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INFLUENCE OF PHYSICAL MODIFICATION ON STRUCTURAL, THERMAL, PASTING AND IN- VITRO DIGESTIBILITY PROPERTIES OF PEARL MILLET STARCH

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Abstract
Starch, the major constituent of pearl millet grain (70 percent) can be an excellent alternative for popular industrial starches like corn, rice and potatoes. Heat moisture treatment (HMT) of pearl millet (*Pennisetum typhoides*) starch at different moisture levels, i.e., 20% (HMT-20), 25% (HMT-25), and 30% (HMT-30), was carried out for 8 h at 110 °C and its structural and in vitro digestibility properties were studied. Amylose content, swelling power and solubility of starches decreased after HMT. HMT also induced cavity and some dents on starch granules surface. The X-ray pattern of native and HMT starches exhibited A-type diffraction pattern and less crystallinity than native starch. Short range ordered molecules had similar absorption bands with no new peaks detected between native and heat moisture treated (HMT) starches. HMT starches exhibited higher To, Tp, Tc than native starch and ΔH decreased in HMT than native starch. Heat moisture treated starches exhibited decreases in PV, TV, FV, while increasing pasting temperature. The HMT-30 sample had almost twofold increase in RS concentration compared to native starch. While RS and SDS content increased after modification, RDS content declined. The thermal stability and nutritional value of starches improved after HMT modification, allowing for more food uses.
Key words: *Physical modification, Heat Moisture Treatment, Pearl millet starch, In-vitro digestibility, Pasting characteristics*

1. Introduction

Starch is an important food product and a functional biopolymer, which has versatile applications in both food and non-food industries. Globally, Maize, Cassava, Sweet potato, Potato and Wheat are the major sources for starch. Recent trends in the food industry have created renewed and rapid expansion in the demand for starch (1). Hence, the search for alternate sources of starch becomes inevitable, focusing attention on a very less utilized crop like Pearl millet. Pearl millet or Bajra belongs to the *Poaceae* family and is a potential source of starch with up to 70% starch content (2). Pearl millet is the most extensively planted type of millet in the world and it is a drought tolerant crop that requires minimal growth conditions and less fertile soils, which further improves its suitability as an industrial crop (3,4). Its extraction and application as native and modified starch can give a new direction to starch-based industries (5).

Starch in its native form has limited functionality and application. The versatility of starch in industrial applications is defined by its physicochemical and functional characteristics, which

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necessitates the modification of the starch for different purposes. Because starches are unstable with heating, shearing, acidity and storage, they are frequently changed to increase their use in the food and non-food industries (6). There is a need to increase the qualities of native starches without utilising chemicals to modify starches in order to provide more natural dietary components (7). Hoover and Manuel (8) and Maache-Rezzoug et al., (9) recommended HMT as an essential modification method for improving the functional qualities of native starch. Physical methods of modification has gained popularity because of their low cost, safety, and effectiveness, as well as the fact that they are a green alternative (no chemical reagents required) to improve starch applicability for specific sorts of applications (10).

Heat Moisture Treatment (HMT) is a type of physical modification that involves a period of time and a low moisture level (10–35%) as well as a high temperature (80–140 °C) for certain durations (11,12,9&13) and safer than chemical modifications (14). After HMT, starch undergoes structural reorganization resulting in granular swelling power, solubility, and crystallinity, amylose leaching parameters, gelatinisation, retrogradation, thermal stability, and changes in paste properties. Hydrogen bonds produced between water and molecular chains within starch granules are attributed for the treatment's effect (15&16). HMT is directly influenced by the botanical source of the starch granule with respect to its composition and organization of amylose and amylopectin (17).

HMT tends to provide an increase in the temperature range, an increase in the gelatinisation temperature, and better thermal stability depending on the technological interest (12). These behaviours are more evident when HMT is applied at more severe conditions, such as a longer holding time or higher moisture level (18). Punia et al., (10) and Sandhu et al., (19) found that swelling power of HMT starch was less (12.64 g/g) than native starch (15.86 g/g), which may be attributed to the amylose–lipid interactions, and/or interactions involving amylose–amylose and/or amylose–amylopectin chains

HMT is also used as a pre-treatment because of the structural modification into amorphous and crystalline regions on the granules (17). According to Zavareze and Dias (12), the grain size of HMT granules is unchanged when compared to native ones (20), partial gelatinization of starch caused by HMT causes cavities, fissures, and holes on the surface of starch granules. Punia et al., (10) and Sandhu et al., (19) observed that peak viscosity of native starch was higher than HMT starch. As a result, it can be a better substitute for chemically modified starches in retort foods, confections, salad dressings and batter products (20). However very little research information is available on HMT modification of pearl millet starch. Hence, this study was taken up with the objective of understanding the structural characteristics of pearl millet starch under HMT modification.

2. Materials and Methods

2.1. Materials

Pearl millet variety CO10 was purchased from Center for Plant Breeding and Genetics, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India. The

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grains were cleaned, mold- and weathering-free. They were stored in polyethylene bags to prevent the loss and absorption of moisture during transportation to the laboratory of Department of Food science and Nutrition, Periyar University, Salem to carry out the experiments. Analytical-grade chemicals were employed, which were acquired from Hi-Media chemicals, Mumbai.

2.2. Methods

2.2.1 Isolation of starch

Starch was isolated from pearl millet grains according to the method explained by (22) with some modifications. Pearl millet grains were cleaned and steeped for 24 hours at 40°C in a solution of 0.5% lactic acid and 0.3% sodium metabisulfite. The steeping solution was completely removed from the grains by three washings. In a Waring blender, coarsely ground grains were blended for five minutes at 8000 rpm. The resulting slurry was sieved through a stack of 80 and 300 wire mesh sieves starting at the top and working down. The leftovers from the sieves were sieved after being processed in a blender for two minutes. This process was carried out twice. The resulting slurry was brought to pH 9.0 by adding 0.5M NaOH solution, agitated for 2 hours with a magnetic stirrer, and allowed to settle at 4°C. A spatula was used to scrape the protein coating off. To get rid of the remaining protein, the sedimented starch was homogenised once more after being washed with water. After being dried at 45°C in a forced air oven, the starch was employed for the investigation. Because of their low ash, lipid, and protein contents, the samples are immaculate (23).

2.2.2 Heat Moisture Treatment (HMT)

The method followed by Sun et al., (24) was employed for the heat moisture treatment with slight modification. The starch moisture level was adjusted to 20, 25, and 30% after starch samples were weighed into various glass containers. The sealed samples (in glass jars) were heated in a hot air oven at 110± 2 °C for 8 h followed by drying at 40±5 °C to uniform moisture level (~10 %).

2.2.3 Analysis for physicochemical characteristics

Physicochemical characteristics of native (NS) and heat-and-moisture-treated (HMT) starches, including their moisture content (as determined by the hot air oven method), ash content, and amylose content, were determined using standard method (25). The protein and fat contents of the starch were examined using the Micro-Kjeldahl and Soxhlet extraction methods, respectively.

2.2.4 Water absorption capacity (WAC) and Oil absorption capacity (OAC)

Water absorption capacity (WAC) and oil absorption capacity (OAC) were determined according to procedures followed by (26). 20 mL of distilled water or 20 mL of mustard oil were combined with 2.5 g of starch and swirled at room temperature for about 30 minutes. It was then centrifuged at 3000 rpm for 10 minutes. Supernatant was then thrown away. Oil and water absorption capacities were used to quantify the weight gain.

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$$\text{WAC and OAC} = \frac{\text{Weight of sample} - \text{weight of sample after centrifuge}}{\text{weight of sample}}$$

2.2.5 Swelling power (SP) and solubility (S)

In a pre-weighed 50 mL centrifuge tube, 20 mL of distilled water was mixed with 0.5 g of starch for 30 minutes at 90 °C in a shaking water bath. After that, the suspension was centrifuged for 10 minutes at 3000 rpm. Carefully decanting the supernatant into a petri dish, it was then dried at 103°C for 12 hours. After decantation, the weighted granules were taken for the study (27). The results were expressed as:

$$\text{Swelling Power (SP)} = \frac{\text{Weight of swollen granules}}{\text{Weight of sample}} \times 100$$

$$\%\ \text{Solubility (S)} = \frac{\text{Weight of dried starch in Petri dish}}{\text{Sample weight}} \times 100$$

2.2.6 Bulk density (BD)

A 50 g sample of pre-weighed starch was placed in a 100 mL measuring container. On a lab bench, the cylinder was repeatedly tapped to maintain a constant capacity. (28). The results were expressed as:

$$\text{Bulk density(BD) } (g/cm3) = \frac{\text{Weight of sample}}{\text{Volume of the sample after tapping}}$$

2.2.7 Scanning Electron Microscope (SEM)

To analyse the granular morphology, a scanning electron microscope (JSM, 6380A, Jeol Japan) was utilised (29). The starch samples were coated with 30 nm of gold and put on a SEM stub using double-sided adhesive tape. The photos were examined at a 3000X magnification.

2.2.8 X- ray diffraction (XRD)

The isolated pearl millet starches were closely packed in a rectangular glass crucible and subjected to an X-ray beam produced by a 40 KV, 250 mA X-ray diffractometer. The diffraction angle regions were selected at 2θ angle range of 5° to 40°, which covered the majority of the important diffraction peaks of the starch crystallites (30). The graph was created using Origin pro 8E (OriginLab, USA) software. The Relative crystallinity (RC) of the starch samples were analyzed using following formula

$$\text{Relative crystallinity (RC\%)} = \frac{AC}{(AC + Aa)} x100$$

Where Ac – Crystalline area, Aa Amorphous area

2.2.9 Fourier Transform Infrared spectroscopy *(FTIR)*

A FTIR spectrophotometer (NEXUS-870, Thermo Nicolet Corporation) was used to

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record the infrared spectra of starch samples as stated in (31). The materials were combined with potassium bromide (KBr) and compressed into pellets to have an average moisture content of 8%. The pellets were then exposed to Attenuated Total Reflectance (ATR) spectroscopy in the 4000-400 cm^{-1} range. The height of the absorbance bands from the baseline was measured to determine the intensity of the spectra. The graph was made by exporting an MS Excel data sheet to the OriginLab, 8E (USA) software.

2.2.10 Thermal characteristics

A differential scanning calorimeter was used to test the thermal properties of starches (DSC 6000- PerkinElmer, USA). In an aluminium pan, a 2.0 mg sample (dry basis) was weighed before 10 mL of deionized water was added. Before doing the study, the pan was firmly sealed and let to stand for an hour. As a guide, an empty aluminium pan was used. A heating programme with a temperature range of 10 to 125 °C and a heating rate of 5 °C/min was applied to the sample. We identified the transition enthalpy (H), onset, peak, and final temperatures (To, Tp, and Tc, respectively).

2.2.11 Pasting characteristics

The pasting properties of starches were studied using a starch cell from a Modular Compact Rheometer, (Anton Paar, Austria). Starch slurries (1.2 g starch in 13.8 g distilled water) were heated from 50 to 95 °C at a rate of 6 °C/min, held for 2.7 min, then cooled to 50 °C at the same rate and held at 50 °C once more for 2 min. Each sample underwent a triplicate analysis. The pasting graph was used to determine the peak viscosity (PV), break-down (BD), setback (SB), final viscosity (FV), and pasting temperature (PT).

2.2.12 In vitro starch digestibility

Based on Englyst et al (32) method, the in vitro starch digestibility assay was estimated to quantify the proportion of digestive starch fractions, including rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) content of the NS, HMT treated starches. A 20 mL solution of sodium acetate buffer (pH 6.0) and 0.3 g of dry starch were combined and cooked in a water bath for 30 minutes. An enzyme solution (5 mL) of -amylase (1400 U/mL) and amyloglucosidase (13 AGU/mL) was added after 15 minutes of 37 °C equilibration. The starch solution was shaken for 120 minutes at 37 °C. The GOPOD kit was used to measure the total glucose concentrations in the hydrolysates after 20 and 120 minutes of digestion, respectively (G20 and G120). The residual residue was extensively hydrolyzed with 7M KOH and then with amyloglucosidase (50 AGU/ml). The total glucose concentration of the final hydrolysate was then determined (TG). The values obtained for G20, G120 and TG were used to calculate the RDS, SDS and RS as follows.

$$RDS = G20 \times 0.9$$
$$SDS = (G120 - G20) \times 0.9$$
$$RS = (TG - G120) \times 0.9$$

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2.2.13 Statistical analysis

All determinations were done in triplicate and the data are reported as the mean values ± standard deviation (SD). ANOVA (analysis of variance) was performed by one way analysis followed by Duncan's multiple range tests ($P<0.05$) to compare treatments' means using SPSS (version 19.0). Correlation analysis was also carried out.

3. Results and Discussion

3.1. Physicochemical characteristics

The physico-chemical characteristics of pearl millet starch and heat moisture treated pearl millet starch are given in Table 1. Yield of pearl millet starch was found to be 53g/100g, similar to the findings of Balasubramanian et al., (33) and Shaikh et al., (34), who reported 53.1g /100g and 56.3 g/100g respectively. The moisture content of native starch was 4.67g/100g, protein (0.23g/100g), fat (0.09g/100g), ash (0.16g/100g), amylose content (20.01g/100g). Based on the similar results reported by Dangi et al. (35), who found that the moisture, fat, protein, and ash contents of the isolated starch were observed to be 8.70, 0.78, 0.21, and 0.19% respectively. Similar findings were made by Bhupender et al. (36) who found that isolated starch had an amylose concentration of 18.71%. The effective isolation of starch with a high level of purity was shown by the low residual protein and ash levels.

Table 1. Physicochemical characteristics of native and heat moisture treated pearl millet starch

Sl. No.	Properties	Native starch	HMT-20	HMT-25	HMT-30
1	Moisture (g/100g)	4.67 ± 1.04^{a}	5.67 ± 0.57^{a}	6.17 ± 0.29^{b}	$9.17 \pm 0.29^{b,c}$
2	Protein (g/100g)	0.23 ± 0.01^{a}	0.27 ± 0.01^{b}	0.26 ± 0.02^{b}	0.25 ± 0.01^{b}
3	Fat (g/100g)	0.09 ± 0.01^{a}	0.08 ± 0.01^{b}	0.07 ± 0.01^{c}	0.06 ± 0.01^{d}
4	Ash (g/100g)	0.16 ± 0.01^{a}	0.14 ± 0.02^{a}	0.14 ± 0.02^{a}	0.13 ± 0.01^{a}
5	Amylose content (g/100g)	20.01 ± 2.31^{a}	19.71 ± 0.51^{a}	$17.72 \pm 0.79^{a,b}$	16.44 ± 1.16^{b}

[a-d] Mean values having different superscript in rows are significantly different at $p \leq 0.05$.

The study revealed that heat moisture treated (20, 25, 30%) pearl millet starch had moisture (5.67, 6.17, 9.17g/100g), protein (0.27, 0.26, 0.25g/100g), fat (0.08, 0.07, 0.06g/100g), ash (0.14, 0.14, 0.13g/100g) and amylose content (19.71, 17.72, 16.44g/100g). The amylose content of heat moisture treated starches (20, 25, and 30%) were significantly ($P<0.05$) less than native starch.

Amylose content, which impacts the physicochemical properties and determines the usability of the starches in diverse applications, is the most significant aspect of starch (37). Amylose

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content of heat moisture treated (20, 25, 30%) pearl millet starches were (19.71, 17.72, 16.44 g/100g), the highest for HMT-20, while the lowest was observed for HMT-30. The diminished amylose content upon HMT can be attributed to increased interactions between the amylose-amylose and amylose-amylopectin chains that modify the crystalline starch matrix, making amylose more insoluble and unavailable for quantification (12) or due to an increase in the quantity of lipid complex amylose chain (13). Similar results were obtained by Sandhu and Siroha (23); Sandhu et al., (19) and Punia et al., (3), who reported a reduction in amylose content after HMT modification as compared to their native counterpart starches.

3.2 Water absorption capacity (WAC) and oil absorption capacity (OAC) and Bulk density (BD)

The water absorption capacity of heat moisture treated samples (20, 25 & 30%) was higher 2.26, 2.37 and 2.58 mL/g, respectively when compared to native starch (1.10 mL/g) at. Heat moisture treatment linearly increased water absorption capacity of these starches, which implies that hydrophilic tendency increased with increasing level of moisture treatment. Similar results were also reported by Adebowale et al., (38).

The oil absorption capacity of heat moisture treated samples (20, 25 & 30) was higher than native starch (1.67 mL/g) at 2.55, 2.66, and 2.74 mL/g, respectively. The oil absorption capacity of heat moisture treated starches were significantly ($p<0.05$) higher than native starch and it was almost twice than that of native starch. This implies that after heat moisture treatment, the lipophilic potential of pearl millet starch increased. Sharma et al., (2) reported that oil absorption capacity (OAC) of pearl millet starch increased by up to 10% after HMT. Similar observations have been reported for potato and wheat starches by Kulp and Lorenz (39) and for autoclaved corn starch by Dundar and Gocmen (40).

Table -2 Functional characteristics of native and heat moisture treated pearl millet starch

Sl. No	Properties	Native starch	HMT-20	HMT-25	HMT-30
1	Water absorption capacity (mL/g)	1.10 ± 0.01^{a}	2.26 ± 0.23^{b}	$2.37 \pm 0.10^{b,c}$	2.58 ± 0.18^{c}
2	Oil absorption capacity (mL/g)	1.67 ± 0.16^{a}	2.55 ± 0.14^{b}	2.66 ± 0.07^{b}	2.74 ± 0.09^{b}
3	Bulk Density (g/cm^3)	0.79 ± 0.02^{a}	0.68 ± 0.04^{b}	0.56 ± 0.02^{c}	0.50 ± 0.10^{c}
4	Swelling power (g/g)	3.48 ± 0.09^{a}	3.36 ± 0.13^{a}	$3.26 \pm 0.09^{a,b}$	3.07 ± 0.23^{b}
5	Solubility (%)	5.51 ± 1.72^{a}	4.22 ± 0.51^{a}	3.72 ± 1.35^{a}	3.50 ± 0.60^{a}

HMT- Heat moisture treatment, a-dMean values having different superscript in rows are significantly

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different at $p \leq 0.05$.

The moisture content of starch has an impact on bulk density (BD). The moisture content of heat moisture treated (20, 25, & 30%) starches were 5.67, 6.17 and 9.17g/100g, respectively when compared to native starch (4.67g/100g). The BD of heat moisture treated starches were 0.68, 0.56 and 0.50 g/cm^3 respectively when compared to native starch (0.79 g/cm^3), similar to the findings of Abdalla et al.,(28), who reported that bulk density of pearl millet starch were 0.63g/cm^3. BD reflects the stability of starch granules to continuous heating and agitation.

3.3. Swelling Power (SP) and Solubility (S)

Swelling power of HMT (20, 25, & 30%) starches decreased from 3.36 to 3.07 g/g. Similarly the solubility of HMT starches was also found to be decreasing from 4.22 to 3.50 g/g. During heat moisture treatment, there is creation of cross linkage in the amorphous region and therefore an increase in crystallinity, which could be the cause for decreased swelling power and solubility (2). The variations in the swelling power of the modified starch are also accounted by the rising gelatinization temperature. This is supported by the findings of Sandhu et al., (19) and Punia et al., (3), who reported that the SP and S of HMT starches were found to be lower than those of their native counterparts. SP and S of heat moisture treated starches was significantly lower ($P<0.05$) than native starch. HMT also influences the functional characteristics (such as SP and S) of millet starches. The amount of leached amylose during hydration and swelling affects starch solubility (41). During heat moisture treatment, there is creation of cross linkage in the amorphous region and therefore an increase in crystallinity, which could be the cause for decreased swelling power and solubility (2). According to Adebowale (42) and Zavareze and Dias (12), the decreased swelling power and solubility of HMT starch compared with that of native starch can be attributed to internal reordering of starch granules.

3.4. Scanning Electron Microscope - SEM

Wang et al. (43) explained that the morphology of starch granules is an important element to consider when examining the relationship between starch structures and features. It plays a major part in the processing of starches. Hence, the scanning electron micrographs (3000× magnification) of native and HMT pearl millet starches were carried out and are presented in figure-1. Starch granule sizes were similar (NS, HMT-20, HMT-25, HMT-30), with diameters ranging from 2 to 12µm in diameter. The shape of starch granules varied from polygonal, to round/oval granule structure. In addition, some granules were irregular and polygonal. HMT caused various dents/holes on the surface of starch granules (Fig.1), possibly due to disintegration and molecular rearrangement. Significant alterations on the surfaces of the starch granules were detected after HMT. Liu et al., (44), indicated that HMT causes partial gelatinization of starch, which leads in the creation of cavities, fissures, and holes on the surface of starch granules. Sharma et al.,(2) reported that in the case of HMT samples, physical integrity was lost along with granular surface degradation (typical of partial gelatinization). During HMT, the severity increased as the moisture content increased which is evident from Fig.-1. In

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HMT-modified starches, more moisture makes starch granules active because of water absorption during treatment, encouraging their expansion and resulting in morphological changes induced by thermal force (45).

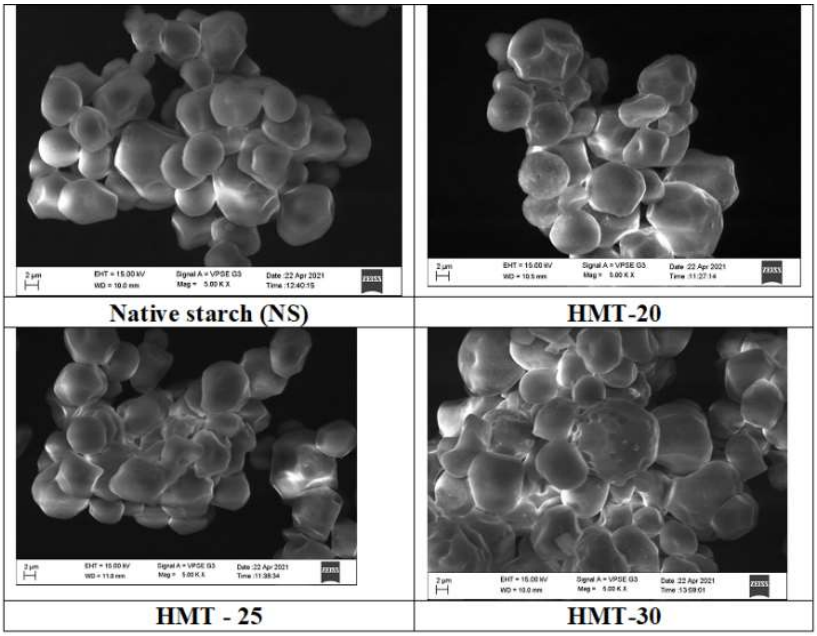

Fig.-1. SEM image of native and heat moisture treated starch

3.5. X- ray diffraction (XRD)

Table 3 displays the relative crystallinity (RC) and X-ray diffractogram intensity of native and HMT starches. All native and HMT starches displayed the A-type diffraction pattern, which is characterised by two single peaks at 2 θ values of 15.0 and 23.1 and a strong doublet-centered diffraction peak at 2 θ values of 17.0. (Fig. 2). Similar findings were made by Punia et al., (3) who observed that increasing moisture content and/or heating time lengthened the peak intensity for all HMT starches but had no effect on the natural diffraction pattern of native starches. HMT resulted in a visible reduction of crystallites and this phenomenon became more prominent with the increased water content (Table 3), similar to the findings of Klein et al., (46). Varatharajan et al., (47) also reported that water promotes the effects of HMT on starch crystallite disruptions by inducing breakage of hydrogen bonds and RC decreases upon increase in water content. Similar findings were reported in potatoes and cassava (48). Gunaratne and Hoover (49), reported that RC of potato and true yam decreased by 9 and 8%, respectively after HMT. The DSC results are consistent with the decrease in RC of the treated starches. The greater increase in Tp and reduction in Δ_H as a function of moisture content could be explained by a higher number of effective bonds in the crystalline phase and broken hydrogen bonds in the crystalline area. As a result, as previously reported by Sui et al., (50) the amount of moisture in the starch was discovered to be a larger driver of thermal characteristics. The connection of neighbouring double helices and an increase in hydrogen bonds brought on by thermal energy affect the X-ray intensities of cereal starches (12).

Table - 3. Relative crystallinity of native and heat moisture treated pearl millet starch

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Sl. No.	Properties	"d" spacing	2 theta (2Θ)	Relative Crystallinity (%)
1	**Native**	15.0 17.0 23.1	5.7 5.0 3.9	24.62
2	**HMT-20**	15.0 17.0 23.1	5.8 5.1 4.1	23.98
3	**HMT-25**	15.0 17.0 23.1	5.8 5.1 4.1	23.02
4	**HMT-30**	15.0 17.0 23.1	5.8 5.1 4.1	22.17

*HMT- Heat moisture treatment

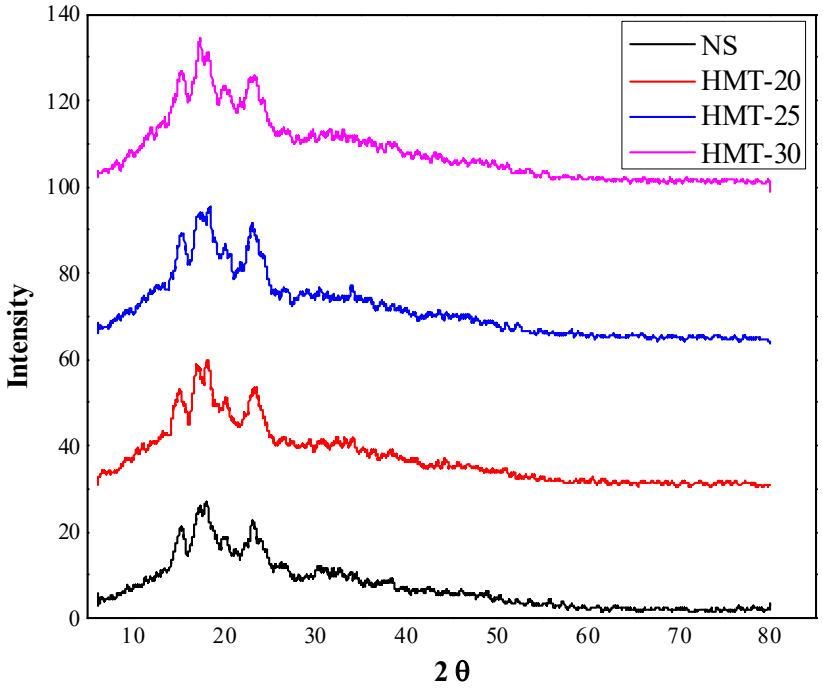

Fig. 2 XRD pattern of native and heat moisture treated starches

3.6. Fourier Transform Infrared spectroscopy (FTIR)

FTIR spectra provide information on the chemical functionality and short-range order of molecules. FTIR spectra for native and HMT starches at 20%, 25% and 30% moisture content for 8 h are illustrated in Fig. 3. Table 4 shows the functional groups discovered by FTIR of native and heat moisture treated starches. Wavelengths ranging from 3,500 to 1,500 cm^{-1} were employed to determine functional groups.

The FTIR spectrum of heat moisture treated starch displayed stretched bands in the

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region 861, 928 cm-1, indicating the presence of C=C stretching bands. No significant differences in the spectrum pattern were observed between the native and HMT starches (Fig. 3). Similar observations have been reported for potato, pea and lentil starches (47). Peaks in the spectral range 3200–3400 cm-1 corresponded to the stretching of the OH bond in water, which could be linked to the starch's O–H bond stretching and its width to the development of inter and intra molecular hydrogen bonds, which were found at their highest. HMT produced varying effects on the intensity ratio at different levels of moisture content. While the intensity ratio increased for HMT-20, it decreased for HMT 30, whereas there was no effect on the intensity ratio for HMT-25. It appears that sufficient water enhanced the formation of hydrogen bonds between amylose – amylose and amylose –amylopectin starch chains, which is consistent with the pasting and thermal properties results as denoted by Sui et al., (50). The asymmetrical stretching of the C-H of the –CH2 bond was visible in the peak about 2928 cm^{-1} in HMT-30 starch. The absorption bands at 1647 cm^{-1} support the existence of water as reported by (51). The vibrations of C-H stretching, C-H, and C-O-H bending had peaks at 1316–1416, 1140–1171, and 927–1010 cm-1, respectively. Amir et al., (52) observed that the FTIR spectra of starch showed broad absorption between 3000-3600 cm^{-1} and 1500-1700 cm^{-1} due to stretching frequency of the –OH group and C–H group, respectively.

Table - 4. Functional group of FTIR native and heat moisture treated starch

Peak (cm^{-1})	Nature of bond	Functional group
3399-3420	-O-H	Carboxyl
2927-2925	-C-H	Alkanes
2853	-N-H	Amines
1640-1638	-C-C	Alkanes
1412-1415	-O-H	Carboxyl
1153	-C-O	Alcohol
1077	-C=O	Anhydride
861	-C-C	Alkanes
763	$-NH_2$& N-H	Amines
573	-C-I	Aliphatic iodo
529	-C-I	Aliphatic iodo

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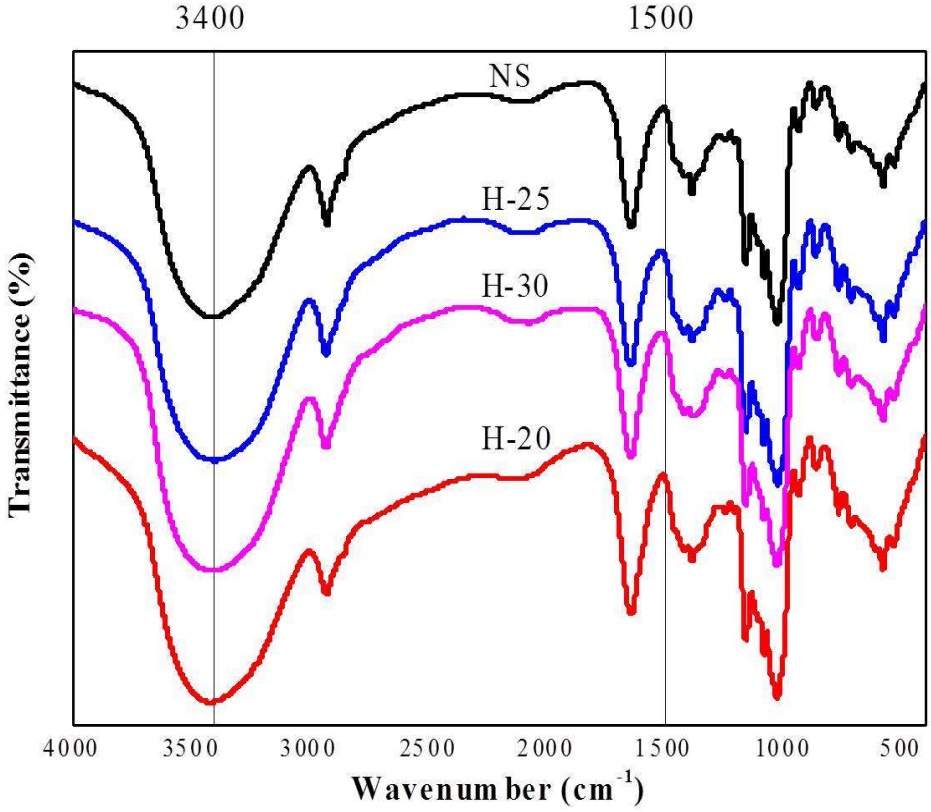

Fig.- 3. FTIR spectra of native and heat moisture treated starch

The results show that all spectra had similar absorption bands with varying intensities at their peaks. There were no new peaks detected, implying that no new covalent bonds were formed between native and heat moisture treated starches, indicating that only hydrogen bonds were formed in starch molecules, as the spectra of starch exhibited the same characteristics as native starch with a little change in peak intensities. Similar results have been reported by (53); Yin et al., (54) who used FTIR to investigate the interactions of guar and xanthan gum with maize starches. HMT treated starch had stretching band 1383cm-1 indicating the presence of –S=O group. Wu *et al*., (55) attribute the signal at 1380-1398 cm-1 to the polysaccharide (1-4) glycosidic bond stretching vibration. ATR-FTIR observations of structural changes at the molecular level at the surfaces of starch granules during HMT could provide further information for the field of heat moisture treated starch.

3.7. Thermal characteristics

The onset (To), peak (Tp), conclusion (Tc) temperatures and enthalpy (ΔH gel) of pearl millet starch as determined using DSC are given in Table 5. From the table it could be inferred that HMT enhanced the thermal characteristics of pearl millet starch. In the case of native starch the gelatinization peak (Tp) was found at 68.5°C, whereas for HMT (20, 25, and 30%) starches, it was found at 72.56, 81.07, and 85.05°C, respectively. Similar increase was witnessed with HMT starches in the case of To and Tc. The onset temperature (To) for native starch was 62.5°C, whereas for HMT (20, 25, and 30%) samples it increased to 68.01, 76.16, and 79.13°C, respectively. Similarly, Tc for HMT (20, 25, and 30) samples were 81.85, 84.50, and 89.05°C,

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respectively while for native starch it was only 74.2 °C. Increase in To, Tp and Tc of HMT starches arises mainly from amylose – amylose and amylose –lipid interactions. The increase in gelatinization temperatures can be associated to decrease in swelling power and also attributed to a strengthening of interactions between amylose and amylopectin branching due to heat moisture treatment. Similar findings were also reported by Sharma et al., (2). The stability of the crystalline domains of starch is correlated with the enthalpy of gelatinization. Thus, the collapse in the crystal structure of starch granules, which was assessed by XRD (Table 3), can be used to explain the lowered enthalpy. Similar findings were also reported by Oliveira et al (56).

Table-5 Thermal characteristics of native and heat moisture treated pearl millet starch

Sl. No	Properties	Native starch	HMT-20	HMT-25	HMT-30
1	T_o ° C	62.5 ± 2.26^{a}	68.01 ± 0.46^{b}	76.17 ± 2.22^{c}	79.13 ± 1.23 ^{c}
2	T_p ° C	68.5 ± 0.10^{a}	72.56 ± 1.03^{b}	81.07 ± 0.87^{c}	85.05 ± 3.09^{d}
3	T_c ° C	74.2 ± 1.71^{a}	81.85 ± 2.07^{b}	84.50 ± 2.67^{b}	89.05 ± 0.96^{c}
4	Δ_H J/g	10.30 ± 0.27^{a}	10.27 ± 06^{a}	9.43 ± 0.21^{b}	7.93 ± 0.21^{c}
5	PHI	1.73 ± 0.06 ^{a}	1.03 ± 0.04^{b}	1.37 ± 0.06^{c}	1.76 ± 0.06 ^{a}
6	R	11.7 ± 0.36 ^{b}	9.92 ± 0.25^{a}	11.56 ± 0.19^{b}	9.78 ± 0.35^{a}

HMT- Heat moisture treatment, $^{a-d}$ Mean values having different superscript in rows are significantly different at $p \leq 0.05$.

To - onset temperature of gelatinization, Tp - Peak temperature of gelatinization, Tc - Conclusion temperature of gelatinization; R - Gelatinization range; ΔH - Enthalpy of gelatinization, PHI - Peak height index.

The gelatinization range (R) of HMT-30 was significantly ($P<0.05$) lower than native starch. When the peak's height changes in relation to its width, PHI, a gauge of gelatinization uniformity, changes. The PHI of native starch was 1.73, but the PHI of heat-moisture-treated starch ranged from 1.03 to 1.76. According to Gunaratne and Hoover (49) a reduction in ΔH during HMT is caused by the disruption of double helices that are present in both the crystalline and non-crystalline sections of the granules.

3.8. Pasting characteristics

Table 6 and Fig. 4 show the pasting properties of native and HMT starches as measured by RVA. The peak, final, and trough viscosities of all HMT starches were considerably lower than those of native starches (Table 6). Different reaction conditions had varying degrees of impact on the pasting qualities of HMT products. Peak and final viscosities of pearl millet starch decreased more significantly as a function of moisture content during HMT when the length of heating was constant. However, following treatment, the Peak Viscosity of starch reduced, indicating a decrease in starch water solubility and swelling power during gelatinization, and the PT of starch increased, which was compatible with the change in DSC gelatinization temperature (57).

The peak viscosity for HMT (20, 25 & 30%) starches were less 2604, 2349 and 2178

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(cP) respectively than native starch (2644 cP). This indicated a decrease in the peak viscosity upon increasing moisture percentage in HMT. Increased intra- and intermolecular hydrogen bonding in starch chains, as well as the breakdown of amylose in HMT starches, may be responsible for the decrease in PV (58). In the case of final viscosity also a decrease upon heat moisture treated starches than native starch. The final viscosity values for the HMT (20, 25 & 30%) starches were 3881, 3703, 3312 (cP) respectively when compared to native starch 4019 cP. In earlier research, Dhull et al.(59) found that black rice starch was HMT at 100°C for 16 hours with 30% moisture content, which decreased peak and final viscosities while increasing breakdown viscosity and pasting temperature. As the moisture content of the HMT increased, the SP values of the samples declined significantly, which is consistent with the decline in peak viscosity in the curves (Fig. 4). They explained the modifications in pasting properties as the result of a protective shell that developed after HMT around the exterior of partially gelatinized starch granules, acting as a water-repellent barrier and preventing gelatinization and pasting (60).

Pasting temperature increased as the moisture content of the paste increased during HMT. The native starch pasting temperature was 75.1°C, and for HMT-20, HMT-25, and HMT-30, it increased by 3.7, 4.8, and 5.6 °C, respectively. Sharma et al. (2) speculate that an increase in pasting temperature may be caused by the production of greater crosslinking, decreased swelling power, and increased crystallite perfection following HMT, all of which increase the amount of heat necessary for granule breakdown and paste formation.

Other pasting parameters i.e., BD, TV and SB showed significant differences ($p<0.05$) after HMT. The BD ranged from 442 to 1635 cp while TV ranged from 543 to 1907cp for HMT treated samples. In the case of SB, the values ranged from 1795 to 2769 cp for HMT treated starches.

Table-6 Pasting characteristics of native and heat moisture treated pearl millet starch

Sl. No	Properties	Native starch	HMT-20	HMT-25	HMT-30
1	PV(cP)	2644.00 ± 119.00^{a}	2604.67±212.50^{a}	2349.67 ± 161.50^{a,b}	2178.67 ± 68.50^{b}
2	BD(cP)	787.67 ± 12.50^{a}	834.67±83.50^{a}	442.00 ± 48.00^{b}	1635.67 ± 22.50^{c}
3	TV (cP)	1856.33 ± 131.50^{a}	1770.00 ± 129.00^{b}	1907.67 ± 113.50^{b}	543.00 ± 46.00^{b}
4	SB (cP)	2163.00 ± 83.00^{a}	2111.00 ± 61.00^{a}	1795.67 ± 58.50^{b}	2769.00 ± 22.00^{c}
5	FV(cP)	4019.33 ± 214.50^{a}	3881.00 ± 68.00^{a}	3703.33 ± 172.00^{b}	3312.00 ±24.00^{c}
6	PT (ºC)	75.10 ± 0.00^{a}	78.85 ± 0.35^{b}	79.90 ± 0.10^{c}	80.75 ± 0.00^{d}

HMT- Heat moisture treatment, $^{a-d}$Mean values having different superscript in rows are significantly different at $p \leq 0.05$.

PV - Peak viscosity, BD - Breakdown viscosity, TV-Trough viscosity, SB - Set back viscosity,

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Final Viscosity (FV), PT - Pasting temperature

After HMT, the pasting characteristics alter, resulting in a decrease in granular swelling and amylose leaching, as well as an increase in starch chain bonding and granular rigidity (57& 49).

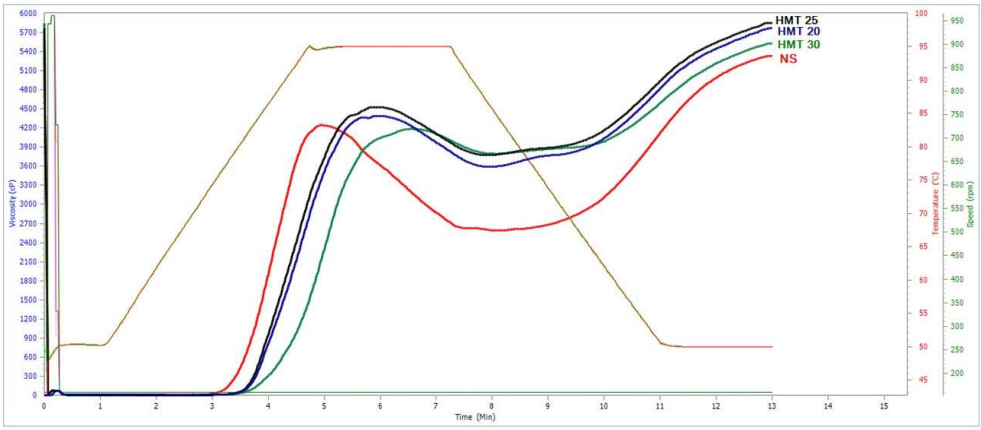

Fig.- 4. Pasting characteristics of native and heat moisture treated starch

3.9. In vitro digestibility

By increasing the SDS and RS contents of starch-based commodities, HMT has been linked to changes in starch digestibility that improve the nutritional value of those items (61) The starch botanical source, starch qualities including crystallinity, granule size, amylose and amylopectin content, interactions and organisation between those two macromolecules, and process parameters are the specific factors directly related to the influence of HMT on digestibility (62).

The RS content of native and HMT treated samples are presented in Table 7. Native starch had an RS content of 11.48 %. The RS content of the samples increased significantly ($p < 0.05$) during HMT, and the rise was bigger as the moisture content of the samples increased. HMT-30 had the greatest RS (19.40 %), which was almost higher than native starch. RS possesses qualities that are similar to dietary fibers and as a result, it provides numerous health benefits. The creation of hydrogen bonds between molecular chains within starch granules, which results in a more compact region that is resistant to enzyme hydrolysis, may be responsible for the greater RS content following HMT (63). Changes in resistant starch content are associated with structural alterations in starch, such as gelatinization and retrogradation, as well as molecular reorganisation and crystallinity (64). The shattering of the double helices, which is evidenced by the reported decrease in gelatinization enthalpy, is responsible for the alterations in SDS and RS (Table- 5). The samples' RDS content decreased from 50.72 for native starch to 42.20 for HMT 30. (Table - 7).

Earlier studies in maize, corn, pea, lentil and pearl millet starches reinforce the above finding that HMT resulted in increased RS and SDS content in starches while decreasing RDS content. (65,19 & 3).

Industrially, the increase in SDS and RS content of starch following HMT could be a promising digestibility factor in developing low carbohydrate foods for consumers with chronic

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diseases such as diabetes.

Table - 7. In vitro digestibility of native and HMT treated pearl millet starch

Sl. No	Treatments	RS	SDS	RDS
1	Native	11.48 ± 0.16^{a}	$37.80 \pm 1.16^{a,b}$	50.72 ± 0.75^{a}
2	HMT-20	12.79 ± 0.16^{b}	39.57 ± 0.52^{c}	47.64 ± 0.08^{b}
3	HMT-25	16.81 ± 0.23^{c}	36.20 ± 0.73^{a}	46.99 ± 0.28^{b}
4	HMT-30	19.40 ± 0.18^{d}	$38.40 \pm 0.86^{b,c}$	42.20 ± 0.33^{c}

HMT- Heat moisture treatment, a-d Mean values having different superscript in columns and rows are significantly different at $p \leq 0.05$.

RS – Resistant Starch, SDS – Slowly Digestible Starch, RDS – Rapidly Digestible Starch

3.10. Correlation Analysis

Amylose content has a prominent effect on the physicochemical characteristics and determines the utility of the starches in various applications like cooking, pasting, hardness, stickiness, nutritional and eating qualities (66&37). Hence, correlation analysis was taken up to understand the relationship between the amylose content and functional characteristics, thermal characteristics and gelatinization characteristics and the results are illustrated as a heat map (Fig- 5).

Amylose content exhibited a positive and significant relationship with swelling power ($r = 0.613$), Solubility ($r = 0.706$), PV ($r = 0.735$), TV ($r = 0.606$), FV ($r = 0.701$) and RDS ($r = 0.700$). Meanwhile, properties like T_0 ($r = -0.754$), T_p ($r = -0.787$) T_c ($r = -0.666$) and RS ($R= -0.733$) exhibited a negative and significant relationship with amylose content. Similar results were reported by Han *et al*., (67), who stated that amylose content positively correlated with FV and SB and negatively correlated with relative crystallinity, To, Tp, Tc, and ΔH for proso millet starch. The strong correlation between amylose, SP, S, RDS than thermal characteristics and RS, indicates that the functional characteristics of starch are not easily damaged in food processing. Moreover, the relationship between amylose, RDS and RS reflect the utility of pearl millet starch in food industry as a probiotic, because HMT resulted in decrease in amylose content (Table-1).

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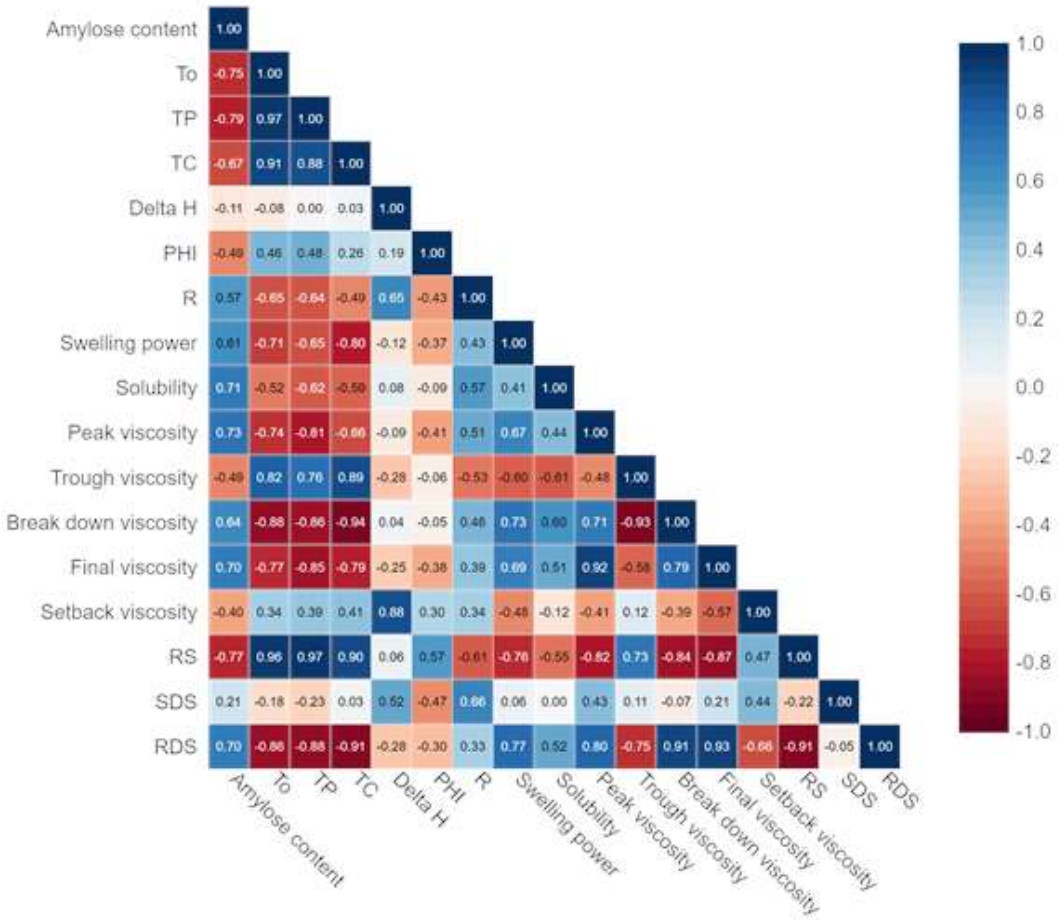

Fig.- 5. Pearson's correlation coefficients between amylose content and functional, thermal and pasting characteristics of HMT treated pearl millet starch

4. Conclusions

In the present study, pearl millet starch was subjected to different levels of heat moisture treatment (20, 25 & 30%). The study revealed that heat moisture treated starches had an increased WAC and OAC compared to its native counterpart, whereas, in the case of amylose content, SP, and S, a profound decrease was witnessed. Thermal characteristics (To, Tp & Tc) of pearl millet starch increased while gelatinization range (R) decreased upon HMT. Heat moisture treatment significantly ($p < 0.05$) lowered the pasting characteristics (PV, FV & TV), when compared to the native starch. RS and SDS content of the starches significantly increased upon HMT, while the RDS content decreased. Morphological studies revealed that the heat moisture treated starches underwent partial gelatinization manifested by the changes on the granule surface like formation of dents, cavities and swelling with a visible reduction of crystallites. Amylose content was found to have a positive and significant ($p < 0.05$) relationship with SP, S, PV, FV, SDS and RDS meanwhile negative and significant relationship with To, Tp, Tc, and RS. The study endorses that heat moisture treatment modifies the physiochemical, thermal, pasting and in vitro characters of pearl millet starch to suite the

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demanding food industrial utility, thereby promoting its potential use.

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