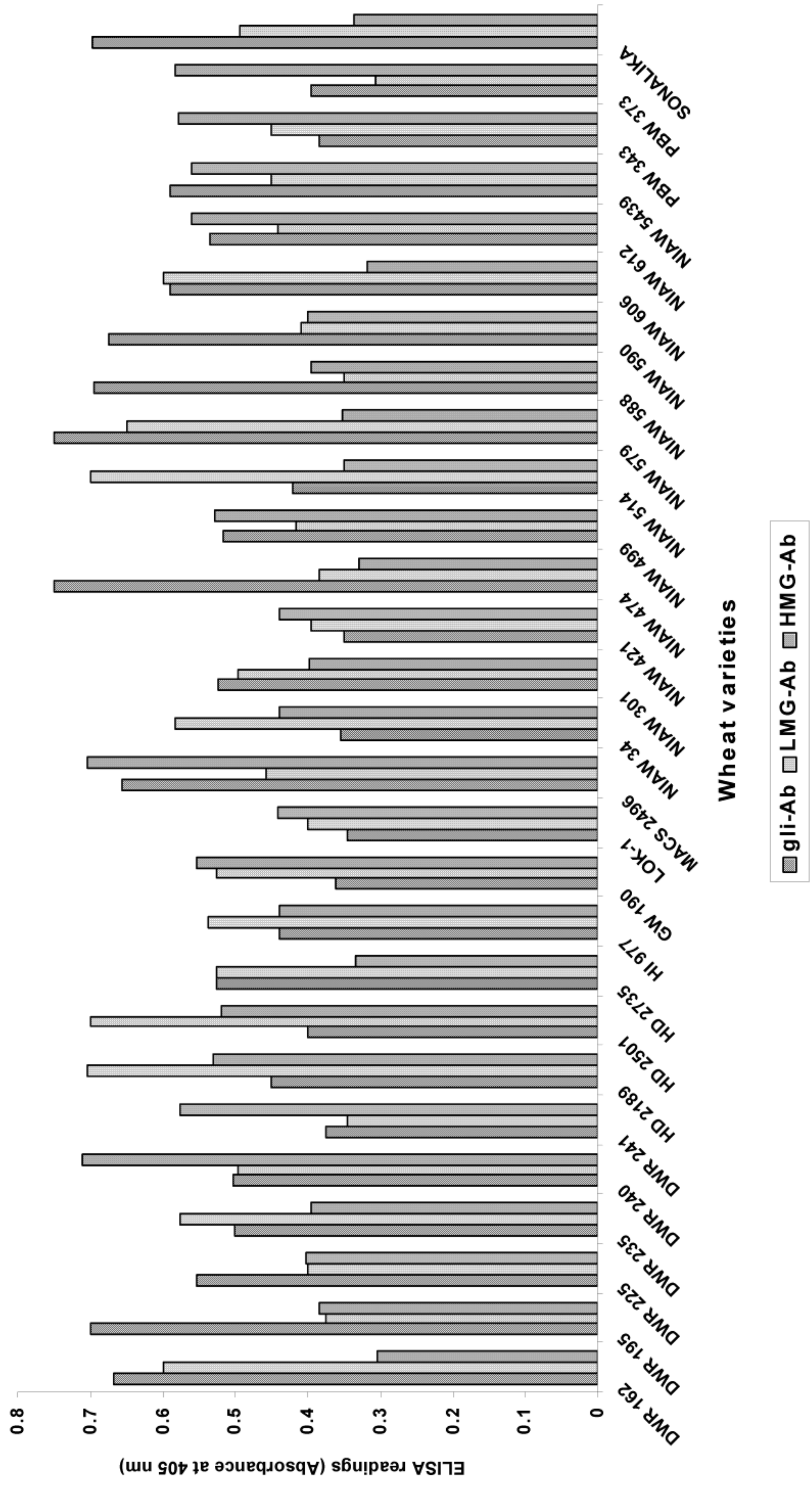

Figure 1. Antibody responses of Indian wheat varieties.

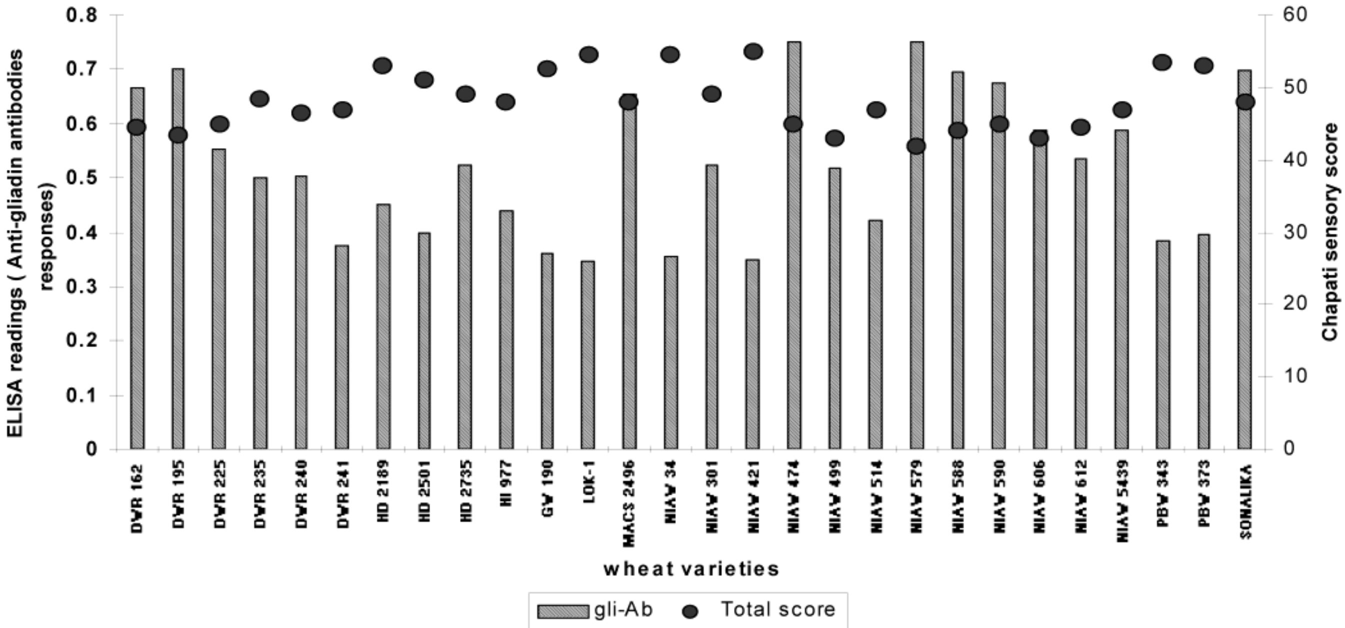

Figure 2. Chapati-making quality and antigliadin antibody responses of Indian wheat varieties.

LITERATURE CITED

(1) Haridas Rao, P.; Leelavathi, K.; Shurpalekar, S. R. Test baking of chapatis: development of a method. *Cereal Chem.* **1986**, *63*, 297−303.

(2) Andrew, J. L.; Blundell, M. J.; Skerritt, J. H. A simple antibody-based test for dough strength. III. Further simplification and collaborative evaluation for wheat quality screening. *Cereal Chem.* **1993**, *70*, 241−246.

(3) Brett, G. M.; Mills, E. N. C.; Tatham, A. S.; Fido, R. J.; Shewry, P. R.; Morgan, M. R. A. Immunochemical identification of LMW subunits of glutenin associated with bread-making quality of wheat flour. *Theor. Appl. Genet.* **1993**, *86*, 442−448.

(4) Hill, A. S.; Giersch, T. M.; Loh, C. S.; Skerritt, J. H. Immunoassay for wheat processing quality: Utilization of a sandwich assay incorporating and immobilized single-chain fragment. *J. Agric. Food Chem.* **1999**, *47*, 4484−4490.

(5) Shurpalekar, S. R.; Kumar, G. V.; Rao, G. V.; Ranga Rao, G. C. P.; Vatsala, C. N.; Rahim, A. A Physcico-chemical, rheological and milling characteristics and bread and chapati making quality of Indian wheat. *J. Food Sci. Technol.* **1999**, *13*, 79−86.

(6) Haridas Rao, P.; Leelavathi, K.; Shurpalekar, S. R. Objective measurements of the consistency of chapati dough using a research water absorption meter. *J. Texture Stud.* **1986**, *17*, 401−420.

(7) Austin, A.; Ram, A. Studies on chapati making quality of wheat. Tech. Bull. No. 31. Indian Council of Agricultural Research: New Delhi, India, 1971.

(8) Sharma, H.; Bains, G. S. Effect of grinding on the quality of whole wheat meal. *J. Food Sci. Technol.* **1976**, *13*, 92−96.

(9) Shurpalekar, S. R.; Prabhavathi, C. Brabender farinograph, research extensometer and Hilliff chapati press as tool for standardisation and objective assessment of chapati dough. *Cereal Chem.* **1976**, *53*, 457−462.

(10) Ram, B. P.; Nigam, S. N. Puffing and textural characteristics of chapati in relation to varietal difference in gluten composition. *J. Food Sci.* **1981**, *47*, 231−233.

(11) Prabhasankar, P. Electrophoretic and immunochemical characteristics of wheat protein fractions and their relationship to chapati making quality. *Food Chem*. **2002**, *78*(1), 85−91.

(12) Pomeranz, Y. In *Wheat Chemistry and Technology*; American Association of Cereal Chemists: St. Paul, MN, 1988; Vol. I, pp 17−21.

(13) AACC Approved Methods. Association of Cereal Chemists: St. Paul, MN, 2000.

(14) Weiss, W.; Vogelmeier, C.; Gorg, A. Electrophoretic characteristics of wheat grain allergens from cultivars involved in baker's asthma. *Electrophoresis* **1993**, *14*, 805−816.

(15) Curioni, A.; Pogna, N. E.; Pasini, G.; Spettoli, P.; Voltarel, M.; Dal Belin Peruffo, A. Characterisation of the glutenin fraction from eikorn wheat (Triticum monococum SSP. Mononcoccum) with different bread making qualities. *Ital. J. Food Sci.* **2000**, *12*, 91−102.

(16) Prabhasankar, P.; Ragupathi, G.; Sundaravadivel, B.; Annapoorani, K. S.; Damodaran, C. Enzyme-linked immunosorbent assay for the phytotoxin thevetin. *J. Immunoassay* **1993**, *14*, 279−296.

(17) Skerritt, J. H. A simple antibody-based test for dough strength. II. Genotype and environmental effects. *Cereal Chem.* **1991**, *68*, 467−474.

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