Chapter 4

Characteristics of rice starches modified by single and dual heat moisture and osmotic pressure treatments

4.1. Introduction

Rice starch is the smallest starch with a mean size of 3-8 μm and polygonal shape. Native starch exists as discrete and semi-crystalline granular form, and typically consists of linear chains of amylose and branched chains of amylopectin [31], and their ratio may vary when exposed to different modification processes. The semicrystalline radial growth rings in starch granules are made up of crystalline and amorphous lamellae with amylopectin double helices in the former and amylopectin branch points with amylose molecules in the latter. This produces a highly ordered structure in the starch granule that can be degraded by specific enzymes, chemicals, or high temperature [7], and the disruption of the ordered structure brings about many changes in the starch functionality.

In Chapter 3C, HMT and OPT for rice starch were optimized. HMT and OPT cause many changes during the modification process that are not limited to the physicochemical properties of starch. HMT and OPT have been reported to enhance paste stability of starch at high temperature, reduce peak viscosity, breakdown and increase final viscosity in sago starch [55]; increase gelatinization temperature, increase amylose: amylopectin ratio after modifications in potato starch [56]; decrease crystallinity [8, 43, 52, 57]; cause changes in granule morphology and rheological properties [22]; and increase resistant starch (RS) content [27, 63].

Recently, improvement in starch characteristics by dual modification have gained novel starch modification and bring new functionalities. The combination of several technologies can potentially influence the outcomes, yet occasionally those outcomes may be unpredictable. Dual modification of starch is done to change the properties of single modified starch further by using the same or different ways of modification in an attempt to improve the functionality of single modified starches [4]. Dual modified starch by HMT with other chemical and physical modification methods have been reported [14-15]. However, the impact of OPT and HMT as a combined process on the physicochemical and other properties of rice starch have not been reported so far. Therefore, the goal of the current research was to elucidate how OPT, HMT single modifications, and HMT-OPT/OPT-HMT dual modifications affected the pasting, thermal, rheological, morphological, crystalline, and in-vitro digestibility of rice starch.

4.2. Materials and methods

4.2.1. Starch isolation

Starch was isolated from medium broken rice of *Ranjit* variety of Assam by alkaline steeping method as described previously in the section 3C.2.1. of Chapter 3C.

4.2.2. Single modified starches

4.2.2.1. Heat moisture treatment of starch

Heat moisture treated starch (HMTS) was prepared under the previously optimized process condition as a result of the study in Chapter 3C. Moisture content of rice starch (100 g) was adjusted to 29% by adding calculated amount of distilled water. The moistened starch was equilibrated overnight before being heated in a hot air oven at 111 ^{o}C for 45 min. Finally, the starch sediment was dried at 40 ^{o}C for 24 h, powdered with a mortar and pestle, and sieved through 150 μm sieve, packed in an airtight glass container and kept in a refrigerator until further analysis.

4.2.2.2. Osmotic pressure treatment of starch

Osmotic pressure treated starch (OPTS) was prepared at the optimal process condition obtained in the Chapter 3C investigation. Hundred gram of sample was mixed with 200 ml of saturated sodium sulfate solution. Rice starch and salt solutionmixture was heated at 117^{o}C for 35 min. Starch samples were washed with distilled water several times and centrifuged after each wash to remove the residual salt. Presence of saltresidue was tested by 0.1 M Barium Chloride solution. Starch sediment was dried at 40 °C for 24 h, ground using mortar and pestle, sieved through 150 μm sieve, packed in an airtight glass container and kept in a refrigerator until further analysis.

4.2.3. Dual modified starches

Rice starches modified at optimized process conditions of OPT and HMT were subjected to dual modification to obtain two types of dual modified rice starches as follows:

4.2.3.1. HMT-OPT starch

Fifty gram of rice starch was first modified by HMT followed by OPT according to the methods as described above in the section 4.2.2.1 and 4.2.2.2 respectively. The resultant modified starch was named as HMT-OPT starch (HMT-OPTS).

4.2.3.2. OPT-HMT starch

Fifty grams of rice starch was first modified by OPT followed by HMT as per the procedures described above in the section 4.2.2.2 and 4.2.2.1 respectively. The resultant modified starch was named as OPT-HMT starch (OPT-HMTS).

4.2.4. Chemical composition and functional properties

Fat, protein, ash, and amylose content of the native rice starch were determined a per the methods described in the section 3A.2.6. in Chapter 3A. The sodium content of native and modified starches was measured according to the procedure is given in section 3C.2.2. in Chapter 3C. SP and SOL were determined as per the method described in the section 3A.2.7.3. (Chapter 3A).

4.2.5. Pasting properties and gel texture

The pasting profile of modified rice starches and gel texture of the RVA gels were evaluated as per the method described in Chapter 3A, section 3A.2.8.

4.2.6. Morphological observations

4.2.6.1. Polarized light microscopy

Native starch and the modified starches were dispersed on glass slides using distilled water followed by covering with a cover slip. Slides were viewed under polarized light microscope (Olympus BX51, Japan) (magnification 1000X, 20 μm scale) equipped with real-time view and camera.

4.2.6.2. Scanning electron microscopy (SEM)

The surface morphology of starch granules was observed using a field-emission scanning electron microscope (Sigma 300, ZEISS, Germany), operated at an acceleration voltage of 5kV. Starch sample was fixed on SEM stub with adhesive tape and coated with gold. Representative micrographs of the rice starch granules were obtained at magnifications 5000X, 3 μm scale.

4.2.7. Fourier Transform Infrared Spectroscopy (FTIR)

The compressed alkali metal halide pellet method was utilized to determine the functional groups present in rice starch samples using FTIR spectroscopy [26]. To obtain a homogeneous mixture, 0.1 g starch sample was ground with 0.1 g anhydrous KBr in a crucible. In a hydraulic press, the mixture was pressed to form a translucent pellet. In an

Infrared Spectrum, the pellet was scanned at an infrared absorption area of 450-4000 cm^{-1} (IMPACT I-410, Nicolet, USA).

4.2.8. Wide-angle X-ray Diffraction (XRD)

The wide-angle XRD data of the starch samples were recorded using a D8 Focus and Minifiex X-ray diffractometer (Bruker AXS, Germany) that was operated at 40 kV and 40 mA with CuKα radiation (λ=0.1542 nm). X-ray difractograms were obtained at the Braggs angle (2θ= 5^o to 40^o) with a step size of 0.033^o and a step rate of 0.5s per step. The relative crystalinity (RC) of each of the starch samples was calculated in OriginPro 8.5 software [72] using the formula given below,

$$Relative\ crystallinity\ (\%) = \frac{Area\ under\ the\ peaks}{Total\ area} \times 100 \qquad \text{Eq. (4.1)}$$

4.2.9. Thermal properties

Differential Scanning Calorimeter (DSC, NETZSCH, Germany) was used to evaluate the thermal characteristics of native and modified starch [72]. Briefly, 3 mg starch was weighed in an empty aluminum pan (30 µl), 12 µl distilled water was added to it using a micropipette and mixed using a needle. Pan was hermetically sealed and equilibrated at 4 ^oC for 24 h. Samples were heated from 2 5^oC to 150 ^oC at 10 ^oC/min. An empty pan was used as the reference. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures of gelatinization of dry starch were recorded.

4.2.10. Rheological properties

Rheological properties of the rice starch gels were determined with a controlled stress rheometer (MCR72, Anton Par, Austria) using a parallel plate geometry (50 mm diameter) with slight modification in the method as described elsewhere [51]. Starch suspension (10%, w/w) was cooked in boiling water bath for 30 min with occasional stirring to prevent agglomeration. Pre-cooked paste was transferred onto the ram of rheometer and excess sample was trimmed off. Dynamic oscillatory tests for frequency sweep tests were performed at 25 ^oC and 0.5% strain (in the linear viscoelastic region) was kept constant with a gap of 1 mm. The prepared starch gels were scanned at angular frequency range of 0.06283 rad/s to 62.83 rad/s (0.01-10 Hz). Experimental data of storage modulus (G'), loss modulus (G''), complex viscosity (η*) and phase shift angle (**δ**) were obtained.

4.2.11. *In vitro* starch digestibility

The native and modified rice starches were analyzed for rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) fractions through *in vitro* starch digestibility assay according to the method of Englyst et al. [25] and Vanhung et al. [68] with some modification. Briefly, 300 mg (db) of each starch sample was weighed in a 50 ml centrifuge tube and 25 ml of 0.1 M sodium acetate buffer (pH 5.2) was added. The tubes were vortexed vigorously before placing in boiling water bath for 30 min with occasional shaking. The tubes were transferred to water bath operated at 37 ^{o}C for equilibration (15-20 min). Then, 5ml enzyme solution containing α-amylase (1400 U/ml) and amyloglucosidase (13 AGU/ml) were added to each tube and incubated at 37 ^{o}C in a shaking water bath. Exactly after 20 min, 0.5 ml aliquot of hydrolyzed solution was removed and added to 4 ml 80% ethanol in a centrifuge tube and analyzed for glucose content (G_{20}) by phenol sulphuric acid method. Again, after 100 min (total digestion time 120 min), 0.5 ml hydrolysate was taken out and analyzed for glucose content (G_{120}) in the similar way as above. The remaining digested residue was vortexed vigorously followed by boiling water incubation for 30 min. The contents were mixed manually, cooled to 0 ^{o}C before adding 10 ml 7 M potasssium hydroxide and held at 0 ^{o}C for 30 min with occasional shaking. Thereafter,1 ml digested sample was added to 10 ml 0.5M acetic acid in a centrifuge tube and mixed well, and 0.2 ml amyloglucosidase solution (50 AGU/ml) was added, mixed, and incubated at 70 ^{o}C for 30 min and then at 100 ^{o}C for 10 min. Tubes were cooled to room temperature and 40 ml water was added and analyzed for total glucose concentration (TG). RDS, SDS and RS were calculated by using the data of G_{20}, G_{120} and TG as follows,

$$RDS = G_{20} \times 0.9 \quad \text{Eq. (4.2)}$$

$$SDS = (G_{120} - G_{20}) \times 0.9 \quad \text{Eq. (4.3)}$$

$$RS = (TG - G_{120}) \times 0.9 \quad \text{Eq. (4.4)}$$

4.2.12. Statistical analysis

Using the statistical software program SPSS 20.0 (SPSS Incorporated, Chicago), one-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to evaluate the data. Statistical significance was defined as $p < 0.05$. Results are presented as the average value ± standard deviation of triplicate experiments.

4.3. Results and discussion

4.3.1. Chemical composition and functional properties

Table 4.1 presents the results of chemical composition of native and modified rice starches. It can be seen that there was no significant difference in the protein, fats, ash and sodium content between the starches. The results of sodium content confirmed the absence of sodium sulphate residue in native (2.6 mg/100g) and modified starches (2.6-2.7 mg/100 g) as there is no statistical difference in their values [55-57]. Pukkahuta et al. reported no significant change in sodium content of OPT potato starch which had sodium content of 101.8 mg/kg and 105.3 mg/kg before and after treatment respectively [56]. Any food item containing less than 5 mg/100 g sodium content is considered as sodium free.

Table 4.1 Chemical composition of native and modified starches.

Starch samples	Protein (%)	Fat (%)	Ash (%)	Sodium content (mg/100 g)
NRS	0.1 ± 0.01^{a}	0.04 ± 0.01^{a}	0.17 ± 0.05^{a}	2.6 ± 0.05^{a}
HMTS	0.1 ± 0.01^{a}	0.04 ± 0.01^{a}	0.17 ± 0.05^{a}	2.6 ± 0.04^{a}
OPTS	0.1 ± 0.02^{a}	0.03 ± 0.01^{a}	0.18 ± 0.05^{a}	2.7 ± 0.01^{a}
HMT-OPTS	0.1 ± 0.02^{a}	0.04 ± 0.01^{a}	0.19 ± 0.04^{a}	2.7 ± 0.02^{a}
OPT-HMTS	0.1 ± 0.02^{a}	0.03 ± 0.01^{a}	0.18 ± 0.03^{a}	2.7 ± 0.05^{a}

Results are represented as mean ± SD, Significant differences ($p \leq 0.05$) exist between the mean values with different superscript in a column.

Amylose content significantly increased after HMT and OPT (Fig 4.1a). Increase in amylose content after HMT [20, 56-57] and OPT [27, 39] have been reported. HMT can cause changes in amylose and amylopectin conformations in the starch granule in a dissimilar approach and interaction between them might occur due to their different molecular architecture and arrangement of amylose and amylopectin [38]. The increased amylose content may be due to the amylopectin's hydrolysis and depolymerization during heating, which allows longer linear segments of starch to be exposed more effectively. However, the increase in amylose content during OPT was lower than HMTS. This could be due to less amount of free water that limits the gelatinization during OPT, thus lesser number of broken amylopectin chains are formed [49]. However, Fasuan at el. reported depolymerization or debranching of α 1-6 glycosidic bonds during OPT that led to increase in amylose content [27]. The dual modified starches showed greater increase in amylose content than single modified starches (Fig 4.1a).

The results presented in the Fig 4.1b showed significant decrease in SP of HMTS, OPTS, HMT-OPTS and OPT-HMTS. Decrease of SP in HMT indicates the inhibitory effect of amylose to granule swelling [57, 66]. The molecular weight, branching pattern, and chain-length distribution of amylopectin were the primary determinants of the swelling behavior of starch [17, 66]. The reorganization of starch chains and the repositioning of their linkages induce this decrease in SP. HMT also increases the granule's rigidity and heat resistance, resulting in a more hydrophobic granule [28, 50]. The amorphous regions containing amylose and non-ordered amylopectin branches are more susceptible to undergo change during OPT [57] and most probably have strengthened the intermolecular hydrogen bonding that led to reduced starch hydration. The reduction in SP of OPT starch is associated with the presence of SO_4^{2-} ions during treatment that inhibited the water penetration into granules [48, 56]. Presence of higher amylose content is also responsible for decrease in the swelling power. The severity of both modification methods must have promoted further reduction in SP in HMT-OPTS and OPT-HMTS which might also have been affected by their higher amylose content and decreased crystallinity.

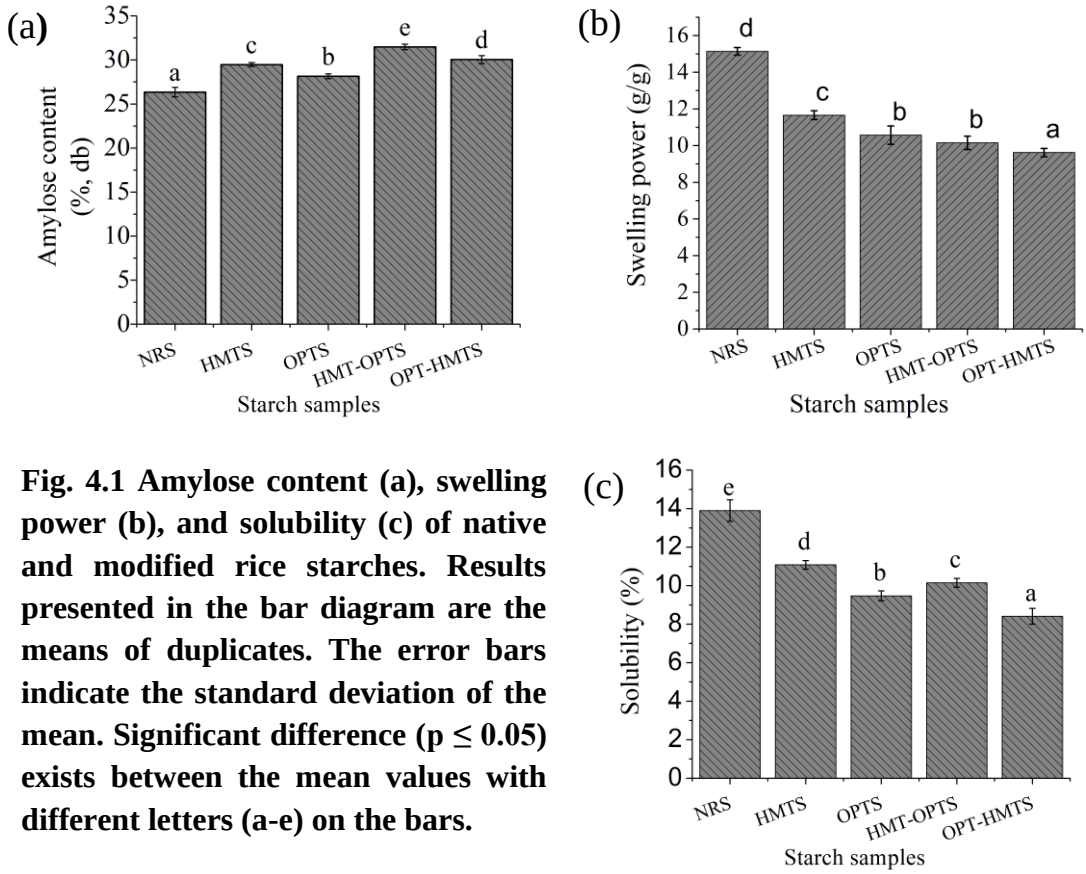

Fig. 4.1 Amylose content (a), swelling power (b), and solubility (c) of native and modified rice starches. Results presented in the bar diagram are the means of duplicates. The error bars indicate the standard deviation of the mean. Significant difference ($p \leq 0.05$) exists between the mean values with different letters (a-e) on the bars.

Similarly, SOL decreased significantly during single and dual modification of

starches (Fig 4.1c). At lower temperatures of treatment, smaller amylose fractions release, whereas larger fractions leach at higher temperatures. Decreased SOL during single and dual modification indicates better molecular stability in the modified starches. Formation of amylose-amylose, amylose-amylopectin and amylose-lipid inclusion complexes that develop newer polymorphism could be considered another aspect for lower SOL values [75]. Low SOL of dual modified starch could be due to the use of high treatment temperature of HMT and OPT at which the granules show more rigidity possibly due to rearrangement between amylose and amylopectin [67]. On the contrary, increased solubility of OPTS was reported by some authors in corn starch [48, 57]. Reduced SP and SOL are important for applications in food products such as noodles [28].

4.3.2. Pasting properties and gel texture

The RVA pasting profiles of single and dual modified rice starches are presented in Table 4.2. The modification methods promoted significant ($p < 0.05$) changes in the modified rice starches. Higher pasting temperature (PT) was observed in all the modified starches than native starch. Higher PT seen in HMT starches imply that the starch granule contains more forces and cross-links, necessitating a higher heating temperature to cause structural breakdown and paste formation [17, 43]. PV decreased in all the modified starches. Among the single modified starches OPT had high PV next to NRS. The lowest PV was observed in HMT-OPTS and it was followed by OPT-HMTS and HMTS that is attributed to their limited swelling capacity [33]. This decrease in PV will reduce the destabilization effect of crystallite melting on the amorphous region, thereby starch swelling will necessitate a greater temperature (higher PT) [56]. Increased molecular binding forces in the starch chains may have contributed to the drop in peak viscosity, limiting hydration and, hence the swelling capacity was reduced [3].

Native starch had lower HPV and higher BD, indicating less paste stability of swollen granules. OPTS showed a narrow peak viscosity range similar to native starch indicating its apparently low stability at high temperature heating. But it had higher hot paste viscosity than NRS. However, HMTS showed wider peak viscosity range with much higher hot paste viscosity value. The hot paste stability was much enhanced by HMT alone and in combination with OPT, as seen by the relatively constant paste viscosity in HMTS, HMT-OPTS, and OPT-HMTS. Similar changes were reported in potato starch [56]. Breakdown viscosity decreased in all modified starches. BD viscosity indicates the fragility of starch paste. HMT increased the mechanical and thermal resilience of rice starches, as evident by a decrease in breakdown. As interactions between molecules of

Table 4.2 RVA pasting properties and gel hardness of native and modified starches.

Starch samples	Pasting temperature (°C)	Peak viscosity (cP)	Hold viscosity (cP)	Breakdown (cP)	Setback (cP)	Final viscosity (cP)	Gel hardness (g)
NRS	67.32 ± 0.02^{a}	3422 ± 15.0^{e}	2561 ± 33.5^{b}	861 ± 18.5^{e}	2000 ± 37.0^{c}	4561 ± 3.5^{c}	50.60 ± 0.20^{a}
HMTS	79.96 ± 0.31^{c}	3132 ± 21.5^{c}	2781 ± 16.5^{e}	351 ± 5.0^{c}	2107 ± 64.0^{e}	4888 ± 45.5^{d}	64.20 ± 0.14^{d}
OPTS	77.51 ± 0.19^{b}	3286 ± 2.0^{d}	2647 ± 11.5^{d}	539 ± 13.5^{d}	2092 ± 52.0^{d}	4739 ± 41.0^{e}	63.80 ± 0.20^{c}
HMT-OPTS	81.58 ± 1.59^{c}	2528 ± 9.0^{a}	2486 ± 10.5^{a}	42 ± 1.5^{a}	1137 ± 45.5^{a}	3623 ± 56.0^{a}	61.43 ± 0.25^{b}
OPT-HMTS	80.15 ± 1.43^{c}	3006 ± 20.5^{b}	2694 ± 26.0^{c}	312 ± 5.5^{b}	1327 ± 40.5^{b}	4021 ± 14.5^{b}	61.85 ± 0.39^{b}

Results are represented as mean ± SD, Significant differences ($p \leq 0.05$) exist between the mean values with different superscript in a column.

amylose and amylopectin increased due to HMT; a more stable structure had evolved [3]. OPT is known to reduce granular swelling at higher temperatures, resulting in a lower BD. However, the reduction in BD by OPT was comparatively less than HMT alone and dual form which is suggestive of relatively low strength of OPTS hot paste. However, unlike the two single modified starches, BD values were much lower in the dual modified starches which could be attributed to degradation of starch caused by the action of both treatments, which induced starch granule degradation [20]. The much low BD in HMTS, HMT-OPTS and OPT-HMTS implies better stability during continuous heating and shearing process. Even though some swollen granules were ruptured, the paste viscosity generated upon cooling remained high in HMTS and OPTS. This could be due to the amount of leached starch fragments that barely changed or caused limited granule deformation, resulting in tightly packed swollen granules that increased the viscosity of the paste when cooled. This led to the formation of the continuous gel matrix and thus increasing FV [16, 20]. However, the dual modified starches showed lower FV. The synthesis of an amylose–lipid complex during the treatment processes is responsible for the decrease in PV, BD, and FV in the dual modified starches [57]. HMT and OPT caused increase in SB while dual modifications tended to decrease the same. The increase in SB is plausibly due to the presence of rigid starch granules that did not rupture during single HMT and OPT; whereas the decrease in SB during dual modification is most likely because of the rising amylose content at prolonged high temperature treatment which enhanced the interaction with amylose and amylopectin that reduced the amylose exudation [19,64]. Moreover, the long treatment duration in dual modification favors the formation of lipid-amylose complexes, which restricts retrogradation and thus reduces setback [3, 24].

The higher gel hardness observed in all modified starches than NRS (50.60 g) is most likely because of their lower SP and SOL (Table 4.2). The starch gel is a network of solid-liquid phase where the manner in which the liquid phase is entrapped, the reduction in SP of starch increased hardness of gel. Low SOL reflects rearrangement of granule components promoting increased interactions among functional groups of starch [43]. The rise in gel hardness in HMTS (64.20 g) and OPTS (63.80 g) is attributed to higher amylose content and higher interactions between amylose chains of starch forming higher junction zones in the continuous gel phase [12]. Long treatment time of OPT-HMTS and HMT-OPTS might have caused partial gelatinization of the starch resulting in collapse of starch structure and less rigid gel [43]; also, formation of lipid-amylose complex may reduce

retrogradation tendency which might have decreased their gel hardness as compared to single HMTS and OPTS [3, 24].

4.3.3. Morphological characteristics

4.3.3.1. Polarized light microscopy

The micrographs of all the modified starches shown in the Fig 4.2. The amorphous region of native starch granules contains amylose, amylopectin branching points, linear amylopectin branches, and some amylose units interact in crystalline double helixes that arrange in parallel order to create the crystalline structure. These starch granules under

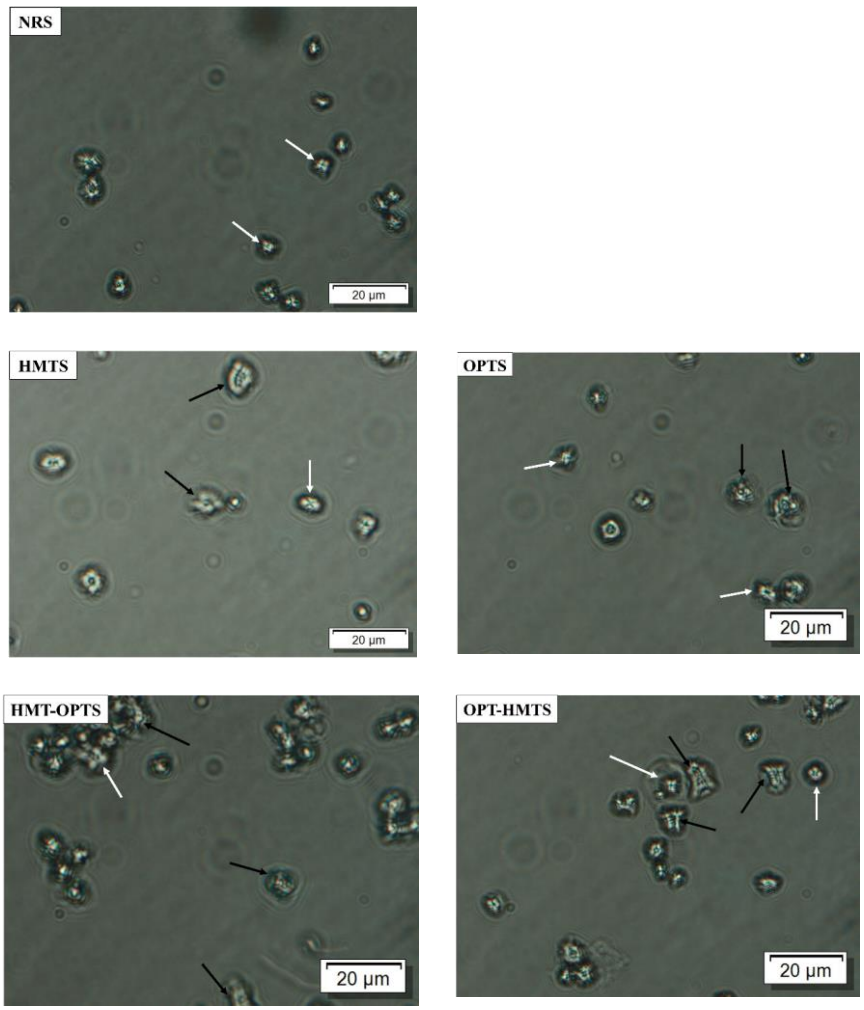

Fig. 4.2 Plane polarized light micrographs (1000X) of native rice starch and modified starches at 20 µm scale bar.

polarized light exhibit birefringence due to the orderly radial arrangement of amylopectin crystallites, with an interference pattern visible as a Maltese cross with the hilum at the center [11]. NRS showed small granules with distinct Maltese cross with prominent

quadrants (indicated by white arrows), whereas some starch granules of modified starches exhibited weaker birefringence patterns having less well-defined quadrants and voids in the center (indicated by black arrows) as presented in the Fig 4.2. Similar observations were reported in potato starch and corn starch during HMT [2, 14-15, 40], and OPT potato starch [56]. This could be explained by a transfer or reorganization of the molecular structure of the starch components at the center of the granule because of the heating process. The decrease in HMTS and OPTS birefringence intensity indicates possible loss in radial alignment of amylopectin crystallites as a result of increased amylopectin chain flexibility when subjected to thermal energy [2, 14]. The presence of larger void with weaker birefringence was observed in the order as OPT-HMTS>HMT-OPTS>HMTS>OPTS with decreasing order of birefringence pattern strength (Fig 4.2). The severity of the two treatments have caused more reduction in the birefringence strength of of HMT-OPTS and OPT-HMTS due to further starch chain mobility and loss of molecular order. The reduction in clarity of the Maltese cross or the birefringence in all the modified starch granules indicates a decrease in the crystallinity of the starch [40]. Some of modified starch granules exhibited birefringence that confirms the presence of non-gelatinized starch granules and the heterogeneity of modified starches.

4.3.3.2. Scanning electron microscopy

The surface morphology of the native and modified rice starches was observed by SEM as shown in the micrographs in Fig 4.3. NRS are apparent, small angular, polyhedral shaped granules with smooth surface. HMTS granule surface seems rough, eroded (indicated by white arrow) and uneven. The center of the granules of HMTS shows hollow depression or indentation at the center, slightly folded inward structure (indicated by black arrows), which suggest that the center part was disintegrated. HMT caused the rearrangement of the starch components at the granule center where the starch structure was comparatively weak [44]. Similarly, OPTS also showed eroded and rough surface (indicated by white arrow) with more irregular granule structure and shape. Plasmolysis or the formation of deep groove at the central core region of the granule (indicated by black arrows) because of osmotic pressure was noted as reported in OPT potato starch [56] and sago starch [57]. However, the extent of groove formation is less in OPT. The reason may be the reduced swelling of starch during OPT due to lack of free water and restricted gelatinization. Moreover, the dual modification process of HMT and OPT evidently affected the resultant rice starches. Both HMT-OPTS and OPT-HMTS showed loss of

physical integrity with distension of granular surface and aggregation (some starches are seen to have fused together) attributed to partial gelatinization [19]. The starch granules seemed to have more eroded and rougher surface and loss of physical integrity as evident from the presence of irregular granule shape can also be accepted owing to the severity of two treatments. Moreover, the OPT-HMTS modification caused visible agglomeration of the granules with no sign of distinct polygonal shape of typical rice starch, and presence of more inward folding grooves at the center of the starch granule and eroded uneven

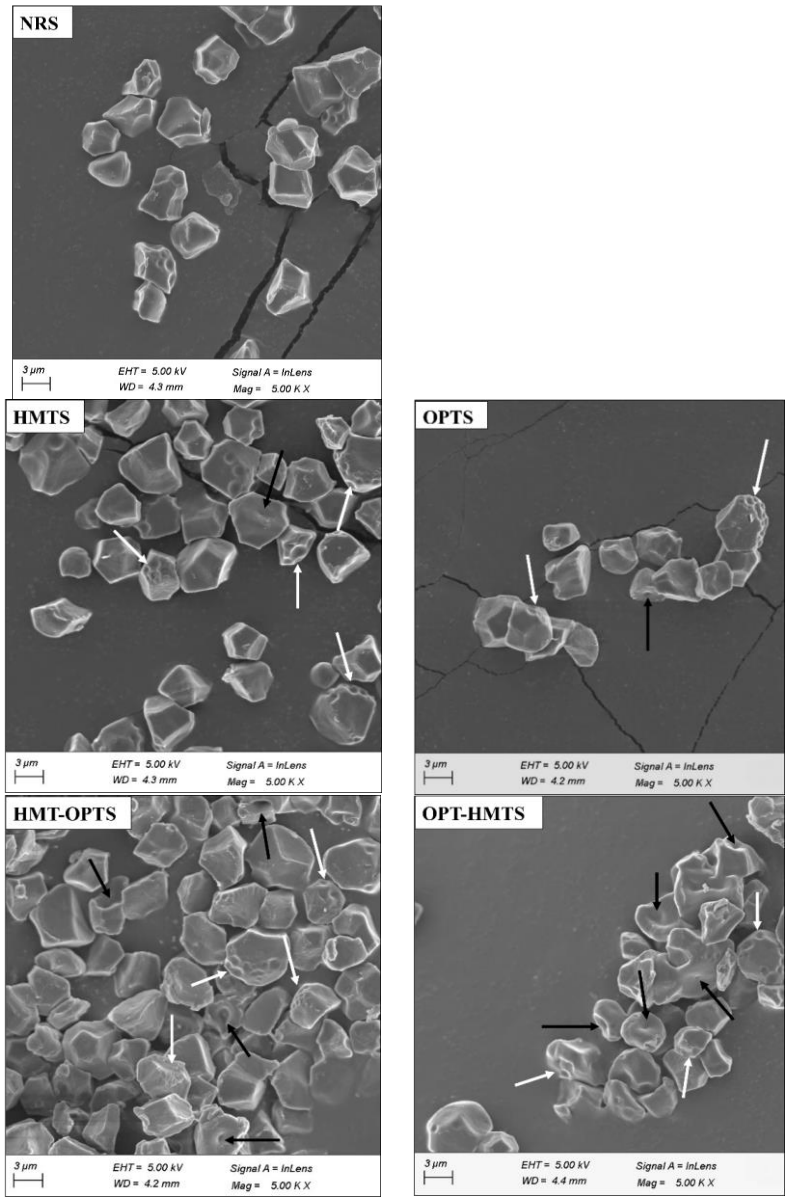

Fig. 4.3 Scanning electron micrographs of native and modified rice starches at 3 µm scale bar; 5 kV acceleration voltage and 5000X magnification; black arrow shows inward folding or indentation; white arrow shows eroded or rough surface of the modified starches.

surface was noticed. The formation of groove is due to the reason that the hilum at the central part is believed to be less organized which undergoes disintegration and consequent rearrangement during hydrothermal treatment [45]. The results of SEM are consistent with the loss of birefringence in the starches after modification.

4.3.4. Fourier Transform Infrared Spectroscopy

Infrared spectroscopy is effectively used to characterize the structural changes at molecular level (short range ordered structure or double helical order of starch) viz. starch chain conformation, helicity, crystallinity retrogradation, etc [10]. The double helix's short-range order structures can be detected in the FTIR spectrum between 1050 and 950 cm^{-1} [53, 70]. Three primary vibrational modes have been identified in the FTIR spectrum of starch, with maximal absorbance occurring at 995, 1022, and 1047 cm^{-1}. The C-O-H bending vibrations at 1047 cm^{-1} and 1022 cm^{-1} represent the ordered structure (increases with crystallinity) and unordered or amorphous domain of starch respectively [9], whereas the 995 cm^{-1} band is related to the hydrated crystalline domain [71].

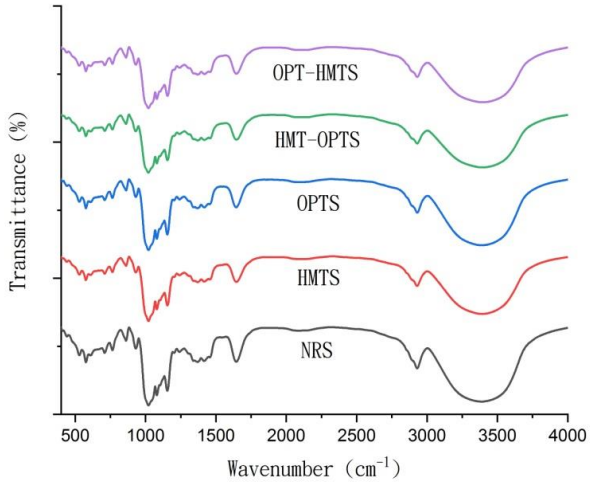

Fig. 4.4 FTIR spectra of native and modified rice starches.

Table 4.3 Short-range molecular order of native and modified starches.

Starch samples	1047/1022 ratio	995/1022 ratio
NRS	1.59	2.00
HMTS	1.26	1.34
OPTS	1.43	1.66
HMT-OPTS	1.27	1.33
OPT-HMTS	1.27	1.33

The band at 995 cm^{-1} include interaction of starch with water by hydrogen bonding, which modulates the COH bending modes [71]. Estimation of the degree of ordered structure within starch granules has been done by comparing the FTIR spectra of different starches based on the absorbance ratios 1047/1022 and 995/1022 [70-71]. The ratio between the FTIR bands at 1047 and 1022 cm^{-1} serves as a measure of the crystallinity (amount of the short-range order of double helices) of starch. This intensity ratio is typically used to illustrate how physical and chemical modifications affect the crystallinity of native starch. The results (Table 4.3 and Fig 4.4) showed that the absorbance ratio of 1047/1022 and 995/1022 decreased as a result of single and dual modifications. The ratio of 1047/1022 decreased in the order of NRS > OPTS > HMT-OPTS = OPT-HMTS > HMTS whereas the 995/1022 ratio decreased in the order of NRS > OPTS > HMTS > OPT-HMTS = HMT-OPTS, which clearly indicates that HMT and dual modification methods had more pronounced effect on the crystallinity of rice starches than OPT. Decrease in both 1047/1022 and 995/1022 ratio is also reported in corn starch after OPT [49]. This change is attributed to reorientation of double helices within the crystalline array and disruption of a few hydrogen bonds, which links the adjacent double helices [14, 5]. The decrease in 1047/1022 cm^{-1} ratio in the modified starches is suggestive of the loss in crystallinity on modification.

4.3.5. Wide-angle X-ray Diffraction and relative crystallinity

The intensity of the five main peaks as well as the RC of rice starches are shown in Table 4.4. The numbers 1–5 (15°, 17°, 17.8°, 20° and 23°, respectively) in Fig. 4.5 and Table 4.4 represent the highest peaks detected in the XRD. Native rice starch showed a typical A-type pattern with strong reflection at 2θ= a single peak 15°, a doublet at 17° and 17.8° and a single peak 23°. The other four modified rice starches also exhibited the typical A-type XRD patterns, which is suggestive of no alteration in the crystalline pattern of the starch granules after modification.

The RC of the rice starches reduced in the order of NRS > OPTS > OPT-HMTS > HMT-OPTS > HMTS (Table 4.4). Higher RC of the native starch implies its higher amylopectin content than the other modified starches. This result positively correlates with the FTIR results as discussed above (Fig 4.4). Both single and dual modifications caused a decrease in RC and intensity of the peaks at the peak number 1-3. HMT caused significant loss in RC as a single modification as well as in combination with OPT.

However, the reduction in RC was not significant when the HMTS was modified by OPT. The disintegration of the crystalline structure, the conversion of semi-crystalline components into amorphous parts because of partial gelatinization following the single and dual modifications are the plausible causes of the decreased RC [65]. The moisture content (29%) of starch during HMT was responsible to cause disruption and subsequent reorientation of the double helices, resulting in reduced crystallinity [75]. Moreover, partial gelatinization of the starch molecules during single and dual modifications is evident from the results of SEM (Fig 4.3).

Table 4.4 Intensity of major peaks and RC of native and modified starches.

Starch samples	1	2	3	4	5	RC (%)
NRS	2000	2207	2216	1416	1818	31.54
HMTS	1703	1862	2077	1574	1708	28.04
OPTS	1710	2034	2117	1420	1731	30.01
HMT-OPTS	1791	2039	2030	1503	1637	28.05
OPT-HMTS	1667	2169	2223	1654	1920	28.70

The numbers 1 to 5 represent the highest peaks detected in the X-ray diffractograms at 2θ=15°, 17°, 17.8°, 20° and 23°, respectively.

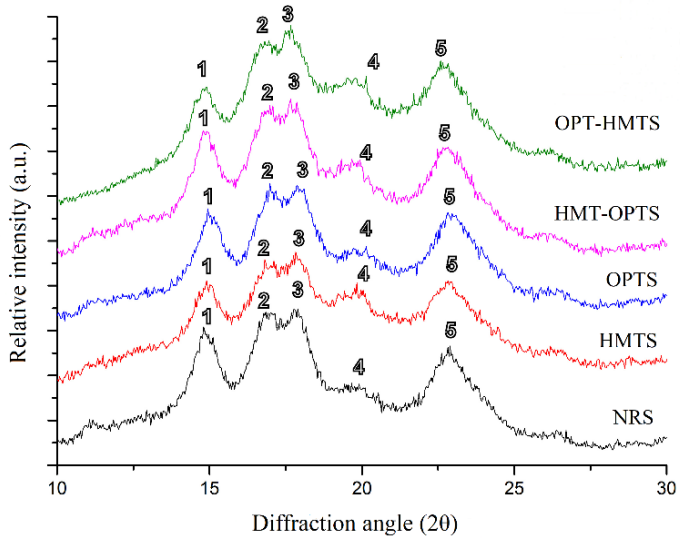

Fig. 4.5 X-ray diffractograms of native and modified starches. The numbers 1 to 5 represent the highest peaks detected in the X-ray diffractograms at 2θ=15°, 17°, 17.8°, 20° and 23°, respectively.

The peak intensity of all the modified starches showed apparent rise at peak 4 (2θ =20°), in the order OPT-HMTS > HMTS > HMT-OPTS > OPTS suggesting the formation of amylose-lipid complex (V-type pattern) which also known as resistant starch, type 5 [72]. HMT showed higher impact on the changes in the XRD peak intensities both in single and dual modification as compared to single OPT as evident by the values of peak intensities and RC (%) (Table 4.4). The peak intensity at peak number 5 (2θ=23°) decreased when the starch was treated by HMT, OPT and HMT-OPT whereas increased in OPT-HMT treated starch. A-type starches possess four water molecules between the double helices and the nearby double helices are bonded by hydrogen bonding. The drop in the XRD peak intensity indicates loss of the crystalline array, which is caused by the rupture of those hydrogen bonds, that results in displacement of nearby double helices leading to imperfect parallel rearrangement in the crystalline array [43]. It is plausible that when HMTS was OPT treated, the lack of free water restricted hydrogen bonding, preventing crystallites from perfect ordering. OPT-HMT might have facilitated higher complexing of glucose units with residual lipids that led to higher V-type formation in it.

4.3.6. Thermal properties

The DSC thermograms of native and modified rice starches are presented in Table 4.5. T_o, T_p and T_c increased significantly in all the modified starches with an increase in gelatinization temperature range (T_c-T_o). Increased gelatinization temperature range was reported in literature [18, 58] and decreased values were also reported elsewhere [43]. Broader T_c-T_o in modified starches indicates increased granular changes after modification [24]. Highest value of T_o was observed in HMT-OPTS followed by the other modified rice starches in the order: OPT- HMTS > HMTS > OPTS. T_o is a measure of temperature of the conversion of starch semi-crystalline structure to amorphous structure [30] and the more perfect crystallites would show higher onset temperatures [13, 36]. The T_p represents the temperature for optimum gelatinization which increased after modification in the order of HMT-OPTS > OPT-HMTS > HMTS > OPTS. Accordingly, modified starches also showed quiet higher T_c values than native counterpart in the order of HMT-OPTS > OPT-HMTS > HMTS > OPTS. Thus, the extent of post modification changes is greater in both dual modified starches than single modified starches. Among the single modified starch HMTS showed higher extent of changes in gelatinization parameters than OPTS.

The increase in T_o of starch in HMTS was caused by the conversion of the intercrystallite parts into amorphous phases imparting more freedom to short chains in the

crystalline structure. Eventually, the short chains on the edges of crystalline micelles form partial helices with the amylose chains, resulting in the higher T_o [42, 46]. The increase in the T_p and T_c in HMTS can also be ascribed to the melting of crystallites and double helices of ordered amylopectin side chains during gelatinization because of swelling of amorphous region. Swelling of amorphous area imparts stress on crystalline region requiring high temperature to strip off polymer chains from surface of starch crystallites [1, 37, 72].

Table 4.5 Gelatinization parameters of native and modified rice starches.

Starch Samples	Gelatinization temperatures (°C)			
	T_o	T_p	T_c	$T_c - T_o$
NRS	73.3	94.2	109.7	36.4
HMTS	89.1	117.5	127.3	38.2
OPTS	84	115.9	122.6	38.6
HMT-OPTS	91.4	119.6	131.2	39.8
OPT-HMTS	90.2	118.1	130.8	40.6

Furthermore, starch to water ratio and heating rate are considered to effect DSC endotherm [21, 23, 35]. Higher T_c was reported for starches which were treated at high temperature and moisture during hydrothermal treatments [62]. During DSC, if starch contains low water level, it restricts the swelling and tends to widen the endotherm requiring higher temperature for the melting of crystallites in the starch granule where both the starch chains and the crystallite become disordered [23]. During OPT, SO_4^{2-}, having more symmetry structure decrease the amount of free water and repel starch molecules thus inhibit starch gelatinization and strengthen the hydrogen bonds among the starch molecules, which makes them more rigid [73]. Starch granules form a rigid entangled structured envelope at the periphery consisting chiefly of amylopectin that act like a semi-permeable membrane (to the interior components of the granules) during the early stage of gelatinization [6]. The internal starch components get released only when the granule rupture and collapse at critical stress point at higher temperature as the gelatinization is complete. Since there is limited free water to facilitate swelling, the gelatinization is restricted during OPT. At higher temperature OPT must have caused melting of the amylopectin chains. Thus, the resultant OPTS tends to have lower swelling tendency and cause increase in gelatinization temperature [49, 57].

Thus, shifting of transition temperatures towards a higher temperature range can be attributed to amylose-amylose (suppresses the amorphous region mobility), amylose-amylopectin interactions (reduce the mobility of the amylopectin chains) [29], strengthened chemical bonding [74], and formation of amylose-lipid complex (detected by XRD) [73]. However, it is difficult to say how much lipid inclusion complex with amylose was formed with the residual lipids in the starch during the treatments. Both dual modified starches and single HMTS and OPTS had higher amylose content [Fig 4.1a] as compared to native starch and this supports the amylose-amylose and amylose-amylopectin interactions occurring as stated above.

4.3.7. Viscoelastic characteristics of native and modified starches

Rheological information about how a product will behave during storage and application can be provided by frequency sweep curves. G' and G" indicate the solution's solid-like and liquid-like responses, respectively. G' is a measure of the deformation energy that is stored in the sample during the shear process and depicts the sample's elastic behavior; G″ is a measure of the deformation energy that is used up and lost during the shear and depicts the sample's viscous behavior [61]. In general, starch paste is a composite of swollen starch granules, particularly the amylopectin fraction, that remain dispersed in an amylose biopolymer matrix. Hence the overall rheological properties of starch gel can be determined by the viscoelastic properties of the swollen granules (dispersed phase) and the amylose network (continuous phase) and the interaction between the two phases.

Value of G' greater than G" indicates the presence of gel network. The G' of all the modified starches was greater than their G" indicating the solid characteristics of starch gels and the elastic character prevailed over viscous nature (Fig 4.6a). The changes in G' and G" remained in the same trend throughout the deformation period of gel structures without any crossover during the observed frequency range signifying the stability of the gels [61]. HMTS increased G' and G" accounts for improvement in elasticity due to a rise in cross-linking among starch chains enabling the development of more junction zones in the gel's continuous phase [12, 32]. OPTS increased G' to a lesser extent than HMTS attributed to its relatively weaker starch chain interactions resulting in lesser enhancement of elasticity while it showed higher G" than other modified starches. The G' was substantially increased in HMT-OPTS and OPT-HMTS as compared to OPTS, HMTS and NRS indicating further enhancement of elastic properties. HMT-OPTS showed moderate increase in G" whereas OPT-HMTS showed higher increase in G". Higher G' and G" value

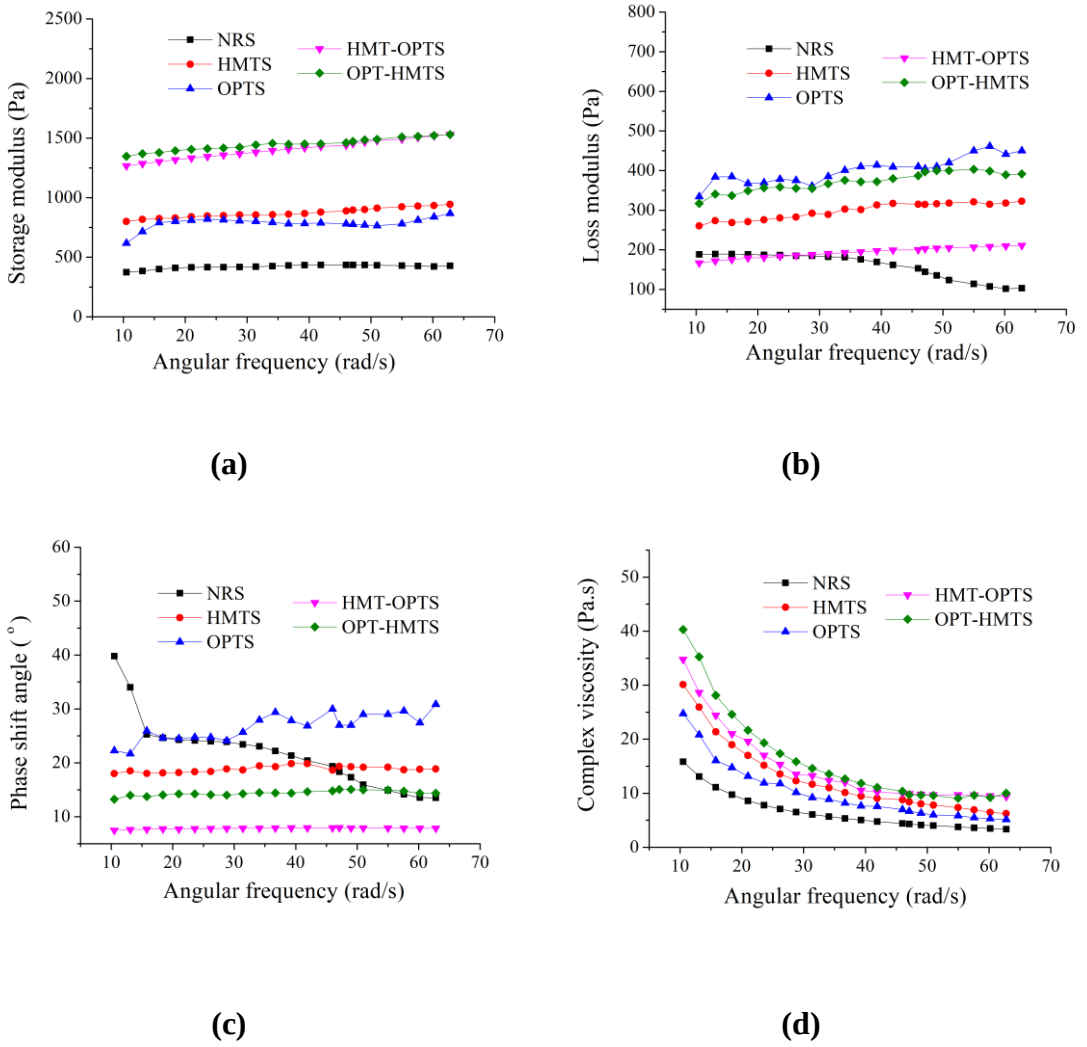

Fig. 4.6 Angular frequency (ω) dependence of storage modulus (G') (a), loss modulus (G'') (b), phase shift angle (δ) (c) and complex viscosity (η*) (d) for NRS, HMTS, OPTS, HMT-OPTS and OPT-HMTS gels at 25 ºC.

could be attributed to the stronger interactions of amylose-amylose and amylose-amylopectin of swollen granules due to higher amylose content, decreased swelling power and higher granular rigidity [61]. This result is consistent with the results of amylose content and SP (Fig 4.1b). Higher G' is desirable for food product such as rice noodles such as semi-dry and dry noodles where relatively stronger and more elastic gel is required [12].

The loss tangent (tan δ = G''/G') values of all the samples were within the range of 0.122-0.44 (tan δ < 1) which indicates all the samples were more elastic than viscous and suggested their solid behaviour. OPTS showed higher tan δ than other modified starches. Dual modified starches showed lower tan δ, HMT-OPTS in particular signifying higher elasticity attributed to the improved and well cross-linked or entangled gel network [51].

Lower values of phase shift angle of the modified starches were observed (Fig 4.6c). Minimum phase shift angle (towards 0^o) showing more solid behaviour is desirable for food applications that require higher gel hardness. OPTS showed highest phase shift angle; whereas HMT-OPTS and OPT-HMTS had lower phase shift angle revealing higher gel strength desirable for application in noodles.

The complex viscosity is a measure of overall resistance to flow depicting the overall system viscosity that decreased linearly with increasing angular frequency in the order OPT-HMTS>HMT-OPTS>HMTS>OPTS>NRS, depicting the shear thinning property of the modified starch gels (Fig 4.6d). Higher amylose content, lower SP and G' of modified starches seems to be positively related which contributes to the higher complex viscosity of modified starches. Viscoelastic properties of the modified starches showed that HMTS and dual modified starches showed higher desirability for their usage in noodle processing than OPTS.

4.3.8. *In vitro* starch digestibility

The results of starch fractions obtained through *in vitro* digestibility of native, single, and dual modified starches are presented in Fig 4.7. NRS had 81.75% RDS decreased in all the single and dual modified starches possibly due to less accessibility of starch granules to enzyme hydrolysis. NRS had 12.94% SDS and only 5.3% RS, which significantly increased after all four modifications. OPTS had higher RDS and lower SDS and RS than HMTS. HMT-OPTS and OPT-HMTS showed further decrease in RDS and increase in SDS and RS as compared to single HMTS and OPTS.

Many researchers have reported that a moisture content of 30% caused extensive increase in RS content in starch [2, 34, 72]. In presence of sufficient moisture (29%), HMT in the current study might have increased starch chain mobility for molecular rearrangement, facilitating the retrogradation of HMT starch by a mechanism involving disruption of the crystalline structure, dissociation of the double helices, and reorganization of the damaged crystals through various interactions: amylose-amylose and amylose-amylopectin [68, 75]. This led to synthesis of retrograded starch (RS3) which are resistant to enzyme hydrolysis hence decreased RDS and increased RS in HMTS [63]. OPTS did not cause RS3 formation due to lack of free water and limited gelatinization but produced amylose-lipid complex (RS5) [27]. Formation of RS5 during HMTS is also reported [68-69]. Amylose content has a negative correlation with RDS and a positive correlation with SDS and RS [41]. The modified starches had higher amylose content than

native starch and amylose can contribute to RS formation [54]. HMTS have higher amylose content and produced higher RS5 than OPTS as evident from XRD result which might have led to lower accessibility of amorphous region for enzymatic digestion resulting in lower RDS and higher RS values in HMTS than OPTS.

Decrease in RDS and increase in SDS and RS in HMT-OPTS are indicative further interactions among starch components of HMTS during OPT. Similarly, HMT must have caused more disruption of crystalline structure of OPTS to form partially disrupted crystalline mass causing higher SDS and rearrangement of their molecular structure contributing to the formation of RS3 and RS5 resulting in higher RS level in OPT-HMTS. Moreover, HMT-OPTS and OPT-HMTS had higher amylose content than OPTS and HMTS which must have undergone higher interaction with amylose, amylopectin and lipid bodies leading to their low accessibility for enzyme action as indicated by their low RDS and higher SDS and RS.

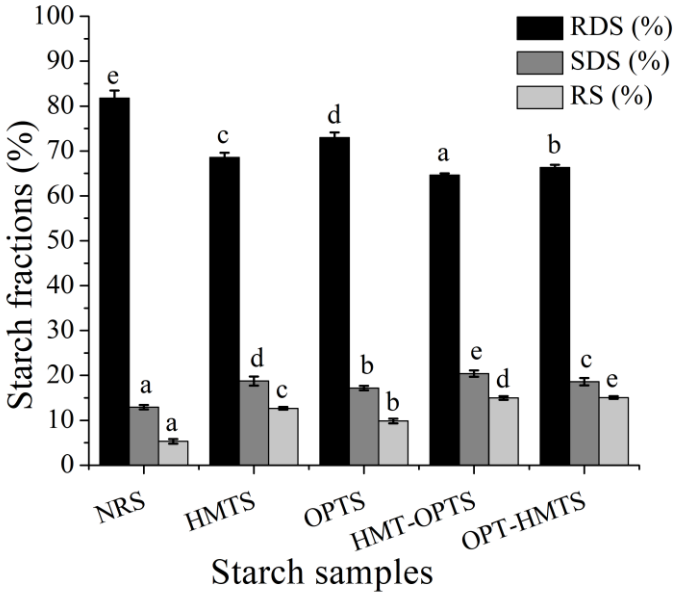

Fig. 4.7 Estimation of the RDS, SDS and RS of native and modified rice starches. Results presented in the bar diagram are the means of duplicates. The error bars indicate the standard deviation of the mean. Significant difference ($p \leq 0.05$) exists between the mean values with different letters (a-e) on the bars.

SDS was significantly increased by single and dual modification. SDS is mainly derived from changes in amylopectin [47] and thus, higher content of SDS in single and dual modified starches than NTS could be due to the presence of large amounts of amorphous components and partially disrupted crystalline components [34], whose

interactions formed during modifications have survived the process [14]. OPTS had relatively low SDS than HMTS, HMT-OPTS and OPT-HMTS. This could be attributed to the relatively lower amylose content and higher RC in OPTS than other modified starches. Again, partial gelatinization during OPT is also limited due to limited free water and presence of SO_4^{2-} ions thus low partially disrupted crystalline components formed.

RS can lower cholesterol and blood sugar levels, reduce gallstone formation, can serve as substrate to probiotic microorganisms and decrease epithelial atrophy of colon [60], can help control diabetes and obesity [59]. In the current study, HMT, HMT-OPTS and OPT-HMTS gave comparatively better digestibility results than OPTS. Therefore, incorporation of those modified starch containing reduced RDS and increased SDS and RS will be helpful in developing products such as rice noodles to improve their digestive properties.

4.4. Conclusion

Lower swelling power, solubility and higher amylose content, improved pasting stability and harder gel texture were the notable changes observed in the rice starches subjected to single and dual HMT and OPT modifications. The intensity of birefringence at the center of starch granules decreased after dual modifications. Dual modifications tended to cause more eroded and rougher surface, hollow depression, or groove; slightly folded inward structure as observed under SEM. HMT and OPT as a single treatment and combined in dual form resulted in decreased crystallinity, 1047/1022 ratio, PV, BD, and SB and increased PT; however, these changes were comparatively lower in OPTS. Gelatinization temperature increased significantly in all modified starches but to a lesser extent in OPTS. The elasticity of HMT-OPTS and OPT-HMTS were improved to higher extent than HMTS and OPTS as evident by the higher storage moduli. The modified starch gels depicted the shear thinning property, which was higher in HMTS among single modified starches and OPT-HMTS among dual modified starches. Significant increase in RS after modifications was noted by in vitro digestibility study where HMT, HMT-OPTS and OPT-HMTS showed comparatively higher RS than OPTS. Thus, the findings of the current study indicated more desirability of HMTS, HMT-OPTS and OPT-HMTS in rice noodle processing than OPTS.

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