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Physicochemical and biochemical characteristics of Indian durum wheat varieties: Relationship to semolina milling and spaghetti making quality

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Abstract

Six Indian durum wheat varieties were examined for their physical, chemical, biochemical, and semolina milling properties. Semolina samples were evaluated for their physicochemical, rheological and spaghetti making properties. Results showed that these varieties had relatively high values for vitreousness, hardness and test weight. Semolina milling yield varied from 57.3% to 63.7%. Some strong relationships existed between semolina characteristics such as total protein, wet gluten, acetic acid insoluble protein, scanning electron micrographs, farinograph, pasting properties and spaghetti quality. Spaghetti prepared from durum wheat MACS 1967 with the highest protein content showed the lowest cooking loss, higher firmness value and lower surface stickiness. Similarly, spaghetti made from variety PDW 215; having relatively lower protein content also produced very good quality spaghetti. Variety PDW 274 that had the lowest amount of wet gluten and acetic acid insoluble protein content, showed poor spaghetti quality. Results of SDS–PAGE showed that a 45 kDa polypeptide was absent in poor durum varieties. A peak with retention time of 36–37 min was also absent in RP-HPLC profile of gliadin proteins from poor durum varieties.

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Keywords: Durum wheat; Semolina milling; Spaghetti; SDS–PAGE; RP-HPLC

1. Introduction

Pasta is a traditional cereal-based food product that is becoming increasingly popular worldwide because of its convenience, nutritional quality, and palatability [\(Cub](#page-10-0)[adda, 1994\)](#page-10-0). Durum wheat (Triticum durum) is the best raw material for processing into pasta products due to its unique colour, flavour and cooking quality [\(Feillet & Dex](#page-11-0)[ter, 1998](#page-11-0)). Pasta made from durum wheat varieties of superior quality results in a bright yellow colour and it retains, after cooking, firmness and is resistant to surface disintegration and stickiness. However, not all durum wheat semolina produces pasta of good cooking quality; many variables are involved in pasta manufacturing and their role is not completely understood [\(D'Egidio, Mariani,](#page-11-0) [Nardi, Novaro, & Cubadda, 1990](#page-11-0)).

Many researchers have established that content and composition of proteins, gluten strength in particular, are important for the cooking quality of pasta ([Grzybowski](#page-11-0) [& Donnelly, 1979; Novaro, D'Egidio, Mariani, & Nardi,](#page-11-0) [1993; Walsh & Gilles, 1971](#page-11-0)). The suitability of a durum wheat cultivar for pasta products is determined to a large extent by its seed protein composition ([MacRitchie, 1992\)](#page-11-0).

Studies have indicated that apart from gluten proteins, starch also plays an important role in determining the cooking quality of pasta. [Resmini and Pagani \(1983\)](#page-12-0) showed that pasta cooking quality was highly influenced by both starch gelatinization and protein network forma-

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tion. The role of starch in pasta cooking quality has been better understood only in recent years ([Delcour, Vanstee](#page-11-0)landt, Hythier, & Abécassis, 2000; Delcour et al., 2000; [Sung & Stone, 2003\)](#page-11-0).

Besides wheat components, the physical characteristics of durum wheat, such as test weight, kernel weight, kernel size, and degree of vitreousness have also been known to influence the milling performance of durum wheat and also pasta quality directly or indirectly [\(Dexter, Matsuo, &](#page-11-0) [Martin, 1987; Dexter, Williams, Edwards, & Martin,](#page-11-0) [1988; Troccoli, Borreli, De Vita, Fares, & Di Fonzo, 2000\)](#page-11-0).

India is a major durum wheat producer with around 2.5 million tonnes per year and has the potential to export this wheat to the world market. On the other hand, pasta products are becoming quite popular in this subcontinent. However, there is still limited data available on the suitability of Indian durum wheat for pasta production. In the present study, six Indian durum wheat varieties were analyzed for their physicochemical, biochemical, and semolina milling properties to examine their suitability for spaghetti production.

2. Materials and methods

2.1. Materials

Six Indian durum wheat varieties, namely, DWR 2006, MACS 1967, MACS 2694, PDW 215, PDW 274, and WH 896 (2002 harvest) were obtained from various wheat breeding stations and Agricultural Universities in India. Two biotypes of an Italian durum wheat variety, Lira, namely, Lira 42 and Lira 45 were used as reference varieties in biochemical studies.

Sodium dodecyl sulphate, Coomassie brilliant blue R-250, bromophenol blue, protein markers, Tris, acrylamide, N, N -methylene bis acrylamide, N, N, N', N' -tetramethyl-1,2diaminoethane (TEMED), and β -mercaptoethanol were obtained from Sigma Chemical Co. (St. Louis, MO, USA). HPLC grade acetonitrile was purchased from Merck Ltd., Mumbai, India. All other chemicals were of analytical grade and obtained from Qualigens Fine Chemicals, Mumbai, India.

2.2. Physico-chemical properties of durum wheat samples

Moisture, ash, protein, yellow pigment content, and hectoliter weight of different wheat varieties were determined according to the [AACC approved methods \(2000\)](#page-10-0). Vitreousness of durum wheat kernels was determined by visual examination of 100 kernels and the experiment was carried out three times. Average value was expressed in terms of percent vitreousness. Thousand-kernel weight was determined using a seed counter (Numigral, Falling Number AB, Stockholm, Sweden). Length and breadth of the kernels were determined by placing the kernels either horizontally or vertically, on a graph paper, in such a way that the edge of one kernel touched the edge of the next kernel. For

each measurement, 10 kernels were taken and average value of three experiments was reported in mm. Hardness of durum wheat kernels was measured using 'Instron' Universal Testing machine equipped with a special rod shape plunger with 50 mm diameter (Model 4301, High Wycombe, UK). Fifty sound kernels of each variety were taken randomly and the force required to crush individual kernel was noted and the average value calculated and expressed in terms of Newton (N). The cross-head speed of the plunger was maintained at 100 mm/min with a load cell of 500 kgf at 50% compression.

2.3. Durum wheat milling

Durum wheat samples were cleaned for any foreign material and conditioned to 17% moisture for 18 h prior to milling. Milling process was carried out using Buhler laboratory mill (Model MLU-202, Uzwil, Switzerland) according to the method of [Rahim, Haridas Rao, and](#page-11-0) [Shurpalekar \(1974\)](#page-11-0) with minor modifications. The gap between break rolls 1 (B1) and break rolls 3 (B3) were set at 0.4 and 0.2 mm, respectively, to obtain maximum semolina yield. The reduction rolls were kept disconnected from the break rolls during the milling operations. Bran contamination in semolina was removed using an air classifier (Petkus, Thurm, Germany) having air speed adjustability from 0 to $200 \text{ m}^3/\text{h}$.

2.4. Physico-chemical characteristics of semolina

Moisture, ash, protein, wet gluten, yellow pigment content and particle size distribution of semolina samples were determined according to the [AACC approved methods](#page-10-0) [\(2000\)](#page-10-0). Acetic acid insoluble protein of semolina was determined according to [Sgrulletta and De Stefanis \(1989\)](#page-12-0). Semolina colour was evaluated as per [Manthey and Hareland](#page-11-0) [\(2001\)](#page-11-0) with a colorimeter (Minolta CM, 3500d, Osaka, Japan) using the CIE colour scale and b^* value, which measures yellowness of semolina, was used in this study.

Farinograph characteristics of semolina were determined according to [Irvine, Bradley, and Martin \(1961\)](#page-11-0) using Brabender Farinograph-E (Brabender OHG, Duisburg, Germany) at constant water absorption of 35%. A modified farinograph software version 2.3.2 (Brabender OHG, Duisburg, Germany), specific for the measurement of farinograph characteristics of semolina, was used in this study.

Pasting properties of semolina samples were measured using a micro visco-amylograph (Brabender OHG, Duisburg, Germany). Semolina was powdered in a laboratory mill (Type 3100, Perten Instruments, Huddinge, Sweden) to facilitate better interpretation of results [\(Dexter, Mat](#page-11-0)[suo, & Kruger, 1990](#page-11-0)). Fifteen grams (on 14% moisture basis) of the powdered semolina were suspended in 100 ml of distilled water and heated in the visco-amylograph from 30 to 92 °C at a rate of $5 \degree C/\text{min}$, held at 92 °C for 5 min, cooled to 50 °C and then held at 50 °C for 1 min under constant stirring (250 rpm). Torque measuring range was 300 cmg. The viscosity was expressed in Brabender units (BU).

Scanning electron microscopy of semolina samples was carried out using LEO 435 VP scanning electron microscope (Leo Electron Microscopy Ltd., Cambridge, UK). Semolina samples were crumbled on to carbon coated double sticky tape on specimen holder and excess semolina was blown off. The samples were coated with gold and SEM was done at an accelerating voltage of 20 kV using a 35 mm Ricoh camera.

2.5. Spaghetti preparation

Semolina and distilled water (40 $^{\circ}$ C) were premixed in a Hobart mixer (Model N-50, Richmond Hill, Ont., Canada) at speed 1 (60 rpm) for 5 min to facilitate uniform distribution of water. The premixed dough (500 g) was transferred to a laboratory pasta machine (La Monferrina, model Dolly, Asti, Italy) and further mixed and kneaded for 10 min. The dough was then extruded through a 36 strand, 1.7 mm diameter die to obtain the spaghetti strands. Spaghetti was dried using a fermentation chamber with humidity control (National manufacturing Co., Lincoln, Nebraska, USA) held at 55 \degree C. Relative humidity of the chamber was reduced gradually from 95% to 65% during 10 h drying period.

2.6. Colour measurement of dry spaghetti

Colour of dry spaghetti was measured using a colorimeter (Minolta CM, 3500 d, Osaka, Japan) according to [Manthey and Hareland \(2001\)](#page-11-0). Colour readings were expressed as Hunter values and 'b' value, which measures yellowness of spaghetti, was used in this study.

2.7. Cooking quality of spaghetti

Dry spaghetti (10 g) was cooked in 200 ml of boiling distilled water for 10 min. Cooking loss was determined according to Bureau of Indian standards [\(IS 1485, 1993\)](#page-11-0). After cooking, the sample was drained on a Buchner funnel and rinsed with distilled water $(\sim 50 \text{ ml at room tempera-}$ ture) for 30 s and allowed to drain for 2 min. Total volume of the gruel and the rinsed water were measured. The gruel was shaken well for even distribution of the solid content. Twenty millilitres of the above gruel were pipetted out into a tarred petri dish and evaporated to dryness on a water bath. The petri dish was transferred to a hot air oven maintained at 105 ± 2 °C and dried to constant weight. Difference in weight of the gruel before and after drying was expressed as percent of solid loss. Cooked weight was determined by weighing the drained and rinsed spaghetti.

Firmness of cooked spaghetti was measured according to [Walsh and Gilles \(1971\)](#page-12-0) using a universal texture measuring system (LLOYDS Instruments, LR-5K, Hampshire, UK). Two cooked spaghetti strands were sheared at a 90° angle. The shear was performed at a cross-head speed of 10 mm/ min and load cell of 5 kg. The force (gf) required to shear the spaghetti was measured in triplicate, and the average value reported. A higher value indicates a firmer product.

Surface stickiness of the cooked spaghetti was determined according to [Dexter, Kilborn, Morgan, and Matsuo](#page-11-0) [\(1983\)](#page-11-0) with some modifications according to [Grant, Dick,](#page-11-0) [and Shelton \(1993\).](#page-11-0) A universal texture measuring system (LLOYDS Instruments, LR-5K, Hampshire, UK) with special plunger and sample holder was used for the measurement of stickiness.

2.8. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

Whole durum wheat flour proteins were extracted and fractionated by SDS–PAGE according to the method explained by [Du Cros \(1987\).](#page-11-0) SDS–PAGE with 10% acrylamide gel was used for separation of the high molecular weight (HMW) glutenin bands, and 12% acrylamide gel was used for separation of the low molecular weight (LMW) proteins. HMW glutenin subunits in 10% gel were numbered according to [Payne and Lawrence \(1983\)](#page-11-0) nomenclature using wheat varieties with known HMW glutenin subunits.

2.9. Reversed-phase high-performance liquid chromatography (RP-HPLC)

RP-HPLC of gliadins was done using acetonitrile $(CH₃CN)$ –water–trifluoroacetic acid (TFA) system as per the procedure explained by [Burnouf and Bietz \(1984\)](#page-10-0) with some modifications. Durum wheat kernels were ground with a mortar and pestle and the gliadins were extracted by continuous agitation with ethanol 70% (v/v) (250 mg flour/4 ml 70% ethanol) at room temperature for 30 min. The solution was centrifuged (10 min, $10,000g$) and 20 μ l of the supernatant were chromatographed in RP-HPLC Supelco C18 column $(250 \times 4.6 \text{ mm})$; Supelcosil LC-318; bead size 5μ ; pore size 300 A°) (Bellefonte, PA, USA). Chromatography was carried out using mixtures of two solvents, designated as solvents 'A' and 'B'. Solvent 'A' contained 15% (v/v) CH₃CN and 0.1% (v/v) TFA, and solvent 'B' contained 80% (v/v) CH₃CN and 0.1% (v/v) TFA. The column was equilibrated with a mixture containing 80% solvent 'A' and 20% solvent 'B'. The proteins were eluted using a linear gradient (20% 'B' to 55% 'B') for 55 min, which was initiated upon sample injection. Column temperature was maintained at 31 \degree C and the flow rate at 1 ml/min. Protein in the column effluent was monitored continuously by measuring the absorbance at 210 nm. Re-equilibration to initial solvent conditions was achieved in 20 min. Each sample was analyzed in duplicate.

2.10. Statistical analysis

The data were statistically analyzed using Duncan's New Multiple Range Test [\(Duncan, 1955\)](#page-11-0). Correlation

coefficients were determined using Microsoft Office Excel 2000 software.

3. Results and discussion

3.1. Physico-chemical properties of durum wheat

The moisture contents of PDW 215 and WH 896 were 10.8% and 10.2%, while their ash content was 1.93% and 1.92%, respectively. The protein content of PDW 215 was 12.7% while that of WH 896 was 10.7%. The yellow pigment content of PDW 215 was 5.0 ppm and that of WH 896 was 6.15 ppm. All values are expressed on a dry weight basis. The chemical properties of varieties DWR 2006, MACS 1967, MACS 2694 and PDW 274 have been reported earlier ([Aalami, Leelavathi, & Prasada Rao,](#page-10-0) [2007\)](#page-10-0).

Physical characteristics of six Indian durum wheat varieties are presented in Table 1. Test weight of variety PDW 274 was significantly higher than the other five varieties. This was followed by the varieties WH 896 (83.3 kg/hl) and PDW 215 (83 kg/hl). Kernels of MACS 1967 had the lowest test weight (79.75 kg/hl). These values are comparable with those of two Canadian amber durum wheat composites, which had test weights of 75.9–83.1 and 77.6– 83.3 kg/hl, respectively [\(Dexter et al., 1987](#page-11-0)). These test weights are also much higher than those for other Indian durum varieties reported earlier by [Rahim et al. \(1974\)](#page-11-0). [Dick and Matsuo \(1988\)](#page-11-0) reported that durum wheat having a test weight of 82 kg/hl is invariably sound and undamaged. Test weight also exhibits a strong linear relationship to kernel weight and therefore a good predictor of semolina milling ([Dexter, Tkachuk, & Tipples, 1991\)](#page-11-0). Based on the above results, it can be predicted that all the above durum varieties with their relatively high test weight have the potential for good semolina yield on milling. On the other hand, [Dexter et al. \(1987\)](#page-11-0) have also pointed out that the lone beneficial effect of low test weight

was an improvement in cooked spaghetti firmness and resilience, because of a strong negative relationship between test weight and wheat protein. Accordingly, it was observed that MACS 1967 which had the highest amount of protein content (15.9%) among the varieties, showed the least amount of test weight.

Another test that is complementary to the test weight is the kernel weight. The 1000-kernel weight is a measure of average kernel size. Since the ratio of endosperm to bran is greater in larger kernels, a higher milling yield can be expected from these kernels ([Matsuo, 1988\)](#page-11-0). In the present study, 1000-kernel weight varied from 40.31 (WH 896) to 48.42 g (PDW 274). These values compare well with 24 Indian and 4 Canadian durum wheat varieties reported by [Haridas Rao, Rahim, Prabhavati, and Shurpalekar](#page-11-0) [\(1976\)](#page-11-0). [Dexter and Matsuo \(1978\)](#page-11-0) reported values of 42.0 and 42.5 g for two Canadian durum wheat cultivars. In the present study, PDW 274 that had the highest test weight also had the highest 1000-kernel weight. On the contrary, WH 896 that had the second highest test weight showed the least 1000-kernel weight among the varieties tested. [Cubadda \(1988\)](#page-10-0) explained that there is no valid study which demonstrates that durum wheat varieties with small kernels and hence with low 1000-kernel weight, have a potentially lower capacity to produce high semolina yields.

Measurement of the length and breadth of kernels of wheat varieties showed DWR 2006 followed by MACS 1967 to have the longest kernels. Both varieties, as seen earlier, had lower test weight. On the other hand, varieties PDW 274 and WH 896 had relatively shorter kernels. These two varieties recorded significantly higher test weight. [Matsuo and Dexter \(1980\)](#page-11-0) found significant correlation between semolina yield and kernel size.

Kernel vitreousness is another aspect for evaluation of durum wheat quality and do not show any trace of starchy endosperm [\(Dexter et al., 1988\)](#page-11-0). In the present study, MACS 1967 had 100% vitreous kernels followed by DWR

A Data are expressed as means \pm SD from three determinations. Means within a row followed by different letters are significantly different (p < 0.05).
^B Drv basis.

 C Acetic acid insoluble.

2006 with 99.3% vitreous kernels. Varieties MACS 2694 (86.6%) and WH 896 (87.5%) showed a significantly lesser degree of vitreousness. It has been found that semolina yield was reduced slightly with a decrease in vitreous kernels ([Dexter et al., 1988](#page-11-0)). Starchy durum wheat is softer than vitreous durum wheat, and gives a lower yield of semolina and a higher yield of flour, thereby reducing milling potential ([Matsuo & Dexter, 1980](#page-11-0)). Vitreous kernels are also considered to have a positive effect on the colour and cooking quality of pasta. However, that does not necessarily mean that semolina derived from vitreous grains always produces pasta of good cooking quality ([Cubadda, 1988\)](#page-10-0).

Objective measurement of the kernel hardness showed that the highest kernel hardness (173.5 N) belonged to the variety MACS 1967 followed by DWR 2006 (146.0 N). As discussed earlier, these two varieties also had significantly higher vitreous kernels than other varieties. Hard vitreous kernels are desirable for production of semolina, whereas kernels appearing white, starchy or opaque are considered undesirable for semolina milling ([Dick](#page-11-0) [& Matsuo, 1988\)](#page-11-0).

3.2. Semolina milling

Results of semolina milling are shown in Table 2. Wheat variety WH 896 which had the lowest kernel weight, vitreousness, size and hardness, had lower semolina yield (57.3%) . Percentage of flour $(-150 \text{ }\mu\text{m})$ produced from this variety during milling was significantly higher than that of other varieties. On the other hand, variety DWR 2006, which had the highest kernel length and vitreousness and relatively high kernel hardness, had a higher semolina yield (63.6%) and a lower flour content. PDW 215 and PDW 274 with significantly higher test weight and kernel weight and similar vitreousness and hardness, showed significantly second highest semolina yield among the varieties. Statistical analysis showed that kernel vitreousness ($r = 0.61^{**}$) and 1000-kernel weight $(r = 0.81^{**})$ were significantly correlated (1% confidence level) to semolina yield, whereas relationship between test weight and semolina yield was not significant. There is no general consensus among researchers on the value of test weight as an indicator of wheat milling potential ([Dexter et al., 1987\)](#page-11-0). [Troccoli and Di Fonzo](#page-12-0)

Table 2 Milling yield and particle size distribution of semolina^A [\(1999\)](#page-12-0) did not find any correlation between test weight and kernel weight and consequently on semolina milling yield. However, [Dexter and Matsuo \(1978\)](#page-11-0) demonstrated that semolina extraction rate of laboratory milled durum wheats does not alter spaghetti cooking quality.

3.3. Physico-chemical characteristics of semolina

The chemical characteristics of semolina milled from different durum varieties are shown in [Table 1.](#page-3-0) The moisture content varied from 13.22% to 14.41% and the ash content from 0.79% to 0.86%. Protein content of semolina was significantly more in MACS 1967 (13.83%) followed by DWR 2006 (12.7%). Protein content of semolina is important because it influences the functional quality of pasta. Adequate amounts of gluten protein are necessary to impart desirable attributes of mechanical strength and cooking quality to pasta ([Kulkarni, Ponte, & Kulp, 1987](#page-11-0)). [Irvine](#page-11-0) [\(1971\)](#page-11-0) explained that semolina samples with protein levels of 11.5–13.0% can be processed with little difficulty and expected to give satisfactory results. Too low a protein is likely to produce pasta with relatively poor mechanical strength in the dried product and less than optimum quality with respect to cooking stability and cooked firmness ([Grzybowski & Donnelly, 1979\)](#page-11-0). Among the six varieties studied WH 896 had significantly the lowest protein content of 9.30% followed by PDW 215 that had a protein content of 10.84%. However, percentage of wet gluten in semolina, which is one of the indicators of protein quality, was not in the same order of semolina protein content. Variety MACS 1967 that had the highest protein content also had the highest amount of wet gluten (34.4%). Though PDW 274 had considerably high amount of protein, its wet gluten content was the lowest (25.2%) among the samples. Low wet gluten content in WH 896 (25.6%) can be attributed to low amount of protein in semolina. Though protein content of other three varieties DWR 2006, MACS 2694 and PDW 215 was significantly different, there was no significant difference among their wet gluten content. Earlier, [De Stefanis and Sgrulletta \(1990\)](#page-11-0) found wet gluten of 22– 34% in semolina from five Italian durum varieties whose average value (26.6%) was less than that of six Indian durum varieties reported here (28.7%).

A Data are expressed as means \pm SD from three determinations. Means of the same column followed by different letters are significantly different $(p < 0.05)$.

^B Purified semolina.

Results of the present study showed the highest amount of acetic acid insoluble protein (6.93%) in the variety DWR 2006 and the lowest (4.30%) in PDW 274. Even though the wheat variety MACS 2694 had the same amount of total protein as PDW 274, its acetic acid insoluble protein content was significantly higher. On the other hand, WH 896 which had the lowest protein content had significantly higher level of acetic acid insoluble protein than few other varieties which had a higher protein content. [Sgrulletta and](#page-12-0) [De Stefanis \(1989\)](#page-12-0) found that acetic acid insoluble protein was more efficient than total protein content for predicting pasta cooking quality.

Content of yellow pigment in semolina is another important parameter determining the pasta making potential of semolina samples. In the present study, semolina from DWR 2006 had the highest yellow pigment content (6.35 ppm), while the variety MACS 2694 had the lowest (3.12 ppm). Semolina from WH 896 contained the second highest pigment content (5.46 ppm). The present study showed that there was a pigment loss ranging from 12% (DWR 2006) to 20% (PDW 215) during the milling process. Earlier, [Dexter and Matsuo \(1978\)](#page-11-0) reported pigment loss of 17.7% and 20.3% for two Canadian amber durum wheat varieties.

Yellowness $(b^*$ value) of semolina from DWR 2006 was significantly higher than that of other semolina samples (Fig. 1), which can be attributed to its higher yellow pigment content. However, [Dexter and Matsuo \(1978\)](#page-11-0) indicated that yellow colour of semolina is due to differences in light reflectance from fine and coarse semolina and not necessarily to yellow pigment content; i.e. coarser the semolina higher the yellowness. The particle size distribution of semolina samples in this study ([Table 2](#page-4-0)) did not show much variation among the samples, except for WH 896, which had significantly more of finer particle size. Probably due to this reason, yellowness of semolina from WH 896 was comparable to that of PDW 274, in spite of its higher pigment content.

Fig. 1. Yellowness (b^*) of semolina samples. Data are expressed as means \pm SD from three determinations. Bars carrying different letters are significantly different ($p < 0.05$) from each other.

Scanning electron micrographs (SEM) of semolina samples from different durum varieties are shown in [Fig. 2](#page-6-0). They appear to be composed of both large lenticular and small round starch granules embedded in a protein matrix. SEM of semolina from all varieties except WH 896 appeared to be tightly and compactly packed with no air spaces. Similar results were also observed by [Dextor, Dron](#page-11-0)[zek, and Matsuo \(1978\).](#page-11-0) On the other hand, SEM of the variety WH 896 did not show a tight and compact structure instead exhibited more number of exposed and damaged starch granules. The reason for the above difference has been explained as due to the hardness of the wheat kernels [\(Hoseney, 1992\)](#page-11-0). When hard wheat is broken, it breaks at the cell wall rather than through the cell and under the electron microscopy the strong adherence of protein to starch granules results in the appearance of tight and compact structure. On the other hand, when soft wheat is broken it fractures through the cell content rather at the cell wall hence exposing more of the embedded starch granules. As was discussed earlier, the kernels of WH 896 variety had the least hardness value than the other varieties studied. This variety also had the least protein content. SEM of semolina from PDW 274 was also slightly different from those of other samples in which, the majority of large lenticular shaped starch granules not covered by the protein matrix with a size of around $30 \mu m$ were visible, whereas in other varieties, the maximum granule size was around $25 \mu m$.

Results of farinograph experiment of semolina samples which was carried out at 35% water absorption are shown in [Table 3](#page-6-0). The results showed that varieties MACS 1967 (3.3 min) and DWR 2006 (3.7 min) had the lowest dough development time (DDT). The maximum consistency (MC) of these two varieties was 372 and 366 Farinograph units (FU), respectively. These two varieties had significantly higher protein content than the other varieties. On the other hand, semolina from variety WH 896 with the lowest amount of protein content showed the highest value for DDT (10.6 min) and the lowest MC (270 FU). This is supported by the work of [Irvine et al. \(1961\)](#page-11-0), which showed that, as protein content in semolina increased, farinograph DDT decreased and MC increased. Statistical analysis of the present data showed a highly significant inverse relationship $(r = -0.81^{**})$ between semolina protein content and DDT. Protein content of semolina and MC were also significantly correlated $(r = 0.88^{**})$. Similarly, there was significant negative correlation ($r = -0.68^{**}$) between semolina wet gluten and DDT; and positive correlation $(r = 0.69)$ between semolina wet gluten and MC. Though a negative correlation between acetic acid insoluble protein and DDT; and a positive correlation between acetic acid insoluble protein and MC were noticeable, they were not significant at 1% or 5% confidence level. All the semolina samples in the present study showed low values for tolerance index. [Irvine et al. \(1961\)](#page-11-0) showed that utilization of semolina with increased particle size or with heterogenous particle size resulted in a decrease in maximum consistency

Fig. 2. Scanning electron micrographs of semolina from six Indian durum wheat varieties.

Data are expressed as means \pm SD from three determinations. Means of the same column followed by different letters are significantly different (p < 0.05).

and tolerance index. Our results agree with their findings as around 50% of semolina samples were retained on $425 \mu m$ mesh [\(Table 2\)](#page-4-0).

Pasting properties of semolina samples are shown in Table 4. Onset gelatinization temperature of MACS 1967 followed by MACS 2694 was slightly higher than the other four varieties. Durum variety WH 896 showed the highest peak viscosity (912 BU) compared to other varieties. It has been reported that higher content of starch in flours, to some extent, may contribute to higher pasting viscosity ([Ragaee & Abdel-Aal, 2006\)](#page-11-0). Therefore, lower protein content in this variety might have resulted in higher starch concentration and hence higher peak viscosity. It can also be inferred that the starch granules in this variety had the ability to swell more. With more swelling of the granules, the tendency is greater to leach their contents into the surrounding liquid during cooking of pasta, leading to an increase in cooking loss [\(Sissons & Batey, 2003](#page-12-0)). Differences in protein composition are also known to affect pasting viscosities and properties ([Batey & Curtin, 2000](#page-10-0)). On the other hand, varieties DWR 2006 and MACS 1967 also had higher peak viscosities. However, these two varieties

Data are expressed as means \pm SD from three determinations. Means of the same column followed by different letters are significantly different (p < 0.05).

had significantly higher protein contents compared to WH 896. Breakdown values for viscosity showed varieties WH 896, MACS 2694 and MACS 1967 having significantly higher values than the other three varieties. This indicates that starch from these varieties had less ability to withstand heating at high temperature and the shear stress, which is an important factor in many processes [\(Ragaee](#page-11-0) [& Abdel-Aal, 2006](#page-11-0)). On the other hand, the other three varieties (PDW 215, PDW 274, and DWR 2006) had significantly lower values for breakdown with variety PDW 215 having the lowest value. The stability of hot starch pastes is described by breakdown viscosity. High values for breakdown are usually correlated with the degree of swelling of the starch granule on heating. [Sissons and](#page-12-0) [Batey \(2003\)](#page-12-0) found significant but small negative correlations between cooking loss of pasta and breakdown viscosity of semolina.

3.4. Colour of dry spaghetti

The yellow colour of pasta products, rather than cooking behaviour and taste, is reported to be one of the most important considerations in assessing durum wheat quality [\(Borrelli, Troccoli, Di Fonzo, & Fares, 1999](#page-10-0)). A yellow pasta is considered mark of quality by many consumers [\(Dexter, Matsuo, Preston, & Kilborn, 1981\)](#page-11-0). Yellowness (b value) of spaghetti samples is shown in Fig. 3. Though yellowness of semolina from WH 896 was significantly lower than DWR 2006, yellow colour of spaghetti prepared from these two varieties was not significantly different from each other. According to [Turnbull \(2001\)](#page-12-0), it is possible to have two semolina of different particle size which look different in colour, but when converted to pasta is very similar in colour. In the present study, two commercial spaghetti samples, one Indian and another Italian, were used for comparison. Yellowness of spaghetti samples from DWR 2006 and WH 896 was higher than other four varieties and also the two commercial samples.

Fig. 3. Yellowness (b) of spaghetti samples (*commercial samples). Data are expressed as means \pm SD from three determinations. Bars carrying different letters are significantly different ($p \le 0.05$) from each other.

3.5. Spaghetti cooking quality

Results of evaluation of spaghetti cooking quality are shown in [Table 5.](#page-8-0) Measurement of cooking loss of spaghetti is one of the important parameters in assessing its overall quality. Cooking loss has been associated with both starch pasting properties and protein quality ([Batey & Cur](#page-10-0)[tin, 2000](#page-10-0)). In the present study, the lowest cooking loss was seen in spaghetti from MACS 1967 (5.21%) and PDW 215 (5.82%). The amylograph characteristics of these two samples had shown that while the former had higher peak and breakdown viscosities, the latter had significantly lower peak and breakdown viscosities. The protein content of these varieties was also significantly different, while MACS 1967 had a higher protein content (13.83%); PDW 215 had a lower protein content (10.84%). A strong negative correlation ($r = -0.88$) was found between cooking loss of spaghetti samples and wet gluten of semolina. It can probably be speculated that while starch properties influenced the cooking loss in PDW 215, it was either the protein content or protein quality that would have influenced the cooking loss of spaghetti made from MACS 1967. Spaghetti made from variety PDW 274 had the highest cooking loss (7.04%) amongst the samples tested. Amylograph properties had shown that it had significantly lower peak and breakdown viscosities and had relatively higher protein content (11.5%), from which one could speculate lower cooking loss. Then some other factors such as protein quality or starch quality can be considered. Nevertheless, higher cooking loss of spaghetti from PDW 274 can be partly attributed to structural properties of its semolina as observed by scanning electron microscopy. Lenticular starch granules in this variety were larger than those of other varieties and were not completely covered by the protein matrix. [Hill and Dronzek \(1973\)](#page-11-0) studying the gelatinization of wheat starch indicated that swelling and leaching of material (amylose) is first observed in the lenticular large granules. On the other hand, presence of a strong protein matrix might prevent semolina starch from binding water and subsequent swelling. Hence, due to lack of such a protein matrix, starch has more potential to swell and breakdown the continuous gluten network during cooking resulting in higher cooking loss [\(Sung & Stone, 2003\)](#page-12-0). However, in all probability a cooking loss of 7.0% cannot be considered as too high.

Spaghetti from WH 896 showed the highest amount of cooked weight (30.4 g). Higher cooked weight in this spaghetti might be due to the higher swelling ability of starch in this variety as discussed earlier in pasting properties of semolina. [Dexter, Matsuo, and Morgan \(1983\)](#page-11-0) found a strong relationship between degree of swelling and cooked weight of spaghetti. Cooked weight of spaghetti from other varieties did not vary much. [Grzybowski and Donnelly](#page-11-0) [\(1979\)](#page-11-0) did not find any significant relationship between protein content and gluten strength and cooked weight.

Measurement of firmness of cooked spaghetti showed that spaghetti from MACS 1967 and PDW 215 had the

Table 5 Cooking quality characteristics of spaghetti samples^A

Variety	Cooking loss $(\%)$	Cooked weight $(g)^B$	Firmness (gf)	Stickiness $(N/m2)$
DWR 2006	$6.20 \pm 0.04^{\circ}$	$27.8 \pm 0.28^{\circ}$	$65 + 1.2^b$	$405.5 + 10.4^{\circ}$
MACS 1967	5.21 ± 0.03^e	27.6 ± 0.28 ^c	$84 + 2.9^{\rm a}$	$383.7 + 7.4^{\circ}$
MACS 2694	$6.32 + 0.03^b$	27.8 ± 0.17^c	$66 + 2.8^{\rm b}$	$476.0 \pm 7.8^{\rm a}$
PDW 215	$5.82 + 0.07^{\rm d}$	$28.4 + 0.35^{\rm bc}$	$82 + 2.6^{\rm a}$	$416.7 + 7.7^b$
PDW 274	$7.04 + 0.05^{\rm a}$	$28.8 \pm 0.49^{\rm b}$	$52.5 + 1.2^{\circ}$	$485.5 + 9.8^{\rm a}$
WH 896	$6.30 + 0.03$ ^{bc}	$30.4 + 0.42^{\rm a}$	$64.5 + 0.87^b$	$362.5 + 4.9^d$

^A Data are expressed as means \pm SD from three determinations. Means of the same column followed by different letters are significantly different $(p < 0.05)$.

 B 10 g dry spaghetti was used for cooking.

highest firmness values. On the other hand, spaghetti from variety PDW 274 had the least firmness. As discussed earlier, the highest amount of protein and wet gluten and the second highest amount of acetic acid insoluble protein was present in semolina from MACS 1967, while PDW 215 also had significantly higher level of wet gluten. On the other hand, PDW 274 had the lowest amount of wet gluten and acetic acid insoluble protein. Firmness in cooked pasta is related to gluten strength of durum wheat [\(Walsh, 1971\)](#page-12-0). [Sung and Stone \(2003\)](#page-12-0) indicated that coagulated gluten network plays an important role in imparting firmness to cooked pasta. Statistical analysis of the present data showed a significant correlation $(r = 0.79^{**})$ between wet gluten content of semolina and firmness of spaghetti samples. [Dexter and Matsuo \(1979\)](#page-11-0) pointed out that starch is the major component of semolina, and firmness in cooked spaghetti must, in par, be influenced by gelatinized starch properties. In the present study, statistical analysis indicated that firmness of cooked spaghetti was negatively correlated to cooking loss ($r = -0.94$). A small but significant negative correlation ($r = -0.49^{\circ}$) (5% confidence level) was also found between firmness and stickiness of cooked spaghetti. In other words, softer the cooked spaghetti higher the solid loss and vise versa. Earlier, [Sung and Stone](#page-12-0) [\(2003\)](#page-12-0) studying on starch pasta also found negative correlation between firmness of starch pasta and cooking loss and stickiness.

Evaluating the stickiness properties of the cooked spaghetti, results showed that spaghetti made from MACS 1967 had significantly lower values for stickiness (383.7 N/ m²). On the contrary, that made from PDW 215 had significantly higher stickiness value. This was in spite of similarities in the cooking loss and firmness of spaghetti made from these two varieties. On the other hand, spaghetti made from low protein variety WH 896 with higher cooking loss and lower firmness value had significantly low surface stickiness (362.5 $N/m²$). Highest stickiness values were recorded for varieties PDW 274 and MACS 2694. [D'Egi](#page-10-0)[dio et al. \(1982\)](#page-10-0) reported that surface stickiness of spaghetti need not necessarily be related to total cooking loss. It has been shown that spaghetti stickiness is not strongly influenced by protein content [\(Dexter et al.,](#page-11-0) [1983](#page-11-0)). In the present study, a significant negative correlation $(r = -0.91^{**})$ existed between amylograph peak viscosity of semolina and stickiness of spaghetti. A significant correlation ($r = 0.62^{**}$) was observed between amylograph setback viscosity of semolina and stickiness of spaghetti.

In this investigation, though the variety MACS 1967 with the highest maximum consistency and lowest dough development time in farinograph exhibited excellent cooking quality characteristics, no significant correlation was found between farinograph data and cooking quality properties. [Dexter and Matsuo \(1979\)](#page-11-0) have explained that true dough development might not be attained until about 45% absorption is reached in farinograph. Accordingly, [Mat](#page-11-0)[suo, Dexter, Kosmolak, and Leisle \(1982\)](#page-11-0) pointed out that mixing properties at higher water absorption where gluten is fully developed might better predict the textural characteristics of cooked spaghetti.

Based on the evaluation of cooking quality of six spaghetti samples, it can be concluded that two Indian durum varieties MACS 1967 and PDW 215 were excellent, three varieties DWR 2006, MACS 2694 and WH 896 were good, and variety PDW 274 was poor for spaghetti production. Though variety WH 896 with significant low amount of protein showed relatively good cooking properties after 10 min cooking, it could not maintain its overall quality for longer time. Probably factors such as protein quality or starch characteristics would have played a significant role in controlling the spaghetti quality of this durum variety.

3.6. Biochemical characteristics

3.6.1. SDS–PAGE

A 12% gel was used for fractionation of total flour proteins to provide better separation of subunits in the low molecular weight region ([Fig. 4\)](#page-9-0). Lira 42 and Lira 45, Italian durum wheats known for their poor and good spaghetti making quality, respectively [\(Masci, Lew, Lafiandra, Por](#page-11-0)[ceddu, & Kasarda, 1995\)](#page-11-0), were used as reference in this study. It is clearly observed in the SDS–PAGE analysis of eight varieties that a polypeptide with molecular weight of 45 kDa was absent in poor varieties, PDW 274 and Lira 42, whereas polypeptides with molecular weight of 52 and 58 kDa appeared in higher intensity in these two varieties as compared to good varieties.

Fig. 4. SDS–PAGE fractionation of total endosperm proteins of: (1) DWR 2006, (2) MACS 1967, (3) MACS 2694, (4) PDW 215, (5) PDW 274, (6) WH 896, (7) Lira 42, (8) Lira 45. M: molecular weight marker. Arrows show the 45 kDa polypeptide which is absent in poor durum varieties.

HMW glutenin subunits of six Indian and two Italian durum wheats were identified on 10% SDS–PAGE using wheat varieties with known combinations of HMW glutenin subunits. These subunits are encoded by genes at the Glu-B1 locus of chromosome 1B which numbered according to [Payne and Lawrence \(1983\).](#page-11-0) Four main subunit combinations including $6 + 8$ (DWR 2006, WH 896), 13 + 16 (MACS 1967, MACS 2694), 20 (PDW 215), and $7 + 8$ (PDW 274) were found in six Indian durum wheats, whereas Italian variety Lira had subunit 20. There are contradictory reports regarding the relationship between glutenin subunit composition and cooking quality of pasta [\(Feillet, 1988\)](#page-11-0). No clear relationship was found by [Du](#page-11-0) [Cros, Wrigley, and Hare \(1982\)](#page-11-0) or by [Autran \(1981\)](#page-10-0), with regard to HMW glutenin subunits and pasta quality. However, [Galterio, Grita, and Brunori \(1993\)](#page-11-0) and [Fares,](#page-11-0) [Novembre, Di Fonzo, Galterio, and Pogna \(1997\)](#page-11-0) showed the positive effect of LMW and HMW glutenin subunits on pasta quality. [Autran and Feillet \(1987\)](#page-10-0) demonstrated that HMW subunits $6 + 8$ were positively associated with quality, whereas subunits $13 + 16$ were negatively associated. However, in the present study it was observed that PDW 274 with HMW subunits $7 + 8$ showed a poor cooking quality spaghetti, whereas MACS 1967 with combination of $13 + 16$ showed the best cooking quality characteristics among the varieties studied. Therefore, according to the literature reports and the results of the present investigation, it seems that HMW glutenin subunits do not have a direct

Fig. 5. Typical RP-HPLC profiles of gliadin proteins from two Indian durum varieties, MACS 1967 (good variety) and PDW 274 (poor variety); and two Italian durum varieties, Lira 45 (good variety) and Lira 42 (poor variety). Arrows indicate the peak which is absent in poor varieties.

effect or do not play a major role in determining the spaghetti quality.

3.6.2. RP-HPLC

Gliadins extracted from eight different durum wheat varieties were subjected to RP-HPLC column. These proteins were clearly separated into 9–10 peaks depending on the variety ([Fig. 5](#page-9-0)). Both qualitative and quantitative differences existed among the chromatograms and each possessed a unique pattern. However, a peak obtained around 36–37th minute (designated as GliPK36-37) was found to be present in varieties with good pasta making quality, while it was absent in the variety which yielded poor quality pasta (PDW 274). A similar trend was observed between the two Italian durum wheats. Lira 45 which is reported to be good for pasta making, showed the presence of GliPK36-37, while this peak was absent in Lira 42 which is reported to be poor for pasta making. The area percentage of the mentioned peak in Indian varieties was varied from 14.6% (PDW 215) to 23.2% (MACS 1967), while in Lira 45, its percentage was 26.2%. Thus, the differences in RP-HPLC patterns of durum wheat varieties can be used as a marker to differentiate good and poor durum varieties for pasta making.

4. Conclusions

Assessment of the physical characteristics of durum varieties showed that those having the highest test weight did not necessarily give the highest semolina yield. MACS 1967 which had significantly highest protein content produced good quality spaghetti, in terms of firmness and less cooking loss and stickiness. On the other hand, variety PDW 215 which had significantly low protein content also produced good spaghetti. Otherwise, the above two varieties were not similar in any of the physical or chemical properties. It is probable that along with protein, starch would also have influenced the quality of spaghetti. Variety PDW 274 which had the lowest amount of wet gluten and acetic acid insoluble protein content, showed poor spaghetti quality. Biochemical studies also showed that this variety lacked a 45 kDa polypeptide and a specific peak (GliPK36-37) in RP-HPLC. Scanning electron micrograph of this variety was also different from the other varieties. Variety WH 896, in spite of having good results for spaghetti firmness and stickiness could not maintain its wholesome appearance for longer time. This could be because of the higher starch content as a result of lower protein content. This variety, due to its lower kernel hardness, had resulted in lower semolina yield with higher percentage of flour fraction. On the other hand, WH 896 had significantly higher yellow pigment resulting in spaghetti with higher yellowness. It can be contemplated that if the protein content of this variety was increased, either at breeding stage or during the stage of processing, it would probably become more suitable for spaghetti preparation. The results showed that Indian durum varieties compared well, in one property or the other, in their physico-chemical, biochemical, semolina milling and spaghetti making properties, with some of the well known Canadian and Italian durum varieties that have been reported in literature.

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