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Effect of Dual Modification on the Properties of Native Pearl Millet Starch

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ABSTRACT

Background: A combination of heat-moisture treatment and citric acid was used to modify pearl millet starch in this study. Changes in structural, thermal and *in vitro* digestibility of the treated starches were investigated.

Methods: Native starch was isolated using alkaline steeping method. Native starch was dual modified (Heat moisture treatment and citric acid) used for further analysis. To examine the granular morphology of dual modified starch using SEM, DSC, RC and in vitro starch digestibility assay was assessed to quantify the proportion of digestive starch fractions, including rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS).

Result: Granules of the native starches of pearl millet varied from polygonal to circular or oval in shape. Indentations were observed on the surfaces of dual modified starch. Relative crystallinity was less than native starch. Profound increased gelatinization temperature was witnessed, meanwhile enthalpy of gelatinization was decreased. In the dual modified pearl millet starches, the percentage of RDS contents was significantly decreased. The resistant starch (RS) content of the treated starches, however, significantly increased from 11.48% in native starches to 19.40% in HMT starches and 28.3% in dual modified starches.

Key words: Citric acid, Heat moisture treatment, Polygonal, Relative crystallinity, Resistant starch.

INTRODUCTION

Starch is a vital biopolymer, found in a wide range of food products. Pearl millet grains are primarily composed of starch (55-70%), whereas their exploitation for industrial uses has been very limited. Pearl millet can thrive in challenging terrain and endure inclement conditions and agronomic circumstances (Shaikh *et al*., 2016). Hence, pearl millet grains may be a useful and inexpensive alternative for starch isolation. Native starches have limited industrial food applications due to their less stability during heating, low shear resistance, non-solubility in cold water, thermal decomposition and high tendency to retrograde (Hui *et al*., 2009). In order to enhance functionality a large portion of the starch used for industrial reasons is often altered chemically, physically, or by combining the two (Siroha *et al*., 2020). Modifications cause desirable changes in the structure of starch, which improves its functionality in food products (Tharanathan, 2005). The dual modification of starch structure has received the most attention recently since studies have shown that single modification methods are typically insufficient to enhance the processing and application capabilities of native starches (Li *et al*., 2017). Recent research has demonstrated that the combination of citric acid and HMT can both increase the amount of RS present in starches derived from various sources and improve their thermal stability (Huang *et al*., 2016). Hence, this study was taken up to investigate the effect of dual modification using heat moisture treatment and citric acid, on the structural, thermal and *in vitro* digestibility properties of pearl millet starch.

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MATERIALS AND METHODS

The study was carried out during 2022 at the Department of Food Science and Nutrition, Periyar University. Good quality of Co10 pearl millet was purchased from Center for Plant Breeding and Genetics, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India. Glucose oxidase-Peroxidase (GOD-POD) kit was obtained from Beacon Diagnostics, Navsari, India. All reagents were purchased from SigmaAldrich. Native starch was isolated according to the alkaline (using aqueous sodium metabisulphite and lactic acid) steeping method previously reported by Ali and Hasnain (2011).

Heat moisture treatment (HMT) of isolated starch

The native starch was adjusted to 30% moisture content, placed in a baking pan, covered with aluminium foil and equilibrated overnight at 4-6°C. The starch was kept in the oven for 3 h at 110°C. To ensure that the heat was distributed

evenly, the samples were occasionally shaken. Thereafter, the samples were cooled and dried at 50°C for 4 h, sieved through 100-mesh and sealed in double polyethylene bags until used (Sun *et al*., 2014).

Preparation of acid modified starch

The technique described by Zambrano and Camargo (2002) was used to produce starch that had been treated with citric acid (CA). To make the starch slurry, 40 g of HMT-treated starches (30% moisture content) were mixed with a 5% citric acid solution and placed in a 45°C water bath while being constantly stirred for 3 hours. The pH was brought down to 5.5±0.2 after hydrolysis test by gradually adding 5 g/100 ml of aqueous sodium hydroxide. The starches were dried in a convection oven at 45°C for 48 hours before sieving. After that, they were cleaned three times with a two-fold volume of deionized water. In order to use the dried starches in the future and increase yield, they were ground into a powder and kept in airtight containers.

Scanning electron microscope (SEM)

A scanning electron microscope (JSM, 6380A, Jeol, Japan) was used to examine the granular morphology (Lopez *et al*., 2008). The starch samples were coated with 30 nm of gold and mounted on SEM stubs using double-sided adhesive tape. The images were studied at a magnification of 3000X.

X-ray diffraction (XRD)

X-ray diffractometer (XRD) (Philips, X'pert MPD high resolution XRD, Almelo, Netherlands) using CuK radiation (Ni filter), the crystalline properties of native and modified pearl millet starch samples were examined. The samples were moulded flat onto a sample holder's cavity and the scanning data was collected at a diffraction angle of (2)= 4-40°.

Fourier-transform infrared spectroscopy (FTIR) analysis

The vibrational spectra of the compounds were taken from a Perkin Elmer FTIR spectrometer (Spectrum RX1, Perkin Elmer, Norwalk, CT, USA). The spectra were recorded in the wave number range of 400-4000 cm^{-1} using a diffused reflectance accessory (DRA) and the background spectrum was the KBr.

Thermal properties

The samples' thermal properties were examined using a differential scanning calorimeter (Q100, TA Instruments, New Castle, DE, USA) and the results were modified slightly to follow Huang *et al*. (2020). 3 mg of the sample (dry weight basis) were combined with six microliters of distilled water and placed in a sealed aluminium pan. The pans were equilibrated for 24 h at room temperature and later heated from 30 to 150°C at a rate of 10°C/min. An empty aluminium pan was utilised as the reference to calibrate the DSC instrument.

In vitro starch digestibility

In vitro starch digestibility assay was found by employing the method described in Englyst *et al*. (1992).

Statistical analysis

All determinations were made in triplicate and the data are presented as mean values±standard deviation (SD). ANOVA (analysis of variance) was performed in one way by using the Duncan's multiple range tests to compare treatments' means. Significance level was defined at $P<0.05$. IBM SPSS software was used for the analysis.

RESULTS AND DISCUSSION

Structural properties

The scanning electron micrographs of native and modified pearl millet starches at 3000 magnifications are shown in Fig 1. The native starch granules varied from polygonal to circular or oval. In addition, few of the granules had irregular and polygonal shapes. Earlier studies by Huber and BeMiller (2000) on native corn and sorghum starches support these findings. HMT modified the surface of the starch granules, causing widespread dents and holes (Fig 1) that might be resulted from disintegration and molecular rearrangement. After HMT, significant changes were observed on the starch granule surfaces. Liu *et al*. (2019) reported that in the case of HMT samples, the development of cavities, fissures and holes on the surface of starch granules is a result of the partial gelatinization of starch caused by HMT.

The surfaces of the dual modified starch granules displayed a disorganised structure with more collapses and

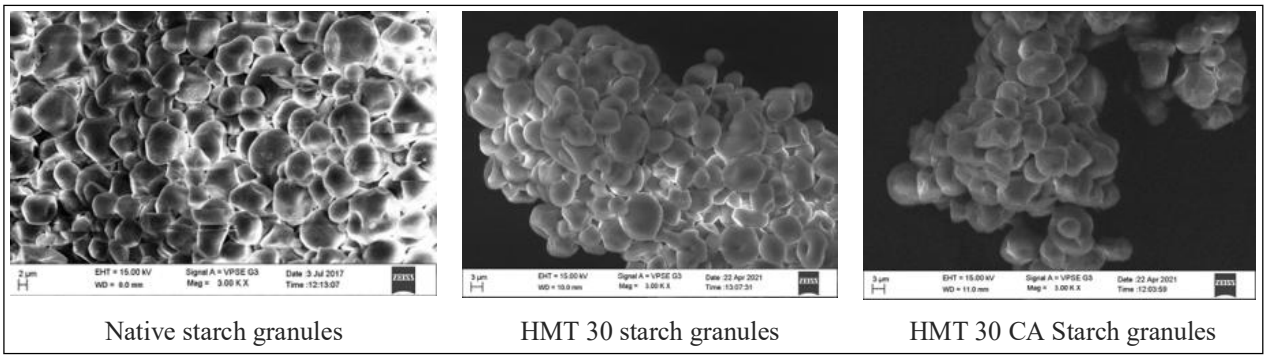

Fig 1: Scanning electron micrographs of native, HMT and dual modified (HMT-CA) pearl millet starches.

concavities. Dimples that have been seen on the surfaces of the citric acid-modified starches may be the result of the acid hydrolysis's weathering effect on the starch granules. Similarly, dimples have been noticed on the surface of acid-modified lentil starch by Kaur *et al*. (2011). Hung *et al*. (2016) also reported starch granule disintegration due to citric acid treatment, which is in conformity with the present findings. Hence, it could be inferred that the deformities on the surface of the dual modified starch granules, which were witnessed in the SEM studies might be due to the dual action of HMT and citric acid.

XRD

The X-ray diffraction pattern and relative crystallinity of native and dual modified (HMT- CA) pearl millet starches are depicted in Table 1 and Fig 2. The modification induced by the addition of CA seemed to reduce the diffraction intensity of native and HMT starch. When compared to native starches, citric acid-treated starch samples' diffraction peak intensities were less. As a result, the relative crystallinity of pearl millet starches decreased from 23.62 per cent (Native starch) to 21.89 per cent (HMT-CA-30) (Table 1). Citric acid was added and this resulted in a reduction in the relative crystallinity of the starch, which has also been documented in other investigations (Mei *et al*., 2015). HMT-CA starches with lower relative crystallinity had more resistant digesting capabilities, suggesting that an increase in RS content may be due to the development of a cross-linked structure that restricts starch chain mobility rather than the crystalline structure.

FTIR

FTIR analysis was carried out on the native, single and dual modified starches to look for any changes in the functional groups on the starch molecules, which helped to confirm that the starch and citric acid underwent an esterification reaction can be detected in the starch FTIR spectrum (VanSoest *et al*., 1995).

The starch citrate samples were washed with ethanol prior to analysis to eliminate any free citric acid that might have impaired the assay. As shown in Fig 3, the broad peak at 3381 cm^{-1} was attributed to the vibration of O-H stretching and the distinctive peaks between 2800 and 3000 cm^{-1} were associated to the vibration of C-H stretching. The bending vibration of O-H appeared at 1649 cm^{-1} (Wang *et al*., 2015). FT-IR spectra showed a new absorbance band at 1735 cm^{-1} in HMT CA treated starch while the band at 1649 cm^{-1} weaker in comparison to native starch. The stretching vibration of the C=O bond of the carbonyl group was responsible for the unique absorption peak at 1735 cm^{-1} (Liu *et al*., 2014). These alterations indicated that covalent connections were created between the citric acid's hydroxyl

Table 1: Relative crystallinity of native and dual modified (HMT-CA) pearl millet starches.

Treatments	"d" spacing	2 theta (2θ)	Relative crystallinity (%)
Native	15.017.023.1	5.75.03.9	23.62
HMT-30	15.017.023.1	5.85.14.1	22.17
HMT-CA-30	15.017.023.1	5.85.14.1	21.89

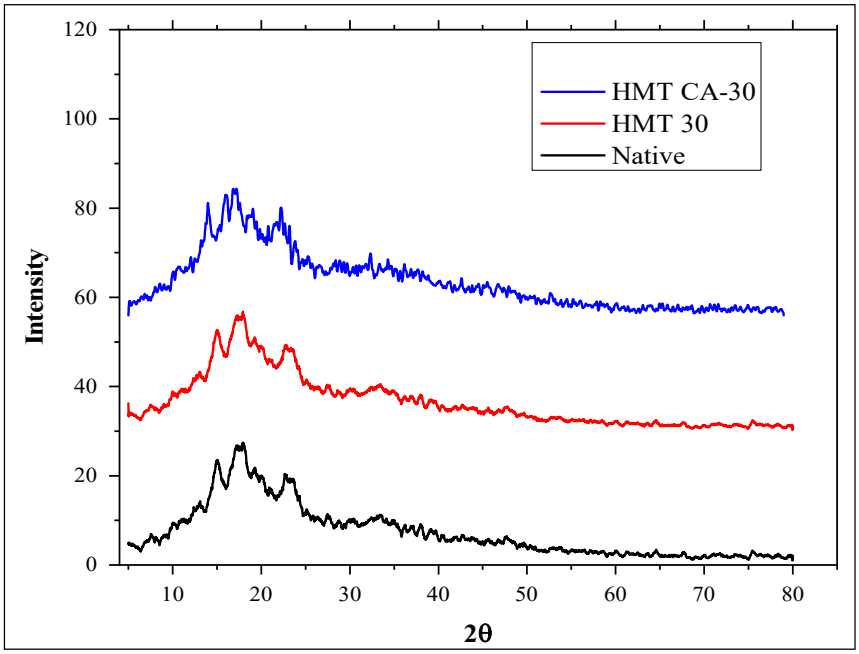

Fig 2: X-ray diffractogram of native, HMT treated and dual modified starches.

and carboxyl groups and the C-O groups of starch. These outcomes demonstrated that citric acid and starch molecules successfully underwent esterification (Jiangping *et al.,* 2019).

The absorbance ratio at 1047 cm^{-1} (representing crystalline portions of starch) and 1022 cm^{-1} (representing amorphous parts of starch) can be used to determine the short-range ordered structure of starch. As shown in Fig 3, the FTIR spectra, deconvoluted FTIR spectra and A1047/A1022 ratio of starch citrates and their controls were represented. Pearl millet starches treated with heat moisture treatment and citric acid had FTIR spectra with peaks at 1022 cm^{-1}. The modification of pearl millet starches resulted in the loss of crystalline structure, which was confirmed by the absence of the 1047 cm^{-1} signal in spectra. Similar to this, Zehra *et al*., (2019), reported that gelatinization caused modified sorghum corn starch to lose its crystalline form. The C-H stretches related to the ring hydrogen atoms are evident in the sharp band at 2927 cm^{-1}. The short-range order of the HMT CA treated starch samples were disrupted, which is consistent with the findings reported by Liu *et al*. (2014).

DSC

The onset (To), peak (Tp) and conclusion (Tc) temperatures of pearl millet starches as determined by DSC are depicted in Table 2. It is evident from the table that the modifications, both HMT and HMT-CA treatments, had a significant ($p \leq 0.05$) impact on the To, Tp, Tc. The onset temperature (To) for native starch was 62.5°C and it reached 79.13°C for HMT- 30 starch and decreased to 67.65°C for HMT CA treated starch sample. Similar trend was seen in both Tp and Tc also. In the case of native starch, the gelatinization peak (Tp) was found at 68.5°C, whereas for HMT-30 it was 85.05°C and HMT CA starches it was 72.16°C. The Tc for HMT CA treated starch was 85.60°C, whereas for HMT 30, it was 89.05°C, while for native starch it was only 74.2°C. The variations in the temperatures which

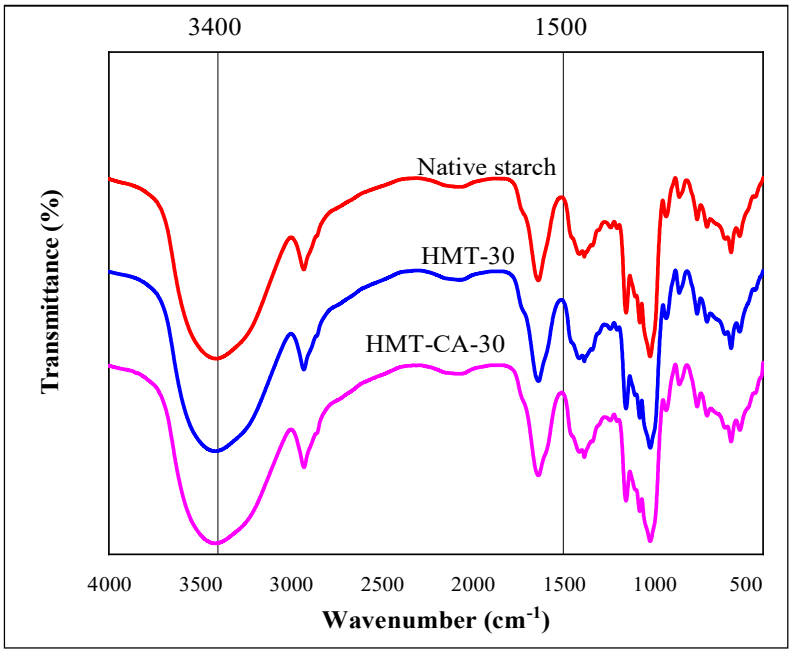

Fig 3: FTIR spectra of modified starches.

Table 2: Thermal analysis of native and dual modified (HMT-CA) pearl millet starches.

Properties	Native starch	HMT-30	HMT-CA-30
To	62.5±2.26^{a}	79.13±1.23^{b}	67.65±0.07^{c}
Tp	68.5±0.10^{a}	85.05±3.09^{b}	72.16±0.04^{c}
Tc	74.2±1.71^{a}	89.05±0.9^{b}	85.60±0.04^{c}
ΔH	10.30±0.26^{a}	7.93±0.21^{b}	6.38±0.03^{c}
PHI	1.73±0.06^{a}	1.83±0.06 ^{b}	1.90±0.02^{c}
R	11.7±0.36^{a}	10.92±0.35^{b}	14.20±0.20^{c}

To= Onset temperature, Tp= Peak temperature, Tc= Conclusion temperature, ΔHgel= Enthalpy of gelatinization (dwb, based on starch weight), PHI- Peak height index, R- Gelatinization range.

The values are expressed as the mean±SD.

Values with similar superscripts in row do not differ significantly ($p>0.05$).

Table 3: Digestive properties of native and dual modified (HMT-CA) pearl millet starches.

Properties	Native starch	HMT-30	HMT-CA-30
RDS	50.70±0.75[b]	42.20±0.33[a]	51.49±0.03[b]
SDS	37.80±1.16[a]	38.40±0.86[a]	20.38±0.03[b]
RS	11.48±0.16[a]	19.40±0.18[b]	28.13±0.07[c]

The values are expressed as the mean±SD.

Values with similar superscripts in row do not differ significantly (p>0.05).

RS- Resistant starch, SDS- Slowly digestible starch, RDS- Rapidly digestible starch

are consistent with the results of short-range range order structure might be explained by the fact that the esterification of citric acid and starch changed the structure of the starch chain and fragmented its crystalline regions (Liu *et al.*, 2014).

Meanwhile, from Table 2, it is evident that HMT-CA decreased the starch's gelatinization enthalpy. Compared with native starch (10.30), the ΔH of the HMT CA treated starch was 6.38 while 7.93 for HMT starch. The starch's gelatinization enthalpy decreased with multiple modifications. This decrease in gelatinization enthalpy (ΔH) can be attributed to either the partial gelatinization of less stable amylose and amylopectin molecules during heating or the dissolution of double helices in the crystalline and amorphous lamellas of the starch granules (Zavareze and Dias, 2011). The highest peak height index (PHI) was observed for HMT CA treated starches (1.90) than HMT and native starch (1.83), (1.73). Gelatinization range (R) of native and modified starches was in the range of 10.92-14.20.

Resistant starch (RS)

The RS content of pearl millet starch increased considerably ($p \leq 0.05$) after dual modification, which is evident from Table 3. It increased from 11.48% in native starch to 28.13% in dual modified starch. In this study, it was found that adding acid to the heat-moisture treatment of pearl millet starches significantly increased (19.40%) the RS, when compared to native and heat-moisture treated pearl millet starches. This indicated that after starch modification with HMT and CA treatment, the breakdown of the ordered structure of the starch chain was more resistant to hydrolysis (Chung *et al.*, 2006). The results concur with Hung *et al.* (2017), who found that cassava and potato starch treated with citric acid produced the highest RS (40.2% and 39%, respectively). When heated, citric acid dehydrates to create anhydride, which esterifies starch to create starch citrate. Crosslinking due to the synthesis of intermolecular di-esters is caused by further treatment at a higher temperature (Olsson *et al.*, 2013). When the starch is treated with citric acid, the cross-linking causes more impediments to the digestion enzymes, which leads to more RS production.

CONCLUSION

Pearl millet starch was subjected to HMT with citric acid and the properties of modified starches were determined. The heat moisture treated starches underwent partial gelatinization, which was obvious in the form of swelling, cavities and dents on the granule surface as well as a clear reduction in crystallites. The surface of the HMT CA treated starch was found to have dimples and it also had less diffraction intensity; a rise in RS content might be caused by the formation of a cross-linked structure that limits starch chain mobility rather than the crystalline structure. In addition, there was no change in functional groups in heat moisture treated pearl millet starch, but new functional groups were formed in chemically modified pearl millet starch at a wavelength of 1022 cm^{-1}. Thus, it could be concluded that dual modification on pearl millet starch would produce high resistant starch, which is more functional and desirable to meet the demands of a health vigilant food industry.

Conflict of interest: None.

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