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Effect of dual modification on chemical composition, structural and pasting properties of black rice flour

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Abstract

Rice *(Oryza sativa L.)* is one of the most common cereal crop consumed as a staple food by more than 50 percentage of the world's population. The composition of rice varies depending upon the variety. The polished white rice is the highly refined version of raw rice. Colored rice varieties have been emerged recently as a potential functional food source because of the nutritional composition and phytochemical compounds. The present study dealt with Native White Rice Flour (NWRF), Native Black Rice Flour (NBRF), Dual Modified White Rice Flour (DMWRF) and Dual Modified Black Rice Flour (DMBRF) with the objective to evaluate effect of dual modification on chemical composition, structural and pasting properties of black rice flour. The dual modifications applied were enzymatic modification and Heat Moisture Treatment. The result shows that the effect of dual modification significantly reduced the nutrient composition of the native samples of both white and black rice flour ($P < 0.05$). All the samples had an A-type X-ray diffraction pattern (XRD). In both the native samples crystallinity percentage decreased after dual modification. Fourier Transform Infrared Spectroscopy (FTIR) patterns showed that similar functional groups were identified in both native and dual modified samples. From the findings of Scanning Electron Microscopy (SEM) analysis, it was observed NWRF granules were polyhedral in form and clustered, while the DMWRF granules were slightly degraded. NBRF had polyhedral shape and was densely packed with sharp angles, whereas DMBRF had slightly degraded granules with soft edges. The results of pasting parameters showed, due to effect of dual modification the final viscosity and setback decreased in both the native samples (NWRF and NBRF). It was evident in this study, the dual modification applied caused significant changes in the chemical composition, structural and pasting properties of sample variety.

Keywords: black rice, composition, dual modification, starch, structural properties

Introduction

Rice *(Oryza sativa L.)* is one of the most common cereal crop consumed as a staple food by more than 50 percentage of the world's population. The composition of rice varies depending upon the variety. The polished white rice, usually consumed, is the highly refined version of raw rice. The aspect of processing of raw rice take away significant parts of the grain namely the bran and the germ, leading to loss of nutrients such as B-vitamins, proteins, minerals and essential fatty acids. In this aspect, the coloured rice is considered as a healthier alternative to white rice. Usually the coloured rice varieties are either dehulled or partially hulled with the bran and germ intact. After the removal of husk, coloured rice still consists of few outer layers such as the pericarp, seed coat, the germ or embryo and the endosperm. Pigments are confined to the pericarp layer [1]. Colored rice varieties have been emerged recently as a potential functional food source because of the nutritional composition and phytochemical compounds. Black rice is packed with high level of nutrients and it is getting popular in recent years because of its nutritional composition and antioxidant properties. The dark colour of the black rice results from the high content of anthocyanins, a phenolic compound which is reported to possess remarkable antioxidant activities [2].

Rice flour obtained from different varieties exhibited variation in terms of proximate composition, total starch, amylose and amylopectin content, which affect the functional, thermal, pasting, cooking properties [3]. Starch is a chief storage constituent of rice grains and made up with macro molecules of amylose and amylopectin. Depending upon the levels of amylose the rice can be classified as waxy (0–2 %), very low (3–9 %), low (10–20 %), intermediate (20–25%) and high amylose rice starches (>25 %). In recent researches, enzymatic modifications have been adopted as partly replacing the chemical and physical methods for the preparation of modified starch [4]. One approach to enzymatic modification is to design a new structure, in which the molecular mass, chain length distribution and amylose/amylopectin ratio can be influenced by enzyme reactions, when the enzymes react with gelatinized starch. These techniques usually produce starches with altered chemical composition, physicochemical properties and modified structural attributes. The enzyme treatment also altered the gelation properties and decreased the viscosity of the rice flour depending on the treatment time, perhaps due to the changes in molecular weight distribution and branched chain length distribution [5, 6]. The enzymatic modification which was performed sequentially through the actions of α-amylase enzymes and amyloglucosidase showed, the enzymatic hydrolysis of the starch granules with α-amylase resulted in 4. 46% hydrolysis and more 8. 63% after the action of amyloglucosidase. Results proved that surface adsorption is more limiting than diffusion. The enzymatic hydrolysis process resulted in a reduction of the amylose content, as well as on chemical composition and phytochemicals. Due to enzyme modification, rice flour granule starches presented surface alterations and tendency to become spherical [7]. The effects of HMT on various aspects of rice starch properties have been reported by several researchers, whereas relatively little work has been done on chemical properties of rice flour. One of the method applied for physical starch modification is Heat Moisture Treatment (HMT). The hydrothermal treatment such as HMT, alters the physicochemical properties of starch by causing significant changes in its molecular structure. HMT technique heats granule at low levels of moisture (<35% slurry in water) and a relatively high temperature (80–140°C) but below the temperature when gelatinization occurs. HMT is considered to be natural and safe compared to chemical modification. HMT decreases starch solubility, swelling power, amylose leaching and peak viscosity, and increases the pasting temperature [8]. Application of the HMT process modified the pasting properties of rice flour and showed improved cooking and textural qualities. Apart from starch, in rice flours the other components, for example protein treated with HMT, will affect the thermal and pasting properties of rice flour [9].

The dual modification applied in this study were, Enzymatic modification and Heat Moisture Treatment (HMT). This study was carried out with the objective to evaluate effect of dual modification on chemical composition, structural and pasting properties of black rice flour.

Materials and Methods

Raw materials and sample preparation

In this study two varieties of rice (Oryza sativa L.) such as Samba -White Ponni and Black Kavuni rice were purchased from local organic shop at Tiruchirapalli, Tamil Nadu, India. White rice was used as control to compare black rice which was used as experimental sample. Each rice variety was ground separately in an analytical mill and then passed through 100 mesh (150µm) sieve. The rice flours were collected and stored in an air tight container at 4˚C. The samples were termed as Native White Rice Flour (NWRF) and Native Black Rice Flour (NBRF). The dual modified rice flour was prepared by applying the method [10] with slight modifications. Each rice flour variety were wet-milled to produce 10% (w/v) rice flour slurry. Then adjusted to pH 4. 5 with a 0. 1M sodium acetate buffer. The enzyme alpha-amylase (0. 2 g) was added into the flour. The solution was incubated at 55 °C for 24 hours in a shaking water bath. The solution was then centrifuged (3000g) for 10 min, the precipitate was washed twice with distilled water and collected by centrifugation. The precipitate was oven dried at 40 °C until the target moisture content (25%) was obtained. The samples were sealed in a screw-cap container and equilibrated at room temperature for 24 hours. The equilibrated containers were then placed in a hot air oven (100 °C) for 1 hour. Then the treated flour was taken out and dried in a hot air oven (40 °C) until 12% moisture content was obtained. The obtained samples were milled and sieved to a particle size of 100 mesh (150µm) sieve, sealed in an air tight container and kept at 4 °C. These samples were termed as Dual Modified White Rice Flour (DMWRF) and Dual Modified Black Rice Flour (DMBRF).

Estimation of chemical composition

Moisture content, ash, crude protein, crude fat and crude fiber were determined by using standard procedures. All measurements were carried out in triplicate. The moisture (method 925. 10), ash (method 923. 03), crude protein (N ×5. 95) (method 992. 23; the Kjeldahl method), crude fat (method 920. 85; the Soxhlet method) and crude fiber content (method 920. 86; by using automatic crude fiber analysis) of the rice flour samples were determined according to the AOAC (2000) [11]. The Total starch content was determined by using the “Total Starch Assay Kit” (AACC 76-13, 2001).

Determination of amylose

A simplified procedure [12] with minor modification was used to estimate amylose content. Rice flour(100 mg) was weighed, transferred into 100 mL volumetric flask, added with 1 ml 95% ethanol and 9 ml 1 N NaOH and left overnight. On the Subsequent day, distilled water was added to the samples to make up the final volume to 100 ml. The mixed solution of 5 ml from 100 ml was pipetted out into another 100 ml volumetric flask. 1N acetic acid (1ml) followed by 2 mL iodide solution was added, and the volume was made up to 100 ml. The content was stirred and allowed to stand for 20 min before absorbance was measured at 620 nm with a UV-spectrophotometer. Amylose concentration was obtained by plotting the absorbance in the potato amylose standard curve. Amylose content was expressed as a percentage of the total quantity of samples taken for analysis.

Determination of amylopectin

Amylopectin content [13] was calculated by applying in the following equation;

$$\text{Amylopectin (\%)} = (\text{Total starch} - \text{Amylose (\%)})$$

Structural Analysis

XRD: X-ray difraction

The X-ray diffraction pattern was carried out with an X-ray diffractometer (XRD) (X' Pert Pro MPD, Panalytical, Japan).

The rice flour samples were subjected to the aluminum sample holders. The X-ray source was Cu K α1 radiation (λ=0. 15405 nm) with an operating the voltage of 40 kV and a current of 30 mA. The diffraction data was collected over an angular range from 5 to 40° at a scanning rate of 0. 1° /min with a step size of 0. 05°. The percentage of crystallinity was computed by the following equation;

$$\text{Percentage of crystallinity} = \frac{\text{Area under peaks}}{\text{Total area}} \times 100$$

FTIR: Fourier transform infrared spectroscopy

Rice flour samples were analyzed using a Fourier transform infrared spectroscopy (FT-IR; Prestige 21, Shimadzu Inc., USA). Rice flour sample (2 mg, dry basis) was mixed with 200 mg of FT-IR grade potassium bromide (KBR) and pressed using a manual press for 20 min. The pellets were transferred into the FT-IR system. Each spectrum was recorded at a resolution of 4 cm^{-1} in a range of 500-4000 cm^{-1}.

SEM: Scanning electron microscopy

The sample morphology was analyzed by using SIGMA scanning electron microscope from CARL ZEISS. The sample was sprinkled on double-sided adhesive tape that attached to a circular specimen stub and coating with gold using a Baltzers SCD004 sputter coater. The samples were viewed and photographed at various magnifications.

Pasting properties

The pasting properties of the rice flour samples were determined using Rapid Visco Analyzer (RVA) equipment (Newport Scientific Pvt. Ltd., Narabeen, Australia). Three grams of the sample was weighed (weight adjusted to 12% moisture basis) and with 25 mL distilled water was blended. Then the rice-water slurry was transferred to the RVA Instrument. The pasting properties were measured on dry weight (dw) basis. The instrument was set at 50°C as the starting temperature and held at the same temperature for 1. 5 min. Then the slurry was heated to 95°C at the rate of 12°C per min while being maintained for 2 min at paste temperature. Then temperature is brought down to 50°C with the decreasing rate of 12°C per min and kept for 6 min at same temperature. The total processing time was about 17 min. The pasting properties of the samples such as peak, trough, breakdown, final, and setback viscosities were recorded.

Statistical analysis

The experiments related to chemical composition were performed in triplicates and the data obtained were expressed as mean ± SD. Comparison of means was performed by one-way analysis of variance (ANOVA) followed by Tukey's method, least significant differences were computed at with Tukey's multiple comparison test ($P<0. 05$) by using Graph Pad prism version 8. 0. 0. for Windows, Graph Pad Software (San Diego, CA, USA). Correlation was used to find out the relationship between amylose and total starch.

Results and Discussion

Chemical composition

Chemical composition of rice flour is shown in Table 1. Moisture content is used to determine the storage stability of flour. Low moisture content results in a longer shelf life and improved storage efficiency, less than 14% moisture, which meets the moisture content criteria for healthy rice storage [14]. In this study, the moisture content of the NBRF was found to be 13. 50 % and followed by DMBRF 12. 50%, NWRF 11. 62 % and DMWRF 8. 41%, respectively. Overall, the NBRF had significantly ($p \leq 0. 05$) higher moisture content than other samples. The amount of ash present in a food sample plays an important role while determining the levels of essential minerals. The ash content of NBRF was significantly higher ($p \leq 0. 05$) than the ash content of NWRF, DMWRF and DMBRF. The protein content of flour samples ranged from 7. 52 -10. 82 % ($P < 0. 05$), and was found to be higher in NBRF (10. 82 %). The obtained values are comparable with the previous study [15]. Moreover, different factors such as cultivar, environmental conditions and processing parameters significantly affect protein content. The protein content in rice will affect the rice texture produced. Rice with high protein levels are usually less tender (hard). In addition, the protein together with the gelatinization temperature also affects the cooking time. Rice with higher protein content requires more water and longer cooking time. Rice protein inhibits water absorption and the development of starch granules when it is cooked, thus limiting the ability to form gelatinization optimally. The lipid content in rice flour ranged from 2. 65% to 3. 24%, Protein and lipid contents affect pasting properties and starch gelatinization [16]. The fat content of rice flour samples ranged from 1. 05- 2. 82% (Table 1), and the highest value was observed in NBRF and followed by that in DMBRF (2. 82 and 2. 65% respectively). The crude fiber content was high in NBRF (2. 32 %). Dual modification significantly reduced the nutrient composition of native samples of both white and black rice flour. Total starch content in rice flour samples ranged from 62. 32 - 85. 10 % and NWRF contained more starch as compared to the other rice flours (Table 1). These results of total starch content are consistent with the previous studies on rice flour [17]. The components amylose and amylopectin are starch-forming glucose polymers. Amylose is composed of a long and unbranched D-glucose residual chain and connected by (α1→4). Whereas amylopectin has higher molecule weight and a lot of branches than amylose. The glycosidic chain that connects between glucose residues on the amylopectin chain is (α1→4), for branching point that occurs every 24 – 30 residue, connected by (α1→6) (Mir *et al*., 2013). Amylose content can influence overall cooking and pasting properties [18]. In this study, the amylose content of the different varieties ranged between 21. 80 and 32. 18%. The NWRF showed significantly lower amylose content than NBRF. In this study the percentage of amylose content in NBRF and DMBRF were 32. 18% and 30. 54% respectively. Whereas NWRF had 23. 80 % and DMWRF had 21. 80 %.

The highest percentage of amylopectin content was observed in both DMWRF. In both native rice flour samples, the amylose/amylopectin also showed decline after dual modification. Molecular, crystalline and granular differences affect the physiochemical as well as functional properties of the different cereal starches. These differences are closely linked to the ratio of amylose to amylopectin present in the starch granules [19]. The findings showed the effect of dual modification had significant impact on chemical composition of native flour. A high degree positive correlation was found between amylose and total starch (r = 0. 95).

Table 1: Chemical composition of native and dual modified rice flour

Chemical composition	NWRF	DMWRF	NBRF	DMBRF
Moisture (%)	11.62 ± 0.03^{c}	8.41 ± 0.01^{d}	13.50 ± 0.16^{a}	12.50 ± 0.07^{b}
Ash (%)	0.42 ± 0.01^{c}	0.40 ± 0.01^{c}	1.24 ± 0.06^{a}	1.01 ± 0.09^{b}
Protein (%)	7.52 ± 0.20^{c}	6.50 ± 0.20^{d}	10.82 ± 0.30^{a}	9.80 ± 0.30^{b}
Fat (%)	1.25 ± 0.21^{b}	1.05 ± 0.25^{b}	2.82 ± 0.04^{a}	2.65 ± 0.06^{a}
Crude fiber (%)	1.20 ± 0.10^{b}	1.20 ± 0.10^{b}	2.32 ± 0.40^{a}	2.02 ± 0.40^{a}
Total Starch (%)	85.10 ± 0.20^{a}	83.22 ± 0.21^{b}	65.20 ± 0.21^{c}	62.32 ± 0.02^{d}
Amylose (%)	23.80 ± 0.02^{c}	21.80 ± 0.01^{d}	32.18 ± 0.14^{a}	30.54 ± 0.18^{b}
Amylopectin (%) Amylose / Amylopectin	61.30 ± 0.18^{a} 0.38 ± 0.0006^{b}	61.42 ± 0.20^{a} 0.35 ± 0.0008^{b}	33.10 ± 0.07^{b} 0.97 ± 0.001^{a}	31.78 ± 0.16^{c} 0.96 ± 0.008^{a}

$^{a\text{-}d}$ Different letter superscripts in the same row indicate statistical difference ($p<0.05$)

Structural Properties

X-ray diffraction pattern

X-ray diffract meter was used for the analysis of crystal structure of starch. Fig. 1 depicts the X-ray diffraction patterns. All samples had an A-type X-ray diffraction pattern, with diffraction at doublet 15°, 17° and 18°, and 23° (2θ). This type of X-ray diffraction patterns were similar to the previous study [19]. Like most cereal starches, all physically and chemically modified black rice starches exhibit the typical A-type X-ray crystalline pattern. The crystallinity percentage of the samples were given in the Fig. 1, in both the native samples (NWRF and NBRF) the crystallinity percentage decreased after dual modification. It has also been described that the presence of non-starch components affected the structure and crystallinity of rice flours [21]. The white rice (3. 0%) and intermediate-amylose rice (20. 1%–21. 8%) had the same A-type diffraction pattern at 15. 0°, unresolved doublet at 17. 0° and 18. 2° [22]. It could be observed that NBRF displayed higher crystallinity (35. 88%) and it also depends on amylose content. Also decrease in percentage of crystallinity was observed in both white and black rice flour due to effect of dual modification. Decrease in the crystallinity of starch sample due to HMT was observed in previous study. This could be attributed to the destabilization of starch granules. A reduction in the relative crystallinity of starch after HMT, who attributed this to the disruption of amylopectin crystallites [23, 24].

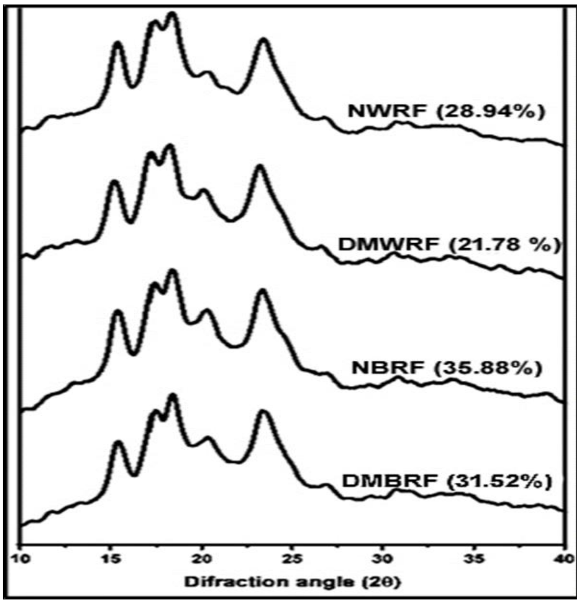


Fig 1: X-ray diffraction patterns of in native and dual modified white and black rice flour

FT-IR patterns

Fig. 2 depicts the FT-IR spectroscopy patterns of native and dual modified white and black rice flour. FT-IR spectroscopy was used to monitor changes in the structure of the starches promoted by dual modification by analyzing the frequency and the intensity of the peaks. Infra-red spectra showed that similar functional groups were identified in rice flours and rice starch [25]. Both native and dual modified samples of white and black rice flour showed similar frequency and the intensity of peaks (3371 – 3416 cm^{-1}) which was assigned to the functional groups of medium O-H stretching with strong, broad intermolecular bond and at 2925-2931 cm^{-1}, which could be attributed to medium O-H stretching with weak, broad intermolecular bond. The band at 1020 cm^{-1} was attributed to amorphous region of the molecules presented in the rice flour which was unchanged after modification. Moreover, there was an increase in intensity of band at 1650 cm^{-1} in both modified starches compared to the respective native starches. The peak of starch at 1650 cm^{-1} was assigned as C-O-C stretching, which could be attributed to the water associated to starch molecules. The increase of this band in modified starches is the result of higher affinity to water as compared with native starches. These results were in accordance with the earlier reports [26].

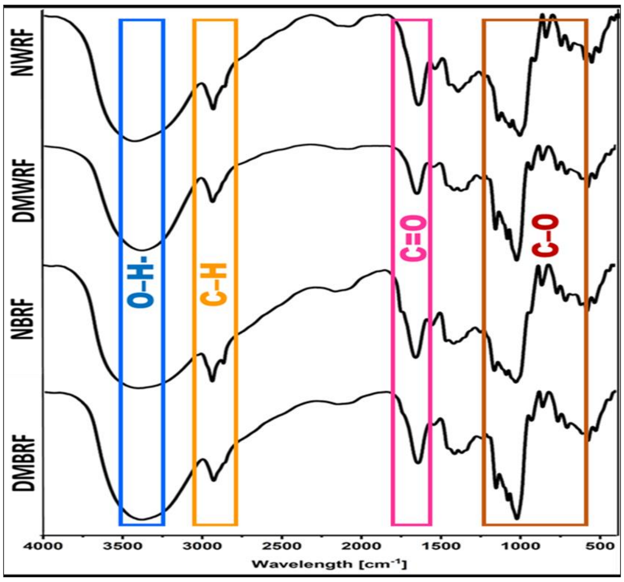
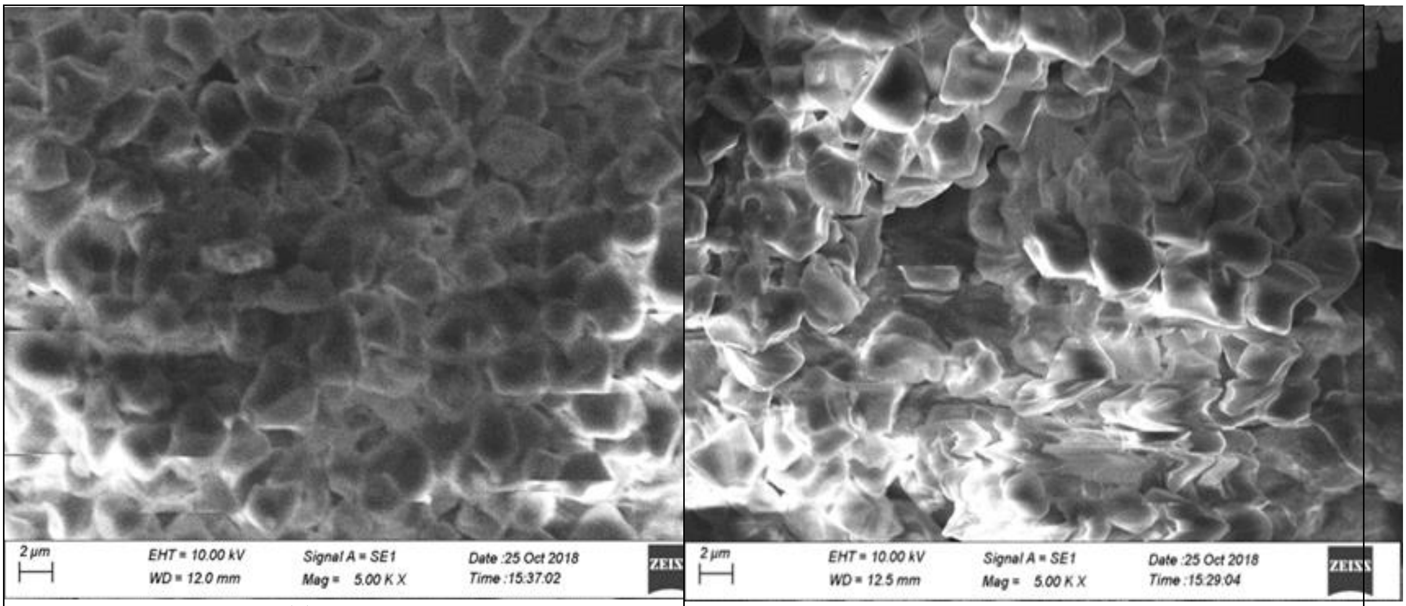


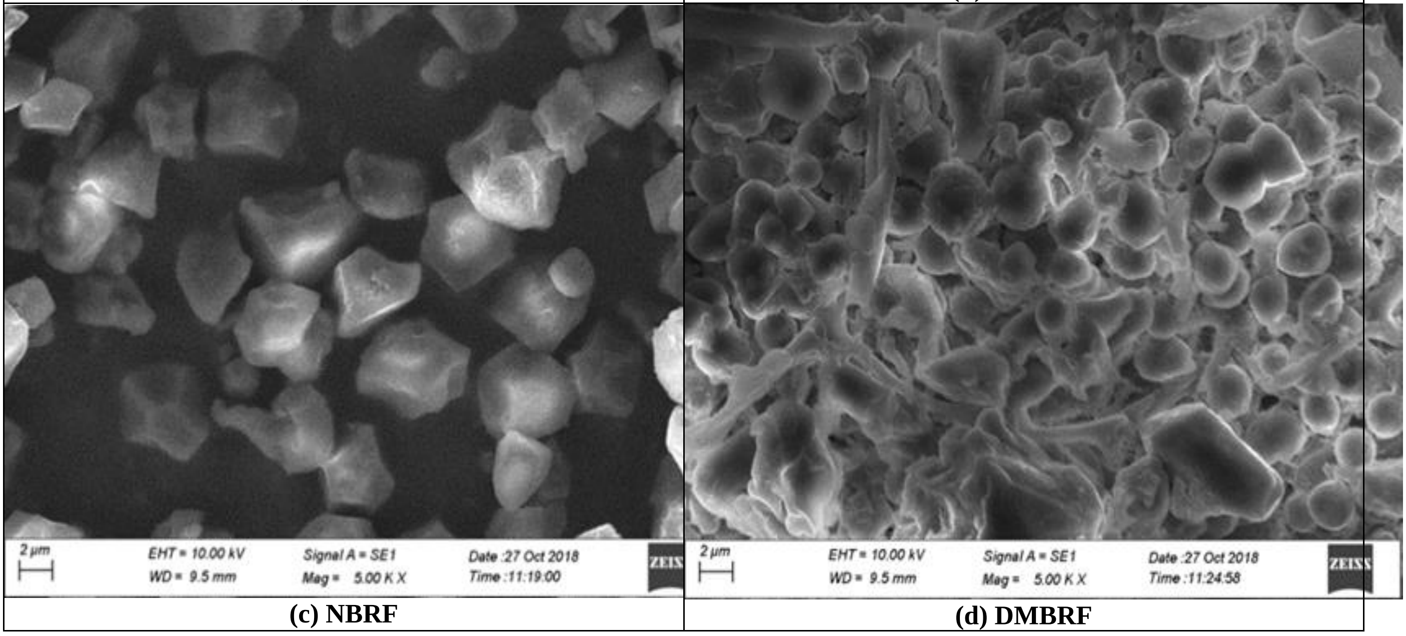
Fig 2: FT-IR patterns of native and dual modified white and black rice flour

SEM analysis

Heat moisture treatment affected the degree of agglomeration of the starch granules. Due to impact of HMT, granules lost their integrity causing flatter and smother surface than the native ones. This effect was pronounced in lower amylose rice when compared to higher amylose sample. It has been reported that the HMT slightly affected the format and degree of agglomeration, making the surface of the granules more irregular, as compared with the native starches [27, 28]. The SEM micrographs are given in the Fig. 3. NWRF granules were polyhedral in form and clustered, while the DMWRF granules were slightly degraded. NBRF had polyhedral shape and was densely packed with sharp angles and edges without pores on the smooth surface, whereas DMBRF had slightly degraded granules with soft edges. Enzyme hydrolysis and heat moisture treatment had a significant impact on the morphology of rice starch granules. The cluster formation of starch granules had been reported after HMT [29]. Native rice starch granules were small in size and irregular in shapes with sharp angles and edges. After modifications, rice starch granules surface lose natural morphology. All native and modified rice starch exhibited the typical A-type X-ray patterns of cereal starches.



(a) NWRF **(b) DMWRF**



(c) NBRF **(d) DMBRF**

Fig 3: Scanning electron micrographs of native and dual modified white and black rice flour

Pasting properties

The pasting properties of rice flour samples are displayed in Fig. 4. High final and set back viscosity were exhibited by NWRF (941. 00 and 635. 00 cP respectively) and followed by that NBRF (663. 00 and 361. 00 cP respectively) (Table 2). This is similar to earlier study reports, that white waxy rice starches had higher swelling volume, resulting in higher viscosity than other rice. Differences in the viscosity parameters have been attributed to the characteristics of the starch granules (amylose/amylopectin ratio, chain length distribution of amylopectin). The native rice flour had highest peak viscosity, but there were significant differences between rice flour varieties. The pasting parameters of rice flour show its pasting performance during cooking and cooling. Peak viscosity reflects the swelling ability of starch during heating. Due to HMT, denaturation of proteins in rice flour exhibited an increase in their surface hydrophobicities, which would retard the swelling of HMT starch granules in rice flour. Also due to HMT, the protein in rice flour played a significant role resulting in a lower pasting viscosity [29, 30].

Table 2: Pasting parameters of native and dual modified rice flour

Sample	Peak (CP)	Trough (CP)	Breakdown (Cp)	Final (CP)	Setback (CP)	Peak time (min.)	Pasting temp. (°C)
NWRF	465.00	306.00	159.00	941.00	635.00	7.00	94.95
DMWRF	178.00	126.00	52.00	378.00	252.00	7.00	96.00
NBRF	416.00	302.00	114.00	663.00	361.00	7.00	94.85
DMBRF	40.00	33.00	7.00	57.00	24.00	6.93	95.00

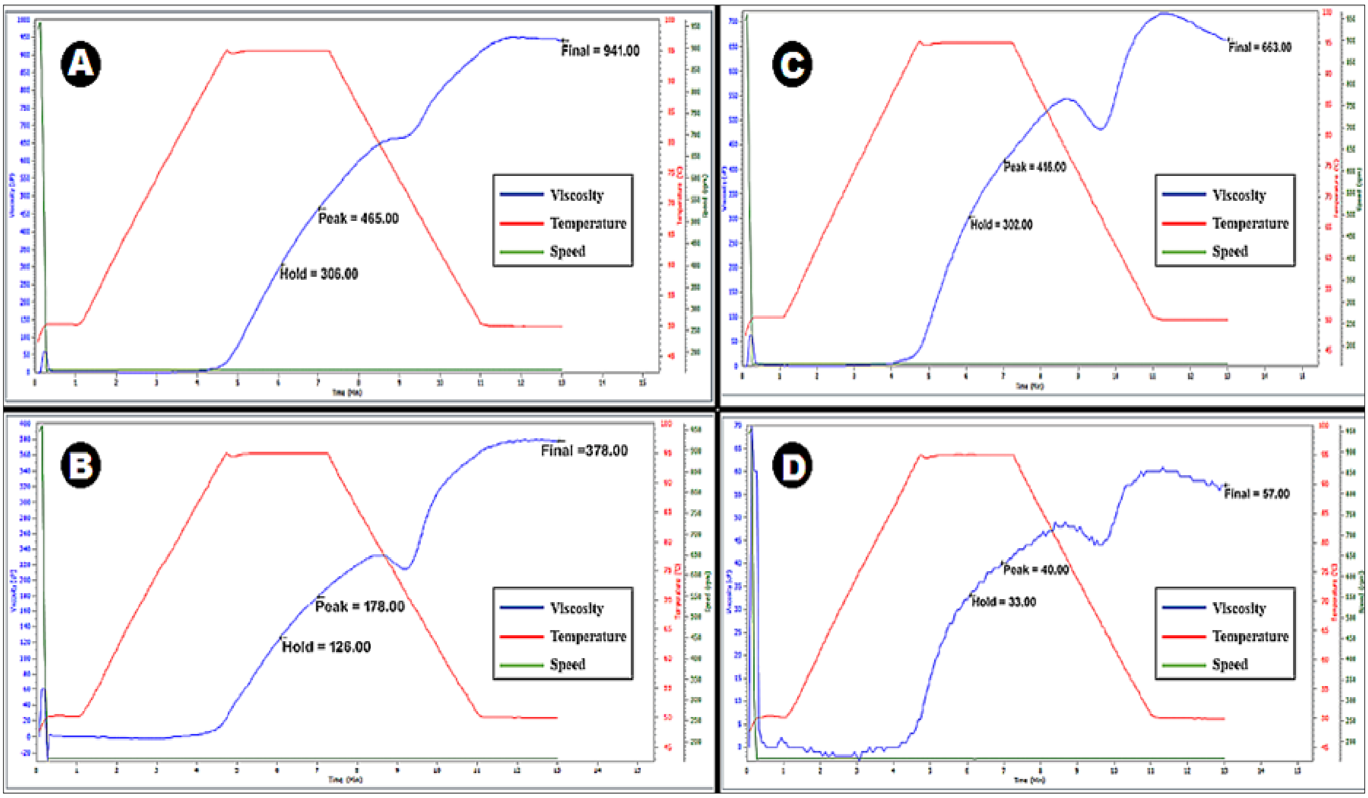


Fig 4: Pasting parameters (A) NWRF, (B) DMWRF, (C) NBRF and (D) DMWRF

Conclusion

Differences were found in the chemical composition, structural and pasting properties of NWRF and NBRF. The differences were also noted due to effect of dual modification.

The results indicated that NBRF had higher moisture, protein, fat, crude fiber and amylose content as compared to the NWRF. In both NWRF and NBRF there were reduction in the chemical composition due to effect of dual modification applied in this study. An A-type X-ray diffraction pattern was exhibited by all the samples. It was also noted, after dual modification decrease in the percentage of crystallinity was observed in both native rice flour samples (NWRF and NBRF). Also dual modification applied had a stronger influence on starch components which attributed decrease in pasting parameters. The impact of dual modification had drastic change in the viscous properties of rice flour samples. Screening of the properties of rice flour is an important quality evaluation parameter for food industry applications. Results of this study could lead to a better appreciation of black rice flour for food product development and food.

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Conflict of interest statement

We declare that we have no conflict of interest

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