



RESEARCH ARTICLE

Tailoring cassava starch properties: A study on Heat-Moisture Treatment (HMT) and Annealing (ANN) for enhanced functional applications

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Received: 29 January 2025; Accepted: 20 May 2025; Available online: Version 1.0: 12 July 2025

Cite this article: Krishna YN, Parveen S, Krishnakumar T, Anand M, Gurusamy K. Tailoring cassava starch properties: A study on Heat-Moisture Treatment (HMT) and Annealing (ANN) for enhanced functional applications. Plant Science Today. 2025;12(sp3):01–10.
<https://doi.org/10.14719/pst.8250>

Abstract

Native cassava starch was subjected to HMT and ANN under varying temperature and treatment duration conditions, as designed using a response surface methodology. The physicochemical properties of the modified starch were compared to those of native starch. The two treatments resulted in distinct alterations of the starch properties. HMT and ANN caused changes in solubility, swelling power, pasting properties, freeze-thaw stability, water and oil absorption capacities, water activity, colour and gelatinization characteristics. Solubility increased by 1.4 % and 3.81 % under HMT 25 % and HMT 30 % respectively, whereas ANN at 1: 3 starch-to-water ratio reduced solubility by 4.02 % compared to untreated starch. Swelling power decreased in the modified starch compared to the control sample. Viscosity studies revealed that peak viscosity decreased from 3812 cP in untreated sample to 3267 cP and 3150 cP after HMT - 25 % and HMT - 30 % respectively. In contrast, ANN increased the peak viscosity to 4014 cP. Freeze-thaw stability was narrowed for both HMT and ANN treated starches compared to the untreated starch. The treatments enhanced water absorption capacity but reduced oil absorption capacity (OAC). The clarity of the modified cassava starch pastes slightly decreased compared to the native starch. Moreover, the whiteness of the treated cassava starch powder was marginally lower than that of the untreated starch, though the differences were not statistically significant. These consequences disclosed that HMT and ANN techniques effectively modified the physicochemical properties of cassava starch.

Keywords: absorption capacity; cassava starch; peak viscosity; physical modifications; physicochemical properties; starch modification; syneresis

Introduction

Tuber crops are preeminent food crops and have abundant starch content. They are extensively cultivated over a large area in different places worldwide and have been a prominent staple food, particularly for low-income people. Cassava (*Manihot esculenta* Crantz) is a crucial crop in the agro-industry due to its high starch content. It is a critical crop worldwide, particularly in tropical regions, serving as a staple for over 800 million people and as a key raw material in various industries. It thrives in poor soils and drought-prone areas, making it a vital resource for food security in developing regions. Cassava applications range from food products like starch, flour and chips to industrial uses such as bioethanol production and non-food sectors such as bioplastics and adhesives. Recent years have seen an increase in cassava yield due to advancements in biofortification and agronomic practices (1). The crop has numerous benefits including its role in reducing hunger, generating income and enhancing soil fertility when

intercropped with legumes. Advantages include its resilience to climate change, low input requirements and high caloric output. However, cassava faces challenges such as low protein content, cyanogenic compounds requiring proper processing and limited mechanization in production (2). Global production has risen, with countries like Nigeria, Thailand and Brazil leading in cassava cultivation. Additionally, innovations in cassava breeding and processing technologies continue to position cassava as an industrial commodity and reliable food source (3). Cassava starch has unique properties, including high paste clarity, low viscosity and excellent gel strength which make it ideal for various applications. Physically modifying cassava starch particularly via HMT and ANN enhances its thermal, textural and physicochemical properties, making it suitable for specialized applications such as resistant starch production and food formulations requiring stability. HMT improves resistant starch levels, benefiting human health by aiding digestion and controlling glucose levels. However, these modifications can alter functional properties such as reducing swelling

capacity or increasing crystallinity, which might limit their application and enhance their functionality, increasing resistant starch levels for health benefits, such as improved glycemic regulation (4). There are numerous reports on the HMT and ANN of cereal and tuber starches. However, efficient studies on the HMT and ANN of cassava starch still need to be examined to improve various industrial applications. Jyothi et al. (5,6) investigated the hydrothermal modifications of tropical tuber starches, specifically examining the effects of heat-moisture treatment (HMT) and annealing (ANN) on their physicochemical, rheological and gelatinization properties. HMT resulted in a marked decrease in peak viscosity, a slight reduction in breakdown viscosity and increased solubility in the treated starches. In contrast, ANN led to a narrowing of the DSC gelatinization patterns across all starches, while solubility decreased notably in cassava starch. The current study aimed at tailoring the effects of HMT and ANN on the physicochemical properties of cassava starch.

Materials and Methods

Raw material

Native starch was extracted using matured cassava (*M. esculenta* Crantz) tubers of Sree Reksha variety, carefully procured from the research farm of Indian Agricultural Research Institute-Central Tuber Crops Research Institute (ICAR - CTCRI), Thiruvananthapuram, Kerala. Analytical chemical grade reagents were used.

Extraction of cassava starch

From freshly harvested cassava tubers, starch was extracted using standard protocol (7, 8). The fresh cassava tubers were washed thoroughly, peeled by hand and cut into small pieces using a cutting machine. These pieces were crushed in a starch extraction unit with enough quantity water. The starch extract obtained was filtered through a mesh sieve -150 μ m and left to settle in a tank overnight. Once starch settled at the bottom, water was decanted and starch was removed from the tank and washed 3 times with plenty of water to make it bright white. Finally, starch was placed in aluminium

trays, dried in a tray dryer at 45 - 50 °C for 12 hr and stored in an airtight container with a moisture content of 10 - 12 % (dry basis) for further treatments and analysis (Fig. 1).

Physical modification of cassava starch

Heat moisture treatment

Heat moisture treatment of cassava starch was conducted (5, 9, 10) with minor modifications. The native starch moisture content was predetermined. The moisture levels of starch sample were adjusted to 25 % and 30 % by adding appropriate volumes of distilled water. The moisture levels were carefully chosen based on primary trials and existing literature. The variables used were moisture content (25 % and 30 % based on dry starch weight), temperature (120 °C and 130 °C) and time (12 hr and 17 hr) respectively. To obtain desired moisture levels, starch was taken in a vessel to a known quantity and uniformly mixed by adding distilled water slowly to avoid lump formation. The air oven is set at a precise temperature (120 °C and 130 °C) and time (12 hr and 17 hr). Now the vessel is allowed to dry. The starch samples were then cooled to room temperature and dried at 50-55 °C until the moisture level reached 6 - 8 %. Samples are indicated as HMT - 25 % and HMT - 30 %. Refer graphical abstract (Fig. 2) for alteration of starch properties after heat moisture treatment.

Annealing treatment

Annealing treatment was carried out (11) with slight modifications. The slurry was made using cassava starch and distilled water at a ratio of 1: 3 (w/v). It was sealed. Subsequently, the slurry was incubated in a water bath at 55 °C for 24 hr and cooled at room temperature. Then sample was dried at 50 - 55 °C until the moisture level reached to 6 - 8 %. The sample was represented as ANN. Refer graphical abstract (Fig. 2) for alteration of starch properties after annealing treatment.

Analytical methods

Determination of moisture content

Moisture content protocol was followed for native and modified starch samples (12, 13). Weighed accurately about 5 g of sample into a pre-dried, cooled and tared moisture Petri plate. The petri plate was placed uncovered in a hot air oven;

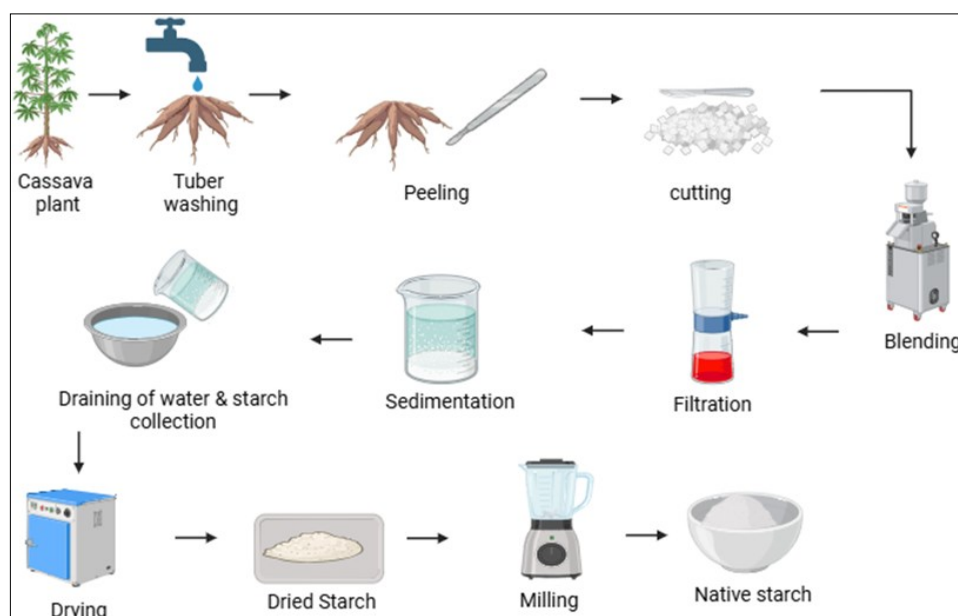


Fig. 1. Graphical representation of cassava starch extraction.

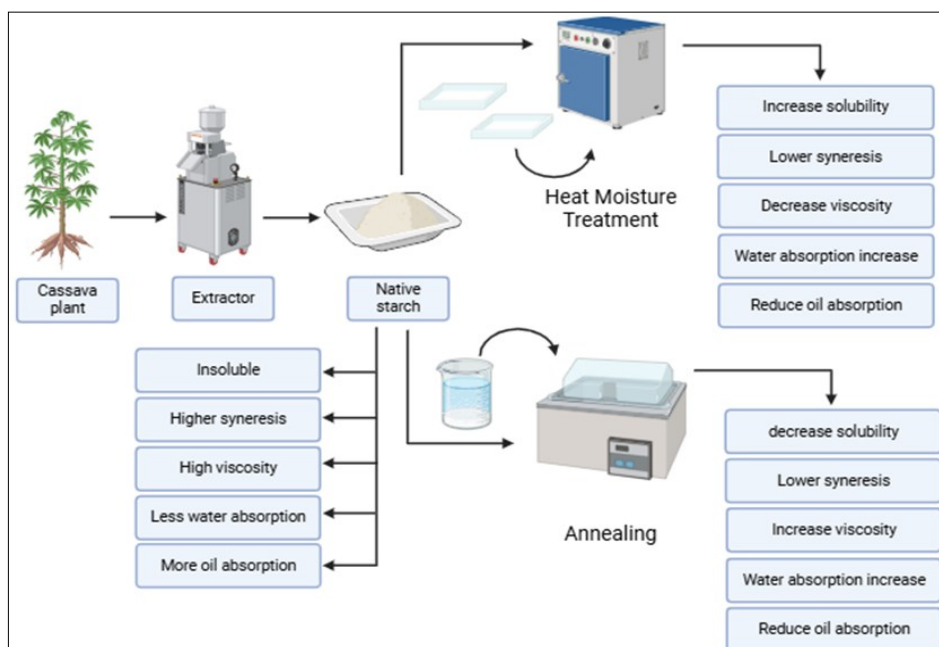


Fig. 2. Graphical abstract of HMT and ANN of cassava starch.

maintained at 105 °C for 3 hr. Immediately after opening the oven, the Petri plate was closed with a lid, placed in a desiccator until it cooled (usually 30 min typically enough) and then weighed.

Moisture (%) =

$$\frac{\text{Weight of sample(g)} - \text{weight of dried sample(g)}}{\text{Weight of sample(g)}} \times 100$$

Estimation of starch content

Starch content estimation was performed for native and modified starch samples (14).

Starch content (%) =

$$\frac{\text{Volume of ferric cyanide} \times \text{make up volume} \times 0.9 \times 100}{\text{Titre value} \times \text{weight of sample} \times 1000}$$

Solubility and swelling power

The solubility and swelling power of native and modified starch samples were determined (6, 15, 16). With slight modification, 400 mg of starch sample was weighed, dispersed in 40 mL distilled water and heated by keeping in a shaking water bath at 95 °C for 20 min and allowed to cool. From that, 10 mL samples were transferred to 50 mL centrifuge tubes with a graduated cylinder and centrifuged at 6000 rpm for 20 min. The supernatant was decanted out carefully after centrifuging and the weight of the sediment was recorded. The supernatant was dried at 105 °C, until constant weight and the weight of the dry solids was measured. Solubility and swelling power were calculated using the following equation:

$$\text{Solubility(\%)} = \frac{\text{Weight of dried supernatant}}{\text{Original dry starch weight}}$$

$$\text{Swelling power (g/g)} = \frac{\text{Weight of sediment}}{\text{Original dry starch weight}}$$

Paste clarity

1 % concentration of starch dispersion was prepared by heating 0.2 g of starch in 20 mL of distilled water in a shaking water bath at 90 °C for 1 hr and cooled to ambient temperature. At 640 nm the transmittance (% T) was measured using (Double Beam Thermo Fisher GENESYS UV-Vis Spectrophotometer) (15, 17, 18).

Pasting properties of starch

The pasting characteristics of the native and modified starch samples of cassava were assessed using the Rapid Visco Analyzer (RVA-4, Newport Scientific, Warriewood, Australia). Viscosity profiles for the starch were recorded by employing starch suspensions. Accurately weighed 2.5 g of dry weight was placed into the aluminum canister; 25 mL of distilled water was added and mixed using a glass rod. The aluminum canister was inserted into the analyzer unit and a heating/cooling cycle was conducted at a constant speed of 160 rpm following the standard I profile. The starch slurry was heated from 50 °C to 95 °C at a rate of 12 °C/min and maintained at 95 °C for a duration of 2 min. The paste was then cooled to 50 °C at 12 °C/min and ultimately held at 50 °C for an additional 2 min. The parameters were evaluated in terms of centipoises (cP); including peak, trough, breakdown, setback, final viscosities, pasting temperature (°C) and peak time were recorded.

Colour of starch

The colour of starch samples of native and modified cassava was analyzed using a colorimeter (Make: Hunter Lab, Model: 45°/0° geometry, D 65 optical sensor, 10° observer). The principal colour parameters L*, a* and b* were measured by inserting starch samples into sample container. The colorimetric measurements of lightness L* parameter denote light/ dark spectrum with a range from 0 (black) to 100 (white), a* indicates green - red spectrum ranging from -60 (green) to +60 (red) and b* signifies blue - yellow spectrum with a range from -60 (blue) to +60 (yellow) dimensions respectively. Moreover, the total colour difference(ΔE) yellowness index (YI) and whiteness index (WI) were determined.

Total colour difference (ΔE) =

$$\sqrt{[(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2]}$$

Where, $L_0 = 99.34$, $a_0 = 0.03$, $b_0 = 0.1$

$$\text{Yellowness Index (YI)} = \frac{142.86 \times b}{L}$$

$$\text{Whiteness Index (WI)} = 100 - \sqrt{[(100 - L)^2 + a^2 + b^2]}$$

Freeze-Thaw stability

The process of freeze-thaw stability was conducted (15, 19). With minor deviations, an aqueous starch suspension (5 % w/v) was heated at 95 °C for 30 min with constant slight agitation in a shaking water bath and then cooled to room temperature. An aliquot paste of 10 mL was then dispensed into the weighed centrifuge tube and subjected to a freeze-thaw cycle: frozen at -16 °C for 24 hr, altered by thawing at 25 °C for 2 hr. Subsequently, it was centrifuged at 3000 rpm for 20 min. After the centrifugation process, the supernatant was carefully decanted. The percentage of syneresis was calculated as the ratio of the weight of liquid decanted multiplied by 100 to total weight of gel before centrifugation.

$$\text{Syneresis (\%)} = \frac{\text{Weight of liquid decanted}}{\text{Total weight of gel before centrifugation}}$$

Water activity

Water activity in starches was assessed using a water activity meter. The starch sample was positioned in the sample holder and readings were documented (20).

Water absorption capacity

The standardized methodology was employed for determining water absorption capacity (11, 16, 21). The density of water is 1 g/mL. With minor adjustments, 10 mL of distilled water was added to 1 g of native and modified starches in a falcon tube of 50 mL capacity. 10 mL of distilled water was added to 1 g of native and modified starches in a falcon tube of 50 mL capacity. The starch suspension was then stirred for 5 min using a vortex shaker. After that, the suspension was left for 2 - 3 min and then centrifuged at 10000 rpm for 10 min. The supernatant obtained was measured using a 10 mL graduated cylinder. The difference between the initial volume of water used and the volume of the supernatant obtained after centrifuging was calculated. The result was expressed as ratio of gram of water absorbed per gram of starch.

$$\text{Water absorption capacity (g/g)} = \frac{\text{Weight of absorbed water}}{\text{Weight of starch}}$$

OAC

The established procedure documented in studies (11, 16, 21) for oil absorption capacity. The density of sunflower oil is 0.92 g/mL. With a minor modification, one gram of native and modified starches was mixed with 10 mL of distilled water in a 50 mL falcon tube. 1 g of native and modified starches was mixed with 10 mL of distilled water in a 50 mL falcon tube. The starch suspension was then agitated for 5 min utilizing a magnetic stirrer at 1000 rpm. Following this, the suspension

was allowed to rest for 2 - 3 min and subsequently centrifuged at 10000 rpm for 10 min. The resulting supernatant was measured with a 10 mL graduated cylinder. The difference between the original volume of oil utilized and the volume of supernatant obtained after centrifugation was calculated. The outcome was expressed as the ratio of grams of oil absorbed per gram of starch.

$$\text{Oil absorption capacity C (g/g)} = \frac{\text{Weight of absorbed oil}}{\text{Weight of starch}}$$

Statistical analysis

All determinations were triplicated; mean values and standard deviations were reported. Data were analyzed using analysis of variance (ANOVA) and treatment means were statistically compared using the Least Significant Difference test at $p < 0.05$ level.

Results and Discussion

Effect of HMT and ANN on moisture and starch content

A moisture level of more than 13 % is considered unstable and unsafe during storage, as it makes the starch prone to microbial contamination. The chemical composition of native and modified cassava samples exposed to different treatments was measured and presented in Table 1. The moisture content (%) ranges from 6.09 ± 0.004 - 10.07 ± 0.39 . The data showed a clear reduction in moisture content for the modified starches compared to the control sample. ANN and HMT modified starch granules exhibited enhanced water release, reducing moisture content. HMT appeared to be more efficacious in lowering moisture content than ANN because the high temperatures (100 °C) used in HMT reduce the ability to hold water in starch. These results agreed with the previous findings (10, 22, 23). This reduction in moisture content is beneficial in improving the stability and shelf-life of starch-based products, particularly in applications where lower moisture retention is desirable. The untreated starch showed a starch content of 93.43 %, while the treated starches (HMT - 25 %, HMT - 30 % and ANN) exhibited no significant difference in starch content compared to the control.

Effect of HMT and ANN on solubility, swelling power and paste clarity of cassava starch

The effect of different treatments on the solubility, swelling power and paste clarity of cassava starch at 95 °C is given in Table 2. The solubility of cassava starch increased after HMT, with values of 20.5 ± 2.34 % for HMT - 25 % and 22.91 ± 3.6 %

Table 1. Moisture content, starch content, water and oil absorption capacities, water activity of native, HMT and ANN starches

Parameter	Samples			
	Native	HMT - 25%	HMT - 30%	ANN
Moisture content (%)	10.07 ± 0.39	6.31 ± 0.17	6.76 ± 0.09	6.09 ± 0.04
Starch Content (%)	93.43 ± 0.99	95.43 ± 1.16	94.74 ± 2.52	94.54 ± 1.70
WAC (g/g)	2.21 ± 0.49	2.55 ± 0.38	2.58 ± 0.31	3.30 ± 0.62
OAC (g/g)	2.61 ± 0.45	1.75 ± 0.27	1.73 ± 0.33	2.22 ± 0.57
Water activity (*w)	0.45 ± 0.01	0.43 ± 0.09	0.412 ± 0.08	0.44 ± 0.02

Values are the means of triplicate determination \pm standard deviation (SD).

Table 2. Effect of different treatments on solubility, swelling power and paste clarity of cassava starch at 95 °C

Parameter	Samples			
	Native	HMT - 25%	HMT - 30%	ANN
Solubility (%)	19.1 ± 2.59	20.5 ± 2.34	22.91 ± 3.60	15.08 ± 0.25
Swelling power (g/g)	7.07 ± 0.57	6.51 ± 0.96	6.35 ± 1.64	7.00 ± 1.47
Paste clarity (%T)	49.94 ± 3.04	45.69 ± 2.62	44.05 ± 5.72	49.37 ± 3.81

Values are the means of triplicate determination ± SD.

for HMT - 30 % compared to the control 19.1 ± 2.59 %. This could be due to partial molecular disintegration of the starch granules during HMT. In contrast, annealing significantly decreased solubility by 15.08 ± 0.25 %, indicating a more ordered granular structure that resists solubilization. The lower solubility of annealed starch occurs because the bonds between amylose and amylopectin or between amylopectin molecules become stronger. This makes starch harder to dissolve and prevents it from leaking out of the granules. These findings are consistent with previous research (5,6,24). Enhanced molecular organization reduced starch swelling power and solubility. The treatments significantly influenced the swelling power of cassava starch. The control starch exhibited the highest swelling power 7.07 ± 0.57 g/g, indicating its greater ability to absorb water at high temperatures. HMT reduced the swelling power, with values decreasing to 6.51 ± 0.96 g/g for HMT - 25 % and further to 6.35 ± 1.64 g/g for HMT - 30 %. This reduction can be attributed to changes in organization of crystalline regions of starch, increased crystallinity, amylose - lipid complexes formation, increased interactions between amylopectin and amylose molecules and hardened intramolecular bonds, leading to restricted water penetration during HMT. These consequences aligned with common buckwheat (10), maize (16), potato and cassava (25), rice (26), sorghum (27) and corn starches (28). Conversely, starch treated by ANN showed a reduction in swelling power 7.00 ± 1.47 g/g comparable to the control, the reduction in swelling power is responsible for increase in molecular organization and minimal disruption of its granular structure. These outcomes are accorded with barley (29), wheat starch (30) and groundnut (31). Paste clarity decreased in % light transmittance of starch samples treated with HMT, with values of 45.69 ± 2.62 % for HMT - 25 % and 44.05 ± 5.72 % for HMT - 30 %, compared to the control 49.94 ± 3.04 %. The reduced clarity is likely due to a slight browning effect and attributed to the development of firm surface coating of granules during HMT (5, 32). Annealing had a minimal impact on paste clarity, with a value 49.37 ± 3.81 % close to the control, this slight reduction might have enhanced the integrity of swollen granules. ANN reduced cassava starch paste clarity (6).

Effect of HMT and ANN on pasting properties of cassava starch paste

The pasting properties of cassava starch subjected to different treatments are shown in Table 3 and graphs illustrated in Fig. 3-6. The treatments significantly influenced the pasting behaviour including peak viscosity, trough viscosity, breakdown viscosity, final viscosity, setback viscosity, peak time and pasting temperature. The control

Table 3. Pasting properties of native and modified starches subjected to different treatments

Parameter	Samples			
	Native	HMT - 25%	HMT - 30%	ANN
Peak Viscosity (cP)	3812 ± 0.33	3267 ± 0.13	3150 ± 0.31	4014 ± 0.11
Trough viscosity (cP)	1358 ± 0.12	1681 ± 0.15	1693 ± 0.06	1779 ± 0.14
Breakdown viscosity (cP)	2454 ± 0.21	1586 ± 0.20	1457 ± 0.05	2235 ± 0.21
Final viscosity (cP)	1939 ± 0.14	2231 ± 0.16	2262 ± 0.08	2191 ± 0.04
Setback viscosity (cP)	581 ± 0.10	550 ± 0.28	569 ± 0.31	412 ± 0.23
Peak time (min)	3.73 ± 0.01	3.73 ± 0.02	3.73 ± 0.06	3.73 ± 0.08
Pasting temperature (°C)	72.65 ± 0.31	71.8 ± 0.24	70.95 ± 0.41	76.7 ± 0.25

Values are the means of triplicate determination ± SD. "cP" indicates rapid viscosity units (centipoises).

starch exhibited a peak viscosity of 3812 ± 0.33 cP, which was reduced after HMT. HMT - 25 % and HMT - 30 % showed peak viscosities of 3267 ± 0.13 cP and 3150 ± 0.31 cP respectively. The reduction in peak viscosity is attributed to structural modifications caused by HMT, such as reduced water absorption capacity and amylose leaching. Conversely, ANN resulted in the highest peak viscosity i.e., 4014 ± 0.11 cP, suggesting that ANN enhanced the swelling capacity of starch granules. Trough viscosity, which indicates the minimum viscosity during heating, increased after HMT and ANN, this suggests improved structural stability of the starch granules under heat and shear. However, breakdown and setback viscosity decreased significantly after both modifications. These results represent better paste stability. ANN treated starch showed a high trough viscosity of 1779 ± 0.14 cP and a moderate breakdown viscosity viz., 2235 ± 0.21 cP, reflecting its improved swelling and partial stability. Final viscosity, indicative of starch's ability to form a gel after cooling, increased in HMT-treated starch, with 2231 ± 0.16 cP (HMT - 25 %) and 2262 ± 0.08 cP (HMT-30%) showing higher values than the control i.e., 1939 ± 0.14 cP. All treatments had a similar peak time. However, pasting temperature decreased slightly in HMT-treated starch (71.80 ± 0.24 °C for HMT - 25 % and 70.95 ± 0.41 °C for HMT - 30 %) compared to the control (72.65 ± 0.31 °C), indicating enhanced gelatinization efficiency due to thermal modification. In contrast, ANN-treated starch exhibited a higher pasting temperature (76.7 ± 0.25°C), suggesting greater resistance to gelatinization, likely due to the formation of a more ordered starch structure and attributed strengthening of bonds. These outcomes are in agreement with cassava (5, 6) and potato starches (33).

Effect of HMT and ANN on colour of the starch powder

The colour properties of native and modified cassava starches are given in Table 4. Colour is a vital parameter in estimating the quality of starch. The lightness value (L^*) of native starch was highest at 94.15 ± 1.12, while it decreased slightly in the modified starches, with the lowest L^* value observed in HMT - 30 % was 92.82 ± 1.53. HMT at higher moisture and temperature conditions may have induced molecular rearrangements or slight browning due to Maillard reaction, reducing the lightness value. Annealing slightly reduced L^* values (93.13 ± 1.49) compared to native starch, consistent with literature cited (34). The a^* and b^* values,

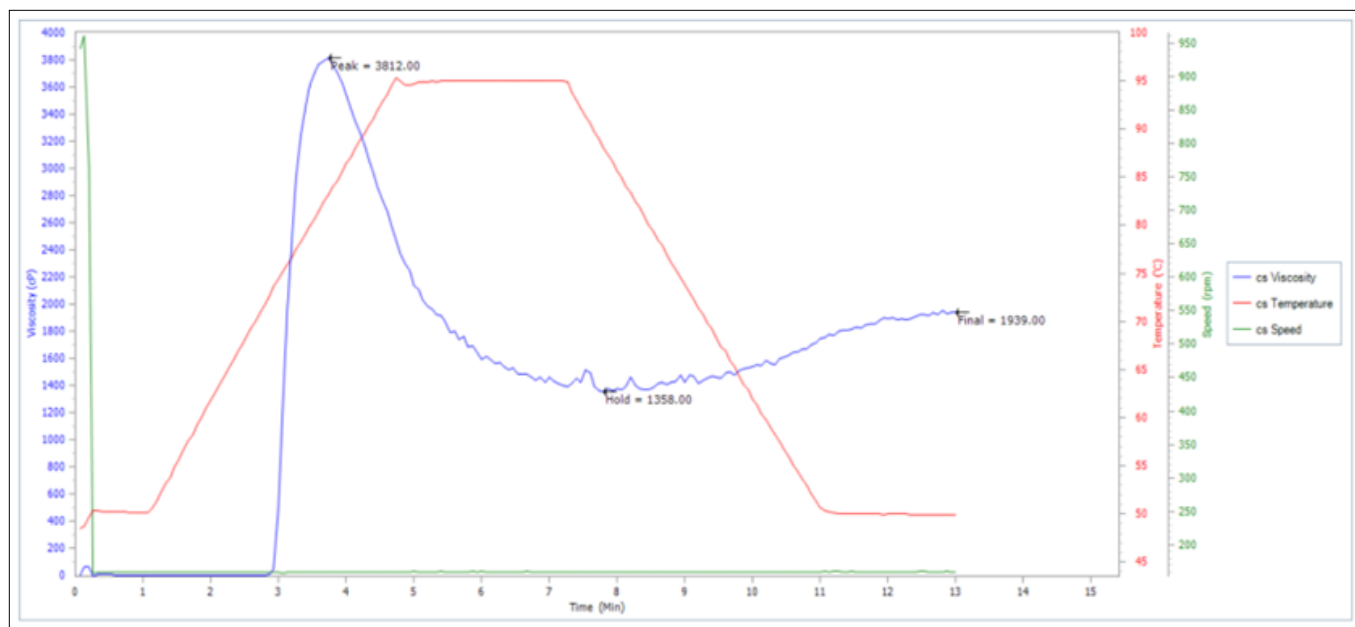


Fig. 3. Native cassava starch.

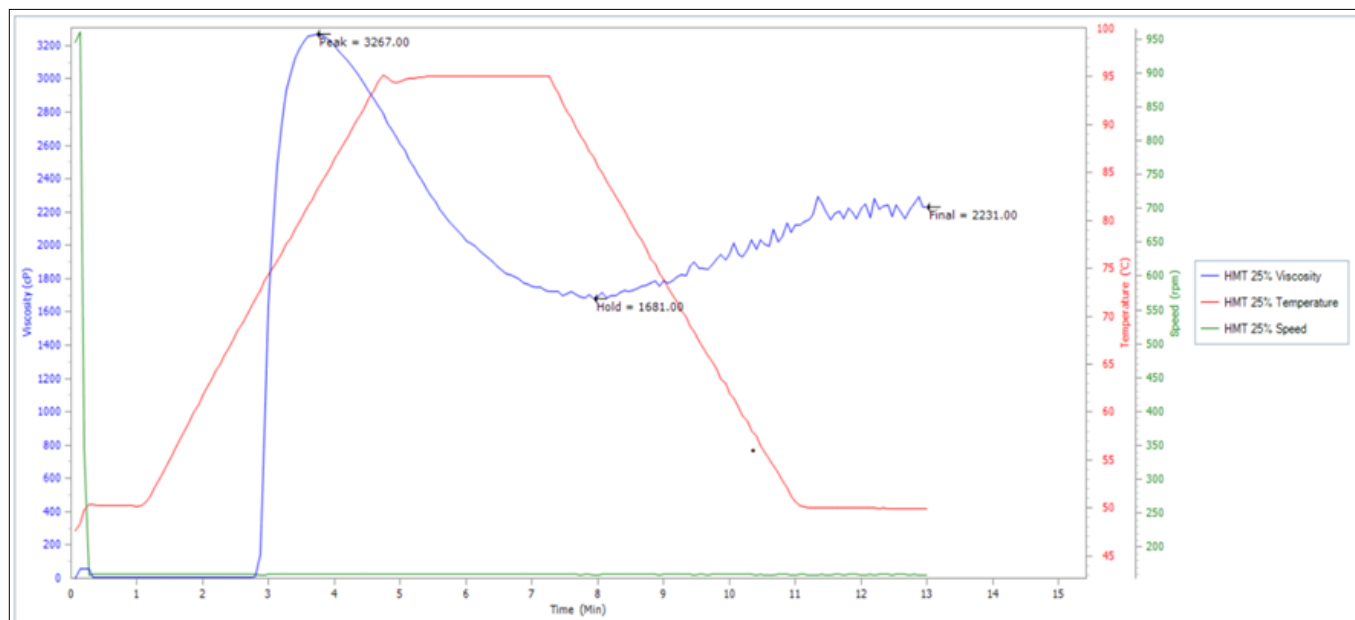


Fig. 4. HMT - 25 %.

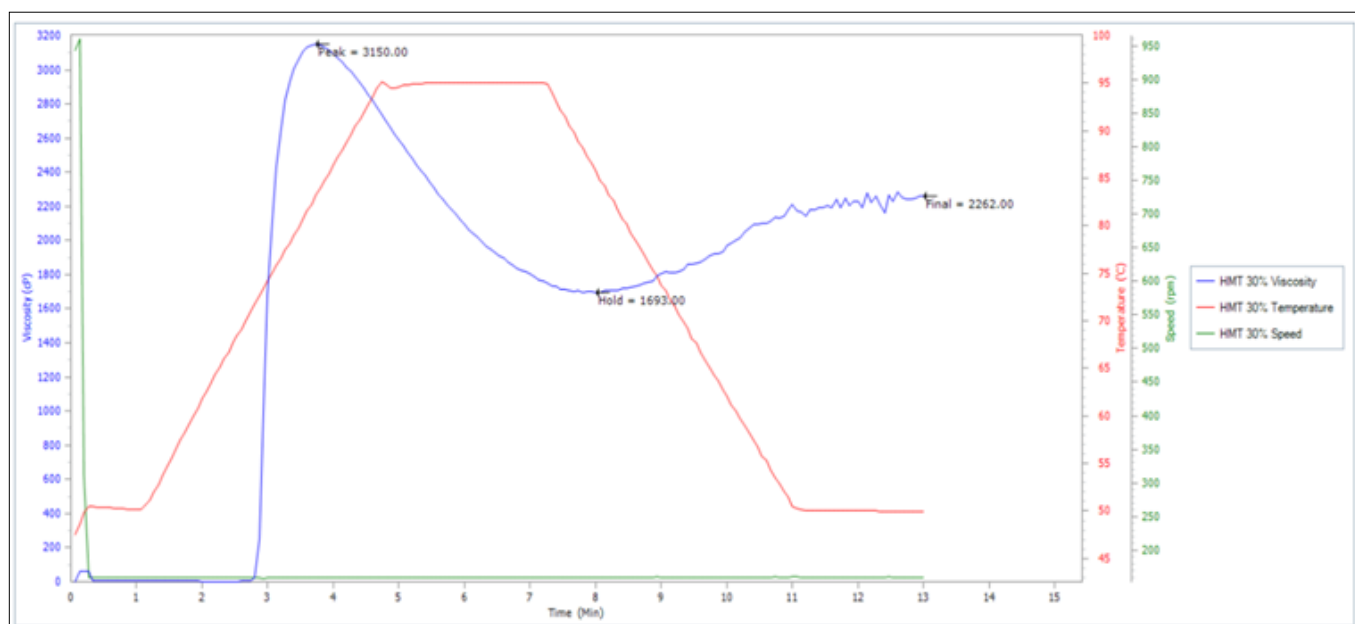


Fig. 5. HMT - 30 %

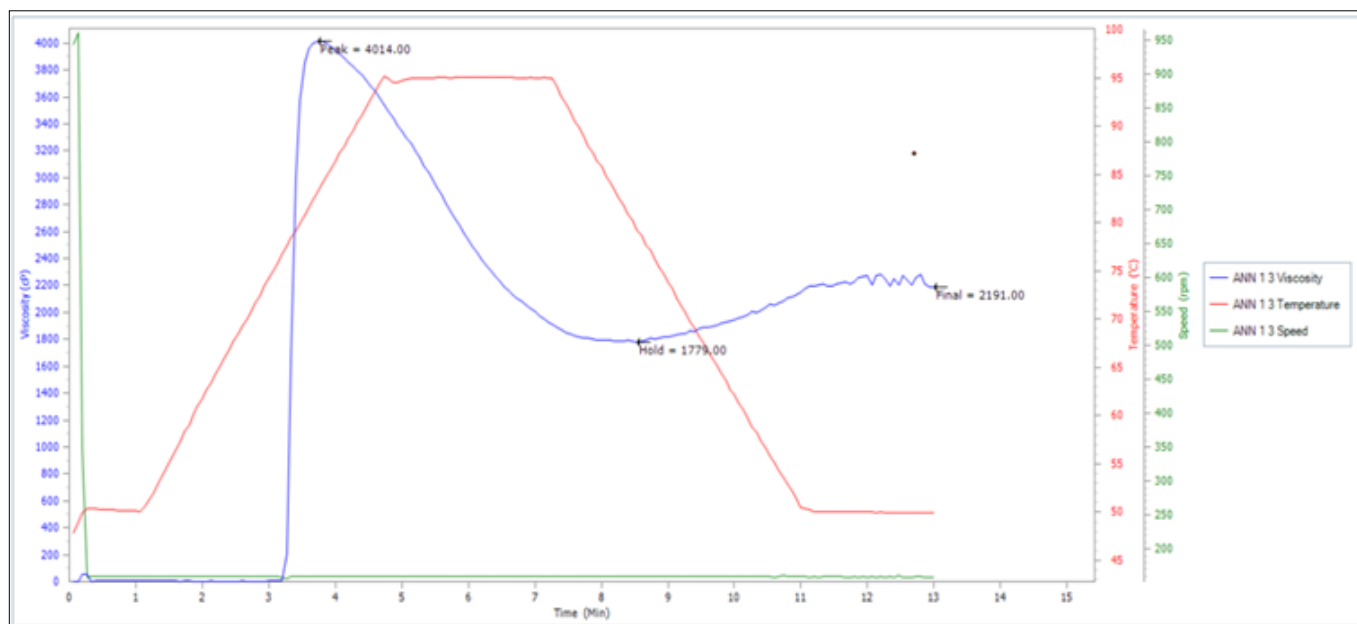


Fig. 6. Annealing.

Table 4. Effect of HMT and ANN on colour of the cassava starch

Parameter	Samples			
	Native	HMT - 25%	HMT - 30%	ANN
L*	94.15 ± 1.12	93.40 ± 1.08	92.82 ± 1.53	93.13 ± 1.49
a*	0.14 ± 0.01	0.10 ± 0.07	0.03 ± 0.01	0.09 ± 0.01
b*	4.22 ± 1.44	3.00 ± 0.11	3.37 ± 0.20	2.66 ± 0.51
ΔE	6.62 ± 0.03	6.61 ± 0.05	7.29 ± 0.04	6.71 ± 0.04
WI	92.78 ± 0.02	92.74 ± 0.01	92.06 ± 0.03	92.63 ± 0.05
YI	6.40 ± 0.06	4.58 ± 0.03	5.18 ± 0.01	4.08 ± 0.04

Values are the means of triplicate determination ± Standard deviation. indicative of red-green and yellow-blue hues respectively, also showed changes. The a^* values ranged from 0.14 ± 0.01 to 0.03 ± 0.01 , indicating reduced redness after HMT, particularly at higher treatment levels. The b^* values declined from 4.22 ± 1.44 (native) to 2.66 ± 0.51 (ANN), suggesting a reduction in yellowness, with ANN showing the most pronounced decrease. The total colour difference (ΔE) values were highest for HMT - 30 % (7.29 ± 0.04), followed by ANN (6.71 ± 0.04), indicating more noticeable changes in colour compared to native starch. The whiteness index (WI) decreased slightly in the modified starches, with the lowest value recorded for HMT 30 %. However, the decrease was not substantial, indicating that the modifications maintained the visual appeal of the starches. The yellowness index (YI) showed a similar trend, where the native starch had the highest YI (6.40 ± 0.06) and ANN resulted in the lowest value (4.08 ± 0.04). The reduction in YI after HMT and ANN could be linked to the removal of pigments or impurities during treatment. Similar consequences were stated for potato starch (35) and cassava starch (23). HMT and ANN may have caused starch disintegration, allowing impurities to bond and reducing whiteness, redness and yellowness.

Effects of HMT and ANN on freeze-thaw stability of cassava starch

The freeze-thaw stability of cassava starch samples, measured as the amount of water released (syneresis) after repeated freeze-thaw cycles is elucidated in Table 5. The

Table 5. Freeze-thaw stability of native and modified starches

Treatments	Samples			
	Native	HMT - 25%	HMT - 30%	ANN
First cycle	28.15 ± 0.12	18.42 ± 1.02	15.08 ± 1.08	20.36 ± 0.57
Second cycle	25.21 ± 1.07	16.37 ± 0.22	14.67 ± 1.04	18.34 ± 0.26
Third cycle	23.36 ± 0.55	15.37 ± 1.25	14.65 ± 0.24	15.32 ± 1.02

Values are the means of triplicate determination ± SD. results indicate that the stability of cassava starch improved significantly after HMT and ANN compared to the control. The control starch showed the highest syneresis across all cycles, with values decreasing progressively from 28.15 ± 0.12 % in the first cycle to 23.36 ± 0.55 % in the third cycle. This indicates poor freeze-thaw stability due to the high retrogradation tendency of native starch, where starch chains re-associate during freezing and expel water upon thawing. HMT significantly improved freeze-thaw stability, with HMT - 30% showing the lowest syneresis values (15.08 ± 1.08 % in first cycle, 14.67 ± 1.04 % in second cycle and 14.65 ± 0.24 % in third cycle). This improvement is attributed to structural changes induced by HMT, such as increased interaction between starch chains and reduced mobility of amylopectin, which hinder retrogradation. Similarly, HMT - 25 % also showed enhanced stability but to a lesser extent than HMT - 30 %. The consequences agreed with a previous study on potato (33), legumes (36) starches. Annealing resulted in intermediate freeze-thaw stability, with syneresis values lower than the control but slightly higher than HMT treated samples. ANN treated starch exhibited 20.36 ± 0.57 % syneresis in the first cycle, which was reduced to 15.32 ± 1.02 % by the third cycle. The improvement in stability can be linked to increased molecular order and reduced amylose leaching caused by the annealing process. Previous studies on barley starch (37) also reported similar. Overall, HMT (especially at HMT - 30%), was most effective in enhancing the freeze-thaw stability of cassava starch, while annealing provided moderate improvements. These results highlight the potential of these treatments to reduce syneresis in starch-based products, making them suitable for frozen and refrigerated food applications.

Effect on absorption capacities and water activity of starch samples at different treatments

Water absorption capacity (WAC) increased significantly after both HMT and ANN. The control starch exhibited a WAC of 2.21 ± 0.49 g/g, which increased to 2.55 ± 0.38 g/g in HMT - 25 % and 2.58 ± 0.31 g/g in HMT - 30 % (Table 1). ANN treated starch showed the highest WAC (3.30 ± 0.62 g/g), attributed to increased starch granule hydration resulting from improved molecular alignment. The study found that modified starch samples had stronger interactions between water and hydroxyl molecules compared to unmodified. During modification process, hydrogen bonds in the starch were broken, causing a slight expansion of the amorphous region. These changes likely made the starch more hydrophilic, leading to better water absorption (38). The previously available literatures are similar to maize (16), red sorghum (38), potato and wheat (39) starches. OAC decreased significantly after HMT, with values dropping to 1.75 ± 0.27 g/g (HMT - 25 %) and 1.73 ± 0.33 g/g (HMT - 30 %) compared to the control (2.61 ± 0.45 g/g). This reduction can be linked to reduced surface hydrophobicity and fewer non-polar binding sites on the granule surface post HMT; which substantiates with the results of red sorghum starch (38). Conversely, ANN treated starch exhibited a moderate reduction in OAC, suggesting partial retention of oil-binding capacity. Earlier consequences showed similar in pea (11) and maize starches (16). The water activity of the starch samples slightly decreased after HMT, with values of 0.43 ± 0.09 for HMT - 25 % and 0.41 ± 0.08 for HMT - 30 %, compared to 0.45 ± 0.01 for the control. Annealing resulted in slightly lower water activity (0.44 ± 0.02), reflecting its higher structural integrity and reduced moisture absorption.

Novelty of the work

The main objective of this study is to tailor cassava starch properties by HMT and ANN for enhanced functional applications in food industry.

1. There are reports on HMT and ANN of cassava starch. However, systematic studies on HMT and ANN of new cassava variety "Sree Reksha" developed by ICAR-CTCRI for starch extraction and modification techniques are scanty.
2. Enhanced Functional Properties: Modifications increased solubility, improved water absorption capacity, reduced syneresis, decreased pasting properties and lowered OAC.
3. The study demonstrates innovative methods to modify starch properties for diverse industrial applications, offering valuable insights for future research and commercial use.

Conclusion

In conclusion, the results of this study demonstrate that both HMT and ANN significantly modify the physicochemical properties of cassava starch. HMT led to an increase in solubility and decrease in swelling power, with peak viscosities reduced under HMT conditions but enhanced under ANN. The treatments enhanced water absorption capacity while declining OAC, indicating altered interactions within the starch matrix. Additionally, changes in colour and clarity were noted, although these differences were not statistically significant. These findings confirm that HMT and ANN effectively enhance the

functional properties of cassava starch, making it suitable for various applications in food and industrial sectors. Moreover, both modifications have advantages that no use of chemical agents thus offers a better scope for a safe and convenient technique for modifying starch for specific applications.

Acknowledgements

I would like to express my sincere gratitude to Agricultural Engineering College & Research Institute, Tamil Nadu Agricultural University, Coimbatore, ICAR - Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, and V.O.Chidhambaranar, Agricultural College and Research Institute, killikulam, Tamil Nadu Institute for providing access to resources and literature necessary for the completion of this research article. Special thanks to Dr. Parveen S, Dr. Krishnakumar T, Dr. Anand M, Dr. Gurusamy K for their insightful feedback and discussions that helped in shaping the direction of this work.

Authors' contributions

YNK and SP did the conceptualization. Data curation and writing of the original draft were carried out by YNK. SP, TK and MA supervised, SP contributed to funding acquisition. SP and MA contributed to writing - methodology and TK did the editing. KG conducted investigation and review. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical issues: None

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