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Application of enzyme-linked immunosorbent assay for the assessment of spring wheat quality

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The present project was undertaken with the key objective to find out relationship of HMW and LMW glutenin sub-units and immunological test with bread making quality. The antibody response assessed through animal modelling by using rabbits as test animals showed significant variation towards high molecular weight glutenin (HMG) and low molecular weight glutenin (LMG) of different spring wheat varieties. A significant and positive relationship of bread sensory attributes with antibody response against HMG was observed. The mastication ($r = 0.64$), texture ($r = 0.69$), taste ($r = 0.72$), aroma ($r = 0.68$), grain ($r = 0.71$) and total score ($r = 0.83$) showed highly significant association with antibody response against HMG. The loaf volume correlated linearly with that of HMW antibody response ($r = 0.51$). The LMG antibody response was negatively correlated with flour characteristics and sensorial attributes with non significant level.

Keywords: ELISA; spring wheats; protein; HMW; LMW; bread; LMG; HMG; wheat

1. Introduction

The quality of wheat is generally assessed on the basis of different physical, chemical, biochemical and rheological properties (Khan et al., 2010). The products prepared from wheat grains require different quality characteristics. The wheat flour's ability to be processed into different food products is mainly determined by the quantity and quality of gluten proteins (Weegels, Pijpekamp, Graveland, Hamer, & Schofield, 1996). The main constituents of the gluten are gliadins and glutenins. Two major classes of glutenin subunits have been identified in wheat endosperm, the high molecular weight (HMW) glutenins (80–130 kDa) and the low molecular weight (LMW) glutenins (10–70 kDa). The HMW-GS are major determinant of the bread-making characteristics of dough, but the LMW-GS also play an important role with respect to dough resistance and extensibility (Cornish, Bekes, Allena, & Martin, 2001).

The quality of wheat is governed by the interaction of many constituents; therefore it is difficult to assess quality of wheat by any single test. Test baking is not always possible, especially for a large number of samples. Therefore, there is a dire need to develop a rapid, sensitive, single test to assess the bread making quality of Pakistani wheat varieties (Khan et al., 2010). The identification of food components has been

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mostly carried out in the last few years by different techniques. Chromatographic and electrophoretic techniques are considered to be very useful in identification of food components (Mayer, 2005) but these methods are not considered convenient for routine testing because they are relatively costly, time consuming, and complex to perform. So immunological assay has been reported to be used as an alternative method to reduce the test time and cost (Asensio, Gonzalez, Garcia, & Martin, 2008).

Pakistani wheat varieties differ in their functionality and suitability for bread making. Enzyme-Linked Immunosorbent Assay (ELISA) may be an effective tool for predicting bread making qualities of different wheats (Khan et al., 2010). ELISA is an immunological technique involving an enzyme (a protein that catalyzes a biochemical reaction) to detect the presence of an antibody or an antigen in a test sample. The use of ELISA has also been reported for the identification of LMW and HMW subunits of glutenins associated with bread-making quality of wheat flours (Brett et al., 1993). ELISA method has not still been reported for assessing bread making quality of Pakistani wheat varieties. The wheat varieties developed in Pakistani need to be tested for the immunochemical characteristics in order to assess their effect on the bread making qualities.

Therefore, the present study was designed to assess the immunochemical characteristics of wheat varieties grown in Pakistan in relation to bread making quality.

2. Materials and methods

2.1 Collection of wheat samples

Sixteen wheat varieties were collected from Wheat Research Institute, Faisalabad grown during the crop year 2005–2006. The wheat varieties were evaluated for various physico-chemical characteristics and immunochemical properties.

2.2 Physical tests

Thousand kernel weight of each variety was recorded in grams/1000 kernel by counting and weighing the clean, unbroken and sound kernels. The test weight of each wheat variety was measured according to the procedure given in American Association of Cereal Chemists ([AACC] 2000) method No.55–10. The test weight was expressed in Kg/hL.

2.3 Milling

Two kg grains of each wheat variety were tempered to 16.0% moisture level. The tempered wheat grains were milled through a Brabender Quadrumate Senior Mill (C.W. Brabender Instruments, Inc.) The straight grade flour was prepared by blending the two flour fractions i.e. break roll flour and reduction roll flour.

2.4 Protein analysis

The Kjeldhal's method as described in AACC (2000) method No. 46–10 was used for protein estimation. Wet and dry gluten contents in different flour samples were determined by Glutamic system (Model, GM 2015) from Perton Instruments AB.

2.5 Pelshenke value

The Pelshenke value gives an estimate of the wheat gluten strength through gas production and retention capacity of the wheat carbohydrate/protein complex. The flour of each wheat variety was tested for Pelshenke value according to AACC (2000) method No 56–50.

2.6 Protein fractionation

The protein fractions i.e. HMW and LMW glutenin proteins from flour samples of each wheat variety were fractionated, using a modified sequential extraction standard methods developed by Curioni et al. (2000).

2.7 Immunochemical studies

2.7.1 Production of polyclonal antibodies

The immunization of rabbits with LMG and HMG was performed according to the method of Prabhasankar and Manohar (2002). New Zealand white rabbits were purchased from National Institute of Health, Islamabad and were injected with LMG and HMG. The initial immunization in three-time concentrated Freund's complete adjuvant (250 ug LMG and HMG in 500 uL of 16 mM acetic acid per rabbit) was followed by two further immunizations of 150 ug LMG and 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 rested for 2 months. These were given a booster of 100 uL intradermally. One week later, blood was collected; serum was separated and stored at -20°C for further studies. Antibodies were purified from antiserum by using ammonium sulfate precipitation method and purified antibodies were stored in small aliquots along with 10% bovine serum albumin and 0.01% sodium azide (McKinney & Parkinson, 1987).

2.7.2 Development of indirect ELISA for LMG and HMG

Indirect ELISA method was developed for quantification of LMG and HMG according to the method of Skerritt, Diment and Wrigely (1985). Indirect ELISA method was developed by using anti HMG and anti LMG antibodies and commercially available anti-rabbit IgG-Alkaline Phosphatase Conjugate as enzyme tracer. The optimal dilutions of antibody and enzyme conjugate were 1:15,000 and 1:10,000, respectively. The antibody responses for different wheat varieties were recorded. Accordingly, 200 uL of protein (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 micro titer plates and incubated for 2 h at 37°C . After washing the plates three times with wash phosphate buffer saline (PBS-containing 0.5 g/L Tween 20), the empty sites were blocked with a 200 uL 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 LMG and HMG antibodies, incubation for 1 h at 37°C , followed by addition of anti-rabbit IgGAKP and incubation of the plates at 37°C for 1 h. The reading was recorded on ELISA reader at 405 nm.

2.8 Preparation of bread

The breads were prepared according to the AACC (2000) straight dough method No 10–10B. The ingredients were mixed for 5–10 minutes in a Hobart A-200 Mixer to form dough and allowed to ferment at 30 °C and 75% R.H. for 180 minutes. First and second punches were made after 120 and 150 minutes, respectively. The dough was moulded and panned into 100 g test pans, and final proofing was done for 45 minutes at 95°F (35 °C) and 85% R.H. The bread was baked at 232 °C for 13 minutes.

The sensory scores for external characteristics (volume, crust colour, symmetry, evenness of bake, break and shred) and for internal characteristics (grain, crumb colour, aroma, taste, mastication and texture) were recorded for each loaf assigned by a panel of trained judges according to the bread score method developed by the American Institute of Baking and reported by Matz (1960).

2.9 Statistical analysis

The data obtained for each parameter was analyzed through Statistical Package Co-Stat-2003 (Cohort v-6.1) (Steel, Torrie, & Dickey, 1997). The experiment was performed using completely randomised design and the analysis of variance was applied to determine the level of significance. Moreover, Duncan multiple range test was applied to explore the significant ranges in different cultivars for their various quality attributes. Correlation matrix was generated to check the interdependence of variables over each other with special reference to immuno-chemical properties of cultivars.

3. Results and discussion

3.1 Physical characteristics of wheat varieties

The results presented in Table 1 indicated that 1000 kernel weight varied between 40 and 25.98 g among different wheat varieties. Significantly the highest 1000 kernel weight (40.00 g) was exhibited by the grains of wheat variety Uqab 2000 followed by Punjab 81 (39.25 g), while significantly the lowest 1000 kernel weight (25.98 g) was yielded by the grains of wheat variety GA-02.

The present study revealed existence of wide variation with respect to 1000 kernel weight among different wheat varieties. However, the results pertaining to 1000 kernel weight in the present study are similar to the earlier findings of Pasha (2006) who found variation in 1000 kernel weight from 24.28 to 52.7 g among different Pakistani wheat varieties grown during two different crop years. Anjum, Ahmad, Butt and Ahmad (2002) also observed 1000 kernel weight of different Pakistani wheat varieties exceeding 30 g which also supports to the findings of the present study in which 11 varieties out of 16 exceeded the 1000 kernel weight to 30 g. The 1000 kernel weight is a useful tool used for assessment of the potential milling yield i.e. flour yield. The present study suggests that all the wheat varieties possessing better grain weight i.e. 30 g offer great potential for better milling yield.

The results presented in Table 1 indicated that significantly the highest test weight was yielded by the wheat variety C-273 (78.00 kg/hL) followed by Iqbal 2000 (76.90 kg/hL). The grains of Kohinoor 83 possessed significantly the lowest test weight (66.47 kg/hL). The test weight per unit volume is considered to be one of the most

Table 1. Thousand kernel and test weight of different Pakistani spring wheat varieties.

Varieties	1000 K wt (g)	Test wt (Kg/hL)
C-273	34.29b	78.00a
Faisalabad 83	34.60b	70.30cde
Faisalabad 85	34.17b	75.45abc
GA-02	25.98e	69.98cde
Inqlab 91	35.71b	77.02ab
Iqbal 2000	34.76b	76.90ab
Kohinoor 83	28.99d	66.47e
Kohistan 97	28.42d	72.48bcd
Manthar 03	28.28d	73.90abcd
MH-97	30.21cd	69.91de
Pak-81	34.85b	72.49bcd
Pasban 90	34.19b	73.96abcd
Punjab 81	39.25a	70.00cde
Punjab 85	31.89c	72.47bcd
Punjab 96	35.87b	73.00abcd
Uqab 2000	40.00a	74.00abcd

Note: Means carrying same letters in a column are not significantly different from one another.

important physical criteria in all wheat grading systems. The test weight is dependent on both grain size and shape. There is a rapid decrease in milling flour yield with decrease in the test weight. The results of the present study are in consistent with the earlier findings of other researchers Anjum et al. (2002), Randhawa, Anjum and Butt (2002) and Zahoor (2003), who reported variations from 68.30 to 81.00 kg/hL in different Pakistani wheat varieties.

3.2 Crude protein

The results given in Table 2 indicated that the protein content ranged from 10.01 to 11.60% between the straight grade flours (SGF), respectively among different wheat varieties. It is obvious from the results that significantly the highest protein content was found in the SGF of Iqbal 2000, Pasban 90 and Uqab 2000 wheat varieties and the lowest protein content (10.01%) was observed in SGF of wheat variety Faisalabad 83.

The results are in consistent with the results reported by Anjum, Ahmad, Butt, Sheikh and Pasha (2005) who reported variation in protein content from 9.68 to 13.45% among Pakistani wheat varieties. The results are also supported by the studies of Ahmad (2001), Mahmood (2004) and Randhawa et al. (2002), who found variation from 9.71 to 15.42% in protein content of different wheats. The protein content is an important criterion for assessing wheat quality. It is evident from the results that wheat varieties Iqbal 2000, Pasban 90, MH-97, Inqlab 91 and Uqab 2000 possessing higher contents of protein are more suitable for bread making.

3.3 Wet and dry gluten contents

The wet and dry gluten contents of different wheat varieties have been presented in Table 2. The wet gluten ranged from 28.47 to 41.09% in SGF of different wheat varieties.

Table 2. Crude protein, wet gluten and dry gluten contents and Pelshenke value of SGF of different Pakistani spring wheat varieties.

Varieties	Crude protein (%)	Wet gluten (%)	Dry gluten (%)	Pelshenke value (minutes)
C-273	10.50bcde	37.10bcd	13.60ab	150.12b
Faisalabad 83	10.01e	38.47abc	12.80c	51.02f
Faisalabad 85	10.46bcde	41.09a	13.80a	64.14e
GA-02	10.45bcde	34.35de	11.20efg	53.07f
Inqlab 91	11.30ab	36.14cde	10.95efg	107.25c
Iqbal 2000	11.60a	38.83abc	12.90bc	98.15d
Kohinoor 83	10.23de	28.47g	10.49fg	51.04f
Kohistan 97	10.46bcde	39.47ab	12.16cd	52.02f
Manthar 03	10.38bcde	33.16ef	11.05efg	50.85f
MH-97	11.20abc	31.00fg	10.40g	54.01f
Pak-81	11.03abcd	36.98bcd	12.33cd	93.08d
Pasban 90	11.51a	39.80ab	11.28ef	223.00a
Punjab 81	10.33cde	31.28fg	10.43fg	56.18f
Punjab 85	10.85abcde	35.18de	11.73de	51.03f
Punjab 96	10.25de	38.31abc	12.77c	55.80f
Uqab 2000	11.45a	35.18de	11.73de	68.09e

Note: Means carrying same letters in a column are not significantly different from each other.

Significantly the highest wet gluten (41.09%) was found in SGF of variety Faisalabad 85 followed by Pasban 90 (39.80%), Iqbal 2000 (38.83%), Faisalabad 83 (38.47%) and Punjab 96 (38.31%). However, significantly the lowest (28.47%) wet gluten content was found in SGF of Kohinoor 83. The results are in consistent with the findings of Miralbes (2003) who found variation from 15.6 to 39.3% in wet gluten of different wheat varieties. The results are supported by the findings reported by Randhawa et al. (2002) who also found similar ranges for wet gluten of different wheats.

The dry gluten content in SGF of different wheat varieties ranged from 10.40 to 13.80%. It is obvious from the results that significantly the highest dry gluten contents (12.89%) was found in the SGF of Faisalabad 85 followed by C-273 (13.60%) while the lowest dry gluten content (10.40%) was recorded in SGF of wheat variety MH-97 followed by Punjab 81 (10.43%).

These results are comparable with the findings of Anjum and Walker (2000) who have reported significant variation in dry gluten contents among different Pakistani wheat varieties, which ranged from 8.88 to 10.09%. The gluten content of different Pakistani wheat varieties has been found similar to those who observed differences among different wheat varieties ranging from 7 to 17% (Pasha, Anjum, Butt, & Sultan, 2007; Randhawa et al., 2002). The wheat varieties Pasban 90, Inqlab 91, Iqbal 2000, Faisalabad 85, C-273 and Faisalabad 83 possessing higher wet and dry gluten content may have better potential for bread making and should be used to explicit their potential in the development of new varieties by the wheat scientists.

3.4 Pelshenke value

The Pelshenke value ranged from 50.85 to 223 minutes in SGF of different wheat varieties (Table 2). The Pelshenke value was found significantly the highest in the

SGF of Pasban 90 (223 minutes). The Pelshenke value (52.03 minutes) was measured significantly lowest in SGF wheat variety Kohistan 97.

The wheats possessing higher gluten contents results in higher Pelshenke value. Williams, El-Haramain, Nakkoul and Rihawi (1986) have described that the Pelshenke value < 60 minutes reflects poor or soft gluten quality and the wheats with Pelshenke value ranging from 60 to 120 minutes are regarded as medium strong gluten while wheat with the Pelshenke values exceeding 120 minutes may exhibit strong gluten quality. The results regarding Pelshenke value obtained in the present study are in close agreement with the earlier findings of Randhawa et al. (2002) who found Pelshenke values varying from 108 to 185 minutes among different Pakistani wheat varieties. The results reported by Zahoor (2003) also support the present findings who reported that Pelshenke value was significantly affected due to differences in varieties. The wheat variety Pasban 90 may have strong gluten proteins and should be used for exploit this trait in new genotypes.

3.5 Sensory characteristics of bread

3.5.1 External characteristics of bread

The data regarding external characteristics has been presented in the Table 3. It is evident from the results that significantly the highest scores (7.80) were assigned to loaf volume of breads prepared from flour of Pak-81 followed by Pasban 90 (7.50), MH 97, Inqlab 91, Punjab 85, Iqbal 2000. Significantly the lowest scores (6.00) for loaf volume was given by the panellist to the breads prepared from wheat variety Manthar 03 and GA 02. The scores for crust colour of breads indicated that significantly the highest scores were given to crust colour of the breads prepared from wheat varieties C-273,

Table 3. Scores of breads for external characteristics prepared from different Pakistani spring wheat varieties.

Varieties	Loaf volume	Crust colour	Symmetry of form	Evenness of bake	crust character	Break and shred
C-273	7.35abc	6.20a	2.25abc	2.27ab	2.25ab	2.20a
Faisalabad 83	6.80cd	5.80abcd	2.15abcd	2.10abc	2.10bc	2.15ab
Faisalabad 85	7.25abc	5.70abcd	2.25abc	2.27ab	2.25ab	2.26a
GA-02	6.25de	5.35d	2.08cd	2.25ab	2.20abc	2.28a
Inqlab 91	7.40abc	5.90abc	2.17abcd	2.25ab	2.22abc	2.23a
Iqbal 2000	7.33abc	5.90abc	2.17abcd	2.19abc	2.19abc	2.16ab
Kohinoor 83	7.00bc	5.45cd	2.17abcd	2.04c	2.17abc	2.15ab
Kohistan 97	6.81cd	5.81abcd	2.15abcd	2.20abc	2.20abc	2.25a
Manthar 03	6.00e	5.60bcd	2.10bcd	2.22abc	2.20abc	2.23a
MH-97	7.32abc	5.74abcd	2.29ab	2.29a	2.24abc	2.29a
Pak-81	7.80a	6.20a	2.30a	2.27ab	2.33a	2.25a
Pasban 90	7.50ab	6.08ab	2.30a	2.27ab	2.22abc	2.25a
Punjab 81	6.80cd	5.50cd	2.17abcd	2.08bc	2.10bc	2.00b
Punjab 85	7.40abc	5.80abcd	2.25abc	2.25ab	2.25ab	2.25a
Punjab 96	7.00bc	5.55cd	1.90e	2.08bc	2.05c	2.10ab
Uqab 2000	7.20abc	5.70abcd	2.00de	2.10abc	2.05c	2.10ab

Note: Means carrying same letters in a column are not significantly different from each other.

Pak-81, Iqbal 2000, Inqlab 91, Kohistan 97, Pasban 90 and Uqab 2000 but all these wheat varieties possessed non significant differences for this sensory parameter of bread. The lowest scores (5.35) were given to crust colour of breads prepared from wheat variety GA-02. It is also obvious from the results that scores assigned to symmetry of breads ranged from 2.30 to 1.90 scores for different wheat varieties.

The wheat varieties Pak-81 and Pasban 90 were ranked at top (2.30) for symmetry by the judges, whereas the wheat variety Punjab 96 was ranked at the bottom with respect to symmetry scores. Scores assigned to evenness of bake to different breads varied significantly from 2.04 to 2.29 scores among different wheat varieties. Significantly the highest scores for this attribute were assigned to breads prepared from MH-97 wheat variety while the lowest scores were assigned to the breads prepared from variety Kohinoor 83. The breads prepared from the flour of Pak-81 wheat variety got significantly the highest scores (2.33) while the lowest scores (2.05) for character of crust were given to the breads prepared from wheat varieties Punjab 96 and Uqab 2000. The results indicated that scores assigned to break and shred of breads of different wheat varieties ranged from 2.29 to 2.00 scores. The wheat variety MH-97 got maximum scores (2.29) by the judges, whereas the wheat variety Punjab 81 was ranked at the bottom by judges with respect to scores assigned to break and shred of breads.

3.5.2 Internal characteristics of bread

The data regarding internal characteristics of bread is presented in Table 4. The scores assigned to mastication of breads prepared from SGF of different varieties ranged from 5.92 to 8.04 scores. The wheat varieties Pasban 90 and C-273 got significantly the highest scores whereas the wheat varieties Punjab 81, GA 02, Punjab 96 were ranked at bottom by judges for mastication of bread. The scores assigned to

Table 4. Scores of breads for internal characteristics prepared from different Pakistani spring wheat varieties.

Varieties	Texture	Grain	Colour of crumb	Taste	Aroma	Mastication
C-273	11.48ab	7.00a	7.00bcd	11.40ab	7.60abc	7.84ab
Faisalabad 83	10.04de	7.00a	7.10abc	10.52bcde	7.00cdef	6.60fg
Faisalabad 85	11.32b	7.20a	6.80cde	11.26abc	7.30bcde	7.20cde
GA-02	9.64e	5.80c	5.60g	10.08de	6.40f	6.24gh
Inqlab 91	11.18bc	7.40a	7.50ab	10.48bcde	7.30bcde	6.84ef
Iqbal 2000	11.16bc	7.50a	7.60a	11.61a	7.60abc	6.80efg
Kohinoor 83	10.84bcd	7.20a	7.20abc	10.84abcd	6.50f	7.02def
Kohistan 97	11.06bc	7.41a	7.41ab	11.30abc	7.01cdef	7.45bcd
Manthar 03	10.64bcd	6.40b	6.45de	11.32abc	6.70ef	7.44bcd
MH-97	11.01bc	7.52a	6.71cde	11.26abc	7.37bcd	7.36bcde
Pak-81	10.96bcd	7.40a	6.40ef	11.66a	7.70ab	7.64abc
Pasban 90	12.28a	7.40a	6.80cde	11.44ab	8.00a	8.04a
Punjab 81	10.24cde	6.00bc	7.25abc	9.64e	6.60f	5.92h
Punjab 85	11.52ab	7.60a	7.40ab	11.04abc	7.40bcd	7.20cde
Punjab 96	10.60bcd	6.40b	5.90fg	10.40cde	7.00cdef	6.44fgh
Uqab 2000	10.56bcd	7.00a	7.00bcd	10.84abcd	6.80def	6.48fgh

Note: Means carrying same letters in a column are not significantly different from each other.

texture of different breads varied from 9.64 to 12.28 scores among different wheat varieties. Significantly the highest scores were assigned to texture of breads prepared from Pasban 90 and Punjab 85 while the lowest scores were given to the breads prepared from wheat varieties GA-02, Punjab 81, Faisalabad 83 by the judges.

The scores given to grain of breads prepared from SGF of different wheat varieties ranged from 5.80 to 7.60 scores. All tested wheat varieties except GA 02, Manthar 03 and Punjab 81 were placed statistically in the same group with respect to scores assigned to grain. The results also revealed that scores for crumb colour of bread prepared from different SGF ranged from 5.60 to 7.60 scores. Maximum scores were given to crumb colour of bread prepared from Iqbal 2000 and bread for GA 02 got lower scores for crumb colour. The breads prepared from wheat variety Pak-81 got the maximum scores (11.66) whereas minimum scores (9.64) for taste were assigned to breads prepared from wheat variety Punjab 81. The scores given to aroma ranged from 6.40 to 8.00 scores among breads prepared from SGF of different varieties. The highest scores were given to bread prepared from wheat variety Pasban 90 and Iqbal 2000. The bread for wheat variety GA-02 was ranked at the bottom by judges with respect to scores given aroma of breads.

3.5.3 Total scores

Significantly the highest total scores (Table 5) were assigned to breads prepared from flour of Pasban 90 followed by Pak 81 (74.85), C-273 (74.72) Punjab 85 (74.35), Iqbal 2000 (74.14) and Inqlab 91 (73.07), wheat varieties while significantly the lowest total scores (64.06) were given to the breads prepared from wheat variety GA-02. The wheat varieties Faisalabad 83, Faisalabad 85, Kohinoor 83, Kohistan 97, Manthar 03, Punjab 81, Punjab 85 and Uqab 2000 were found to fall statistically in the same group with respect to total scores assigned to breads. Wheat flour, water and yeast

Table 5. Total scores of breads prepared from different Pakistani spring wheat varieties.

Varieties	Total scores
C-273	74.72ab
Faisalabad 83	69.30bcde
Faisalabad 85	72.78abcd
GA-02	64.06e
Inqlab 91	73.07abcd
Iqbal 2000	74.14ab
Kohinoor 83	70.53abcd
Kohistan 97	73.00abcd
Manthar 03	69.15bcde
MH-97	73.36abc
Pak-81	74.85ab
Pasban 90	76.02a
Punjab 81	66.90de
Punjab 85	74.35ab
Punjab 96	67.28cde
Uqab 2000	69.90abcde

Note: Means carrying same letters in a column are not significantly different from each other.

are the major ingredients in pan breads (Osuji, 2006). The quality of breads based on sensoric attributes is described by appearance, aroma, texture and flavour (Lawless & Heyman, 1999; Meilgard, Civille, & Carr, 2007). The present study showed that all the sensory attributes were significantly differed among different wheat varieties. The differences in the scores assigned to different attributes may be ascribed to the differences in chemical composition of wheat varieties especially the crude protein, wet and dry gluten content as has been observed in the previous sections.

The differences in bread quality attributes may mainly be due to differences in genetic makeup. Several others researchers like Preston, Lukow and Morgan (1992) and Graybosch, Peterson, Moore, Stearns and Grant, (1993) also reported that difference in chemical composition such as protein and sugar contents of wheat varieties significantly affects the sensory attributes of breads. In the present study existence of significant variation in sensory parameters of breads prepared from different wheat varieties suggested that wheat varieties possessing higher scores such as Pasban 90, Iqbal 2000, C-273, Pak 81, Punjab 85 and Inqlab 91 should be used by the bakers. In bread purposes this information is useful to the wheat scientists to exploit the potential of these good bread quality wheats in their future breeding programmes.

3.6 Immunochemical characterization of wheat varieties

The data pertaining to antibody response evaluated through animal modelling using rabbits as test animal depicted significant variation among different wheat varieties through antibody responses against high molecular weight glutenins (HMGs) and low molecular weight glutenins (LMGs). The antibody response against HMG varied from 0.300 to 0.70 among different wheat varieties. The maximum antibody response against HMG was yielded by wheat variety Iqbal-2000 (0.70) followed by Pasban 90 (0.65) and Inqlab-91 (0.65). The wheat varieties such as Kohistan 97 and Pak-81 exhibited the identical antibody response of 0.600 against HMG. The wheat varieties Kohinoor 83 and Manthar 03 also showed similar antibody response of 0.500 and the wheat varieties MH-97, Punjab-85 and Uqab-2000 showed similar antibody response of 0.460 against HMG. However, the wheat varieties Punjab-96, GA-02 and Punjab-81 exhibited the lowest antibody response against HMG which was 0.300, 0.350 and 0.380, respectively, as compared to other tested wheat varieties (Figure 1).

It is evident from Figure 2 that significant variation existed among wheat varieties with respect to the antibody response against low molecular glutenin (LMG). Maximum antibody response of 0.900 was recorded against LMG for MH-97 followed by FSD-83 (0.85). The wheat varieties Punjab-96 and Punjab-85 possessed identical antibody response of 0.80. The antibody response against LMG was similar for wheat varieties Uqab-2000, Manthar-03 and Pasban-90. The wheat varieties Kohistan-97 and Pak-81 exhibited same value for antibody response of 0.70, whereas the antibody response of GA-02, Kohinoor-83 and Iqbal 2000 was also similar 0.650 against LMG. The lowest antibody response against LMG was observed in wheat varieties, i.e. Punjab-81, C-273 and FSD-85 which was 0.55, 0.55 and 0.58, respectively.

The present study showed higher antibody responses against LMG as compared to HMG for different wheat varieties. The present results are in consistent with the

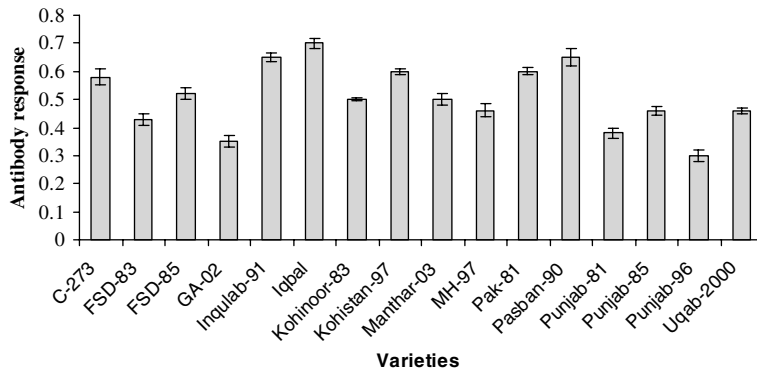


Figure 1. Antibody response of high molecular weight glutenin. Vertical bars shows mean $\pm$ standard deviation ($n = 3$ replicates).

earlier findings of Prabhasankar and Manohar (2002) who developed an indirect ELISA for evaluating chapatti making quality of some Indian wheat varieties and responses against anti-LMG antibodies and anti-HMG antibodies varying from 0.307 to 0.703 and 0.303 to 0.710, respectively. They also reported that anti-HMG response was positively correlated with overall chapatti sensory score. Li et al. (2008) suggested that polyclonal antibodies could be used for rapid prediction and screening of wheat quality. The present results for antibody responses against HMG fall within the ranges reported by Prabhasankar and Manohar (2002), but the antibody response against LMG was higher than reported by these researchers. This variation may be due to differences in the genetic makeup of wheat varieties used in these studies. Existence of wide variation with respect to antibody responses of HMG and LMG indicates differences in the end quality of these wheat varieties.

3.7 Correlation studies between antibody responses against HMG and LMG with different quality attributes

Correlation matrix given in Table 6 showed a significant relationship of HMG and LMG with different quality attributes of straight grade flour and sensory parameters

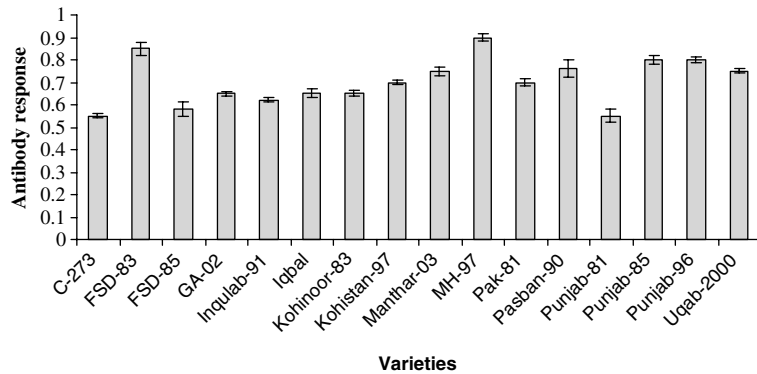


Figure 2. Antibody response of low molecular weight glutenin. Vertical bars shows mean $\pm$ standard deviation ($n = 3$ replicates).

Table 6. Correlation coefficients of HMG and LMG antibody responses with quality attributes in different wheat varieties.

Parameters	HMG antibody response	LMG antibody response
Crude protein	0.60	0.11
Wet gluten	0.28	-0.12
Dry gluten	0.26	-0.20
Pelshenke value	0.61	-0.18
Mastication of bread	0.64	0.09
Texture of bread	0.69	-0.06
Taste of bread	0.72	0.12
Aroma of bread	0.68	0.07
Crumb colour of bread	0.56	-0.21
Grain of bread	0.71	0.22
Crust colour of bread	0.73	-0.07
Loaf volume bread	0.51	-0.04
Symmetry of form of bread	0.58	-0.16
Break and shred of bread	0.39	0.15
Crust character of bread	0.58	-0.24
Evenness of bake of bread	0.48	-0.08
Total score of bread	0.83	-0.01

of bread. It is obvious from the results that a significant and positive correlation existed between HMG antibody response and crude protein content ($r=0.51$), and wet gluten ($r=0.37$).

The correlation studies were also carried out of straight grade flour and sensory attributes of bread with antibody responses to HMG and LMG. The antibody response against HMG showed significant and positive relationship with protein content of flour ($r=0.60$) and pleshenke value ($r=0.61$). A positive correlation existed between wet and dry gluten with that of HMG antibody response. A significant and positive relationship of bread sensory attributes with antibody response against HMG was also observed. There was a significant correlation of HMG with mastication ($r=0.64$), texture ($r=0.69$), taste ($r=0.72$), aroma ($r=0.68$), grain ($r=0.71$) and total scores ($r=0.83$). The loaf volume also showed relationship with that of HMW antibody response ($r=0.51$). It is obvious from the correlation studies that LMG antibody response was negatively and non significantly correlated with flour characteristics and sensory attributes of breads.

The results regarding correlation studies in the present study are in close agreement with the findings of Prabhasankar and Manohar (2002) who developed an indirect ELISA for evaluating chapatti making quality of some Indian wheat varieties. They found that anti-HMG response was positively correlated with farinograph water absorption and overall chapatti sensory scores whereas anti-LMG was negatively correlated with most of the quality attributes studied by them. The present results are further supported by Xi-Yun and Fang (1998) who found that antibodies produced against HMG was positively correlated with protein content, wet and dry gluten and bread volume as has been observed in the present study. Skerritt (1991) reported an assay to predict strength related properties of wheat dough using antibodies against high molecular weight

glutenin subunits (HMW-GS) and concluded existence of high correlations between antibody binding and dough strength parameters. Andrew, Blundell and Skerritt (1993) investigated correlations between antibody binding and dough strength parameters by using antibodies specific for low molecular weight glutenin subunits (LMW-GS) and found similar kind of relationship among some quality parameters. The present study suggests that use of antibodies response against HMG offers good tool for predicting quality of wheat for suitability to bread making quality.

Conclusion

An immunoassay was developed to predict bread making quality of spring wheats. Polyclonal antibodies against LMG and HMG proteins were produced by using New Zealand white rabbits as test animals and utilised in ELISA. Significant variation existed among the wheat varieties with respect to bread making quality. Anti-LMG antibody response is negatively correlated to total scores of breads. Anti-HMG antibody response is positively correlated to total scores of breads. The wheat varieties having good bread making quality exhibited high anti-HMG antibody responses. Anti-HMG antibody can be used as a tool for screening wheats for good quality breads. ELISA is a reliable method and can be used as single tool for the wheat quality assessment therefore it should be developed for all wheat varieties. The varieties Iqbal-2000, Pasban 90, Inqlab-91 had excellent characteristics and are more suitable for bread making.

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