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# Influence of size distribution of proteins, thiol and disulfide content in whole wheat flour on rheological and chapati texture of Indian wheat varieties

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**Abstract**

The influence of protein composition, as measured by size-exclusion high performance liquid chromatography (SE-HPLC), on rheological properties and chapati texture was investigated in the whole wheat flours of eight Indian wheat cultivars grown at a single location. Proteins were extracted using two-step procedure: extraction with buffer containing 0.5% SDS (SDS buffer), followed by sonication. The results showed that SDS buffer extracted 72–90% of the total flour protein in different varieties and 7–11% protein was extracted from the remaining residues by sonication. The proteins extracted were fractionated by SE-HPLC into large polymeric proteins (>130 kDa), small polymeric proteins (80–130 kDa) and monomeric proteins (10–80 kDa). Total polymeric protein content in the flour protein showed a significant positive correlation with dough hardness ($r = 0.71$, $p < 0.05$) and positive correlation with chapati texture ($r = 0.58$, $p < 0.05$). Of the SDS extractable polymeric proteins, large polymeric protein in flour protein had significant positive correlation to dough hardness ($r = 0.89$, $p < 0.05$) and chapati cutting force, which reflects the chapati texture ($r = 0.70$, $p < 0.05$). Protein disulfide content showed positive correlation to dough hardness ($r = 0.66$, $p < 0.05$) and texture of chapati ($r = 0.58$, $p < 0.05$) while protein thiol content showed significant negative correlation to chapati texture ($r = -0.77$, $p < 0.05$). Thus, the results indicate that high proportion of SDS extractable large polymeric protein in flour protein increases the toughness of chapati texture while flours having high thiol content decrease the toughness of chapati.


*Keywords:* Wheat proteins; Dough rheology; Size distribution of proteins; Chapati texture; Thiol and disulfide content of flour

## 1. Introduction

Wheat is an important cereal crop of the world. When wheat flour is mixed with water, unique visco-elastic dough is formed. Due to this unique characteristic, wheat flour can be processed into a variety of food products such as bread, biscuit, chapati and pasta, among others. It has been reported that the rheological and baking properties of dough depend on the quantity of protein and proportion of different types of proteins (Lee, Ng, & Steffe, 2002; MacRitchie, Du cross, & Wrigley, 1990; Veraverbeke & Delcour, 2002). Wheat proteins are classified into gluten proteins and non-gluten proteins. The non-gluten proteins are albumins and globulins, and molecular weights of majority of these proteins were reported to be <25,000 Da (Bushuk & Wrigly, 1971; Meredith & Wren, 1966). Gluten proteins are a heterogeneous class with monomeric gliadins of molecular weights ranging from 30,000 to 80,000 Da (MacRitchie et al., 1990), and a mixture of polymeric glutenin having molecular weights ranging from 80,000 Da to several million dalton (Kasarda, 1989). Although non-gluten proteins are easily extractable in salt solutions, gluten proteins viz., gliadins and glutenins are not extractable in salt solutions and buffers that are

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commonly used for protein extraction. Therefore, a significant amount of insoluble protein remains unextracted depending on the solvent used and the wheat variety.

Gliadins are soluble in aqueous alcohols while both gliadins and glutenins are soluble in dilute acetic acid. Gliadins and glutenins are mainly responsible for the functional properties such as dough extensibility and elasticity. Ratios between gliadins and glutenin are used to correlate the quality of bread or chapati prepared from different wheat cultivars (Ram & Nigam, 1981; Uthayakumaran, Gras, Stoddard, & Bekes, 1999). However, these correlations were not successfully used because of incomplete extraction of particular protein fraction due to variation in extraction conditions, and also overlap of these protein fractions. For example, when Osborne extraction method is used more often gliadins are contaminated with glutenins (Prasada Rao & Nigam, 1987). As different wheat protein fractions differ in their molecular weight, separation of individual protein fractions based on size distribution is an alternate method. Size exclusion HPLC has been used to evaluate the impact of protein size distribution on bread quality (Ciaffia, Tozzi, & Lafiaandra, 1996; Gupta, Khan, & MacRitchie, 1993).

Major part of the wheat produced in Indian subcontinent is used for the preparation of chapati, a flat and unleavened baked product made from whole wheat flour, and it is the staple diet of a majority of population in this region (Haridas Rao, Leelavathi, & Shurpalekar, 1986). These are also widely consumed in the UK and other countries by the Asian ethnic community. The chapatis are generally consumed hot along with other adjuncts. Soft and pliable texture is the most important quality attribute of chapati. The chapati should be soft to chew and should not be either leathery or brittle (Haridas Rao, 1993; Srivastava, Prasada Rao, & Haridas Rao, 2003). These properties may depend on chapati dough characteristics as well as flour protein characteristics. The physicochemical characteristics of wheat greatly affect the quality of dough and chapati. Whole-wheat flour contains bran and germ along with endosperm and they constitute 15%, 3% and 82% of the grain, respectively. As bran contributes 20% and germ 8% to the total grain protein (MacMasters, Hinton, & Bradbury, 1978), the protein profile in whole wheat flour may be different compared to wheat flour (refined flour) used for bread making. Although few reports are available on the protein composition and their size distribution in relation to bread quality (Singh, Donovan, & MacRitihie, 1990; Southan & MacRitchie, 1999), no study has been carried out on the quantitative and qualitative differences in the whole wheat flour proteins and their relation to dough rheology and chapati texture. The aim of the present investigation was to study the effect of whole wheat flour protein size distribution, and disulfide and thiol content on the rheological properties of dough and texture of chapati quality of eight Indian wheat varieties.

## 2. Materials and methods

### 2.1. Materials

Eight *Triticum aestivum* wheat varieties, namely GW322, HD2189, HD2501, K9644, HD2781, MACS2496, NIAW34 and NI5439 were procured from Agharkar Research Institute, Pune, India. Sodium dodecyl sulphate (SDS), triflouroacetic acid, 5,5′-dithio-bis-(2-nitrobenzoic acid) and protein molecular weight standards were from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of analytical grade. Solvents used in this study were either analytical or HPLC grade.

### 2.2. Sample preparation for SE-HPLC

Proteins from the flours were extracted with a two-step extraction procedure according to Gupta et al. (1993) with modification. Flour samples (55 mg) were stirred for 30 min in 5 ml of sodium phosphate buffer (pH 6.9) containing 0.5% SDS. Extractions were followed by centrifugation for 10 min at 10,000*g*. Residues were resuspended in the same buffer (2.5 ml) and sonicated for 90 s at a frequency of 50 Hz using a Julabo bath type ultrasonic device (model USR-1). Sonication was followed by centrifugation for 10 min at 10,000*g*. The supernatant of the first step contains SDS-extractable proteins (proteins soluble in dilute sodium dodecyl sulphate-buffer solution) while the supernatant from the second step contains SDS-unextractable proteins (proteins soluble only after sonication).

### 2.3. SE-HPLC

SE-HPLC was performed using LC 10 A Shimadzu HPLC system (Shimadzu corporation, Kyoto, Japan) and a Biosep–SEC–S–4000 Phenomenex column (300 × 7.8 mm, 5 μm, Phenomenex, Torrence, CA, USA). All the samples were filtered through a 0.45 μm filter (Millipore, Type HA). Each sample (15 μl) was injected to the column and the protein eluted was monitored at 280 nm. The mobile phase was 50% acetonitrile containing 0.1% triflouroacetic acid with a flow rate of 0.5 ml/min. Column was calibrated using the following standard proteins, β-amylase (200,000 Da), alcohol dehydrogenase (150,000 Da), bovine serum albumin (66,000 Da), carbonic anhydrase (29,000 Da) and cytochrome *c* (12,400 Da). The percentages of large extractable polymeric protein (LEPP), large unextractable polymeric protein (LUPP) and total polymeric protein (TPP) were calculated using the following equations as described by Kuktait, Larsson, and Johansson (2004):

$$\%\ \text{of LEPP in flour protein} = \frac{\text{SDS extractable (FI)} \times 100}{\text{Flour protein}}$$

$$\%\ \text{of LUPP in flour protein} = \frac{\text{SDS unextractable (FI)} \times 100}{\text{Flour protein}}$$

$$\%\ \text{TPP in flour protein} = \frac{[\text{SDS extractable (FI} + \text{FII)} + \text{SDS unextractable(FI} + \text{FII)}] \times 100}{\text{Flour protein}}$$

$$\%\ \text{of LEPP in TPP} = \frac{\text{SDS extractable (FI)} \times 100}{[\text{SDS extractable (FI} + \text{FII)} + \text{SDS unextractable (FI} + \text{FII)}]}$$

$$\%\ \text{of LEPP in TPP} = \frac{\text{SDS unextractable (FI)} \times 100}{[\text{SDS extractable (FI} + \text{FII)} + \text{SDS unextractable (FI} + \text{FII)}]}$$

### 2.4. Protein estimation

Protein contents in the SDS-extractable and SDS-unextractable flour proteins were determined according to the procedure of Lowry, Rosebrough, Farr, and Randall (1951). Total protein content in wheat flours was determined according to the standard AACC methods (1995).

### 2.5. Disulfide and sulphydryl content estimation

The protein disulfide and sulphydryl content in the flours was estimated by solid phase assay using $NTSB^{2-}$ according to the method of Chan and Wasserman (1993). Non-protein thiol content in the flours was estimated after precipitating the flour proteins with perchloric acid according to the method described by Jocelyn (1987).

### 2.6. Texture profile analysis of chapati dough

Doughs were prepared from whole-wheat flour by adding an adequate quantity of water (equal to Farinograph water absorption value) and were analyzed for texture profile analysis using a Universal Texture Measuring System (Lloyds, LR5K, Fareham, Hampshire, UK). Dough was cut into cylindrical pieces of 2.2 cm diameter and 2 cm height and measured for dough hardness (peak force during the first compression cycle or first bite), cohesiveness (ratio of the positive force area during the second compression to that of the first compression) and adhesiveness (negative force area for the first bite). Triplicate measurement was taken for each variety. Texture profile analysis was measured with cross head speed of 50 mm/min, load cell of 5 kg, compression of 50% of sample height and probe diameter of 3.5 cm.

### 2.7. Texture (cutting force) measurement of chapati

Cutting force of chapati was determined to measure objectively the texture of chapatis. It was evaluated using a texture analyzer (Model TAHDi, Stable Micro Systems, Godalming, UK) using the Warner Bratzler blade (HDP/BSW) with a speed of 1.70 mm/s according to the method described by Hemalatha, Manu, Bhagwat, Leelavathi, and Prasada Rao (2007). Chapatis were packed in polypropylene pouches and were removed before measuring the texture. For measuring the chapati texture, four chapatis for each variety were taken and each chapati was cut into three strips measuring 4 cm × 2 cm. One strip at a time was placed on the sample holder and the blade was allowed to cut the strip. The force ($N$) required to cut the chapati strip into two pieces was recorded and the average of twelve values was reported.

### 2.8. Statistical analysis

Correlation coefficients of different parameters were determined using MS excel software and the level of significance was determined according to the procedure described by Bailey (2004).

## 3. Results and discussion

### 3.1. Flour protein extractability

The main constraint in studying the properties of wheat proteins and relating them to dough characteristics and chapati quality is the difficulty in completely extracting them without disturbing native state. Dilute acetic acid and aluminum lactate buffer have been used to solubilize the proteins, but their extractability was much less (Danno, Kanazawa, & Natake, 1974). Subsequently, Gupta et al. (1993) reported that maximum extractability of wheat flour proteins could be achieved by extracting the proteins in 0.5% SDS buffer followed by sonication and they further reported that using this procedure a maximum amount of glutenin proteins (polymeric proteins) could be extracted. Therefore, in the present study, proteins from the whole wheat flours of different varieties were solubilized in SDS containing buffer in two-step extraction procedure. In the first step, flours were extracted in SDS buffer, and in the second step, the unextractable protein was resuspended in SDS buffer and sonicated. Fig. 1 shows the protein extractability from the flours on the basis of total flour protein. Maximum amount of whole wheat flour protein (70–92%) was extracted by SDS buffer and the 10% of the proteins in the remaining residue were solubilized by

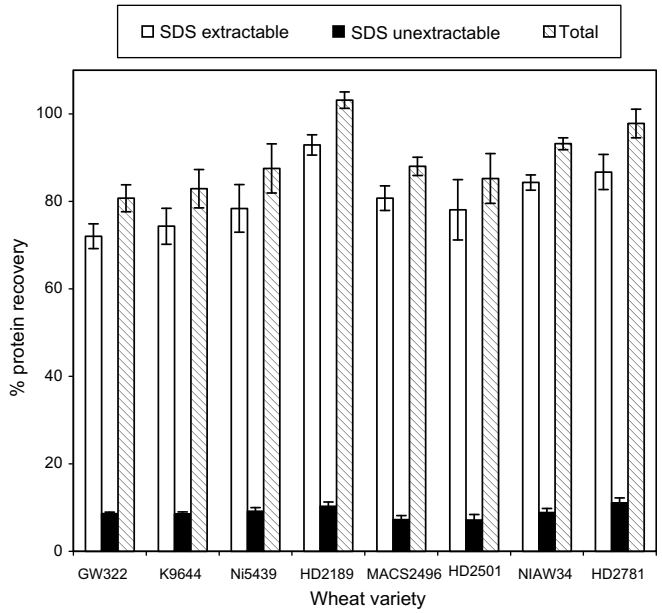


Fig. 1. Extractabilities of flour proteins (based on Lowry protein estimation) in SDS containing phosphate buffer from 8 flours without and with sonication.

sonication. Earlier, Singh et al. (1990) reported the protein extractability of 61–78% in different varieties of bread wheat flours (refined wheat flours) using 2% SDS buffer. Changes in extractability of proteins can occur due to variations in their molecular size and conformation (Phillips, Whitehead, & Kinsella, 1994). The whole wheat flours used in the present study may exhibit different protein characteristics in terms of size distribution and functional properties as they contain bran and germ fractions, which contribute significant amount of proteins (MacMasters et al., 1978).

### 3.2. Fractionation of proteins on SE-HPLC

Proteins are the most important constituents responsible for differences in baking quality of flours of different varieties. Polymeric protein content in wheat flour has been reported to influence dough rheology and bread making quality of wheat (Singh et al. 1990). However, studies on the polymeric proteins in determining the quality of whole wheat flour products like chapati are not reported. Hence, the proteins extracted with SDS buffer as well as solubilized by sonication were fractionated based on their size on SE-HPLC.

The protein profiles obtained were similar for both extracts. However, there was a difference in the quantitative distribution of these fractions between the two extracts. The chromatograms were divided into four parts, fractions I, II, III and IV in the order of their molecular weight decrease (Fig. 2). Fraction I had proteins of molecular weight >130 kDa (designated as large polymeric protein), fraction II had proteins between 80 and 130 kDa (designated as small polymeric protein), fraction III had proteins between 10 and 80 kDa (designated as monomeric proteins). Fraction IV had very low molecular weight components (<5 kDa) indicating that there may be some peptides or phenolic compounds that absorb at 280 nm. This low molecular weight fraction is expected, as whole-wheat flour contains bran and germ which are rich in polyphenols, pigments, peptides and other low molecular weight compounds (Fortmann & Joiner, 1978; Hemalatha et al. 2007; Liyana-Pathirana & Shahidi, 2006).

The percentage recovery of these protein fractions for different varieties is given in Table 1. It should be noted that in most cases the percentage yield of large polymeric protein fraction extracted by SDS buffer is much higher (26–52%) than that of small polymeric protein fraction

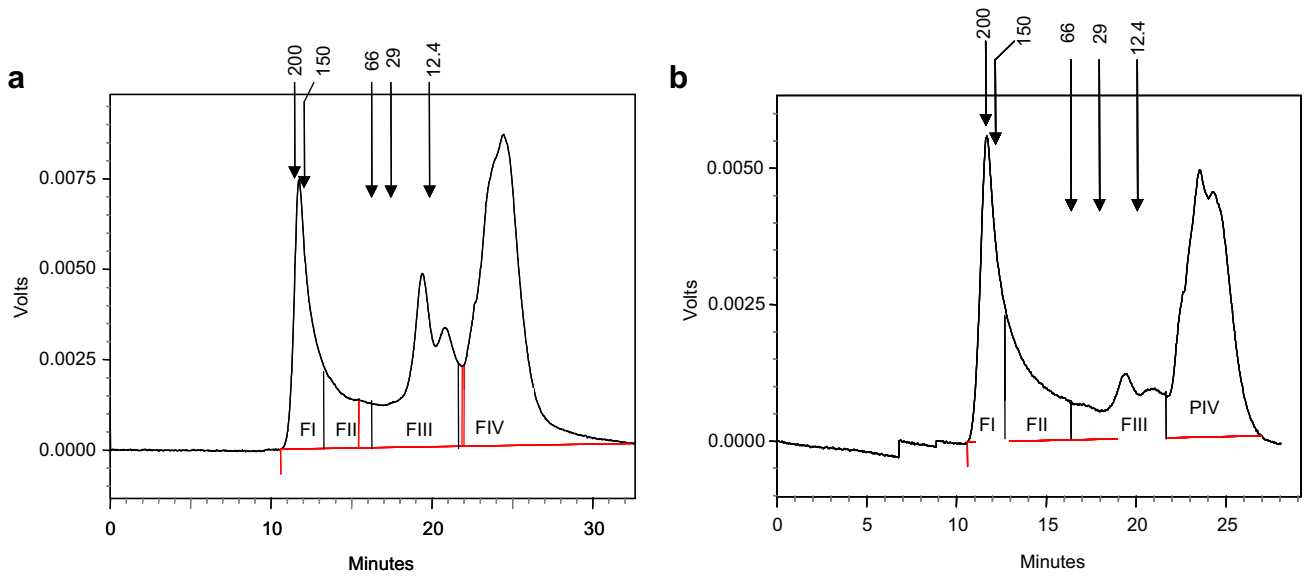


Fig. 2. Representative SE-HPLC chromatograms: (a) SDS extractable proteins; (b) SDS-unextractable proteins; FI, large polymeric proteins; F II, smaller polymeric proteins; FIII monomeric proteins.

Table 1
Area percent distribution of different protein fractions in flours of different wheat varieties obtained from SE-HPLC

| Wheat varieties | Total protein[a] (%) | SDS-extractable | | | SDS-unextractable | | |
|---|---|---|---|---|---|---|---|
| | | FI | FII | FIII | FI | FII | FIII |
| GW322 | 13.3 ± 0.05 | 34.4 ± 2.8 | 18.5 ± 0.8 | 47.1 ± 3.5 | 46.7 ± 2.4 | 27.4 ± 3.5 | 25.9 ± 1.4 |
| K9644 | 13.3 ± 0.16 | 32.3 ± 1.3 | 18.8 ± 2.3 | 48.9 ± 2.2 | 37.5 ± 4.1 | 24.0 ± 0.9 | 30.9 ± 2.1 |
| NI5439 | 13.5 ± 0.13 | 47.8 ± 1.3 | 15.9 ± 4.3 | 33.8 ± 1.0 | 16.4 ± 2.6 | 33.8 ± 2.2 | 49.8 ± 2.3 |
| HD2189 | 12.0 ± 0.27 | 51.8 ± 3.6 | 21.0 ± 3.5 | 27.2 ± 3.2 | 14.9 ± 1.5 | 49.2 ± 1.5 | 35.9 ± 3.0 |
| MACS2496 | 14.6 ± 0.16 | 42.5 ± 2.9 | 19.2 ± 2.7 | 43.0 ± 0.6 | 42.8 ± 1.5 | 17.6 ± 2.5 | 39.6 ± 1.0 |
| HD2501 | 12.7 ± 0.23 | 36.5 ± 3.8 | 16.5 ± 1.4 | 47.0 ± 2.8 | 36.9 ± 1.8 | 26.1 ± 2.3 | 37.0 ± 1.0 |
| NIAW34 | 13.7 ± 0.08 | 32.5 ± 0.8 | 15.6 ± 2.2 | 52.9 ± 2.5 | 14.6 ± 2.4 | 38.5 ± 2.6 | 46.8 ± 3.5 |
| HD2781 | 11.6 ± 0.18 | 25.7 ± 2.6 | 34.5 ± 3.8 | 39.8 ± 1.3 | 42.2 ± 3.0 | 21.1 ± 1.9 | 36.7 ± 1.5 |

FI, FII, FIII represent peak areas shown in Fig. 2a and b.Data expressed as mean ± SD of three experiments.
[a] Values expressed as 14% moisture basis, (Hemalatha et al., 2007).

(16–35%). Earlier, Hemalatha et al. (2007) extracted whole-wheat flour proteins with dilute acetic acid and reported that high molecular weight protein fraction content was around 10%. The difference in these two studies could be attributed to the solvent used for solubilization of proteins. Earlier, Danno et al. (1974) as well as Mecham, Cole, and Pence (1962) reported that dilute acetic acid could extract only 62% of total proteins. In the present study, SDS buffer extracted as high as 90% of total proteins.

### 3.3. Dough characteristics of different wheat varieties

Flour quality can be determined by the rheological properties of dough and recently, Instron Universal testing machine has been used for the determination of rheological properties of bread and biscuits doughs (Angioloni & Rosa, 2007; Gujral, Metha, Samra, & Goyal, 2003; Sudha, Srivastava, Vetrimani, & Leevathi, 2007). The chapati dough characteristics of different wheat varieties are shown in Table 2. The results indicated that dough hardness varied from 5.6 to 7.5 N and was the highest for HD2189 followed by HD2781 and it was the lowest for MACS2496 dough. Cohesiveness of the dough varied from 0.19 to 0.31 while adhesiveness varied from 0.66 to 1.46 N. Both the values were higher for the dough obtained from HD2501, HD2189, and HD2781 varieties. However, dough made from MACS2496 had the lowest adhesiveness.

Table 2
Texture profile of the dough and chapati cutting force

| Wheat variety | Dough hardness (*N*) | Cohesiveness | Adhesiveness (*N*) | Chapati cutting force (*N*)[a] |
|---|---|---|---|---|
| GW322 | 5.96 ± 0.14 | 0.19 ± 0.01 | 0.89 ± 0.04 | 5.10 ± 0.41 |
| K9644 | 6.08 ± 0.39 | 0.22 ± 0.02 | 0.93 ± 0.04 | 4.22 ± 0.51 |
| NI5439 | 6.45 ± 0.29 | 0.24 ± 0.01 | 1.22 ± 0.04 | 5.49 ± 0.20 |
| HD2189 | 7.52 ± 0.09 | 0.26 ± 0.04 | 1.42 ± 0.21 | 6.67 ± 0.15 |
| MACS2496 | 5.6 0 ± 0.25 | 0.24 ± 0.02 | 0.66 ± 0.11 | 4.51 ± 0.58 |
| HD2501 | 6.11 ± 0.35 | 0.20 ± 0.02 | 1.46 ± 0.06 | 5.59 ± 0.18 |
| NIAW34 | 6.87 ± 0.35 | 0.31 ± 0.02 | 1.07 ± 0.11 | 5.49 ± 0.18 |
| HD2781 | 7.38 ± 0.33 | 0.22 ± 0.01 | 1.34 ± 0.05 | 6.96 ± 0.21 |

Mean ± SD of three experiments.
[a] Hemalatha et al. (2007).

### 3.4. Effect of dough characteristics on the texture of chapati

Earlier studies indicated that texture of chapati could be predicted objectively by determining the cutting force (Haridas Rao, 1993; Sidhu, Seibel, & Bruemmer, 1988). As shown in Table 4, dough hardness was positively correlated with chapati cutting force ($r = 0.89$, $p < 0.05$) indicating that higher the hardness of the dough tougher will be the chapati texture. Among these varieties HD2189 and HD2781 had higher dough hardness and higher chapati cutting force while GW322, K9644 and MACS 2496 had lower dough hardness and lower cutting force. Cutting force reflects the texture of the chapattis and it simulates the biting action of human teeth on chapatis (Sidhu et al. (1988). Earlier, Sidhu et al. (1988) determined various sensory properties like appearance and hand feel, mouth feel, flexibility and over all scores of chapattis prepared from whole wheat flours of different quality, and reported that cutting force was negatively but significantly correlated with the overall sensory properties of chapattis.

### 3.5. Thiol and disulfide contents in flour and their relation to chapati texture

Baking quality of wheat is governed by total protein content, type of proteins, and thiol (SH) and disulfide (SS) content in flour (Pomeranz, 1978). In the present study, protein thiol and disulfide, and non-protein thiol

Table 3
Thiol and disulfide contents in flour

| Wheat variety | Protein disulfide (μmol/g) | Protein thiol (μmol/g) | Non-protein thiol (μmol/g) |
|---|---|---|---|
| GW322 | 4.66 ± 0.30 | 12.59 ± 0.12 | 0.16 ± 0.01 |
| K9644 | 3.00 ± 0.14 | 14.52 ± 0.15 | 0.20 ± 0.01 |
| NI5439 | 4.66 ± 0.30 | 11.75 ± 0.11 | 0.15 ± 0.03 |
| HD2189 | 6.50 ± 0.15 | 12.04 ± 0.13 | 0.19 ± 0.01 |
| MACS2496 | 4.66 ± 0.20 | 13.29 ± 0.17 | 0.24 ± 0.01 |
| HD2501 | 5.00 ± 0.22 | 11.99 ± 0.15 | 0.21 ± 0.02 |
| NIAW34 | 9.16 ± 0.21 | 13.06 ± 0.12 | 0.16 ± 0.01 |
| HD2781 | 6.66 ± 0.12 | 11.91 ± 0.10 | 0.15 ± 0.01 |

Data expressed as Mean ± SD of three experiments.

Table 4
Correlation between physical, rheological properties, thiol-disulfide and polymeric protein contents of flour

| Parameters | Protein disulfide content | Protein thiol content | Non-protein thiol content | Dough hardness | Chapati texture |
|---|---|---|---|---|---|
| Dough hardness | 0.66 | −0.49 | −0.59 | – | **0.89** |
| Chapati texture | 0.58 | **−0.77** | −0.53 | **0.89** | – |
| % of large extractable polymeric protein in flour protein | **0.72** | −0.43 | −0.66 | **0.89** | 0.70 |
| % of large unextractable polymeric protein in flour protein | −0.55 | 0.10 | −0.16 | −0.33 | −0.24 |
| %Total polymeric protein in flour protein | **0.71** | −0.27 | **−0.74** | **0.71** | 0.58 |
| % of large extractable polymeric protein in total polymeric protein | 0.52 | −0.49 | −0.63 | **0.81** | 0.64 |
| % of large unextractable polymeric protein in total polymeric protein | −0.69 | 0.17 | −0.23 | −0.45 | −0.38 |

Figures in bold are significant at $p < 0.05$.

content in different wheat varieties were determined (Table 3). The protein SH content varied from 11.91 to 14.52 μmole/g flour, while SS content varied from 3 to 9.16 μmole/g flour. Though there are few reports on SS and SH contents in refined wheat flours, no report is available on whole wheat flour (chapati flour) with regard to SS and SH contents. The SS and SH contents in different refined wheat flours were reported to range from 8.5 to 16.9 μmole/g and 1.0–2.65 μmole/g flour, respectively (Beveridge, Toma, & Nakai, 1974; Tsen & Bushuk, 1968). However, results in the present study indicate that SH content is very high in whole wheat flours while SS content is lower compared to refined wheat flours, which are used for bread making.

Wheat based products like chapati and bread are prepared after mixing the flour with water to form the dough. Interchange of thiols and protein disulfide bonds of the gluten proteins has been demonstrated during the dough mixing (Kasarda, Nimmo, & Kohler, 1978). Protein thiol content in the flour negatively correlated to chapati texture ($r = -0.77$ at $p < 0.05$) (Table 4). Non-protein thiol content varied from 0.15 to 0.24 μmole/g (150–240 nmol/g) (Table 3). Wheat flour contains non-protein thiol compounds or free thiols like glutathione (GSH) and cysteine. Although the free thiol contents are low, they have been considered to play an important role in redox reactions in flour. Content of free thiols like GSH and cysteine was reported to influence the rheological and bread properties of flour (Grosch, 1986; Lambert & Kokini, 2001; Sarwin, Laskawy, & Grosch, 1993; Tkachuk, 1970). Most SH and SS interchange in gluten proteins with low molecular weight SH compounds takes place during dough mixing resulting in depolymerization of gluten proteins (Dong & Hoseney, 1995). Thus, high free thiol content in flour decreases the toughness of chapati.

The non-protein thiol and protein disulfide content in flour also influenced the content of polymeric proteins. The SS content in flour was positively correlated to large extractable polymeric protein and total polymeric protein content while free thiol content was significantly correlated to total polymeric protein content in flour. The Content of both GSH and cysteine in six wheat flours were reported to be between 16 and 46 nmol/g (Sarwin et al. 1993), while in the present study it ranged from 150 to 240 nmol/g whole wheat flour. Thus, the high content of free thiols may change the polymeric protein content and thus, product quality. Earlier studies also report that the levels of reducing agents in wheat flour are important as these compounds modify the structure and functional properties of gluten proteins, which affects the product quality (Lavelli, Guerrieri, & Cerletti 1996; Li & Lee, 1996).

### 3.6. Interrelationships between molecular weight of different protein fractions, dough rheology and chapati quality

The correlation between relative quantities of these protein fractions and quality attributes are given in Table 4. The percentage of large extractable polymeric protein in flour protein was significantly positively correlated with dough hardness ($r = 0.89$, $p < 0.05$) and chapati texture ($r = 0.70$, $p < 0.05$). Earlier, Gupta et al. (1993) have shown that percentage of unextractable polymeric proteins were significantly correlated to dough strength and Kuktait et al. (2004) have shown that strong flours contained higher percentage of large unextractable polymeric proteins. The present study has clearly shown that no significant correlation was observed between unextractable polymeric proteins and dough rheology as well as chapati texture. Chapati dough is prepared from whole wheat flour and the quality requirements of flour for chapati may vary from bread flour.

## 4. Conclusions

SDS extractable proteins constitute the major part of the protein (72–90%) in whole wheat flour. Therefore, variation in SDS-extractable protein components may have significant effects on the quality of the product. The present study indicates that the percentage of large extractable polymeric protein in flour protein influences the dough and the textural properties of chapati. Higher the content of large polymeric protein in flour protein harder the chapati texture. As the SE-HPLC provides a simple and an objective type test for measuring relative size distribution of polymeric proteins, knowledge of optimum molecular

weight distribution for a given end-use requirement can be used to improve product quality during processing or can be used in breeding programmes. The results also indicated that flours containing more amount of thiols yielded dough with less hardness, and chapati with soft texture.

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## References

American Association of Cereal Chemists (AACC) (1995). *Method 44-19; method 46-12; method 80-60; method 76-30A; approved methods of the AACC* (9th ed.). St. Paul, MN.: The Association.

Angioloni, A., & Rosa, M. D. (2007). Effects of cysteine and mixing conditions on white/whole dough rheological properties. *Journal of Food Engineering, 80*, 18–23.

Bailey, N. T. J. (2004). *Statistical methods in biology*. Cambridge: Cambridge University Press.

Beveridge, T., Toma, S. J., & Nakai, S. (1974). Determination of SH- and SS-groups in some food proteins using Ellman's reagent. *Journal of Food Science, 39*, 49–51.

Bushuk, W., & Wrigly, C. W. (1971). Glutenin in developing wheat grain. *Cereal Chemistry, 48*, 448–455.

Chan, K. Y., & Wasserman, B. P. (1993). Direct colorimetric assay of free thiol groups and disulfide bonds in Suspensions of solubilized and particulate proteins. *Cereal Chemistry, 70*, 22–26.

Ciaffia, M., Tozzi, L., & Lafiaandra, D. (1996). Relationship between flour protein composition determined by size-exclusion high-performance liquid chromatography and dough rheological parameters. *Cereal Chemistry, 73*, 346–351.

Danno, G., Kanazawa, K., & Natake, M. (1974). Extraction of wheat flour proteins with sodium dodecyl sulfate and their molecular weight distribution. *Agricultural Biological Chemistry, 38*, 1947–1953.

Dong, W., & Hoseney, R. C. (1995). Effects of certain bread making oxidants and reducing agents on dough rheological properties. *Cereal Chemistry, 72*, 58–64.

Fortmann, K. L., & Joiner, R. R. (1978). Wheat pigments and flour colour. In Y. Pomeranz (Ed.), *Wheat chemistry and technology* (pp. 493–522). St. Paul, MN, USA: American Association of Cereal Chemists, Inc.

Grosch, W. (1986). Redox systems in dough. In J. M. V. Blanshard, P. J. T. Frazier, & T. Galliard (Eds.), *Chemistry and physics of baking* (pp. 155–169). London: Royal Society of Chemistry.

Gujral, H. S., Metha, S., Samra, I. S., & Goyal, P. (2003). Effect of wheat bran, coarse wheat flour, and rice flour on the instrumental texture of cookies. *International Journal of Food Properties, 6*, 329–340.

Gupta, R. B., Khan, K., & MacRitchie, F. (1993). Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. *Journal of Cereal Science, 18*, 23–41.

Haridas Rao, P. (1993). Chapati and related products. In R. Macrae, R. K. Robinson, & M. J. Sadler (Eds.). *Encyclopedia of food science, food technology and nutrition* (Vol. II, pp. 795–801). London: Academic Press.

Haridas Rao, P., Leelavathi, K., & Shurpalekar, S. R. (1986). Test baking of chapati-development of a method. *Cereal Chemistry, 63*, 297–303.

Hemalatha, M. S., Manu, B. T., Bhagwat, S. G., Leelavathi, K., & Prasada Rao, U. J. S. (2007). Protein characteristics and peroxidase activities of different Indian wheat varieties and their relationship to chapati-making quality. *European Food Research and Technology, 225*, 463–471.

Jocelyn, P. C. (1987). Spectroscopic assay of thiols. In Jakoby, W. B., Griffith, O. W. (Ed.), *Sulfur and sulfur amino acids*, *Methods in enzymology* Vol. 143, (pp. 44–67).

Kasarda, D. D. (1989). Glutenin structure in relation to wheat quality. In Y. Pomeranz (Ed.), *Wheat is unique* (pp. 277–302). St. Paul, MN, USA: American Association of Cereal Chemists, Inc.

Kasarda, D. D., Nimmo, C. C., & Kohler, G. O. (1978). Proteins and amino acid composition of wheat fractions. In Y. Pomeranz (Ed.), *Wheat chemistry and technology* (pp. 227–300). St. Paul, MN, USA: American Association of Cereal Chemists, Inc.

Kuktait, R., Larsson, H., & Johansson, E. (2004). Variation in protein composition of wheat flour and its relationship to dough mixing behavior. *Journal of Cereal Science, 40*, 31–398.

Lavelli, V., Guerrieri, N., & Cerletti, P. (1996). Controlled reduction study of modification induced by gradual heating in gluten proteins. *Journal of Agriculture and Food Chemistry, 44*, 2549–2555.

Lambert, I. A., & Kokini, J. L. (2001). Effect of L-cysteine on rheological properties of wheat flour. *Cereal Chemistry, 78*, 226–230.

Lee, L., Ng, P. K. W., & Steffe, J. F. (2002). Biochemical studies of proteins in Nondeveloped, partially developed, and developed doughs. *Cereal Chemistry, 79*, 654–661.

Li, M., & Lee, T.-C. (1996). Effect of extrusion temperature on solubility and molecular weight distribution of wheat flour proteins. *Journal of Agriculture and Food Chemistry, 44*, 763–768.

Liyana-Pathirana, C. M., & Shahidi, F. (2006). Importance of insoluble-bound phenolic to antioxidant properties of wheat. *Journal of Agriculture and Food chemistry, 54*, 1256–1264.

Lowry, O. H., Rosebrough, N. J., Farr, A., & Randall, R. L. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–271.

MacMasters, M., Hinton, J. J. C., & Bradbury, D. (1978). Microscopic structure and composition of the wheat kernel. In Y. Pomeranz (Ed.), *Wheat chemistry and technology* (pp. 51–113). St.Paul, MN, USA: American Association of Cereal Chemists, Inc.

MacRitchie, F., Du cross, D. L., & Wrigley, C. W. (1990). Flour polypeptides related to wheat quality. In Y. Pomeranz (Ed.). *Cereal science and technology* (Vol. X, pp. 79–145). St. Paul, MN, USA: American Association of Cereal Chemists, Inc.

Mecham, D. K., Cole, E. G., & Pence, J. W. (1962). Extractable protein and hydration characteristics of flours and doughs in dilute acid. *Cereal Chemistry, 39*, 81–93.

Meredith, O. B., & Wren, J. J. (1966). Determination of molecular weight distribution in wheat flour proteins by extraction and gel filtration in a dissociating medium. *Cereal Chemistry, 43*, 169–186.

Phillips, L. G., Whitehead, D. M., & Kinsella, J. (1994). Protein stability. In L. G. Phillips, D. M. Whitehead, & J. Kinsella (Eds.), *Structure-function properties of food proteins* (pp. 25–62). New York: Dekker.

Pomeranz, Y. (1978). Composition and functionality of wheat-flour components. In Y. Pomeranz (Ed.), *Wheat chemistry and technology* (pp. 585–674). St. Paul, MN, USA: American Association of Cereal Chemists, Inc.

Prasada Rao, U. J. S., & Nigam, S. N. (1987). Gel filtration chromatography of glutenin in dissociating solvents: Effect of removing non-covalently bonded protein components on the viscoelastic character of glutenin. *Cereal Chemistry, 64*, 168–172.

Ram, B. P., & Nigam, S. N. (1981). Puffing and textural characteristics of chapati in relation to varietal differences in gluten composition. *Journal of Food Science, 47*, 231–233.

Sarwin, R., Laskawy, G., & Grosch, W. (1993). Changes in the levels of glutathione and cysteine during the mixing of dough with L-threo- and D-erythro-ascorbic acid. *Cereal Chemistry, 70*, 553–557.

Sidhu, J. S., Seibel, W., & Bruemmer, J. M. (1988). Measurement of chapati texture using Zwick universal testing machine. *Lebensmittel-Wissenschaft und-Technologie, 21*, 147–152.

Singh, N. K., Donovan, R., & MacRitihie, F. (1990). Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. II. Relative quantity of glutenin as a measure of bread making quality. *Cereal Chemistry, 67*, 161–170.

Southan, M., & MacRitchie, F. (1999). Molecular weight distribution of wheat proteins. *Cereal Chemistry, 76*, 827–836.

Srivastava, A. K., Prasada Rao, U. J. S., & Haridas Rao, P. (2003). Studies on protein and its high-molecular-weight subunit composition in relation to chapati-making quality of Indian wheat cultivars. *Journal of the Science of Food and Agriculture, 83*, 225–231.

Sudha, M. L., Srivastava, A. K., Vetrimani, R., & Leevathi, K. (2007). Fat replacement in soft dough biscuits: Its implications on dough rheology and biscuit quality. *Journal of Food Engineering, 80*, 18–23.

Tkachuk, R. (1970). L-Cysteinylglycine: Its occurrence and identification. *Journal of Biochemistry, 48*, 1039–1079.

Tsen, C. C., & Bushuk, W. (1968). Reactive and total sulfhydryl and disulfide contents of flours of different mixing properties. *Cereal Chemistry, 45*, 58–62.

Uthayakumaran, S., Gras, P. W., Stoddard, F. L., & Bekes, F. (1999). Effect of varying protein content and glutenin-to-gliadin ratio on the functional properties of wheat dough. *Cereal Chemistry, 76*, 389–394.

Veraverbeke, W. S., & Delcour, J. A. (2002). Wheat protein composition and properties of wheat glutenin in relation to bread making functionality. *Critical Reviews in Food Science and Nutrition, 42*, 179–208.