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COMPARISON OF PHYSICOCHEMICAL, THERMAL, PASTING AND MORPHOLOGICAL PROPERTIES OF WEED RICE AND RICE STARCHES

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ABSTRACT: A novel starch was isolated from weed rice crop and its starch properties were compared with rice starch. The starch properties studied were physicochemical, pasting, thermal and X-ray diffraction. The amylose content and swelling power (g/g) of weed rice starch was 13.7% and 19.8 g/g, respectively. Light transmittance (%) of starch paste from weed rice was observed higher as compared to rice starches. Pasting properties (PV, TV, SB, and FV) of weed rice starch was within the range of those earlier reported for rice starches. The transition temperatures (T_o , T_p and T_c) and enthalpy of gelatinization (ΔH_{gel}) were evaluated by using differential scanning calorimetry (DSC). The values of T_o , T_p and T_c were 68.86, 77.45 and 82.05 °C, respectively, for weed rice. ΔH_{gel} value for weed rice starch was 3.80J/g which was significantly lower than that of rice starches (8.09-13.81J/g). X-ray diffractions behavior for both weed and conventional rice starches exhibited a typical A-type pattern which is normally reported for cereal starches. Weed rice starch was also studied for their morphological properties by using a scanning electron micrograph (SEM). SEM of weed rice starch revealed the presence of pores on the surface and varying in size and shape of starch granules from small to large and angular to polyhedral, respectively.

INTRODUCTION: Red rice occurs as wild, weedy, or cultivated types with its kernels covered with dark or light-colored husk ¹. Both weedy and cultivated rice are evolved from wild *Oryza* species. Weedy rice of several *Oryza* species has been in existence, for many years in the vast areas of rice culture in Asia. Weedy rice can be defined broadly and generically as plants of the genus *Oryza* that infests and competes with rice and other crops.

Out of weedy rice, red rice reported being dominant ². The weedy rice is morphologically similar to the cultivated rice in plant and seed characteristics as they share a common gene pool with cultivated rice. Out of the various species of wild rice, the spices known to occur in India include *Oryza granulata*, *O. officinalis*, *O. rufipogon*, and *O. nivara*. *Oryza rufipogon* and *O. nivara* are red pigmented spices used as food and medicine in their native places of their cultivation ³.

The consumption of colored rice varieties is being related to improvement in human health. Due to their antioxidant potency and presence of phenolic compounds, they are classified as functional foods ⁴. Along with its antioxidant properties red rice also a good sources of carbohydrate (75%) and constitute around 28.5% starch ⁵.

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It is the most abundant polysaccharide after cellulose and is deposited in partially crystalline granules differing in molecular structure and morphology among and within plant species. Starch is the only qualitatively important digestible polysaccharide and has been regarded as nutritionally superior to low molecular weight carbohydrates⁶. It is mainly composed of two components: amylose and amylopectin which contribute about 98-99% of the dry weight usually named as “normal” starches; some starches contain high amylopectin levels (98-99%) and are known as “waxy” starches; and third group includes starches having higher percentage of amylose contents (50-70%)⁷. These two starch polymers mainly contribute an important functional role in various food and other industries such as binding and thickening agents, filling and gelling agents, clouding, emulsifier agent, as a dusting powder in cosmetics, for sugar coating in confectionary, expedient for pharmaceutical and photographic paper powder⁸. Nowadays, the interest in rice starch has increased due to its numerous characteristics: white color, easily digestible, hypoallergenic, odorless, and small granular form. These characteristics allocate various applications of starch, in food and non-food industries⁹. Extensive research has been carried out by various researchers on starch obtained from commercially important sources such as cereals, legumes, and tubers; however, no work is reported on starch from weed rice. Therefore, the present study was carried out to isolate starch from weed rice and study of its physicochemical, thermal, morphological and X-ray diffraction properties.

MATERIALS AND METHODS:

Materials: Weed rice was collected from the local market of Sirsa, India. All chemicals used are of analytical grade.

Starch Isolation: Starch was isolated from both weed rice and rice by following the alkali extraction method as described by Sodhi and Singh, (2003)¹⁰. Milled rice was steeped in 5 to 6 volumes of sodium hydroxide (0.2-0.3%) solution at 25 °C for 24 h to soften the endosperms. The steep liquor was drained off and the endosperms were ground lightly in successive small fractions with a mortar and pestle. The slurry was then diluted to the original volume with NaOH (0.2-0.3%).

The mixture was stirred for 10 min and allowed to settle overnight. The cloudy supernatant was drained off, and the sediment was diluted to the original volume with NaOH solution. The process is repeated until the supernatant becomes clear and gives a negative reaction to the biuret test for protein. Starch was suspended in distilled water, passed through a 100-200 mesh nylon cloth, and repeatedly washed with water until the supernatant no longer showed any pink color with the phenolphthalein. The slurry was centrifuged in wide-mouthed cup centrifuge (Remi, New Delhi, India) at 3000 rpm for 10 min and the upper non-white layer was scraped off. The white layer was re-suspended in distilled water and re-centrifuged 3-4 times. The starch was then collected and dried in an oven (NSW-143, New Delhi, India) at 45 °C for 12 h.

Physicochemical Properties of Starch:

Amylose Content: Amylose content of the isolated starches was determined by using the method of Williams *et al.*, (1970)¹¹.

Swelling Power (g/g): Swelling power was determined, in triplicate, using the method of Leach *et al.*, (1959)¹².

Light Transmittance (%): Transmittance of weed rice and rice starches were measured in triplicate, as described by Perera and Hoover, (1999)¹³. A 1% aqueous suspension of starch from each sample was heated in a water bath at 90 °C for 1 h with constant stirring. The suspension was cooled for 1 h at 30 °C. The samples were stored for 6 days at 4 °C in a refrigerator, and turbidity was determined every 24 h by measuring absorbance at 640 nm (Thermo Scientific, GENESYS 10S UV-VIS, Spectrophotometer).

Pasting Behavior: The pasting measurements were carried out using a starch cell of Modular Compact Rheometer (Model -52, Anton Paar, Austria) by following the method described by Thory and Sandhu, (2017)¹⁴. After sample loading in the starch cell, temperature was maintained at 50 °C for 1 min. It was then increased from 50 °C to 95 °C at a heating rate of 6 °C/min. The test temperature was kept at 95 °C for 2.7 min. Finally, it was decreased back to 50 °C at same rate. The sample was held at 50 °C for 2 min.

X-Ray Diffraction Analysis: X-ray diffractograms of the starch samples was obtained using an X-ray diffractometer (Philips, X'pert MPD high-resolution XRD, Almelo, Netherlands) with CuK radiation (Ni filter) at a target voltage and current of 40 kV and 40 mA, respectively. The scanning range and rate were 4° - 40° (2θ) and $4.0^{\circ}/\text{min}$, respectively.

Differential Scanning Calorimetry (DSC): Thermal transition properties of weed rice starch were measured by a model Q10 differential scanning calorimeter (TA Instrument, New Castle, USA). Starch (3.5 mg, dry weight) was loaded into a 40 ml capacity aluminum pan and distilled water was added with the help of Hamilton microsyringe to achieve a starch-water suspension containing 70 g/100 g water. Samples were hermetically sealed and allowed to stand for 1 h at room temperature before heating in DSC. DSC scanning was performed from 40°C to 100°C at a heating rate of $10^{\circ}\text{C}/\text{min}$ under dry nitrogen purge of 50 ml/min. An empty pan was served as a reference.

Morphological Studies: Morphology from weed rice starch was studied by using a scanning electron microscope (JEOL/EO model JSM-6390, JEOL, Tokyo, Japan) at 10.0 kV by using the method described by Kaur *et al.*, (2004). The dried starch power was evenly coated on a double-sided adhesive tabs mounted on aluminum stubs (Stiftproben-teller, Christine Groepel, Tulln, Austria). The mounted starch precipitates were coated with gold/palladium.

Statistical Analysis: The data reported in the tables were carried out in triplicate. The standard deviation (SD) value was calculated and the data

was subjected to one-way analysis of variance (ANOVA) using Minitab Statistical Software version 14 (Minitab, Inc., State College, USA).

RESULTS AND DISCUSSION:

Physico-Chemical Properties: Amylose content of starches has a significant effect on their functional and physicochemical properties, including pasting, gelatinization, retrogradation and swelling behavior¹⁵. Amylose content of rice and weed rice starches shown in **Table 1**. The amylose content of weed rice was found 13.77% while the amylose content of the rice starch was in the range. The amylose content of rice starch was in the range between 4.1-16.4%¹⁶. The variations in amylose content have been reported due to numerous reasons like the cultivar differences, methods of estimation and cultivation conditions (climate and soil conditions) of the crop^{17, 18, 19}.

The swelling power of starch from both rice cultivars and weed rice are reported in **Table 1**. Swelling power is an indication of the hydration capacity of starch in cooking conditions (90°C for 30 min). The swelling power of weed rice starch was in the range that was observed for rice starch. The swelling power of weed rice starch was $19.80 \pm 0.11\text{g/g}$.

Swelling power and solubility depends on amylose to amylopectin ratio, their structure, length of branching and distribution, and degree of granulation^{20, 21}. Swelling power and solubility provide evidence of the magnitude of the interaction between starch chains within the amorphous and crystalline domains and is influenced by the ratio of amylose to amylopectin²².

TABLE 1: AMYLOSE CONTENT (%), SWELLING POWER (g/g) AND THERMAL BEHAVIOR OF WEED RICE AND RICE STARCHES

Samples	Amylose content (%)	Swelling power (g/g)	T ₀ (°C)	T _p (°C)	T _c (°C)	ΔH _{gel} (J/g)	ΔT (°C)
Weed rice	13.7 ± 0.09	19.80 ± 0.11	68.86	74.45	80.05	8.40	13.19
Rice	4.10-16.40	17.02-38.8	61.1-75.76	66.91-79.21	71.93-84.59	8.09-13.81	7.85-10.89

Mean ± SD, n=3 T₀ = Onset Temperature, T_p = Peak temperature, T_c = Conclusion temperature, ΔH_{gel} = Enthalpy of gelatinization, ΔT = gelatinization range

Light Transmittance (%): Light transmittance of starch paste from weed rice and rice cultivars is illustrated in **Table 2**. The light transmittance of both starches (weed rice and rice) starch paste showed a gradual decrease during a period of storage at 4°C . Weed rice starch paste exhibited a

significantly higher light transmittance values as compared to rice starch pastes. Light transmittance value ranged from 18.92 to 7.57% for weed rice starch while it was observed 5.8-1.3% for rice starch pastes. The difference in the light transmittance values may be due to the variation in

amount of swollen granule remnants in the starches that refract light to different extent and thus give the distorted images²³. Paste clarity provides the

information on the behavior of starch paste when the light passes it and depends on the swollen and non-swollen granule remnants²⁴.

TABLE 2: LIGHT TRANSMITTANCE (%) OF WEED RICE AND RICE STARCH PASTES

Samples	0 day	1 day	2 day	3 day	4 day	5 day	6 day
Weed rice	18.92 ± 0.03	15.65 ± 0.06	14.10 ± 0.09	11.11 ± 0.00	09.89 ± 0.06	08.76 ± 0.03	7.57 ± 0.04
Rice	3.0-5.8	2.8-5.1	2.3-4.8	1.8-4.8	1.6-4.4	1.4-4.2	1.35-4.2

Mean ± SD, n = 3

Thermal Properties of Starches: Differential Scanning Calorimetry (DSC) is a widely applied technique for many thermal studies in starches that can give the information of transition temperatures during gelatinization and enthalpy change of starch (ΔH_{gel})²⁵. **Fig. 1** illustrated a typical DSC thermogram for weed rice starch that gives information of T_o , T_p , T_c , and ΔH_{gel} values and these parameters are enlisted in **Table 1**.

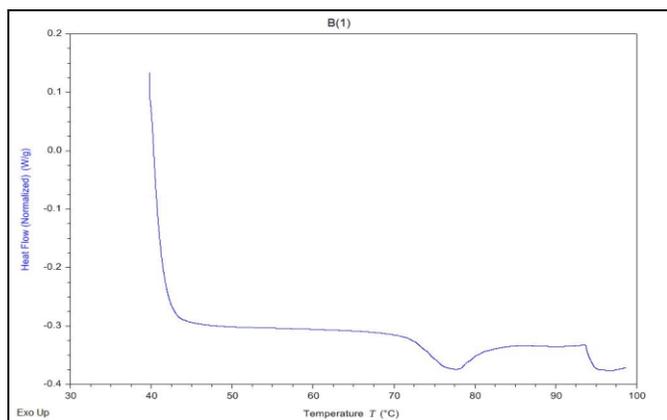


FIG. 1: DSC ENDOTHERMS OF GELATINIZATION OF STARCHES FROM WEED RICE STARCH

Non-significant ($P < 0.05$) difference was observed for transition temperatures among weed rice and rice starches. The values of T_o , T_p , and T_c of weed rice starch were 68.86, 74.45 and 80.05°C, respectively while these ranged between 61.1-75.76, 66.91-79.21 and 71.93-84.59°C for rice

starches. There was no significant difference in ΔH_{gel} value observed for weed rice and rice starches. The variation among the gelatinization temperature ranges may differ according to the botanical sources²⁶.

Pasting Properties of Starches: Pasting is a complex phenomenon which specifically refers to the changes in starch during post-gelatinization heating. It includes further swelling and polysaccharide leaching from the starch granules that increase viscosity due to the application of shear force²⁷. The pasting properties for the weed rice and rice starches were studied by using Rheometer. The results of the pasting properties are reported in **Table 3**. Peak viscosity (PV) is a measure of the water-holding capacity of the starch in terms of the resistance of swollen granules to shear and the swelling performance of granules²⁸. Weed rice starch showed PV, TV, and FV values 1111, 919 and 2296cP, respectively. The pasting properties of starches have been reported to be influenced by size, rigidity, amylose to amylopectin ratio and swelling power of the granules²⁹. The high setback values shown by starch make them unsuitable for food applications where low synthesis rate is required such as in frozen and refrigerated food³⁰. All these properties are known to influence the product texture and palatability³¹.

TABLE 3: PASTING PROPERTIES OF WEED RICE AND RICE STARCHES

Samples	PV	TV	SB	FV	PT (°C)
Weed rice	1111	919	1377	2296	80.0
Rice	1003 ± 20- 2438 ± 20	717 ± 13- 1786 ± 15	674 ± 3- 1761 ± 14	1495 ± 21- 3260 ± 50	71.55 ± 0.5- 79.9 ± 0.8

PV = Peak viscosity, TV = Trough viscosity, SB = Set back viscosity, FV = final viscosity, PT = Pasting temperature

X-Ray Diffraction: Assessment of the crystalline fraction in a variety of starches has been carried out with X-ray diffraction technique³². Analysis with XRD will generate diffraction peaks as the output, which can suggest the specified crystalline pattern in material¹⁴. The typical diffractogram of the

weed rice starch sample was presented in **Fig. 2**. On the basis of X-ray diffraction patterns; starch is of four types, *i.e.*, A, B, C and V³³. The X-ray diffractogram of weed rice starch shows typically A-type X-ray diffraction pattern, which is typically found in cereal starches. Typically, X-ray

diffraction of A-type starch shows first, second, and third peaks at 15, 18, and 23°, respectively. Weed rice starch showed strong reflections at 15 and 23° (2 θ) and an unresolved doublet at 17 and 18° (2 θ) which shows that this starch is of A-type. Singh *et al.*, (2006)¹⁶ and Kaur *et al.*, (2015)³⁴ had earlier reported A-type X-ray diffraction for rice starches. X-ray diffraction pattern is reported to depend on the starch origin as well as environmental growth conditions¹⁸.

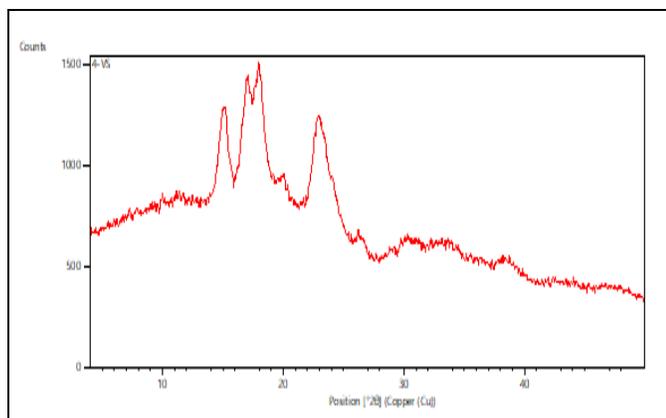


FIG. 2: X-RAY DIFFRACTOGRAMS OF STARCH FROM WEED RICE

Morphological Properties: The morphology of starch granules depends on the biochemistry of the chloroplast or amyloplast, as well as the physiology

of the plant³⁵. The scanning electron micrograph (SEM) of weed rice starch is shown in **Fig. 3**. SEM of weed rice starch granules showed that granules length ranged from 3.12 to 5.62 μ m (with an average value of 6.36 μ m) and breadth in the range from 1.63-6.52 μ m. The scanning electron microscopy of weed rice starch showed the presence of varying size and shape of starch granules from small to large and angular to polyhedral, respectively. Singh *et al.*, (2006)¹⁶ reported a similar variation in the shape and size of 17 varieties of rice starch granules. SEM images of weed rice starch showed the presence of pores on the surface of starch granules.

Lindeboom *et al.*, (2004)³⁶ attributed the differences in the size of starch granules to biological sources. Starch biosynthesis induces natural variability in amylose and amylopectin molecules, which may be responsible for granule diversity. Other factors that affect granule diversity may include the climate and agronomic processing conditions^{37,38}. Sizes and shapes of starch granules have impact in a number of physicochemical, functional and nutritional characteristics as larger granules were developed with high paste viscosity and small granules had higher digestibility³⁹.

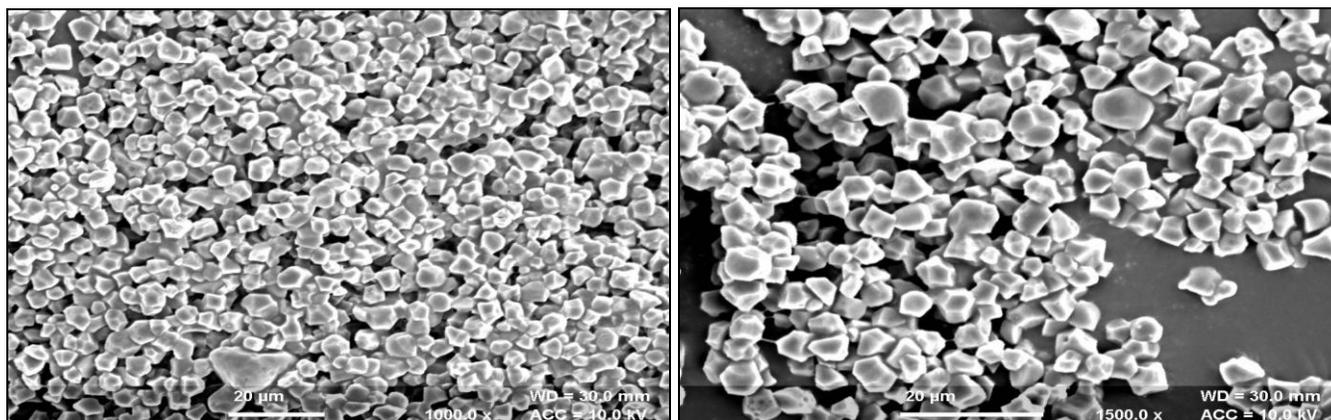


FIG. 3: SCANNING ELECTRON MICROGRAPHS OF STARCH FROM WEED RICE

CONCLUSION: Properties of weed rice starch were comparable to rice starches. Amylose content (%) and swelling power (g/g) of weed rice were within the range of reported values of rice starches; however, light transmittance (%) values of weed rice starch paste were found higher than rice starch pastes. Both the starches showed typical A-type X-ray diffraction patterns and presence of pores on the surface of starch granules with a polyhedral and

angular shape. Pasting temperature (°C) of weed rice starch was observed slightly higher than rice starches. The transition temperatures (T_o , T_p and T_c) of weed rice starch was also within the range of reported values of rice starches. Therefore, the study will improve the prospects of weed rice starch to be utilized as a starch source in food and non-food industries which at present depends mainly on corn, potato, and rice starches.

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