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The Effect of Heat-Moisture Treatment (HMT) on the Structural, Functional Properties and Digestibility of Citric Acid-Modified *Plectranthus rotundifolius* (Hausa Potato) Starch †

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Abstract: In this study, Hausa potato starch was subjected to single and dual modifications by HMT and citric acid. The parameters such as relative crystallinity, pasting, functional properties, and in vitro digestibility were studied. The XRD analysis showed an A-type diffraction pattern for the native starch and was unaffected by all modifications. The relative crystallinity and the gelatinization enthalpy significantly decreased ($p \leq 0.05$) on dual modification. A new peak at 1724 cm^{-1} was observed in the FT-IR spectra of citric acid-modified starch. The peak intensity became stronger in HMT followed by citric acid-modified starch, which was consistent with the results of degree of substitution. The native starch showed a peak viscosity of 3343 cP and significantly decreased on citric acid modification, and HMT treatment increased the effectiveness of the citric acid modification. The in vitro digestibility of Hausa potato starch was significantly affected by both single and dual modifications. The increased DS and RS content of the dual-modified starch suggests that the HMT served as a pre-treatment and favored the production of the citrate starch. The HMT-citric acid dual modification method was shown to modify the starch properties and could be used as a substitute for producing low glycaemic index foods.

Keywords: *Plectranthus rotundifolius* starch; heat-moisture treatment; citric acid-modification; digestibility

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1. Introduction

Plectranthus rotundifolius (Hausa potato) is a nutrient-rich underutilized tuber that has the ability to ensure food and nutrition security [1]. The higher protein, calcium, magnesium, iron, and fiber in the Hausa potato makes it a healthier alternative to sweet potato, cassava, and yam [2]. African and Indian cuisines commonly prepare Hausa potatoes by baking, boiling, and frying them [3]. In general, Hausa potatoes are harvested four to five months after they are sown. Hausa potato has a starch yield of 18%, which is comparable to that of potato starch [1].

Humans consume carbohydrates primarily through the intake of starch comprised of linear amylose and highly branched amylopectin, having α-D- glucopyranose as structural unit. Despite its many advantages, native starch has several downsides, including gelatinization, retrogradation, low solubility, poor texture development and low stability to heat and shear. Starch is limited to some extent by these demerits [4]. Chemical, physical, enzymatic, or dual modifications can be utilized to improve the characteristics of native starch, making it ideal for a variety of industrial applications [5]. Resistant starch (RS) has gained much attention from researchers and businesses in recent decades [6]. The RS is the fraction of starch that remains indigested in the gastrointestinal tract of healthy individuals at the time of digestion [6].

HMT is the cheapest method that alters the crystalline and amorphous area of starch by treating it at high temperature (90–120 °C) with a moisture content of 20–35% for a specific period [5]. Among hydrothermal methods, HMT has become popular for modifying starch properties and increasing levels of SDS and RS while preserving their granular structure [7]. Citrus fruits are rich in citric acid, which possesses one hydroxyl and three carboxyl functional groups. Polysaccharide chemistry uses citric acid to create super-absorbent hydrogels from carboxymethylcellulose and hydroxy ethyl cellulose. In the past few decades, citric acid has been recognized as a nutritionally harmless metabolic byproduct (Krebs cycle) and approved by the FDA for use in food formulations due to its nontoxic nature [8]. The modification of starch with citric acid is one of the green chemical treatment methods for making SDS and RS [5]. This chemical substitution of starch hinders the enzyme's attack, preventing it from being degraded completely [6]. It is possible to make low-glycemic formulations with these modified starches or to use them as ingredients in canned or frozen food products [4].

Hausa potato starch is a potent unconventional source of starch, and the modification of this starch to enhance its functional attributes are yet to be explored. The modified starch could be tailored to meet the requirements of specific food applications by improving its physicochemical properties [8]. Furthermore, the effect of HMT and citric acid treatment on Hausa potato starch have not been reported. Therefore, the current study aimed to examine the impacts of heat-moisture treatment, citric acid, and dual modification on improving the RS, crystallinity, thermal characteristics and pasting properties of Hausa potato starch.

2. Materials and Methods

2.1. Starch Isolation

Hausa potato starch was isolated from the tuber using the procedure described by Akhila et al. [1]. In this experiment, isolated native Hausa potato starch was used as the control (HNS).

2.2. HMT

HMT was performed on HNS as described by Adegbemiro and Workneh [9]. HHM is used to denote the HMT-modified Hausa potato starch.

2.3. Citric acid Modification

A citric acid (20%) modified starch was prepared according to Falade and Ayetigbo [10]. Modified samples were dried, milled, packed and stored until further examination. The citric acid-modified Hausa potato starch is referred to here as HCA.

2.4. HMT- Citric Acid Modification

HMT-Citric acid-modified starch samples were prepared according to Xia et al. [6], and the samples obtained are referred to here as HCA.

2.5. DS

The degree of substitution (DS) was found by the procedure described in Shaikh et al. [11].

2.6. FT-IR and XRD

According to the procedure described by Navaf et al. [12], the FT-IR spectra of Hausa potato starches were captured using an FT-IR spectrophotometer (Nicolet 6700, Thermo Fisher Scientific, Waltham, MA, USA) at room temperature. The crystalline diffraction patterns and RC of the starch samples were analyzed using an X-ray diffractometer (D2 Phaser, Bruker, Karlsruhe, Germany).

2.7. RVA and DSC

A rapid visco-analyzer (Starch master 2, Newport Scientific, Warriewood, Australia) was used to assess the pasting characteristics of HNS and modified starches by the method followed by Aaliya et al. [13]. The gelatinization properties of Hausa potato starches were carried out using DSC (DSC25, TA Instruments, New Castle, DE, USA) by the procedure explained in Sudheesh et al. [14].

2.8. Light Transmittance and In Vitro Starch Digestibility

Light transmittance is the measure of paste clarity and was determined using the procedure of Akhila et al. [1]. The Englyst et al. [15] method was used to test the starches' in vitro digestibility.

2.9. Statistical Analysis

The significance of the difference between variables was calculated using SPSS 20 (Institute Inc., Cary, NC, USA). One-way ANOVA and Duncan's multiple range tests were conducted on the data, and a significance level of $p \leq 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. DS

The DS describes the number of substituted functional groups that exist per unit of anhydrous glucose. During citric acid modification, citrate functional groups were substituted against the free hydroxyl group in the starch chain. A DS of 0.112 was observed for HCA, while HMT enhanced the incorporation of citrate into the starch polymer, and 0.135 was observed for HHC (Table 1). There was a good agreement between the findings of this study and those of Xia et al. [6].

Table 1. Degree of substitution, relative crystallinity, in vitro digestibility of native, single-, and dual-modified Hausa potato starches.

Samples	DS	RC (%)	RDS (%)	SDS (%)	RS (%)
HNS	–	21.63 ± 0.21^{d}	29.31 ± 0.24^{d}	33.61 ± 0.15^{a}	37.07 ± 0.11^{a}
HHM	–	20.73 ± 0.15^{c}	26.12 ± 0.12^{c}	34.03 ± 0.08^{b}	39.82 ± 0.15^{b}
HCA	0.112 ± 0.013^{a}	20.33 ± 0.06^{b}	25.24 ± 0.18^{b}	34.89 ± 0.09^{c}	40.12 ± 0.21^{c}
HHC	0.135 ± 0.051^{b}	18.90 ± 0.08^{a}	20.16 ± 0.11^{a}	36.10 ± 0.14^{d}	44.05 ± 0.03^{d}

Each value represents the average of three measurements ± SD. A significant difference ($p \leq 0.05$) is indicated by different superscript letters in the same column.

3.2. X-ray Diffraction

Figure 1 illustrates the characteristic A-type diffraction pattern of Hausa potato starches with peaks at 15.10°, 17°, 17.96°, and 22.91° and RC 21.63 %. All the modification methods decreased RC in the order of HNS, HHM, HCA, HHC. The citric acid is esterified by starch granules in both the amorphous and partial crystalline phases [11]. As the HMT-citric acid modification increased the citric acid susceptibility of Hausa potato starch, it further decreased the RC of the starch. This result was inconsistent with the outcome of DS.

3.3. FT-IR

The FT-IR spectra of HNS, HMT and citric-modified starches are depicted in Figure 2. An absorption peak at 3365 cm^{-1} and 2932 cm^{-1} corresponds to the stretching vibration of –OH and –CH functional groups in the starch chain. A sharp absorption peak at 1641 cm^{-1} characterizes the bending vibration of H–O–H molecules [16]. Citrate-modified starches showed a significant absorbance band at 1724 cm^{-1} in their FT-IR spectra compared with native starch because of a vibration caused by the C = O symmetry. It was clear from the new absorption band that the starch had undergone esterification. A greater peak intensity at 1724 cm^{-1} indicates a stronger esterification of HHC than HCA. HMT followed by citric acid esterification produces similar results when treating sweet potato starches [6].

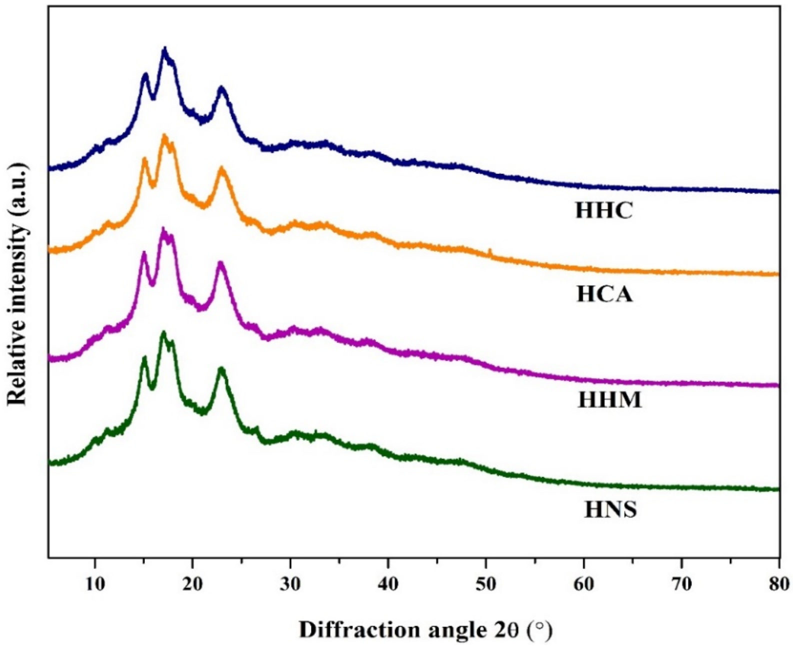

Figure 1. X-ray diffraction pattern of native and modified Hausa potato starches.

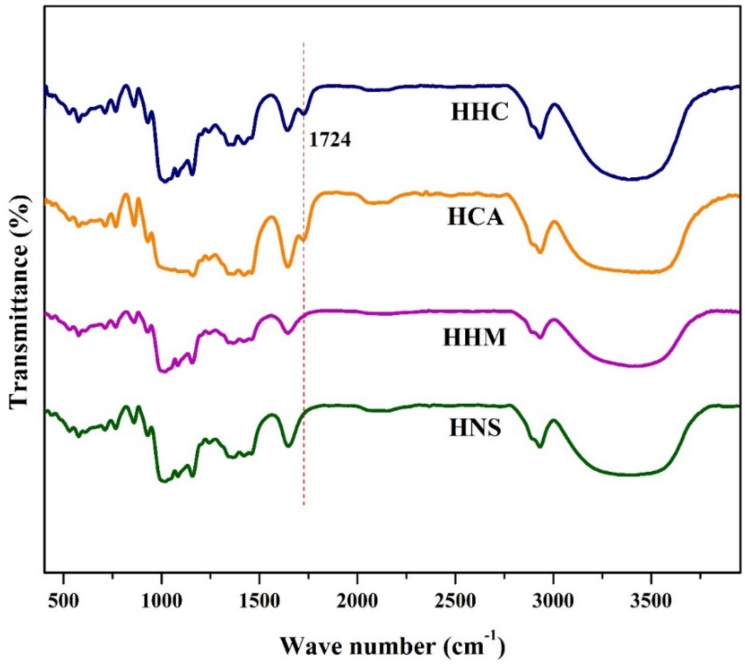

Figure 2. FT-IR spectra of native and modified Hausa potato starches.

3.4. DSC

The gelatinization characteristics such as onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and the gelatinization enthalpy (ΔH) of HNS and modified Hausa potato starches are depicted in Table 2. HHM starch had higher T_o, T_c, and T_p values than native starch while having lower ΔH values compared with HNS. Consistent findings were reported by Hung et al. [17] using potato and cassava starch. The interaction between amylose–amylose, amylose–amylopectin, and amylose–lipid have been implicated in the transition in the gelatinization endotherm of HMT starch towards a higher temperature [18]. After citric acid treatment, the enthalpy of gelatinization was significantly reduced ($p \leq 0.05$) and increased gelatinization transition temperature for

HCA and HHC. A change in enthalpy shows that the granule has lost its molecular order during gelatinization. The dual-modified Hausa potato starch may have caused more loss of ordered structure, which would have reduced the enthalpy [11].

Table 2. Gelatinization parameters of native and modified Hausa potato starch.

Samples	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
HNS	68.18 ± 0.02 [a]	73.04 ± 0.19 [a]	88.23 ± 0.03 [a]	11.12 ± 0.15 [d]
HHM	68.89 ± 0.10 [b]	74.22 ± 0.23 [b]	89.41 ± 0.19 [b]	10.78 ± 0.02 [c]
HCA	69.53 ± 0.06 [c]	74.81 ± 0.04 [c]	90.22 ± 0.07 [c]	8.76 ± 0.09 [b]
HHC	70.18 ± 0.15 [d]	75.06 ± 0.07 [d]	90.94 ± 0.04 [d]	6.57 ± 0.05 [a]

Each value represents the average of three measurements ± SD. A significant difference ($p \leq 0.05$) is indicated by different superscript letters in the same column.

3.5. RVA

Figure 3 illustrates the pasting profiles of native and modified Hausa potato starch viscosity during the heating of starch suspensions. Native Hausa potato starch exhibited remarkably higher ($p \leq 0.05$) peak, breakdown, final, and setback viscosities than the modified starches. The peak viscosity of HNS was 3343 cP, but it was significantly decreased ($p \leq 0.05$) by all modifications, whereas the dual modified starch had the lowest viscosity. The temperature at which starch begins to cook during the heating process is known as the pasting temperature (PT). HHM starches had a higher PT (85.8 °C) than HNS starches (78.40 °C). Ali et al. [8], in their study with HMT-modified lotus seed starch, reported a similar increase in PT. During HMT, the HHM samples exhibited reduced peak viscosities due to amylose–lipid complexes or interaction with amylopectin chains, which in turn inhibit the dispersion and swelling of starch granules [8]. The substitution of citrate prevented starch from swelling and gelatinizing during RVA analysis in the HCA and HHC samples. It was also found that combining citric acid with heat and moisture treatment in maize starch resulted in similar behavior [5]. It was clearly evident from Figure 3 that during RVA analysis, the larger percentage of citrate substitution with accompanied action of HMT limited the swelling and pasting of starch in HHC [14]. The Hausa potato starch's improved thermal stability and decreased viscosity following citric acid modification encourages its usage as an additive in canned foods, as binders in meat and bread, and as a texturizer in confectionery and dairy products [14].

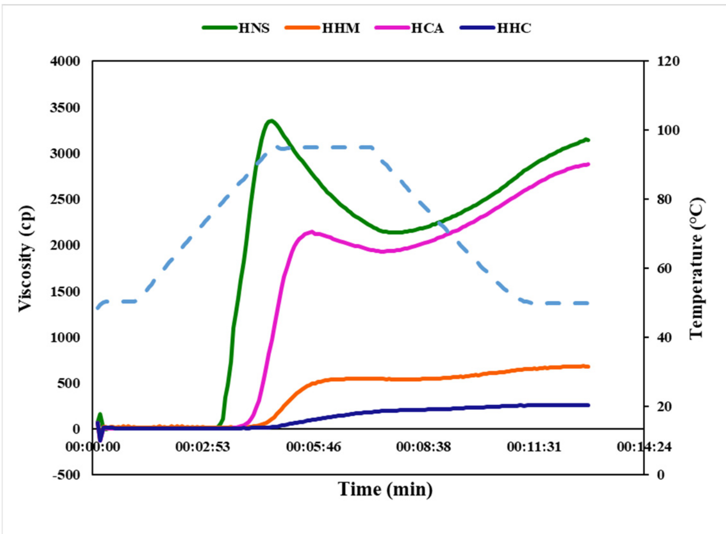

Figure 3. Pasting profile of native and modified Hausa potato starches.

3.6. Light Transmittance

Light transmittance measurements were performed on native and modified Hausa potato starches to assess paste clarity [19]. All the Hausa potato starch samples showed a decreased light transmittance percentage with storage time due to turbidity formation in the starch gel (Figure 4). HHM samples showed a significant reduction ($p \leq 0.05$) in the light transmittance to HNS. HMT starches have a reduced light transmittance because of the increased flexibility of their chains within amorphous areas of granules [20]. Upon citrate esterification of the Hausa potato starches, there was remarkably reduced light transmittance and thereby paste clarity. The HHC samples exhibited the lowest light transmittance among the samples. The bulk of the citrate group, which limits the light flow, may be the cause of the reduced light transmittance of Hausa potato starches treated with citric acid. Furthermore, citric acid could enhanced the hydrolysis of branched-chain molecules into highly cross-linked linear structures, resulting in increasing opacity [14].

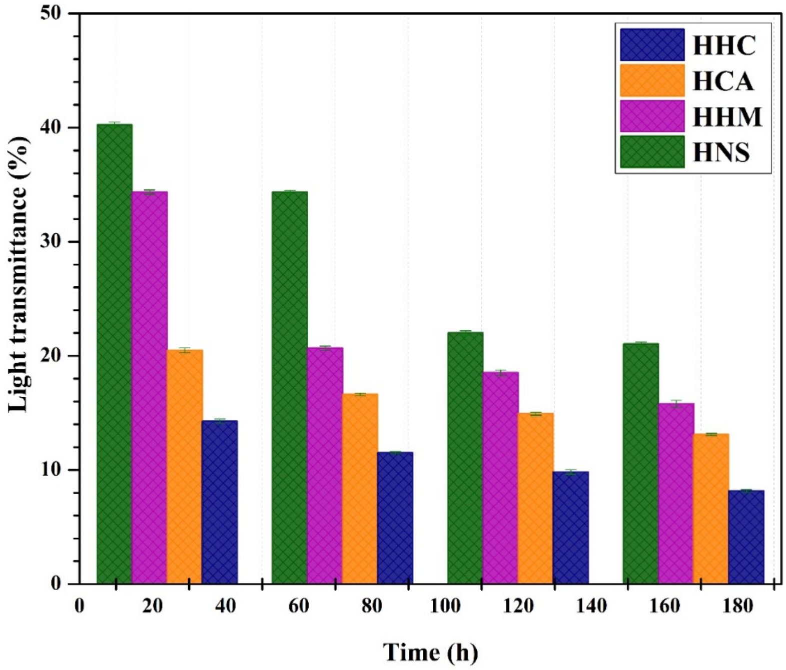

Figure 4. Light transmittance of native and modified Hausa potato starch.

3.7. Starch Digestibility

Table 1 contains the RS, SDS, and RDS of native and modified Hausa potato starches. A noticeable difference was found between the RS contents of the HNS (37.07) and those of HHM (39.82). A retrogradation mechanism by purposeful alteration or processing of the HHM can lead to a higher RS [20]. The citric acid-modified starches, HCA and HHC showed improved SDS and RS and decreases in RDS. Previous research on wheat starches reported that citric acid treatment increased their RS concentration [21]. Starch hydroxyl groups are substituted with citrate groups during citric acid modification of starches. Crosslinking and steric hindrance of the bulkier citrate group leads to resistance to the enzymatic hydrolysis, thereby increasing digestion time and high RS and SDS percentage [14]. Xia et al. [6] concluded that RS and DS had a strong and positive correlation, which was consistent with our findings. The physiological effects of RS are similar to those of dietary fiber [14]. HHC exhibited the highest RS among all the samples, making it suitable for use as a low-calorie functional food.

4. Conclusions

The properties of Hausa potato starch were affected differently by HMT, citric acid, and the combination of these modification methods. However, the starch diffraction pattern was unaffected by any of the modification methods. The DS, thermal analysis, and FT-IR studies suggested that citrate esterification was significantly improved by HMT in HHC. The citrate esterified single and dual modified samples had a lower enthalpy of gelatinization, and light transmittance than that of the native and HMT-modified starches. Reduced viscosities resulting from all the starch modifications are significant quality considerations that can encourage their use in processed meats, sweets, and imitation cheese. These green modifications were effective in improving physiochemical and functional properties of Hausa potato starch, which can be safely applied in food and pharmaceutical industries. The increased DS and RS content of the HHC suggests that the HMT served as a pre-treatment and favored the production of the citrate starch. The dual-modified Hausa potato with a high amount of RS can easily be exploited in food and non-food sectors.

Supplementary Materials: The presentation material of this work is available online at https://www.mdpi.com/article/10.3390/IECBM2022-13392/s1.

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Conflicts of Interest: The authors declare no conflict of interest.

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