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Starch Isolation from Different Cereals with Variable Amylose/Amylopectin Ratio and Its Morphological Study Using SEM and FT-IR

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A B S T R A C T

Starches from the wheat (UP262, PBW343), rice (PB2, PD19) and millets (Finger millet VL Mandua-352, Barnyard millet VL Madira-207, Foxtail millet DHFT-109-3) were isolated by alkali extraction method and characterized for morphologically and biochemical properties. The morphological properties of starch granules were studied by scanning electron microscope. The infrared spectroscopy is sensitive to structural changes on starch macromolecule, such as helicoidal chain conformation, crystallinity, retrogradation and water content. Starch yield was found maximum in rice followed by wheat and millets. High value of amylose-amylopectin ratio indicates low glycemic index. Amylose content is important for food processing in the industry and for quality. The amylose content (%) was found significant in all cultivars. The amylose content (%) was found maximum in rice (PB2, PD19) and wheat (UP262) followed by PBW343 (Wheat) and finger millet. The amylose content in millet varies from 31-33%. Total starch content ranged from 57-70% in cereals in the present study. Total starch content was found maximum in rice followed by wheat and millets. Total starch content in rice (PB2 & PD19) was found 67% and 70% respectively. The proximate content of total starch in barnyard millet (58%), finger millet (57%) and foxtail millet (59%) was found in the present study. Scanning electron microscopy (SEM) has been a useful tool for investigating the microstructures of cereal grains and derived products.

Introduction

Starch is the most abundant storage reserve carbohydrate found in many different plant organs, including seeds, fruits, tubers and roots. It is used as a source of energy during periods of dormancy and re-growth (Roper, 2002). Starch is versatile and useful polymer because of ease of availability with which its

physiochemical properties can be altered through chemical, physical and enzymatic treatment (Jobling, 2004). Starch is important ingredients in various food systems as thickening, gelling and binding agents. It imparts texture to various foodstuffs (Thebaudin *et al.,* 1998). Starch is made up of two polymers of D-glucose: amylose and amylopectin. Amylose is fundamentally a linear molecule of α-1, 4-linked glucan and occupies approximately 15-30% of starch, while amylopectin, the major component (70–85%), is a larger molecule with highly α-1,6 branched chains. Amylose has a molecular weight ranging 10^5 – 10^6 and degree of polymerization is nearly 1000-10,000 glucose units. Amylopectin is a much larger polymer, with a molecular weight about 10^8 and a degree of polymerization is much higher than amylose (Copeland *et al.,* 2009). Starch granules are thought to have alternative layers of crystalline and amorphous regions constructed by amylopectin and amylose (Srichuwong *et al.,* 2005). Amylose and amylopectin make up 98–99% of the dry weight of native granules, with the remainder comprising small amounts of lipids, minerals, and phosphorus in the form of phosphates esterified to glucose hydroxyls. Starch granules range in size from 1 to 100 μm diameters and shape of polygonal, spherical, lenticular, and can vary greatly in content, structure and organization of the amylose and amylopectin molecules, the branching architecture of amylopectin, and the degree of crystallinity (Lindeboom *et al.*, 2004). Wang *et al.,* (1998) reported that chains of amylopectin are organized into double helices and form crystalline structures. Scanning electron microscopy (SEM) is frequently used because of the short wavelength of the electron beam, which makes it possible to determine granule size more accurately. The resolution possible with SEM also provides a more detailed perspective on granule surface characteristics and granule morphology (Chmelik, 2001). Scanning electron microscopy (SEM) has been used to relate granule morphology to starch genotype (Fannon *et al.,* 1992a). SEM has also been used to relate paste structures to paste properties (Fannon and BeMiller, 1992; Fannon *et al.,* 1992b). Hoover (2001) reported that the cereal starch exhibit the A-type crystalline structure whereas, the tuber starches show the B-form and legumes, the mixed state pattern 'C'. Starch granule size, amylose/amylopectin ratio and various physical and chemical characteristics reveals the starch paste behaviours in aqueous system (Madsen and Christensen, 1996). The amylose content of the starch granules varies with the botanical source of the starch and is affected by the climatic conditions and soil type during growth (Morison and Azudin, 1987). Kim and Huber (2008) reported that wheat endosperm contains A-type and B-type starch granules, showing a bimodal granule size. A-type granules are bigger (10–35 μm) and disk- or lenticular-shaped whereas B-type granules are smaller (<10 μm) and spherical or angular (Evers, 1973). Edwards *et al.,* (2002) reported that higher proportion of smaller granules increased dough elastic properties. Hence morphology study using SEM reveals the quality of starch isolated from different botanical origin. Granules size (<10 μm) bind more water, which likely increases dough stiffness and reduces the elasticity (Huang and Lai, 2010). It was reported that the qualities of both dried and cooked starch noodles made from small-sized granule fractions are much better than those made from large-sized granule fractions (Chen *et al.,* 2003) but granule size (about 12 μm) can increase bread weight (Sahlström *et al.,* 1998). The relative amounts of amylose and amylopectin gives starch to unique physical and chemical properties which convey specific functionality (Fergason, 1994). High amylose starches have numerous industrial applications. These starches are used in fried snack products to

create crisp, evenly browned snacks. An added bonus of high amylose starches is that they hamper the penetration of cooking oils, which leads to a decrease in fat intake by the consumer. High amylose starches are widely used as thickeners, are strong gelling agents used in the production of jellies and, owing to their rapid setting properties, are used in the production of gum candies (Slattery *et al.,* 2000). Therefore, the objective of the present study was to characterize the physio-chemical behaviour of starch isolated from different cultivars due to its versatile uses in the food and manufacturing industries. Starch is extensively used in the food and beverage industries as a thickener and a sweetener as well as having some manufacturing applications in the paper and textile industries.

Materials and Methods

Two cultivars of Rice grains (*Oryza sativa L.*) viz., PB2 and PD19 and Wheat grains viz., UP262 and PBW343 were procured from Department of Agronomy and Department of plant breeding and genetics, GBPUA&T, Pantnagar, Uttarakhand respectively. Finger millet (VL Mandua-352) and Barnyard millet (VL Madira-207) grains were purchased from VPKAS, Almora, Uttarakhand. Foxtail millet (DHFT-109-3) grains were taken from ICAR-Indian Institute of Millet Research, Hyderabad. The grains were cleaned and ground in mixture grinder and stored properly at room temperature prior to their use in actual experiment. Other reagents and chemicals used were at minimum of analytical grade.

Isolation of starch

Starch was extracted by using alkaline extraction method of Kim *et al.,* (2012) with some modification. In this procedure, centrifugation steps were at 5000g for 10 min at 20^{o}C. Flour (10 g) was suspended in 60 ml of 0.5% NaOH solution and then stirred for 30 min and kept for 24hr at 4^{o}C. The upper yellowish layer was removed. After that 0.5% NaOH solution was added to form slurry and the alkaline slurry was centrifuged. The sediment was washed with 0.5% NaOH solution and slurry was centrifuged again. This process was repeated until the yellowish layer was completely removed. The alkaline slurry was neutralised with 1.0 M HCl and centrifuged again. The resulting slurry of starch was dried in convection oven at 35-40^{o}C for 48 h and gently passed through sieve using a mortar and pestle and stored at air tight container for future experiment.

Amylose content determination

Amylose content was determined by method described by McCready *et al.,* (1950) with some modification. Weigh 100mg of flour and transfer into 100 ml conical flask. Add 100 ml 1 N sodium hydroxide (NaOH) to disperse sample. Allow dispersion to vortex for 30 min. The mixture must be smooth and free of lumps. Pipet 200 µl of this solution into 100 ml conical flask and add distilled water (dw) to make volume 20 ml. Add 2 drops of phenolphthalein indicator and titrate with 1 N hydrochloric acid solution until the pink indicator colour just disappears. Add 1 ml iodine reagent and finally make up volume 50 ml with distilled water and absorbance of the blue colour was measured at 620 nm against the reference solution. The amylose content was determined from a standard curve with amylose.

Preparation of iodine reagent

Stock iodine solution

Potassium iodide (20 g) was weighed into 100ml beaker together with 2.0 g resublimed iodine. The reagent were dissolved in the minimum of water and carefully diluted to 100 ml in a volumetric flask and stored in amber bottle.

Iodine reagent

10 ml of stock iodine solution was pipetted into a volumetric flask and diluted to 100 ml with distilled water.

Total starch determination

Total starch was determined by method adopted from Dubois *et al.*, (1956) with some modification. Weigh 100 mg sample and crushed in 5 ml ethanol (80%). After crushing the contents were filtered through Whatman filter paper.

Extraction of total starch

The residue left on filter paper is dried. Samples were refluxed for 1 h with 2 ml distilled water in 95 °C water bath.

Add 2 ml of 9.2N 70% perchloric acid and shake well for 15 minute and make up volume 10 ml with distilled water. Samples are allowed for centrifuge at 5000g for 20 minute. The supernatant is collected and the pellet is again refluxed with 2 ml of 4.6N perchloric acid. Samples are allowed for centrifuge.

The supernatant is collected in the same tube and make up volume 25 ml with distilled water for total starch estimation.

Estimation of total starch

Supernatants were analysed for glucose, by the phenol-sulphuric acid method and concentrations determined against soluble starch as a standard. Starch content of the samples was estimated by using this formula.

Starch (%) = (OD of sample/ slope of the standard curve)*(1/1000)*(1/W)*100
1/1000 = Conversion from micrograms to milligrams;
100/W = Factor to express "starch" as a percentage of flour weight; W = weight in milligrams

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) micrographs was recorded with a Jeol-JSM 6601ALV (made in Japan). Starch samples were applied on an aluminium stub using double sided adhesive tape, and the starch was coated with gold (using JFC 1600 gold coater).

The micrographs were obtained with an accelerating potential of 5 kV under high vacuum. In the present study a magnification range 500X, 1000X and 2000X was used for millets starch (Barnyard millet, Finger millet and Foxtail millet) and wheat starch (UP262 and PBW343). For rice starch, 150X, 300X and 500X magnification range was used.

FT-IR

The FT-IR spectra were obtained using FT-IR at IIT Roorkee, Uttarakhand (Perkin Elmer services). The spectra were recorded in transmission mode from 4,000 to 450 cm^{-1} (mid-infrared region). The sample was diluted with KBr (1:100, w/w) before acquisition and the background value from pure KBr was acquired before the sample was scanned.

Statistical Analysis

The experimental data were analysed as mean ± standard error (SE) using Completely Randomized Design. Determinations of starch yield, amylose content and total starch content were done in triplicate. Values with different letters in the same column are significantly different with $p < 0.05$.One-way analysis of variance (ANOVA) was carried out using OPSTAT (O.P. Sheoran Programmer, Computer Section, CCS HAU, Hisar).

Results and Discussion

Isolation of starch from different cereals

Two cultivars of rice grains (*Oryza sativa L.*) viz., PB2 and PD19, wheat grains viz., UP262 and PBW343, finger millet (VL Mandua-352), barnyard millet (VL Madira-207) and foxtail millet (DHFT-109-3) grains were processed in the present investigation for isolation of starch (Fig. 1) and percentage yield of obtained starch was compared (Table 1). The percentage starch yield was found significant in all cereals. The maximum starch yield (%) was found in Pant Basmati 2 followed by Pant Dhan19 (Rice) and UP 262 (Wheat). There are no significant differences in % starch yield of foxtail millet, finger millet and PBW343 (Wheat) *eg.* Comparison of means with CD@ 0.05 is same. The minimum starch yield was found in barnyard millet.

Proximate analysis of amylose and total starch content

Amylose content is important for food processing in the industry and for quality. The amylose content (%) was found significant in all cultivars. The amylose content (%) was found maximum in rice (PB2, PD19) and wheat (UP262) followed by PBW343 (Wheat) and finger millet. The amylose content in millet varies from 31-33%. Amylose contents of the above cereals were analysed and the results are shown in Table 1. The amylose content of rice starch usually ranges from 15-35% (Oko *et al.*, 2012). Blazek and Copeland (2008) reported that 35-43% amylose was found in wheat. Total starch content ranged from 57-70% in cereals in the present study. Total starch content was found maximum in rice followed by wheat and millets. Total starch content in rice-PB2 and PD19 was found 67% and 70% respectively. Li *et al.*, (2016) reported that total starch content in rice varies from 78-81%. The proximate content of total starch in barnyard millet (58%), finger millet (57%) and foxtail millet (59%) was found in the present study. Total starch in millet varies from 60-65% and it was maximum in kodo millet (Shobana *et al.*, 2013; Devi *et al.*, 2014). The results are shown in Table 1. Gerrano *et al.*, (2014) reported on 22 accession of sorghum and found that total starch, amylose varies from 44-68% and 14-18% respectively. Amylose and amylopectin ratio can predict the glycemic index of rice. A high value of amylose amylopectin ratio indicates low glycemic index (Dipnaik and Kokare, 2017). Frie *et al.*, (2003) reported that the rate of hydrolysis of starch is fast which contain high amount of amylopectin. The amylose-amylopectin ratio was determined in all cultivars viz. millet, rice and wheat. It was observed that the amylose-amylopectin ratio was found maximum in Pant Basmati 2 followed by Pant Dhan 19. In case of wheat the ratio was found maximum in UP 262 followed by PBW 343. In millet, the amylose-amylopectin ratio was higher in finger millet followed by barnyard millet. The amylose-amylopectin ratio was found lower in foxtail millet and maximum in Pant Basmati 2 comparatively to all cultivars.

Study of starch by SEM analysis

The morphological properties of starch granules were studied by scanning electron microscope. Scanning electron microscopy (SEM) has been a useful tool for investigating the microstructures of cereal grains and derived products (Orth *et al.*, 1973a, b; Fannon *et al.*, 1993; Gallant *et al.*, 1997). The variation in size and shape of starch granules may be due to their biological origin (Svegmark and Hermansson, 1993). The morphology of starch granules also depends on the biochemistry of the chloroplast or amyloplast as well as physiology of the plant (Badenhuizen, 1969). The scanning images of starch granules of barnyard millet [A B C], finger

millet [D E F] and foxtail millet [G H I] shown in figure 2 at 500X [A D G], 1000X [B E H] and 2000X [C F I]. The scanning images of starch granules of wheat (UP262 [A B C] PBW343 [D E F]) shown in figure 3 at 500X [A D], 1000X [B E] and 2000X [C F]. The scanning images of starch granules of rice (PB 2[A B C] PD 19 [D E F]) shown in figure 4 at 500X [A D], 1000X [B E] and 2000X [C F]. Barnyard millet starch showed large spherical, small polygonal and small spherical shaped granules. Large spherical granules showed relatively smooth surfaces with depressions or indentations due to protein bodies. The size of granules ranged from 6.46µm-12.23µm. Finger millet starch granules are mostly polygonal and the granules size range from 4.60-9.19 µm. Small granules are also present but larger granules were present in higher numbers. The size of granules is smaller than barnyard millet. Foxtail millet starch granules are small spherical and polygonal. Granules size is comparatively smaller than finger and barnyard millet.

The granule size ranges from 4.6-12.02 µm. Malleshi *et al.,* (1986) reported that finger millet starch contained granules of uneven shape spherical, polygonal and rhombic. Hard endosperm of corn has been shown to have nearly polygonal starch granules, whereas the soft endosperm has nearly round granules (Robutti *et al.,* 1974). Pant Basmati 2 starch granules are large irregular and the size range from 30.22µm-177.13µm. Pant Dhan 19 starch granules are small and large asymmetrical shape with size range from 6.22µm -110.95µm.

The cultivars used in the study vary from small to large granules. Small granules (<10 µm) bind more water increases dough stiffness and reduces the elasticity. From the morphology study of starch granules finger millet imparts increased dough stiffness and reduce elasticity. Millet starch binds more water comparatively to wheat and rice starch. Similar result was reported by Huang and Lai (2010).The native starches of different rice cultivars consisted of mixed population of large, medium and small granules with the diameter range of 10µm - 150 µm. The small granules were spherical or ellipsoidal while the medium and large granules were ellipsoidal to irregular or cubical in shape. Basmati starch granules has a intact structure having crystalline surface with pores on some parts of the granules, while other rice starch granules has loose structure with highly rough surface. This irregular rough surface of the granules was due to damage of starch during isolation process (Reddy and Bhotmange, 2013). Bhattacharya (2012) reported that rice starch with small starch granule sizes are suitable ingredient for extruded snack. Pure wheat starch had smooth-walled pores, while an addition of gluten resulted in pores with roughened and torn pore walls (Philipp *et al.,* 2017). Moin *et al.,* (2017) reported that the granular size for both rice varieties ranged between (4.5–7.05) mm.

Rice starch granules for both rice varieties (Basmati and Irri rice) were found to be polygonal and irregular in shape. Singh *et al.,* (2006) reported smaller sizes (1.5–5.8) mm for starches isolated from Indian rice cultivars. However, the shape was irregular, similar to what observed in the present study. Native starches have shiny surface, however due to erosion, starches often clump together and appear in form of clusters.

This phenomenon was found to be more pronounced for rice starches treated with 0.8 M HCl (Hu *et al.,* 2014). UP262 starch granules are small and large spherical with size ranges from 2.56 µm -25.92 µm while PBW343 contained starch granules of anomalous shape with size ranges from 32.2 µm -198.23 µm. The different sizes of the starch granules might be attributed to their different time of formation during grain development (Xie *et al.,* 2008).

Table.1 Proximate analysis of starch yield, amylose content, total starch and amylose –amylopectin ratio

Serial no	Cultivars	Starch yield (%)[1]	Amylose content (%)[2]	Total starch content (%)[3]	Amylopectin[4]	Amylose/ Amylopectin
1	**Barnyard Millet (VL Madira-207)**	35.6 ±.88^{d}	33.33 ± 1.14^{bc}	58.56 ± 0.43^{cd}	66.67 ± 1.14	0.50
2	**Finger Millet (VL Mandua-352)**	37.6 ± 1.45^{cd}	35.10 ± 1.81^{b}	57.25 ± 0.08^{d}	64.9 ± 1.81	0.54
3	**Foxtail millet (DHFT-109-3)**	37.6± 1.45^{cd}	31.33 ± 0.78^{c}	59.62 ± 0.28^{cd}	68.67 ± 0.78	0.46
4	**Wheat (UP 262)**	41.0 ± 2.08^{bc}	43.16 ± 0.56^{a}	63.61 ± 0.22^{b}	54.57 ± 0.12	0.76
5	**Wheat (PBW343)**	39.3 ± 1.76^{cd}	35.76 ± 0.63^{b}	60.34 ± 0.11^{c}	56.23 ± 0.26	0.56
6	**Rice (Pant Basmati 2)**	48.0 ± 1.73^{a}	45.43 ± 0.12^{a}	67.94 ± 1.04^{a}	56.83 ± 0.56	0.83
7	**Rice (Pant Dhan 19)**	44.3 ± 1.20^{ab}	43.76 ± 0.26^{a}	70.68 ± 2.21^{a}	64.23 ± 0.63	0.78

Data represents Mean±SE of triplicates[1, 2, 3, 4]. Values with different letters in the same column are significantly different with $p < 0.05$.

[1]SE (m) 1.553, SE (d) 2.197 CV = 6.639Treatments found Significant at 1% and 5% level of significance.CD (0.01) = 6.540 and CD (0.05) = 4.712

[2]SE (m) 0.928, SE (d) 1.312, CV = 4.199Treatments found significant at 1% and 5% level of significance CD (0.01) = 3.906 CD (0.05) = 2.815

[3] SE (m) = 0.948, SE (d) =1.340 CV= 2.626 Treatments found Significant a t 1% and 5% level of significance CD (0.01) = 3.995 CD (0.05) = 2.878

Table.2 Structural characteristics of different cultivars starches as determined by IR ratio

S. No	Cultivars	1047/1022	1022/995
1	Barnyard Millet (VL Madira-207)	1.00	1.13
2	Finger Millet (VL Mandua-352)	1.02	1.19
3	Foxtail millet (DHFT-109-3)	0.94	1.07
4	Wheat (UP 262)	1.24	0.93
5	Wheat (PBW343)	1.00	0.88
6	Rice (Pant Basmati 2)	1.02	0.89
7	Rice (Pant Dhan 19)	0.99	0.95

Fig.1 Isolated starch from different cereals likes millet (Barnyard millet[A], Finger millet[B], and Foxtail millet[C]), wheat (UP262[D], PBW343[E]) and rice (Pant Basmati 2[F], Pant Dhan 19[G])

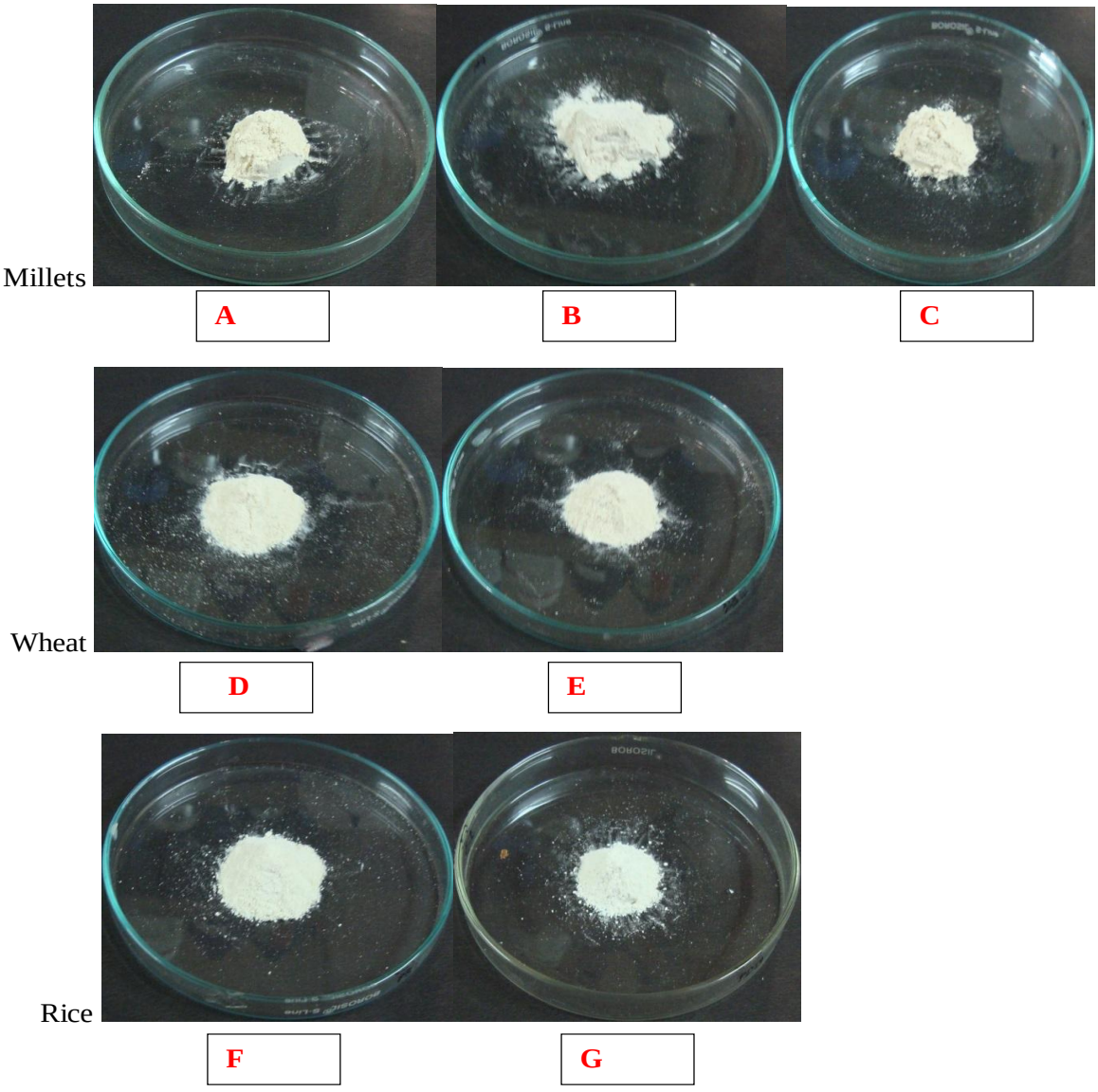
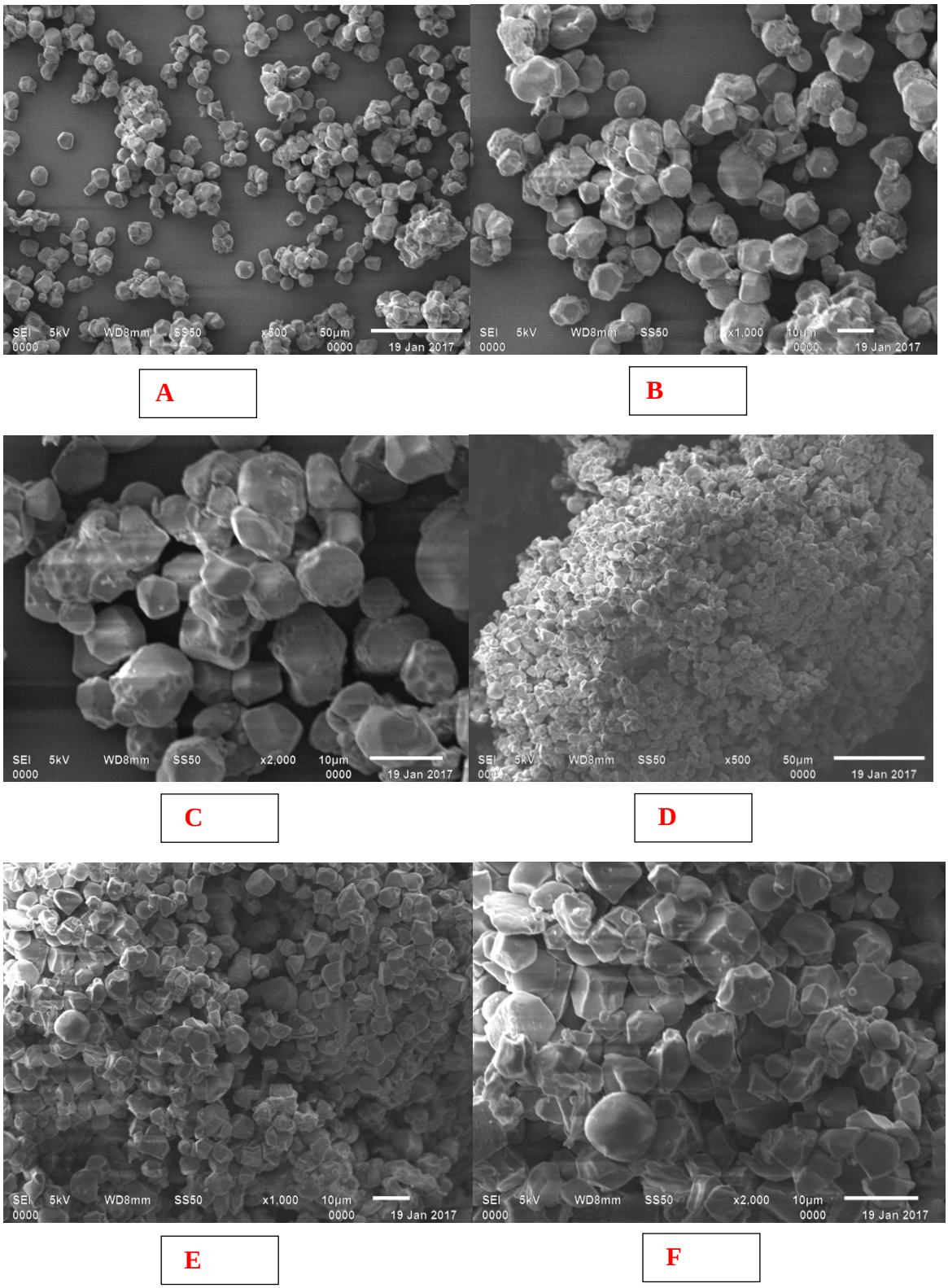
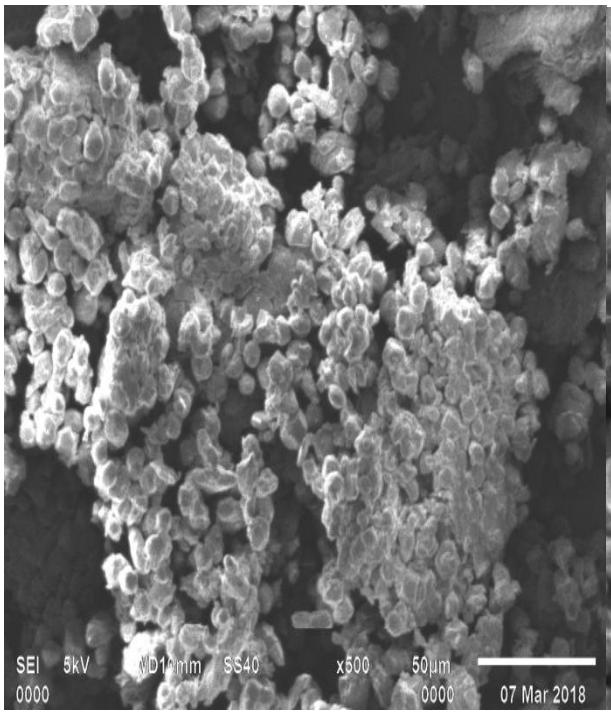


Fig.2 Scanning electron microscope images of millets (Barnyard millet [A B C] Finger millet [D E F] Foxtail millet [G H I]) at 500X [A D G], 1000X [B E H] and 2000X [C F I]

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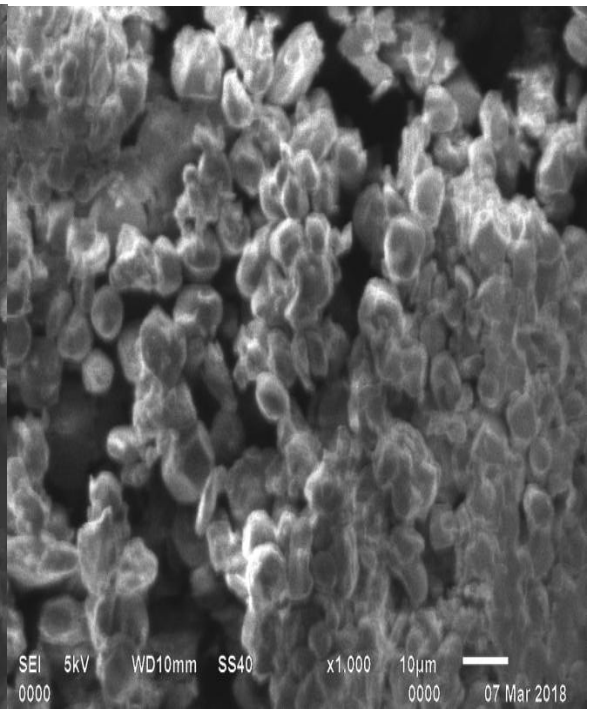
0000 0000 07 Mar 2018

G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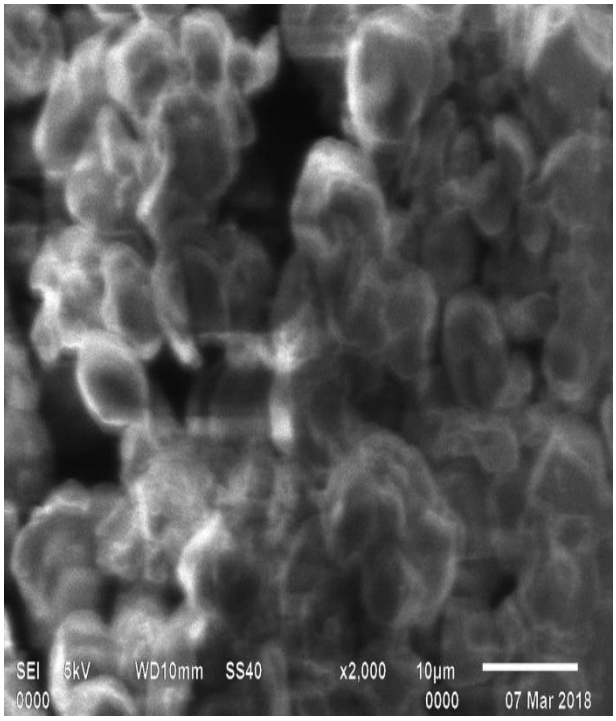
0000 0000 07 Mar 2018

H


SEI 5kV WD10mm SS40 x2,000 10µm
0000 0000 07 Mar 2018

I

Fig.3 Scanning electron microscope images of wheat (UP262[A B C] PBW343[D E F]) at 500X [A D], 1000X [B E] and 2000X [C F]

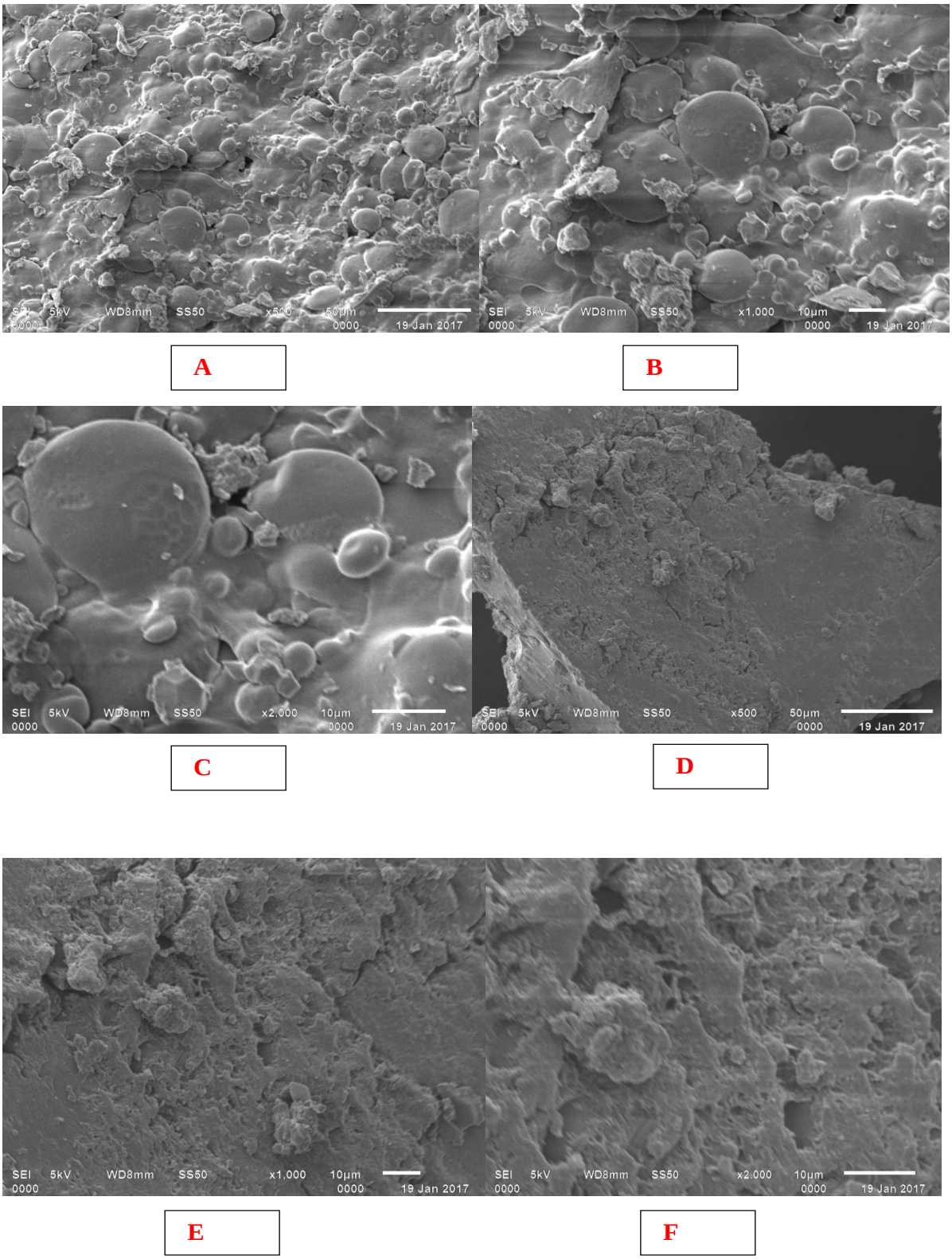


Fig.4 Scanning electron microscope images of rice (Pant Basmati 2 [A B C] Pant Dhan 19[D E F]) at 150X [A D], 300X [B E] and 500X [C F]

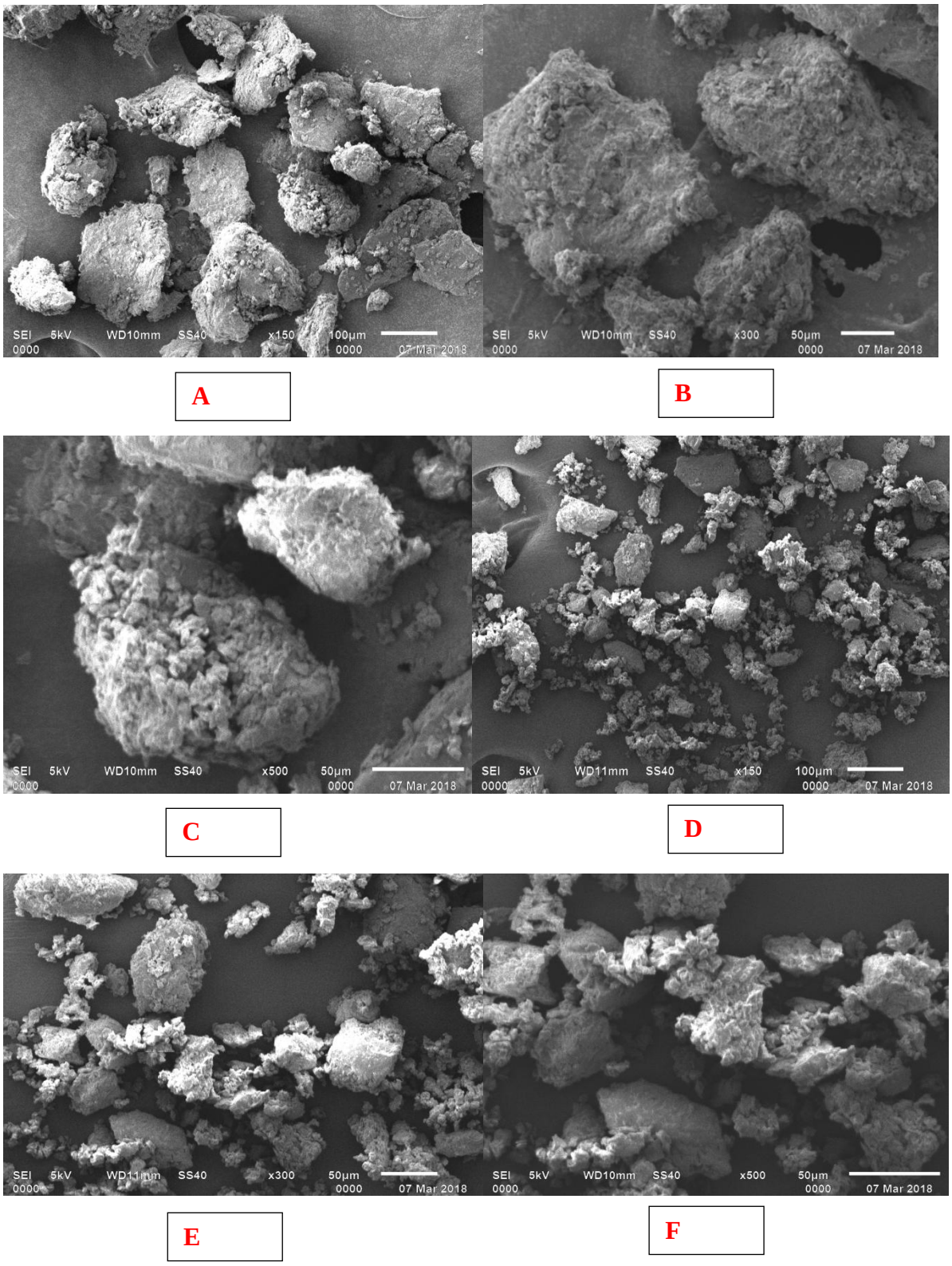


Fig.5 FTIR spectra of millets starch (Barnyard millet, Finger millet, Foxtail millet)

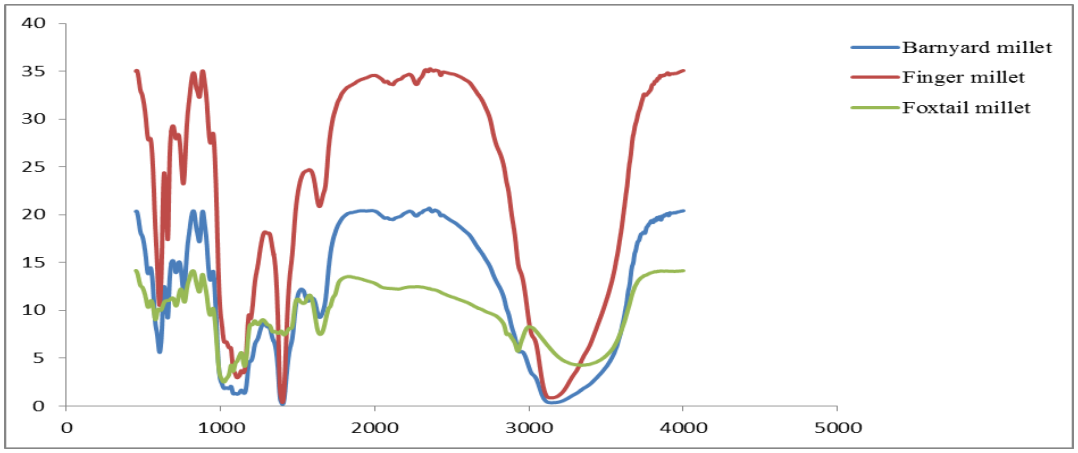


Fig.6 FTIR spectra of Wheat starch (UP262, PBW343)

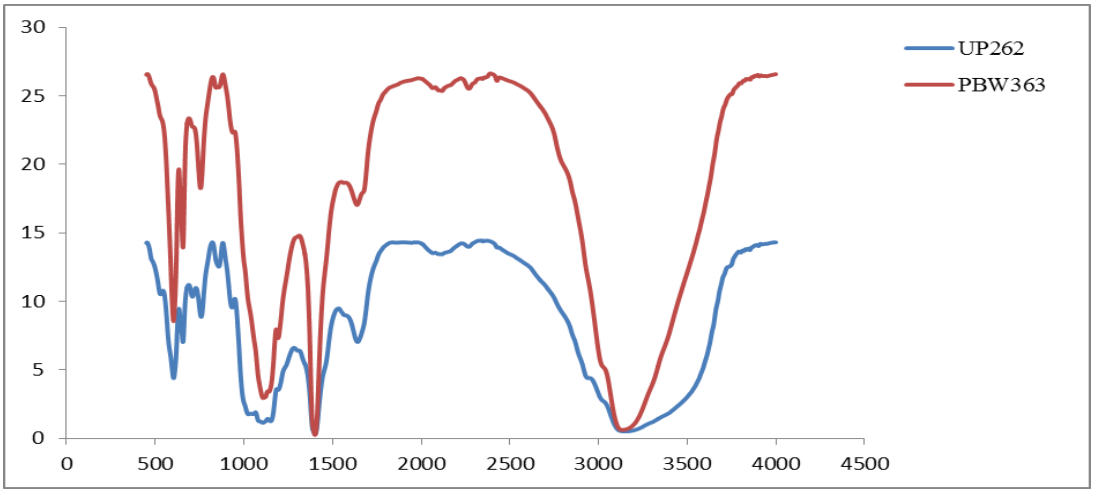
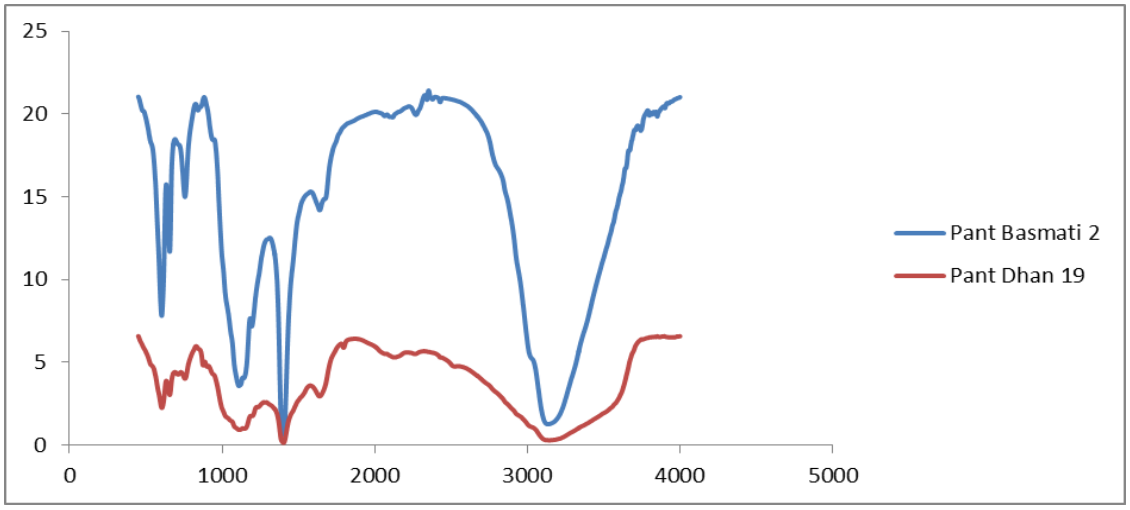


Fig.7 FTIR spectra of Rice starch (Pant Basmati 2 and Pant Dhan 19)



Karwasra *et al.,* (2017) observed that wheat contains generally bimodal size distribution of starch granule populations and has two types of size groups *e.g.* small granules (below 10–14 µm), large granules (above 10–14 µm to 36 µm) and the larger A-type wheat starch granules were generally disc like or lenticular while the smaller B-type granules were spherical and somewhat polygonal in shape. Similar spherical and irregular shapes for B-type starch granules were also observed by Zhang *et al.,* (2013). SEM also revealed that some starch granules were not intact and they were broken into half or more divisions. The damaged starch amount depends on the severity of grinding and the hardness of wheat grains (Hoseney, 1994).

FT-IR spectra of starch

The infrared spectroscopy is sensitive to structural changes on starch macromolecule, such as helicoidal chain conformation, crystallinity, retrogradation and water content (Kumar and Khatkar, 2017). Amir *et al.,* (2011) observed that the FTIR spectra of starch showed broad absorption between 3000-3600 cm^{-1} and 1500-1700 cm^{-1} due to stretching frequency of the –OH group and C–H group, respectively. The major absorption bands of starch observed in between 1000-1200 cm^{-1}, arising from C-O, C-C, and C-O-H stretching and C-O-H bending, were similar to absorption band observed by Warren *et al.,* (2016). The IR spectrums of wheat starches were described by three main regions, with maximum absorbance peaks near 3500–2400, 1700–1000, 1000–400 cm^{-1} (Yoo and Jane 2002; Jane *et al.,* 1994). The FTIR spectra of isolated starch from different cereals like millets (Fig. 5), wheat (Fig. 6), rice (Fig. 7) showed peaks at 3100-3400 and 2200-3000 cm^{-1} corresponding to O–H stretching due to hydrogen-bonded hydroxyl groups and the C-H deformation of the glucose unit, respectively, while the peaks at 1400.99 and 1366 cm^{-1} were attributable to the bending modes of H–C–H, C–H and O–H. The peaks at 1300–1000 cm^{-1} were attributed to C–O–H stretching with some attribute to C–C stretching. The peaks at 1193-1109 cm^{-1} were attributed to C–O–H stretching with some attribute to C–C stretching in PB2. A peak ranging between 840 $cm^{-1}$to 875 cm^{-1} was observed in all samples except PB2 was associated to C-H of residual carbon (Freile-Pelegrín *et al.,* 2007). These results were similar to starch characterization using FTIR by Correia *et al.,* (2012), Luo *et al.,* (2009) and Wu *et al.,* (2009). The peaks at 1191.93-1151 and 1080-1106 cm^{-1} were contributed to C–OH and CH_2 deformations. The bands at 1051.92 and 1022 cm^{-1} were associated with the ordered and amorphous structures of starches, respectively. The bands at 933.78–900 cm^{-1} were attributed to D-glucopyranosyl ring vibrational modes, 861 ± 10 cm^{-1} to the C–H absorbance of the D-glucopyranosyl rings and 759.52 ± 10 cm^{-1} to D-glucopyranosyl ring stretching. The 1641.94–1566 cm^{-1} bands were assigned to the bending vibration of O-H of water absorbed in the amorphous regions of starch. In the present study it was observed that the moisture content of finger millet was found higher followed by barnyard millet and foxtail millet. Similar results were presented by the Zeng *et al.,* (2011). Bands at 1053 cm^{-1} increases with the storage time due to the crystallization of starch chains (Good fellow and Wilson 1990). The absorbance ratios of 1047/1022 and 1022/995 cm^{-1} are the indexes of the short-range order of double helices (Sevenou *et al.,* 2002). IR bands at 1047 cm^{-1} and 1022 cm^{-1} are associated with ordered and amorphous structure of starch respectively (Kumar and Khatkar 2017). The ratio of absorbance 1047/1022 cm^{-1} was used to quantify the degree of order in starch samples. The absorbance ratios of 1047/1022 and 1022/995 cm−1are represented in Table 2. In the

present investigation the maximum short range order of helices was found in wheat (UP 262) followed by Pant Basmati 2. The short range order of helices was found similar in (Pant Basmati 2 and finger millet), (PBW 343 and barnyard millet). It was concluded that short range order of helices in cereals like millet, rice and wheat were approximately similar.

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