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Rice grain and starch properties: Effects of nitrogen fertilizer application

Narpinder Singh[a,*], Nisha Pal[a], Gulshan Mahajan[b], Sandeep Singh[a], Khetan Shevkani[a]

[a] Department of Food Science and Technology, Guru Nanak Dev University, Amritsar 143005, Punjab, India
[b] Department of Plant Breeding & Genetics, Punjab Agricultural University, Ludhiana 141004, Punjab, India

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ABSTRACT

The effects of nitrogen application at different levels (0, 20, 40 and 60 kg/ha) on the characteristics of milled rice and starch from three paddy cultivars were studied. Milled rice was evaluated for physicochemical, cooking and textural properties while starch was evaluated for granule size distribution, structure, thermal and rheological properties. Milled rice from paddy grown with nitrogen application showed lower gruel solids loss and water up take ratio during cooking and higher cooked grain hardness, cohesiveness, and chewiness. Starch from rice grown with application of nitrogen showed lower amylose content and higher pasting temperature, gelatinization transition-temperatures and enthalpy of gelatinization. Principal component analysis indicated that cooked grain hardness and cooking time were closely associated with amylose content and protein content, respectively.

1. Introduction

Different cultivars of paddy are grown in different parts of India, rice milled from these cultivars vary significantly in composition, milling and cooking quality as well as in starch characteristics (Singh, Kaur, Sodhi, & Sekhon, 2005; Singh, Kaur, Sandhu, Kaur, & Nishinari, 2006). Genetic, environmental and agronomical factors have been reported to be mainly responsible for variation in composition and cooking quality of rice. The cooking and eating quality of rice has been related to starch, partially because starch is the main component of milled rice and accounts for up to 95% of the dry matter (Fitzgerald, McCouch, & Hall, 2009). The starch consisted of amylose and amylopectin and the proportion of both varies in different cultivars (Singh et al., 2006). Textural properties of cooked rice and solids loss in the gruel during cooking have been related to amylose content (Singh et al., 2005). The relation between starch structures and cooking properties of rice has also been reported, however little attention has been given to proteins that could explain some properties of cooked rice that starch cannot explain (Martin & Fitzgerald, 2002). It has been reported that the proteins influence pasting properties both through binding water and through the agency of a network linked by disulfide bonds (Martin & Fitzgerald, 2002). Prolamin and glutelin are the two major storage proteins of rice grain. The prolamin increased the hardness whereas glutelin deteriorated the appearance of cooked rice, further suggesting the important role of proteins in determining cooking properties of rice (Furukawa et al., 2003). Using a model rice system consisting of rice starch and proteins (prolamin and glutelin) in different ratios, Baxter, Zhao, and Blanchard (2006) found that two proteins had opposite effects on pasting and textural properties of rice flour. Increased prolamin content caused a decrease in pasting temperature, peak and final viscosities, gel hardness and adhesiveness, but an increase in the breakdown viscosity, while glutelin showed an opposite effect. These contrasting effects mean that the pasting and textural characteristics of rice flour are probably a result of the relative proportions of prolamin to glutelin. Ning et al. (2010) reported that albumin and globulin were controlled more by genotypes than nitrogen treatment whereas prolamin and glutelin were largely determined by nitrogen.

None of the earlier study reports the effect of different doses of nitrogen application during paddy growing on cooking and textural characteristics of milled rice and starch. The objective of the present study was to see the effects of varied doses of nitrogen application on (i) physico-chemical, cooking and textural properties of milled rice and (ii) structure, thermal and rheological properties of starch.

2. Materials and methods

2.1. Materials

An experiment was conducted at the experimental farm, Punjab Agricultural University (30°56′N, 75°52′E and 247 m mean sea level), India, during 2008–2009 to assess genotypic differences in quality and yield of aromatic rice at varied levels of nitrogen. The

* Corresponding author. Tel.: +91 183 2258826; fax: +91 183 2258820.
E-mail address: narpinders@yahoo.com (N. Singh).

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climate of the area is semiarid with an average annual rainfall of 400–700 mm (75–80% received during July–September), the lowest temperature ranging from 0 to 4 °C in January, and the highest maximum temperature of 41–45 °C in June. The soil was loamy sand with pH 7.2, organic nitrogen 224 mg kg^{-1}, organic matter 0.28%, and Olsen P 8.1 mg kg^{-1}. The experiment was laid out in a split plot design replicated thrice. The treatments included four nitrogen rates (0, 20, 40 and 60 kg N/ha) in the main plots and three genotypes (Punjab Mehak 1, Pusa Basmati 1121 and Punjab Basmati 2) as subplots. Nitrogen was applied as urea in two equal splits (21 days after transplanting (DAT) and 42 DAT) as per treatments. The plots for nitrogen treatment were separated by bunds and channels. Seedlings of 30 days old nursery of each cultivar were transplanted with spacing of 20 cm between row and 15 cm between hills with a plot size of 4 m × 2 m. Field was puddled twice by running cultivator in the standing water (75 mm) followed by planking. For controlling weeds, butachlor @ 1.5 kg/ha was applied 2 DAT. Recommended insecticides and fungicides were used periodically to control insect-pests and diseases. Crop was harvested at maturity and grain yield was recorded at 14% grain moisture.

2.2. Dehusking and milling

The paddy was dehusked on a McGill sample sheller (Rapsco, Brookshire, TX, USA). The brown rice samples obtained were polished in a McGill mill No. 2 (Rapsco, Brookshire, TX, USA) to obtain a 6% degree of milling. Milled whole rice kernels were separated from broken rice for the evaluation of physicochemical, cooking, and textural properties.

2.3. Milled rice characteristics

2.3.1. Physicochemical properties

Thousand kernel weight (TKW) was determined by weighing thousand milled head rice. Length–breadth (*L*/*B*) ratio was determined as the ratio of length to breadth using digital vernier calipers for an average of ten grains. Bulk density was measured as the ratio of weight of milled rice kernels to their volume and reported as g/ml. Color parameters (L^*, a^*, b^*) of all the milled rice were determined in triplicates, using a ultra scan VIS Hunter Lab (Hunter Associates Laboratory Inc, Reston, VA, USA). The L^* value indicates the lightness, a^* value gives the degree of red-green color, with a higher positive a^* value indicating redness. The b^* value indicating the degree of the yellow-blue color, with a higher b^* indicating yellowness.

2.3.2. Cooking properties

Head rice were cooked in a test tube containing distilled water (1:10) in a boiling water bath. Few grains were collected after 5 min and thereafter 1 min interval during the cooking operation to determine the extent of cooking by pressing between two glass slides. Cooking time was recorded as the time taken until the disappearance of white core in more than 95% of the collected grains. The sample was drained and rinsed with distilled water on a Buchner funnel, allowed to drain for 2 min and the total volume of the gruel was measured. The gruel was transferred to a hot air oven maintained at 105 ± 2 °C in a tarred Petri dish and dried to constant weight. Weight of dried material was recorded as gruel solids loss (%). The grains were placed between filter papers to remove excess water and weighed. Water uptake was calculated as the ratio of weight of cooked grains to the weight of uncooked grains. Cooked length–breadth ratio was determined as the ratio of cumulative length of 10 cooked kernels and the cumulative breadth of 10 cooked kernels.

2.3.3. Textural profile analysis

Texture profile analysis (TPA) of cooked rice was performed using TA.XT plus Texture Analyser (Stable Micro Systems, England). A single cooked grain was placed at the center of heavy-duty platform (HDP/90). The cooked grain was subjected to compression with a 40 mm diameter aluminum cylinder probe (P/40) at a pre-test, test and post-test speed of 1 mm/s, up to 90% compression, in two cycles using a 1 kg load cell. Five replications were carried out and the textural parameters of hardness, springiness, cohesiveness, chewiness and adhesiveness were calculated as described by Bourne (1978).

2.4. Starch characteristics

2.4.1. Starch isolation

Starch was isolated from milled rice of various cultivars by alkali extraction of the proteins as described earlier (Sodhi & Singh, 2003).

2.4.2. Particle size analysis

Particle size distribution of the starches was measured by laser scattering on triplicate samples using a Malvern Mastersizer Hydro QS-MU (Malvern Instruments Ltd., UK). The sample was added to the sample port until the instrument read ~15% obscuration. The size distribution was expressed in terms of the volumes of equivalent spheres. The selected criteria were the percent volume (% vol.) of granules with a diameter lower than 50 μm and the parameters d (0, 1), d (0, 5) and d (0, 9) expressed in micrometers.

2.4.3. Amylose content

Amylose content of starch samples was determined by the method given by Williams, Kuzina, and Hlynka (1970). Starch sample (20 mg db) was dispersed in KOH (0.5 M) and made up to 100 ml using distilled water. To an aliquot (10 ml) of the solution, 5 ml of HCl (0.1 M) and 0.5 ml of iodine reagent (0.1%) were added, diluted to 50 ml and the absorbance was measured at 625 nm. Amylose content was derived from a standard curve using amylose and amylopectin blends.

2.4.4. X-ray diffraction

X-ray diffractograms of starch samples (equilibrated at 100% relative humidity, at 25 °C for 24 h) were recorded using an Analytical Diffractometer (Pan Analytical, Phillips, Holland), Cu Kα radiation with a wave length of 0.154 nm operating at 40 kV and 35 mA. XRD diffractograms were acquired at 25 °C over a 2θ range of 4–30° with a step size of 0.02° and sampling interval of 10 s.

2.4.5. Thermal properties

Thermal properties of the rice starches from different cultivars were determined using DSC-822^e (Mettler Toledo, Switzerland) equipped with a thermal analysis data station. Starch (3.5 mg, dry weight) was loaded into a 40 μl aluminum pan (Mettler, ME-27331) and distilled water was added to achieve starch–water suspension containing 70% water. The samples were hermetically sealed and allowed to stand for 1 h at room temperature before heating in DSC. The DSC analyzer was calibrated using indium and empty pan was used as a reference. The sample pans were heated at a rate of 10 °C/min from 20 to 100 °C.

2.4.6. Dynamic rheometry

Rheometric measurements were performed in a using a Haake Rheostress-6000 (Thermo Electron, Germany) Rheometer equipped with parallel plate (25 mm diameter). The starch suspension (10%, w/w), after stirring for 30 min with a magnetic stirrer at room temperature, was loaded on the ram of the rheometer and covered with a thin layer of low-density silicone oil to minimize evaporation losses. The gap size, strain and frequency were set to

1.0 mm, 1.0% and 1.0 rad/s, respectively. The starch samples were heated from 50 to 90 °C at a rate of 0.5 °C/min and held at 90 °C for 10 min followed by cooling to 50 °C at same rate. The storage modulus (G'), loss modulus (G'') and loss tangent (tan δ) were measured. Pasting temperature was calculated as the temperature at which the moduli started increasing during the heating process and the maximum values of the moduli during heating and cooling process were designated as the G'_{peak} or G''_{peak} and G'_{final} or G''_{final}, respectively.

2.5. Protein isolation and SDS–PAGE analysis

Milled rice samples were ground in a mortar and pestle and SDS–PAGE analysis was carried out according to the method of Kumagai et al. (2006) with slight modifications. Ground sample (40 mg) was suspended in 1 ml SDS urea solution (8 M urea, 2% SDS, 5% β-mercaptoethanol and 10% sucrose in 50 mM Tris–HCl buffer pH 6.8) and stirred for 60 min. The samples were heated at 95 °C for few min, centrifuged (14,000 × g for 10 min) and 40 μl of the supernatant was carefully loaded into the wells of stacking gel. Gel was run using the method of Laemmli (1970) and resolved at a constant voltage (200 V). Gel was stained overnight using Coomassaie Brilliant Blue R 250 and destained using 20% methanol.

2.6. Statistical analysis

The data reported is the average of triplicate observations and was subjected to analysis of variance (ANOVA) by Duncan's test ($P < 0.05$) using Minitab Statistical Software (State College, PA). Principal component analysis (PCA) was also carried out for determining the relationship between different variables. The PCA results were graphically represented by the projection of the first two principal components.

3. Results and discussion

3.1. Milled rice characteristics

3.1.1. Physicochemical properties

Grain yield of all the cultivars increased with the application of nitrogen. Punjab Mehak 1 had grain yield of 2.6 t/ha which increased to 4.3, 4.8 and 5.4 t/ha, respectively, with application of 20, 40 and 60 kg/ha of nitrogen. Grain yield of Pusa Basmati 1121 was 2.9, 4.3, 4.4 and 4.4 t/ha with application of 0, 20, 40 and 60 kg/ha of nitrogen against grain yield of 2.9, 4.3, 4.6 and 4.0 t/ha for Punjab Basmati 2 with application of similar levels of nitrogen. Pusa Basmati 1121 did not show significant increase beyond 20 kg/ha of nitrogen application. Thousand kernel weight (TKW), bulk density and length–breadth (L/B) ratio of the milled rice obtained from various cultivars as affected by nitrogen applications are shown in Table 1. Pusa Basmati 1121 showed the highest TKW (19.6–21.0 g) followed Punjab Basmati 2 (18.4–19.4 g) and Punjab Mehak 1 (17.9–19.7 g). TKW increased with the increase in level of nitrogen application. Pusa Basmati 1121 and Punjab Mehak 1 showed greater increase in TKW with application of nitrogen as compared to Punjab Basmati 2. The L/B ratio of milled rice from all the cultivars also increased with the increase in level of nitrogen application. The TKW showed a significant correlation with level of nitrogen application ($r = 0.58$, $p \leq 0.05$). L^*-, a^*- and b^*-values of milled rice from different cultivars ranged from 66.2 to 75.3, 0.80 to 2.4 and 13.6 to 17.5, respectively (Table 1). Among the cultivars studied, Punjab Mehak 1 rice had the highest L^* (lightness) value followed by Pusa Basmati 1121 and Punjab Basmati 2. Higher L^* values of Punjab Mehak 1 milled rice indicate lighter color of grains as compared to grains of other rice cultivars. The milled rice from all the cultivars were yellowish with b^* (indicator of blueness and yellowness) ranging between 13.7 and 17.5. Milled rice from Punjab Basmati 2 showed higher b^*-value compared to that from Pusa Basmati 1121 and Punjab Mehak 1. The higher a^*-value of milled rice from paddy grown with the application of nitrogen indicates presence of greater redness than that from the counterpart rice grown without nitrogen application. The variation in a^* value indicates the extent of variation in redness. L^*-value decreased while a^* and b^* value increased with the increase in level of nitrogen application. The changes in color parameters were the greatest up to 20 kg/ha nitrogen application, further increase in nitrogen level caused comparatively less change.

Protein content of rice ranged between 7.7 and 9.6%, milled rice from paddy grown with the application of nitrogen was higher than that produced without application of nitrogen. Milled rice from Pusa Basmati 1121 showed higher protein content as compared to that from other two cultivars. Protein content of milled rice progressively increased with increase in level of nitrogen application up to 30 kg/ha in all the cultivars, and further increase in application level caused a slight decrease in protein content. Rice from paddy grown with application of nitrogen showed higher protein content than those without nitrogen application. Ash content of milled rice also increased with the application of nitrogen. Ash content of milled rice from paddy grown with application of nitrogen ranged between 0.66 and 0.99% for Pusa Basmati 1121, 0.72 and 0.81% for Punjab Mehak 1 and 0.79 and 1.20% for Punjab Basmati 2. Punjab Basmati 2 milled rice from paddy grown with different levels of nitrogen application had higher ash content as compared to corresponding milled rice from Pusa Basmati 1121 and Punjab Mehak 1. Milled rice with higher ash and protein content had lower L^*-value and higher a^* and b^*-values. The protein content of milled rice was significantly negatively correlated with L^* value ($r = -0.58$, $p \leq 0.05$). A similar positive correlation of protein content with L^* has been reported for corn grits and flour (Jamin & Flores, 1998; Sandhu, Singh, & Malhi, 2007).

3.1.2. Cooking characteristics

The cooking characteristics of milled rice from various paddy cultivars as affected by application of nitrogen at different levels are shown in Table 2. Cooking time and L/B ratio of cooked grains from different cultivars varied from 9.5 to 19.0 min and 3.51 to 5.93, respectively. Punjab Basmati 2 had longer cooking time (15.0–19.0 min), followed by Punjab Mehak 1 (9.5–18.0 min) and Pusa Basmati 1121 (10.0–13.0 min). Cooking time and cooked grain L/B ratio of Punjab Mehak 1 and Pusa Basmati 1121 rice showed a progressive increase with the increase in level of nitrogen application, former cultivar showed greater increase. Contrarily, cooking time and cooked grain L/B ratio for Punjab Basmati 2 rice did not change significantly with the nitrogen application. Cooking time between 13.3 min and 24.0 min of different Indian rice cultivars has been reported (Singh et al., 2005). Bulk density and water uptake, respectively, positively and negatively related with level of nitrogen applied, though the correlation of water uptake with nitrogen level was significant ($r = 0.68$, $p \leq 0.01$). Punjab Mehak 1 and Pusa Basmati 1121 showed greater increase in bulk density with nitrogen application and consequently showed greater change in cooking time, and cooked grain L/B ratio. It was reported that a disorganized cellular structure offers the opportunity for increased water absorption during cooking and, thus, a softer cooked grain (Lisle, Martin, & Fitzgerald, 2000). The gruel solids loss showed highly significant correlation with amylose content, consistent with previous studies (Singh et al., 2005). Amylose is known to leach out during cooking and the higher amylose content is liable to leach more into the cooking water (Morris, 1990). The gruel solids loss was negatively correlated with protein content ($r = -0.71$, $p \leq 0.01$) and level of applied nitrogen ($r = -0.86$, $p \leq 0.005$). It can be inferred

Table 1
Effect of nitrogen application on physico-chemical properties of milled rice from different cultivars.

Cultivar	Nitrogen application (kg/ha)	Thousand kernel weight (g)	Bulk density (g/ml)	*L*/*B* ratio	Hunter color parameters			Protein content (%)	Ash content (%)
					*L**	*a**	*b**		
Pusa Basmati 1121	0	19.6^{c}	0.76^{a}	4.61^{d}	72.2^{d}	1.6^{b}	14.9^{b}	7.9^{a}	0.66^{a}
	20	20.0^{cd}	0.77^{a}	4.69^{de}	70.6^{cd}	2.2^{d}	15.6^{b}	8.9^{bc}	0.72^{ab}
	40	20.6^{d}	0.82^{bc}	4.73^{e}	69.7^{c}	2.3^{d}	15.7^{bc}	9.0^{bc}	0.97^{de}
	60	21.0^{d}	0.83^{c}	4.77^{e}	69.4^{c}	2.4^{d}	16.3^{c}	8.2^{ab}	0.99^{e}
Punjab Mehak 1	0	17.9^{a}	0.82^{bc}	4.22^{a}	75.3^{e}	0.8^{a}	13.6^{a}	7.7^{a}	0.72^{ab}
	20	18.1^{a}	0.84^{c}	4.23^{a}	73.1^{de}	1.3^{b}	13.7^{a}	8.8^{b}	0.73^{b}
	40	19.0^{bc}	0.85^{c}	4.25^{a}	72.8^{d}	1.4^{b}	14.3^{ab}	9.1^{c}	0.80^{c}
	60	19.7^{cd}	0.85^{c}	4.48^{c}	72.6^{d}	1.4^{b}	14.4^{ab}	9.0^{bc}	0.81^{c}
Punjab Basmati 2	0	18.4^{ab}	0.75^{a}	4.33^{b}	70.2^{cd}	0.8^{a}	15.1^{b}	8.5^{b}	0.79^{bc}
	20	18.5^{ab}	0.75^{a}	4.39^{bc}	68.1^{b}	1.7^{c}	16.7^{c}	9.6^{c}	0.91^{d}
	40	18.9^{b}	0.78^{ab}	4.41^{bc}	68.0^{b}	2.1^{d}	17.2^{cd}	9.6^{c}	1.20^{g}
	60	19.6^{c}	0.79^{b}	4.46^{c}	66.2^{a}	2.2^{d}	17.5^{d}	9.0^{bc}	1.12^{f}

Means with similar superscript in a column do not differ significantly ($p > 0.05$).

Table 2
Effect of nitrogen application on cooking and textural properties of milled rice from different cultivars.

Cultivar	Nitrogen application (kg/ha)	Cooking properties				Textural properties			
		Cooking time (min)	*L*/*B* ratio	Water uptake ratio	Gruel solids loss (%)	Hardness (N)	Cohesiveness	Chewiness	Adhesiveness (N s)
Pusa Basmati 1121	0	10.0^{a}	5.37^{d}	3.75^{c}	5.64^{c}	2.54^{b}	0.29^{ab}	1.87^{b}	0.05^{bc}
	20	10.5^{ab}	5.48^{d}	3.33^{b}	4.21^{bc}	3.27^{c}	0.49^{c}	2.19^{bc}	0.04^{b}
	40	12.0^{b}	5.66^{de}	2.98^{a}	3.21^{ab}	3.88^{d}	0.57^{d}	3.39^{d}	0.03^{ab}
	60	13.0^{bc}	5.93^{e}	2.97^{a}	3.03^{a}	4.30^{e}	0.62^{d}	3.75^{d}	0.02^{a}
Punjab Mehak 1	0	9.5^{a}	3.51^{a}	4.56^{d}	8.40^{d}	1.67^{a}	0.22^{a}	1.20^{a}	0.13^{d}
	20	14.0^{c}	3.54^{a}	3.32^{b}	3.72^{b}	1.79^{a}	0.32^{b}	1.35^{a}	0.06^{c}
	40	17.0^{d}	4.00^{ab}	3.81^{cd}	2.73^{a}	2.22^{ab}	0.32^{b}	1.38^{a}	0.05^{bc}
	60	18.0^{de}	4.13^{b}	3.34^{b}	2.46^{a}	2.70^{bc}	0.33^{b}	1.91^{b}	0.04^{b}
Punjab Basmati 2	0	19.0^{e}	5.79^{de}	3.72^{c}	5.57^{c}	2.28^{ab}	0.34^{b}	1.52^{ab}	0.03^{ab}
	20	18.0^{de}	4.94^{cd}	3.49^{bc}	3.68^{b}	2.35^{ab}	0.37^{bc}	1.58^{ab}	0.06^{c}
	40	17.5^{d}	4.86^{c}	3.43^{bc}	3.31^{ab}	2.46^{b}	0.45^{c}	1.74^{b}	0.05^{bc}
	60	15.0^{cd}	4.76^{c}	3.41^{b}	3.26^{ab}	3.29^{c}	0.49^{c}	2.69^{c}	0.04^{b}

Means with similar superscript in a column do not differ significantly ($p > 0.05$).

that higher application of nitrogen fertilizer increased the *L*/*B* ratio that contributed to the increase in gruel solids loss. Gruel solids loss has been reported to be influenced by the *L*/*B* ratio (Singh et al., 2005) and the cultivars with higher *L*/*B* ratio offer larger surface to contact with water, hence resulted into greater gruel losses.

3.1.3. Textural properties

The textural properties of cooked rice from different rice cultivars grown under different nitrogen treatments are shown in Table 2. Among the various rice cultivars, Pusa Basmati 1121 cooked grains showed higher hardness, cohesiveness and chewiness. Punjab Mahek 1 cooked rice showed the least value for all the textural parameters. Hardness, cohesiveness, and chewiness progressively increased while adhesiveness decreased with the increase in nitrogen application level. Pusa Basmati 1121 cooked rice obtained with or without nitrogen application had hardness, cohesiveness, chewiness and adhesiveness between 2.54–4.30, 0.29–0.62, 1.87–3.75, and 0.02–0.05, respectively against 1.67–2.70, 0.22–0.33, 1.20–1.91 and 0.04–0.13 for Punjab Mehak 1 cooked rice and 2.28–3.29, 0.34–0.49, 1.52–2.69 and 0.03–0.06, respectively for Punjab Basmati 2 cooked rice. Among the textural properties of freshly cooked rice kernels, Pusa Basmati 1121 rice showed higher hardness and cohesiveness than Punjab Mahek 1 and Punjab Basmati 2 cooked rice. The textural properties of cooked rice from different cultivars grown under different levels of nitrogen were also measured after 2 h of storage at 5 °C. All the textural parameters except chewiness increased during storage for 2 h. The changes in textural properties may be attributed to the retrogradation of starch. The differences in textural properties among the various rice cultivars may be attributed mainly to differences in the amylose content, protein and grain structure. The higher value of hardness in rice cultivars may also be attributed to differences in their granular structure. Punjab Mehak 1 with larger starch granule size showed lower hardness than Pusa Basmati 1121 and Punjab Basmati 2. A higher hardness has been reported for rice cultivars having smallest size starch granules (Singh, Sodhi, Kaur, & Saxena, 2003).

3.2. Starch characteristics

3.2.1. Granule size

The size of the granules in the starch separated from different rice cultivars ranged between 0.5 and 12 μm. Starches from different cultivars showed bimodal distribution profiles for the granules size and each mode had peaks in the range between 0 to 2 μm and >2 to ≤12 μm, respectively (Fig. 1(A)). First mode and second mode showed peak at ∼1.0 and ∼6.0 μm, respectively. The granules of size >2 to ≤12 μm were present in higher proportion i.e. 81.6–85.9% as compared to granules of size 0–2 μm. Punjab Mehak 1 starch had the highest proportion of granules of >2 to ≤12 μm where as Pusa Basmati 1121 showed the lowest proportion of these granules.

3.2.2. X-ray

X-ray diffraction patterns of starches separated from different rice cultivars grown with different level of nitrogen are shown in Fig. 1(B). X-ray diffractions of rice starches showed the expected typical A-pattern (Singh, Nakaura, Inouchi, & Nishinari,

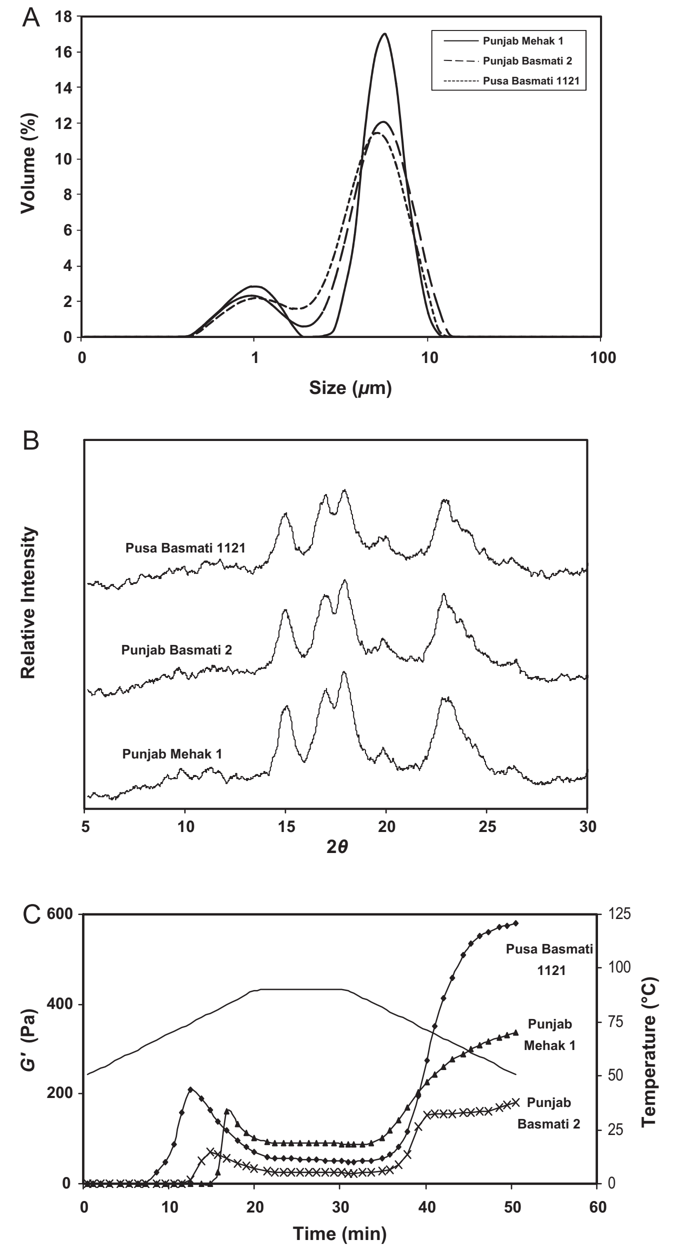

Fig. 1. Characteristics of starches separated from different rice cultivars with different nitrogen application during growth. (A) Particle size distribution, (B) X-ray diffractograms and (C) rheological properties (storage modulus, G').

2007). Starch from all the cultivars showed strong reflections at $2\theta = 15.17^{\circ}$, 17.27°, 18.17° and 23.27°. Punjab Mehak 1 starch showed higher peak intensities compared to other starches, indicating greater crystallinity. An additional peak at $2\theta = 20.07^{\circ}$ was observed in starch from all the cultivars, which has been attributed to amylose–lipid complexes (Singh, Singh, Isono, & Noda, 2010; Zobel, 1988). The intensity of amylose–lipid peak was observed to be higher in Pusa Basmati 1121 compared to the other starches. The difference in crystallinity in different rice starches may be attributed to difference in proportions of amylose, short and long side-chain amylopectin (Singh et al., 2007).

3.2.3. Amylose content

The amylose content of starches ranged from 9.0 to 21.9%, and differed significantly among different rice cultivars (Table 3). The lowest amylose content of 9.0% was observed for Punjab Mehak 1 starch and the highest of 21.9% for Pusa Basmati 1121 starch (Sodhi & Singh, 2003). Amylose content of the starch has been reported to vary with the botanical source of the starch and reported to be affected by the climatic and soil conditions during grain development (Morrison, Milligan, & Azudin, 1984; Yano, Okuno, Kawakami, Satoh, & Omura, 1985).

3.2.4. Thermal properties

Thermal properties (onset temperature, T_o; peak temperature, T_p; endset temperature, T_c; and enthalpy of gelatinization, ΔH_{gel}) of starch from different paddy cultivars grown under different nitrogen treatments are shown in Table 3. Among the starches from different cultivars, gelatinization transition temperatures (T_o, T_p, T_c) varied significantly. Punjab Mehak 1 starch had the highest T_p, T_o and T_c followed by Punjab Basmati 2 and Pusa Basmati 1121. Starch from Pusa Basmati 1121 grown with nitrogen applications at different levels had T_o, T_p and T_c between 60.89–61.48, 64.4–66.75, 68.59–70.49 °C, respectively, against 74.69–76.09, 78.71–80.05, 84.18–84.79 °C for starch from Punjab Mehak 1. The variation in transition temperatures and ΔH_{gel} in starches from different cultivars might be due to differences in amylose and amylopectin ratio and consequently resulting in the variation in crystallinity. Pusa Basmati 1121 and Punjab Mehak 1 starch showed a decrease in gelatinization transition temperatures with the increase in level of nitrogen application while slight increase in Punjab Basmati 2 with increase in nitrogen application was observed. T_o, T_p and T_c of Punjab Basmati 2 starches ranged from 66.93 to 69.22, 71.88 to 74.25, and 76.74 to 79.44 °C, respectively. The ΔH_{gel} of the starches from different rice cultivar also differed significantly. The ΔH_{gel} of the starches from all the rice cultivar showed a decrease with increase in nitrogen application. T_o, T_p and T_c showed significant negative correlation with amylose content ($r = -0.76$, -0.74, -0.73, respectively, $p \leq 0.01$).

3.2.5. Dynamic rheometry

The changes in storage modulus (G') and loss modulus (G'') of starch from different rice cultivars grown with different nitrogen application levels during heating from 50 to 90 °C, holding at 90 °C and followed by cooling to 50 °C are illustrated in Fig. 1(C). G'', a measure of the energy dissipated and lost as heat per oscillation, of starch pastes was greater than G', indicating predominance of viscous character. Both the moduli increased to a maximum followed by a decrease during heating. The initial rise in the moduli (initiation of gelatinization or pasting temperature), during heating among starches, occurred at a temperature between 66.75–68.71 °C, 74.56–77.15 °C and 79.10–81.07 °C, respectively, for Pusa Basmati 1121, Punjab Basmati 2 and Punjab Mehak 1 (Table 3). Starches from Punjab Mehak 1 showed higher gelatinization temperature compared to starches from other cultivars. Pasting temperature was significantly correlated with the gelatinization transition temperatures (T_o, T_p, T_c) measured using DSC. Among the starches from different cultivars, Punjab Mehak 1 showed the G'_{peak} and G''_{peak} of 125–175 Pa and 249–366 Pa, respectively. Loss tangent ($\tan\delta$) ranged between 1.7–2.3, 1.2–1.6 and 1.0–2.9, respectively, for Pusa Basmati 1121, Punjab Mehak 1, and Punjab Basmati 2. Tan δ is the ratio of G'' to G' and is indicative of liquid like behaviour when much higher than 1.0. During heating, the starch granules swell followed by leaching out of amylose molecules and consequently G' and G'' increased to a maximum value. This may be attributed to the formation of a network of swollen starch granules. The difference in G' among starches from different cultivars of rice could be attributed to difference in degree of granular swelling to fill the entire available volume of the system (Singh et al., 2007). Punjab Mehak 1 starch with the largest granules size showed the highest G' and G''. After reaching a maximum, the

Table 3
Effect of nitrogen application on the properties of starches separated from different rice cultivars.

Cultivar	Nitrogen application (kg/ha)	Amylose content (%)	Thermal properties				Rheological properties					
			T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J/g)	G'_{peak} (Pa)	G'_{final} (Pa)	G''_{peak} (Pa)	G''_{final} (Pa)	$Tan_{peak}\,\delta$	Pasting temperature (°C)
Pusa Basmati 1121	0	20.9[e]	61.48[a]	66.75[c]	70.49[b]	11.74[c]	80[a]	88[a]	187[bc]	206[d]	2.3[c]	66.75[a]
	20	18.5[d]	61.24[a]	65.25[b]	70.06[b]	10.34[b]	209[d]	611[f]	345[e]	318[ef]	1.7[b]	66.75[a]
	40	13.8[bc]	61.17[a]	64.74[a]	69.38[a]	10.05[b]	164[bc]	150[b]	283[de]	284[e]	1.7[b]	68.67[b]
	60	21.9[e]	60.89[a]	64.40[a]	68.59[a]	9.24[a]	151[bc]	193[c]	321[e]	364[f]	2.1[bc]	68.71[b]
Punjab Mehak 1	0	14.8[c]	76.09[e]	80.05[g]	84.79[f]	18.17[g]	133[b]	249[d]	156[b]	58[a]	1.2[a]	79.10[e]
	20	10.6[a]	75.86[e]	79.79[g]	84.51[f]	17.47[fg]	162[bc]	337[e]	253[d]	100[b]	1.6[ab]	79.10[e]
	40	9.0[a]	75.73[e]	79.79[g]	83.45[e]	16.97[f]	175[c]	366[e]	233[c]	223[d]	1.3[a]	81.00[f]
	60	12.1[b]	74.69[d]	78.71[f]	84.18[ef]	15.09[e]	125[b]	334[e]	179[b]	60[a]	1.4[ab]	81.07[f]
Punjab Basmati 2	0	20.3[e]	66.93[b]	71.88[d]	76.74[c]	15.34[e]	95[db]	75[a]	276[d]	281[e]	2.9[d]	74.56[c]
	20	17.4[d]	67.07[b]	72.07[d]	76.98[c]	13.68[d]	70[a]	180[bc]	139[ab]	218[d]	2.0[bc]	74.56[c]
	40	14.6[c]	68.49[c]	73.57[e]	78.71[d]	12.00[c]	122[b]	248[d]	216[c]	295[e]	1.8[b]	74.56[c]
	60	12.7[b]	69.22[c]	74.25[e]	79.44[d]	12.38[c]	98a[b]	278[d]	98[a]	162[c]	1.0[a]	77.15[d]

Means with similar superscript in a column do not differ significantly ($p > 0.05$).

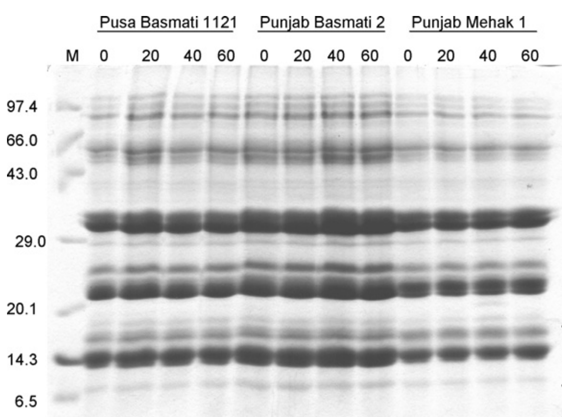

Fig. 2. SDS–PAGE profile of proteins from milled rice of different cultivars grown with varying nitrogen application (0, 20, 40 and 60 kg/ha). M, standard molecular weight marker (×1000 Da).

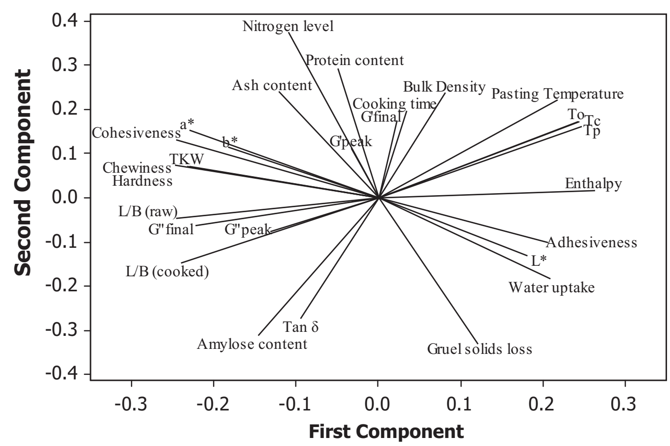

Fig. 3. Principal component analysis loading plot: relationships between properties of milled rice and starches from different rice cultivars.

G' decreased which indicates destruction of the gel structure (Tsai, Li, & Lii, 1997). Starches separated from rice cultivars grown with nitrogen application treatment showed higher moduli than the starch from their counterpart rice grown without nitrogen treatment. G'_{final} of Pusa Basmati 1121, Punjab Mehak 1 and Punjab Basmati 2 starches ranged between 187–345 Pa, 156–253 Pa and 98–276 Pa, respectively. G''_{final} of Pusa Basmati 1121, Punjab Mehak 1 and Punjab Basmati 2 starches ranged between 206–364 Pa, 58–223 Pa and 162–295 Pa, respectively. Both the moduli increased with the increase in nitrogen application. Starches from different rice cultivars grown with higher dose of nitrogen application showed higher moduli as compared to their counterpart starches from cultivars grown without or lower level of nitrogen application. This may be attributed to the increase in protein and concomitant reduction in starch and amylose content. Martin and Fitzgerald (2002) demonstrated that proteins affect the amount of water the rice absorbs, and hence the pasting behaviour of starch.

3.3. Protein analysis

The SDS–PAGE profile of milled rice proteins from all the samples showed similar banding pattern of polypeptide subunits irrespective of cultivar and nitrogen application (Fig. 2). The polypeptide subunits of rice flour proteins ranged between 13,000 and 97,000 Da. Major polypeptide subunits of prolamins (13,000 Da), proglutelins (22,000–23,000 Da) and glutelins (38,000 Da) were observed along with smaller quantities of prolamins (10,000 Da and 16,000 Da), globulin (26,000 Da) and proglutelin (57,000 Da). Similar banding pattern has been reported earlier for Japanese rice (Kumagai et al., 2006). Application of nitrogen fertilizer did not cause any difference in the SDS–PAGE profile of rice proteins.

3.4. Relationship between grain and starch properties

PCA revealed the relationships between different properties of milled rice as well as the starch separated from different rice cultivars (Fig. 3). The loading plot indicated that cooked grain hardness and cooking time, respectively, were closely associated with amylose content and protein content. Similar relationship between hardness and amylose content has been reported earlier (Mestres et al., 2011). The cooking time and amylose content are located in the opposite direction indicating negative relationship between them. Cooking time and bulk density of milled rice are located in the same direction, which indicate that the rice with higher bulk density probably had compact structure that resulted into a slower water uptake and longer cooking time. Paddy grown with application of nitrogen had milled rice with higher L/B ratio and a^*-value and lower L^*-values. L^*-value was located to the opposite side of ash content and protein content. This indicated that rice with higher ash and protein content had lower L^*-value. TKW, bulk density and cooked grain hardness and cohesiveness increased while water uptake decreased with the increase in nitrogen application. Adhesiveness has been associated with the type of rice (*japonica* or *indica*) as well as with the grain size. L/B ratio of raw and cooked grains and adhesiveness was located opposite to each other, indicating negative relation between them. The correlation coefficients of L/B ratio of raw and cooked grains with adhesiveness were −0.61 (p = 0.03) and −0.70 (p = 0.01), respectively. The results showed that increase in nitrogen application resulted into increase in protein content that may have caused a decrease in adhesiveness. Earlier it was demonstrated that proteins affect the amount of water the rice absorbs, and hence the pasting behaviour of starch (Martin & Fitzgerald, 2002). The interactions that take place through a protein network linked by disulfide bonds may have also affected the textural properties. It may thus also be inferred that higher protein content and higher disulfide bonds limit starch/water interaction and consequently adhesion (Martin & Fitzgerald, 2002). Transition temperatures and ΔH_{gel} were located opposite to amylose content. This showed that application of nitrogen reduced amylose content and concomitantly increased the crystallinity that resulted into increase in gelatinization temperature and enthalpy of gelatinization. A decrease in crystallinity with increase in amylose content has been reported (Cheetham & Tao, 1998; Lopez-Rubio, Flanagan, Gilbert, & Gidley, 2008). Patindol, Gu, and Wang (2010) observed a negative correlation between instrumental stickiness and the amylose–amylopectin ratio in material at the surface of cooked rice. Amylose content was positively correlated with loss modulus (G'') and loss tangent (tan δ), however, negatively with storage modulus (G'). This was in consistent with earlier reports that starches in rice with low amylose contents had reduced G'' values (Lii, Tsai, & Tseng, 1996; Sodhi & Singh, 2003). Furthermore, waxy potato starches were reported to have lower G' and G'', and higher tan δ (Kaur, Singh, Sodhi, & Gujral, 2002). It has been suggested that following starch gelatinization, amylopectin molecules form a relatively separate super-globe and clusters, which are included in amylopectin formed gel-balls. The molecular entanglements between gel-balls and super-globe are much less than those between linear polymer chains (amylose) thus accounting for observations of this study (Xie, Yu, Su, & Liu, 2002Xie et al., 2009).

4. Conclusions

The nitrogen application significantly affected the physicochemical and textural properties of milled rice. The changes brought about by the application of nitrogen may be attributed to the increase in protein and decrease in amylose content. Starch separated from the milled rice after nitrogen application showed higher gelatinization and pasting temperature.

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