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BIOCHEMICAL CHARACTERISATION OF GLIADINS IN WHEAT

NATIQUE SIDDIQUE, ARCHANA SACHDEV* AND R.P. JOHARI

Division of Biochemistry, Indian Agricultural Research Institute, New Delhi - 110 012

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SUMMARY

Wheat varieties, *viz*. C-306, HD-2745, HD-2735 (good chapati quality) and Sonalika (poor chapati quality) were selected for the characterization of soluble, storage and gliadin protein. SDS-PAGE pattern of soluble, storage and gliadin showed only qualitative changes among the varieties. PAGE pattern of gliadins was also similar. RP-HPLC analysis of these varieties showed significant differences in the relative concentration of major/minor peaks.

***Key words*: Gliadin, quality, storage protein, wheat**

INTRODUCTION

Wheat is one of the most important crops of the world. It is estimated that more than 35% of the world's population subsists on wheat (Anonymous 1995). In India, approximately 85 to 95 % of wheat is consumed in the form of "chapati" (unleavened pan baked bread made of whole wheat flour). Good physical grain characteristics such as colour, lustre, size and finally good chapati making characteristics are considered important by the Indian consumer. Storage protein (gluten) of wheat plays an important role in determining the rheological properties of wheat dough. During dough formation, the gliadins do not become covalently-linked into large elastic network as the glutenins but act as a 'plasticiser', promoting viscous flow and extensibility which are important rheological characteristics of dough (Shewry and Tatham 1997). Wheat varieties that produce strong elastic doughs are used primarily to make bread, whereas, those with highly extensible doughs are used primarily to make biscuits. Wheat varieties with intermediate properties are used to make chapati (Payne 1987, Austin and Ram 1971). Studies on the subunit composition of glutenin and its relationship to rheological and bread making properties of wheat were carried out by Payne *et al.* (1981). Branlard and Dardevet (1985) also investigated the relationship of HMW glutenin subunits to various bread making quality parameters, such as strength, tenacity, swelling and extensibility to dough. The quantitative differences in specific HMW glutenin subunits have been related with bread making properties and differences among bread wheat cultivars (Lawrence *et al.* 1989). Indian wheat varieties generally have lower levels of the polypeptides which improve loaf volume and texture of bread. Though major part of wheat, in India, is consumed in the form of chapati, not much information is available about the role of gliadins and glutenins in imparting good chapati characteristics. Therefore, the present study was undertaken with an aim to characterize gliadins and to identify polypeptides, if any, in relation to chapati quality in wheat varieties differing in chapati characteristics.

MATERIALS AND METHODS

Seeds of the wheat varieties, viz. C-306, HD-2745 and HD-2735 (good chapati quality) and Sonalika (poor

* Corresponding author, E-mail: arcs_bio@yahoo.com

chapati quality) were obtained from Division of Genetics, Indian Agricultural Research Institute, New Delhi. Total soluble proteins were extracted according to the method of Singh and Shepherd (1985) from endosperm (1:4 w/v) after soaking overnight at 4°C in 50 mM Tris-Cl buffer (pH 6.8) containing 6 mM β-mercaptoethanol and subsequently grinding and homogenizing in a pestle and mortar and centrifuged at 15,000 rpm for 10 min at 4°C using Sorvall RC-5C refrigerated centrifuge with SS-34 rotor. Supernatant (75 µl) was mixed with 25 µl of sample buffer and boiled for 5 min at 100°C. Samples (80 µl) containing uniform protein content were loaded into the wells.

Storage proteins were extracted from single seed of each variety of wheat as described by Kumamaru *et al.* (1988). Seed was ground using a mortar and pestle in 0.5 ml of solvent [50 mM phosphate buffer (pH 6.8) containing 8M urea, 4% SDS, 20% glycerine and 5% β-mercaptoethanol]. The homogenate was sonicated for a few minutes and then centrifuged at 15,000 rpm for 4 min in Sorvall RC5C centrifuge using SS-34 rotor. The supernatant was used for SDS-PAGE.

Gliadin extraction was carried out according to method of Payne *et al.* (1979) and electrophoresed on a 12% SDS-polyacrylamide gel using 0.025 M Tris-glycine buffer (pH 8.3) containing 0.1% (w/v) SDS (Laemmli 1970). Gliadin was also extracted with 70% aqueous ethanol by mixing on a vortex mixer and the mixture was allowed to stand at room temperature for 1 hr. The contents were centrifuged for 10 min at 20,000 g at room temperature. The supernatant was removed and used for electrophoresis on 7.5% polyacrylamide gel (Bushuk and Zillman 1978).

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) of gliadins was performed as described by Scanlon *et al.* (1990). Flours of different varieties (100 mg) were mixed with extracting solvent (400 µl of 70% ethanol) in 1.5 ml micro-centrifuge tubes and vortexed at 5 min interval for 15 min and then centrifuged for 15 min at 10,000 x g at room temperature. The clear supernatant was filtered through 0.45 µm nylon filters. The HPLC apparatus used was of Waters 501, Massachusetts 01757. The column used was Lichroserb RP-8 (5 µm).

RESULTS

SDS-PAGE of soluble proteins

Total soluble proteins isolated from different wheat varieties were analysed on 12% SDS-PAGE. The banding pattern is shown in Plate 1. The banding pattern was within the MW range of 10 kD to 70 kD. No significant qualitative and quantitative differences were observed in the banding pattern of these proteins.

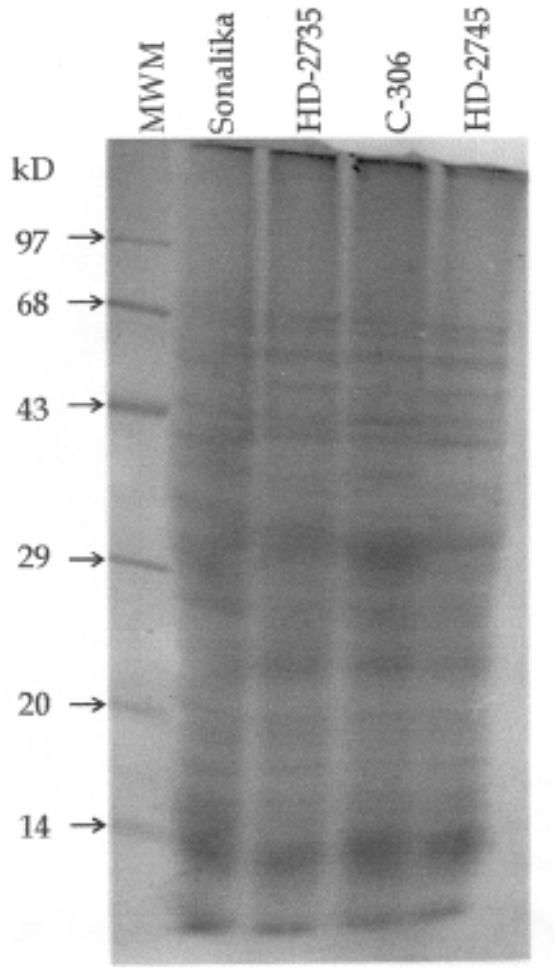

Plate 1. Electrophoretic pattern (SDS-PAGE) of total soluble proteins from different wheat varieties

SDS-PAGE of total storage proteins

Total storage proteins isolated from different wheat varieties were analysed on a 12% SDS-PAGE and the banding pattern is presented in Plate 2. SDS-PAGE

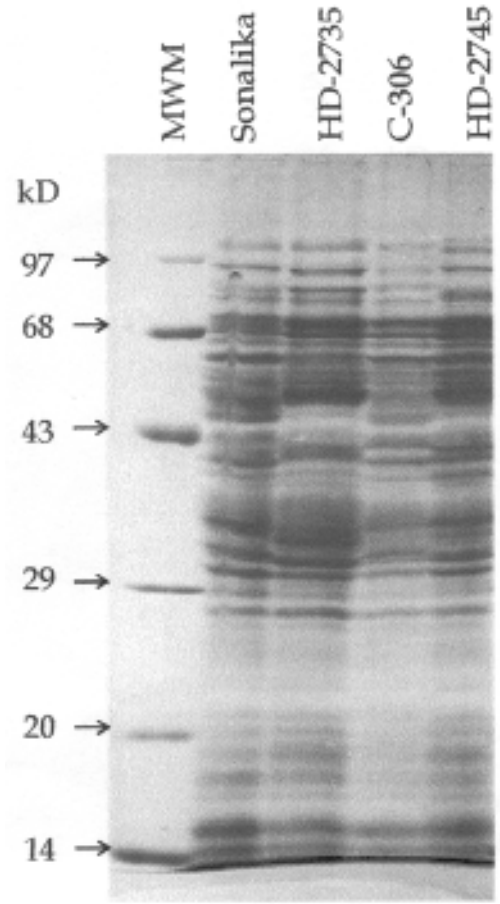

Plate 2. Electrophoretic pattern (SDS-PAGE) of total storage proteins from different wheat varieties

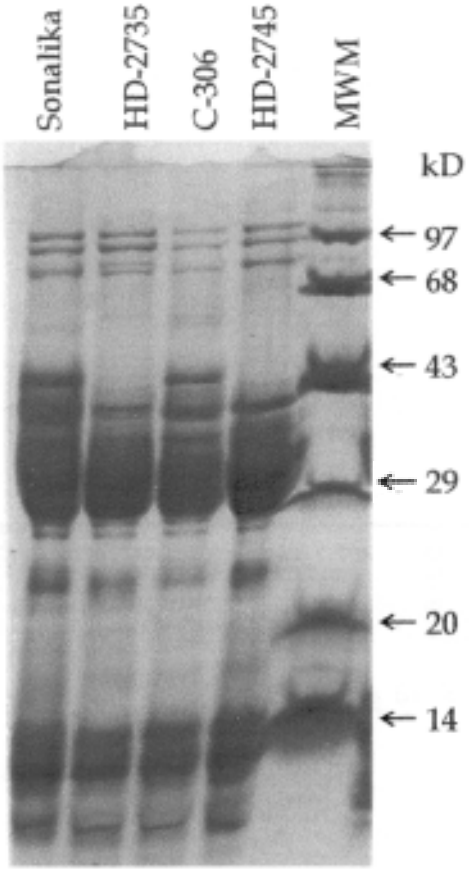

Plate 3. Electrophoretic pattern (PAGE) of gliadin extracted with 70% ethanol from grains of wheat varieties

analysis revealed a MW range of 14 kD to 126 kD for the various polypeptides. The polypeptides with MWs 98, 87, 81, 79, 33, 32, 17, 16 and 14 kD were common in all the varieties and were intense, whereas bands with MWs 117, 103, 85, 76, 34, 31, 30, 28, 27, 26 and 25 kD were also common in all the varieties but were less intense. No major qualitative or quantitative differences were observed.

PAGE analysis of gliadin

Gliadins were isolated from the single grains of different wheat varieties using 70% ethanol and analysed on 7.5% polyacrylamide gel using continuous aluminious lactate system (pH 3.1). The banding pattern of these varieties showed both qualitative and quantitative differences as shown in Plate 3. Six bands with Rm 0.08, 0.20, 0.42, 0.64, 0.78 and 0.85 were present in all the four varieties. A polypeptide at Rm 0.35 was more intense in all the varieties except Sonalika. A single high intensity band at Rm 0.32 was present only in Sonalika.

SDS-PAGE of gliadin

Gliadins isolated from the different wheat varieties were analysed on 12% SDS-PAGE. The pattern of protein bands is shown in Plate 4. Polypeptides of MWs 100, 95, 87, 45, 41, 37, 36, 31, 27, 24, 22, 18, 14, 12, 11, 10 and 9 kD were common in all the varieties. Polypeptide with MW 44 kD was observed only in Sonalika and C-306. In general, the banding pattern of all the wheat varieties was similar. However, intensity of bands showed differences among the varieties.

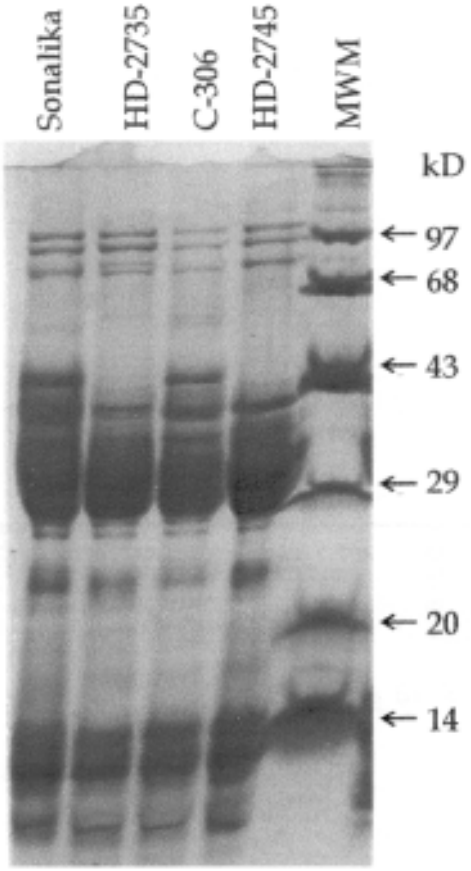

Plate 4. Electrophoretic pattern (SDS-PAGE) of gliadins extracted with ethylene glycol from wheat varieties

Comparative study of gliadin profile by RP-HPLC

Gliadins from different wheat varieties were extracted with 70% ethanol and analysed by RP-HPLC (Fig. 1). The relative concentration of different fractions eluted is shown in Table 1. The results showed both qualitative and quantitative differences amongst the varieties. Each chromatogram was divided into 32 regions. The peaks eluted (at ~ 17.7, 21.4, 22.2 and 23.5 min.) were observed in all the varieties. The relative concentration of these fractions varied and they showed quantitative differences amongst the varieties. The fractions eluted at ~ 21.4 and 22.2 min were almost of similar relative concentration in Sonalika and HD 2735. A single peak was observed only in HD 2735 and Sonalika at 29.7 and 29.5 min with a relative concentration of 22.6% and 42.15% respectively. The fractions eluted at retention time 26.1, 27.5, 29.3, 29.4, 31.3 and 32.1 were observed in C-306 and HD

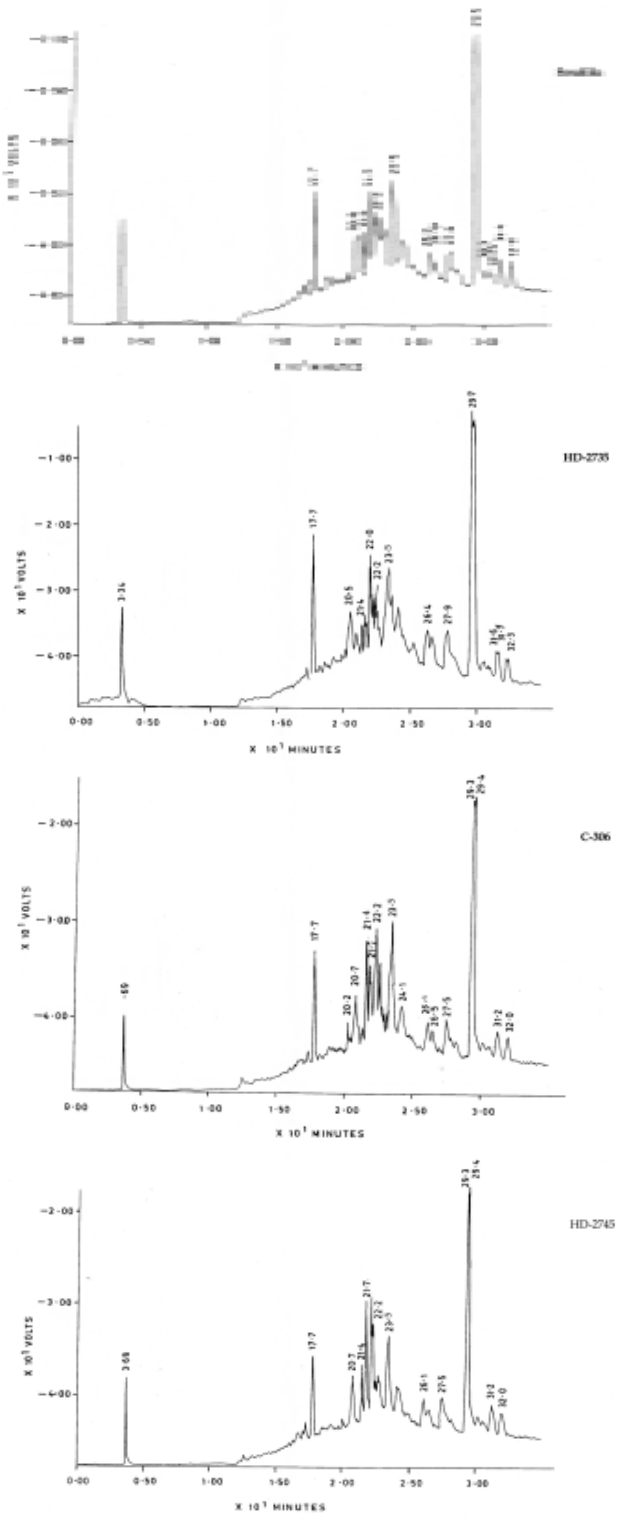

Fig. 1. Reverse phase-High Performance Liquid Chromatograms of gliadin (extracted with 70% ethanol) from wheat varieties

Table 1. Relative concentration of peaks for reverse-phase high performance liquid chromatograms of 70% ethanol-soluble proteins (gliadins)

Peak No.	Retention time (min)	Concentration (%)			
		Varieties			
		Sonalika	HD-2735	C-306	HD-2745
1	17.7	8.54	10.45	6.83	8.11
2	20.2	-	-	-	1.24
3	20.6	-	6.53	-	-
4	20.9	5.31	-	6.12	2.41
5	21.4	2.57	2.57	3.50	6.69
6	21.7	3.88	-	8.39	2.87
7	22.0	-	5.68	-	-
8	22.1	1.97	-	6.01	-
9	22.2	2.60	2.05	4.44	8.89
10	23.5	12.56	4.97	12.14	13.80
11	24.2	-	-	-	4.54
12	26.1	-	-	2.46	3.45
13	26.2	3.99	-	-	-
14	26.4	-	3.35	-	-
15	26.6	2.29*	-	-	1.51
16	27.5	-	-	5.22	3.00
17	27.7	3.37	-	-	-
18	28.0	-	2.65	-	-
19	29.3	-	-	16.58	13.41
20	29.4	-	-	21.01	19.97
21	29.5	42.15	-	-	-
22	29.7	-	22.6	-	-
23	30.4	0.96	-	-	-
24	30.7	0.87	-	-	-
25	31.3	-	-	0.46	3.51
26	31.4	3.61	-	-	-
27	31.6	-	2.23	-	-
28	31.8	-	1.67	-	-
29	32.0	-	-	3.16	2.58
30	32.2	3.24	-	-	-
31	32.4	-	1	-	-
32	32.6	-	1.85	-	-

2745. The fraction eluted at 29.4 min in C-306 and HD 2745 was almost of similar relative concentration. The fractions having very low relative concentration 0.96% and 0.87% eluted at ~30.4 and 30.7 min respectively were observed only in Sonalika.

DISCUSSION

Indian wheat varieties generally have low levels of the polypeptides which improve loaf volume and texture of bread. In India, though major part of wheat is consumed

in the form of chapati, not much information is available about the role of gliadin and glutenin polypeptides in imparting good chapati characteristics. Most of the recently developed high yielding wheat varieties do not have chapati quality like that of old Indian wheat varieties. Gluten the major storage protein imparting quality to wheat consists of two components namely gliadins and glutenins. The glutenin polymers are largely responsible for dough visco-elasticity while gliadins interact non-covalently with each other and with glutenin polymers to plasticise the gluten mass (Shewry *et al.* 1992).

In the present study SDS-PAGE of gliadins of four wheat varieties did not show major differences between good and poor chapati characteristics wheat varieties in the ω-gliadin zone as well as in the α-, β-, γ- gliadin regions. However, the banding pattern showed variation in the intensity. Overall analysis of the polypeptide profile revealed that most of the gliadin components were well within molecular range of 30-44 kD which agrees with the reported values of 30-50 kD (Benerdin *et al.* 1967). It may be possible that the presence of the polypeptide of 68 kD, 47 kD and 37 kD may be contributing to the good chapati quality characteristics or the absence of the polypeptides with MW 83 kD and 48 kD which were present only in poor chapati characteristics wheat (Sonalika) may be contributing to the good chapati characteristics.

Gliadin electrophorogram has been used by Bushuk and Zillman (1978) and Zillman and Bushuk (1979 a, b), for cultivars identification. Gliadin on PAGE resolved into 15 components. Payne *et al.* (1979, 1980, 1981, 1987) studied the subunit composition of glutenins and its relationship to rheological and bread making properties of bread wheat. They correlated the presence or absence of HMW subunits of glutenin to differentiate bread making quality. Therefore, in the present investigation an attempt was made to know exactly the molecular weight of gliadin polypeptides. The low molecular weight polypeptides ((9-18 kD) observed in SDS-PAGE of gliadins exhibited almost similar mobility as the major polypeptides of albumins and globulins. It is possible that these proteins may have been highly bound to the gluten matrix during gliadins extraction and thus appeared as major contaminants of gliadin. The occurrence of such low molecular weight proteins has been reported by Beitz and Wall (1972). Ram *et al.* (1995) isolated gliadins from 13 Indian wheat varieties and analysed them by RP-HPLC. All the varieties could be distinguished by comparing the resulting gliadin profiles showing that RP-HPLC has considerable potential as an analytical tool to identify wheat cultivars. Therefore, further characterisation of gliadins was done by RP-HPLC which provides an excellent resolution and quantification of proteins. The peaks eluted (at ~ 17.7, 21.4, 22.2 and 23.5 min.) were observed in all the varieties. The relative concentration of these fractions varied and they showed quantitative differences amongst the varieties. Gliadin profile by RP-HPLC revealed two major peaks at retention time ~ 29.3 and 29.4 min in C-306 and HD-2745 and one major peak at retention time ~ 29.5 min. in Sonalika and 29.7 min. in HD-2735 with relative concentration of 42.15% and 22.61% respectively as compared to C-306 (21.01%) and HD-2745 (20%). The fractions eluted at ~21.4 and 22.2 min were almost of similar relative concentration in Sonalika and HD-2735 but the concentration was low as compared to C-306 and HD-2745. The relative concentration was highest in C-306 (16.58%) as compared to HD-2745 (13.4%) at retention time 29.3 min. Present study showed that RP-HPLC analysis of ethanol extracted fraction from wheat varieties can be used to predict dough extensibility. This finding is generally consistent with the functional role played by gliadins in wheat gluten. If this method is to be used to predict chapati making quality parameters other than extensibility, more specific fractionation procedures may be required to obviate the masking effect of passive protein components present in the extract that are analysed (Scalon *et al.* 1990).

The results of the present investigation thus showed some qualitative differences in the polypeptide pattern in the SDS-PAGE of gliadins of the selected poor and good quality characteristic wheat. RP-HPLC of these varieties showed significant differences in the relative concentration of major/minor peaks, the difference may be used to predict dough extensibility. If this method is to be used to predict chapati making quality parameters than extensibility more specific fractionation procedure has to be used. However, the exact difference between good and poor chapati characteristics can be made known after further characterization of larger and more varied wheat varieties.

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