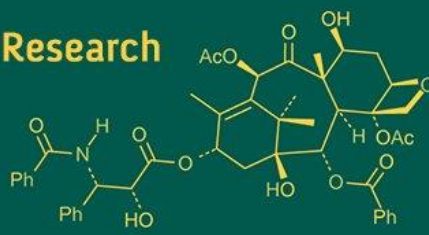


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Functional modification of cassava starch using clean label technologies: Heat moisture treatment, annealing, and atmospheric pressure plasma jet

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Abstract

Native cassava starch exhibits high swelling power, peak viscosity, and retrogradation tendency, but limited solubility, low freeze-thaw stability, and unstable pasting behaviour restrict its use in frozen and processed foods. To overcome these drawbacks, starch from the newly developed high starch yielding cassava cultivar “Sree Reksha” (ICAR-CTCRI) was modified using heat-moisture treatment (HMT), annealing (ANN), and atmospheric pressure plasma jet (APPJ). The process variables selected for the study were: HMT (25% and 30% moisture levels; 100-150 °C; 8-17 h), ANN (starch-water ratios 1:3 and 1:4; 40-60 °C; 12-36 h), and APPJ (gas flow 50-70 L/min; 5-15 min; 21-25 kV). Optimization was conducted using Response Surface Methodology with Central Composite Design. Functional and physicochemical analyses revealed that HMT (25% and 30%) and APPJ enhanced starch solubility by 7.84%, 20.52%, and 23.09%, respectively, while ANN reduced solubility by 20.67% (1:3) and 45.08% (1:4), indicating formation of a more ordered, less soluble structure. Swelling power decreased across all modified starches, showing structural reinforcement. Peak viscosity reduced from 3.812 Pa·s (native) to 3.267 Pa·s (HMT-25%), 3.150 Pa·s (HMT-30%), and 3.024 Pa·s (APPJ), favouring applications like noodles, sauces, and soups; however, ANN increased viscosity, improving thickening potential. Freeze-thaw stability, water absorption, and moisture retention improved, while oil absorption decreased, promoting low-fat texture development. The optimized conditions were HMT-25% at 140 °C/12 h, HMT-30% at 150 °C/17 h, ANN 1:3 at 40 °C/12 h, ANN 1:4 at 40 °C/36 h, and APPJ at 50 L/min, 5 min, 25 kV. Overall, these modifications successfully optimized cassava starch functionality, enhancing its suitability for convenience, bakery, sauce, frozen and low-fat product applications.

Keywords: Functional properties, modified starch, plasma reactive species, starch modification, tapioca starch

Introduction

Tuber crops are vital for global food security as rich sources of starch, offering affordable and sustainable energy to low-income populations. Among them, cassava (*Manihot esculenta* Crantz) holds particular significance as a climate resilient crop capable of thriving in marginal soils and drought prone regions, thereby supporting the livelihoods of more than 800 million people worldwide. Cassava’s remarkable versatility spans food applications such as starch, flour, and chips as well as industrial uses in bioethanol, bioplastics, and adhesives. Its production continues to expand steadily in major cassava growing countries including Nigeria, Thailand, and Brazil [1]. Despite its considerable potential, native cassava starch is hindered by functional limitations including low solubility, poor freeze-thaw stability, and inconsistent pasting properties that limit its application in specialized food formulations. Although various modification techniques have been investigated, clean-label, energy-efficient, and multifunctional starches remain in high demand [2]. Cassava production continues to expand, led by Nigeria, Thailand, and Brazil, with advancements in breeding and processing technologies enhancing its value for both food and industrial applications [3]. Cassava is a major starchy root crop and one of the world’s primary calorie sources, achieved global production of approximately 330 million tonnes in 2022, led by Nigeria, the Democratic Republic of Congo, and Thailand.

In India, it is predominantly cultivated in the southern states of Tamil Nadu, Kerala, and Andhra Pradesh across 1.7-1.8 lakh ha, yielding 6.2-6.9 million tonnes annually at an average productivity of 35-37 t/ha substantially exceeding the global average.

Cassava's high starch content and gluten free nature make it valuable for foods such as boiled roots, tapioca pearls, cassava flour in bakery products, and as a thickener or sweetener. Clean, non-chemical modification methods such as heat-moisture treatment (HMT), annealing (ANN), and atmospheric pressure plasma jet (APPJ) enable precise tuning of cassava starch functionality without damaging granules or introducing reagents. HMT enhances molecular interactions under limited moisture and heat, raising gelatinization temperature, pasting stability, and resistant starch content. ANN, applied below the gelatinization point, refines crystallinity and reduces swelling for improved stability. APPJ alters surface structure and reactivity, boosting water binding and functional properties. Together, these techniques offer environmentally friendly control over solubility, viscosity, and freeze-thaw stability. Applying them to Sree Reksha cassava starch can reveal its unique functional potential and support broader, cleaner applications in the food industry. Jyothi *et al.* (2010) ^[4] investigated the hydrothermal modification of tropical tuber starches through heat-moisture treatment (HMT) to assess its impact on physicochemical, rheological, and gelatinization characteristics. The study reported that HMT markedly reduced peak viscosity, caused only a slight decrease in breakdown viscosity, and significantly increased starch solubility across all treated samples. In a subsequent study, ^[5] investigated the effects of annealing (ANN) on similar tuber starches to evaluate alterations in their physicochemical, gelatinization, and rheological properties. The researchers reported a narrowing of the differential scanning calorimetry (DSC) endothermic transitions across all starch types and a reduction in solubility, most notably in cassava starch, following the annealing treatment.

Kaur *et al.* (2024) ^[6] reported that atmospheric pressure plasma jet (APPJ) treatment significantly altered the rheological and gelling behavior of finger millet starch through mechanisms such as depolymerization, cross-linking, and functional group incorporation. The treatment reduced peak viscosity and enhanced paste clarity, indicating partial structural disruption. Moderate treatment levels (170-200 V) improved gel strength due to the formation of intermediate amylose chains and carbonyl group interactions, whereas excessive treatment (230 V) caused pronounced depolymerization and a weakened gel matrix. Heat-moisture treatment (HMT), annealing (ANN), and APPJ are eco-friendly physical methods that enable precise control over starch functionality without using chemicals. HMT enhances resistant starch content and thermal stability, ANN improves crystallinity and reduces swelling, and APPJ increases surface reactivity and hydration capacity. Applying these clean label modifications to Sree Reksha cassava starch presents opportunities for developing novel, stable, and functional food ingredients with customized physicochemical properties.

These eco-friendly modification techniques maintain native starch integrity and are well suited for developing functional food ingredients, including those derived from the high starch yielding Sree Reksha cassava cultivar ^[7]. The present work systematically examines the comparative effects of

heat-moisture treatment (HMT), annealing (ANN), and atmospheric pressure plasma jet (APPJ) on native starch extracted from the cultivar Sree Reksha (ICAR-CTCRI). The study aims to elucidate how these physical modifications influence key physicochemical and functional attributes such as solubility, swelling power, water and oil absorption capacities, pasting behavior, and freeze-thaw stability to guide targeted food applications.

2. Materials and Methods

2.1 Raw materials

Native starch was extracted from matured cassava tubers of the Sree Reksha cultivar, harvested from the research farm of ICAR-Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram, Kerala (Latitude: 8°18' N to 12°48' N, Longitude: 74°52' E to 77°22' E).

2.2 Extraction of cassava starch

The starch was extracted from freshly harvested cassava tubers following the standard procedure described by Krishnakumar and Sajeew. The tubers were thoroughly washed, hand-peeled, and mechanically cut into small pieces, which were then crushed using ICAR-CTCR mobile starch extraction unit with sufficient water. The slurry obtained was filtered through a 150 µm sieve and allowed to settle overnight in a tank. After sedimentation, the supernatant water was decanted, and the starch cake was washed three times with ample water to obtain a bright white product. The purified starch was then spread on aluminum trays and dried in a tray dryer (LabTech Instruments, LT-DR-50) at 45-50 °C for 12 hours. The dried starch, adjusted to a moisture content of 10-12% (dry basis), was stored in airtight containers for subsequent treatments and analyses. The extraction process is illustrated in Figure 1.

2.3 Experimental Treatments of cassava starch

2.3.1 Heat moisture treatment

Heat moisture treatment of cassava starch was conducted as per the method explained by Jyothi *et al.* (2010) ^[4], Liu *et al.* (2015) ^[9].

Heat-moisture treatment (HMT) was conducted using a Box-Behnken RSM design under different temperature, time and moisture levels: 100, 120, and 140 °C for 8, 10, 12 hours at 25 % (w.b.) moisture, and 110, 130, 150 °C for 13, 15, 17 hours at 30 % (w.b.) moisture levels. Cassava starch samples were prepared at 25 % and 30 % (w/w) moisture contents, selected to achieve optimal viscosity for uniform heat transfer and molecular reorganization, while reflecting concentrations relevant to food applications. The initial moisture content of the native starch was determined and adjusted to the desired level by gradually adding measured amounts of distilled water with thorough mixing to prevent lump formation. The conditioned starch samples were evenly spread in stainless steel containers and subjected to controlled heating in an air oven at the specified temperature and duration according to the experimental design. After treatment, samples were cooled to ambient temperature and subsequently dried at 50-55 °C until the moisture content reached 6-8 % (w.b.). The processed samples were designated as HMT-25 % and HMT-30 %. The experimental workflow is illustrated in Figure 2.

2.3.2 Annealing treatment

The annealing (ANN) treatment procedure was conducted following the method described by Xie *et al.* (2024) ^[11]. The key variables considered were temperature, time, and starch-to-water ratio. The experimental design employed a Box-Behnken response surface methodology (RSM) with three temperature levels (40, 50, 60 °C), three incubation times (12, 24, 36 hours), and a starch-to-water ratio of 1:3 (w/v). Cassava starch was mixed with distilled water to obtain slurries with ratios of 1:3 and 1:4 (w/v) and sealed with perforated covers to allow limited moisture exchange. The samples were incubated at the specified temperatures for the designated durations, followed by cooling to room temperature. Subsequently, the starch was dried at 50-55 °C until the moisture content reached 6-8 % (w.b.). The final samples were labelled ANN (1:3) and ANN (1:4). The experimental process is illustrated in Figure 2.

2.3.3 Atmospheric Pressure Plasma Jet

APPJ procedure followed the method described by Kaur and Annappure ^[6]. Twelve grams of cassava starch were uniformly spread on clean Petri plates and exposed to atmospheric pressure plasma jet (APPJ) treatment (Z/54/14, Zeonics Systech, India). The system was equipped with seven plasma jets to ensure uniform distribution of the plasma field. The independent variables investigated were gas flow rate, applied voltage, and treatment time. The experimental design comprised three gas flow rates (50, 60, and 70 L/min), three voltage levels (21, 23, and 25 kV), and three exposure times (5, 10, and 15 min). Treatment conditions were selected based on preliminary trials and system limitations. The APPJ-treated starch samples, with the electrode positioned at a fixed distance of 2 cm above the thin, even layer of starch, were carried out using argon gas to maintain a stable and inert plasma environment. After treatment, the samples were stored in 75 µm polypropylene bags until further characterization and analysis. A schematic of the APPJ setup is illustrated in Figure 3.

2.4 Functional properties of cassava starch

2.4.1 Solubility and swelling power

The solubility and swelling power of native and treated starch samples were determined following the methods described by Jyothi *et al.* (2011) ^[5], Krishnakumar and Sajeev ^[8], and Liu *et al.* (2016) ^[10]. A 0.4 g starch sample was weighed and dispersed in 40 mL of distilled water, then heated in a shaking water bath at 95 °C for 20 min. After cooling, 10 mL of the suspension was transferred into 50 mL centrifuge tubes using a graduated cylinder and centrifuged at 6000 rpm for 20 min. The supernatant was carefully decanted, and the weight of the sediment was recorded. The supernatant was then dried at 105 °C to constant weight, and the mass of the dry solids was determined. The solubility and swelling power were calculated using the following equation.

$$\text{Solubility (\%)} = \frac{\text{weight of dried supernatant}}{\text{original dry starch weight}} \quad (1)$$

$$\text{Swelling (g/g)} = \frac{\text{weight of sediment}}{\text{original dry starch weight}} \quad (2)$$

2.4.2. Water absorption capacity

The water absorption capacity protocol was adapted from Liu *et al.* (2016) ^[11]. Precisely, 1 g of control or modified

starch was mixed with 10 mL of distilled water (density 1 g/mL) in 50 mL Falcon tubes. The slurry was vortexed for 5 min, allowed to stand for 2-3 min, and then centrifuged at 10,000 rpm for 10 min. The volume of the supernatant was measured using a 10 mL graduated cylinder. The difference between the initial water volume and the supernatant volume was taken as the absorbed water, and the result was expressed as grams of water absorbed per gram of starch.

$$\text{Water absorption capacity (g/g)} = \frac{\text{weight of absorbed water}}{\text{weight of starch}} \quad (3)$$

2.4.3 Oil absorption capacity

The oil absorption capacity was determined using the procedure described by Liu *et al.* (2016) ^[11]. Sunflower oil (density 0.92 g/mL) was used as the medium. Precisely, 1 g of control or treated starch was mixed with 10 mL of sunflower oil in a 50 mL Falcon tube and agitated for 5 min using a magnetic stirrer at 1000 rpm. The suspension was allowed to stand for 2-3 min and then centrifuged at 10,000 rpm for 10 min. The volume of the supernatant was measured using a 10 mL graduated cylinder. The difference between the initial volume of oil and the volume of the supernatant was taken as the absorbed oil and expressed as grams of oil absorbed per gram of starch.

$$\text{Oil absorption capacity (g/g)} = \frac{\text{weight of absorbed oil}}{\text{weight of starch}} \quad (4)$$

2.4.4. Pasting properties of starch

The pasting behaviour of native and modified cassava starch samples was evaluated using a Rapid Visco Analyzer (RVA-4, Newport Scientific, Warriewood, Australia). Viscosity profiles were generated using starch suspensions prepared by accurately weighing 2.5 g of dry starch into an aluminium canister and adding 22.5 g of distilled water, followed by thorough mixing with a glass rod. The canister was placed in the analyzer and the sample subjected to the standard I-profile at a constant paddle speed of 160 rpm. The heating-cooling cycle comprised ramping the temperature from 50 °C to 95 °C at 12 °C/min, holding at 95 °C for 2 min, cooling back to 50 °C at 12 °C/min, and maintaining at 50 °C for a further 2 min. Pasting parameters such as peak, trough, breakdown, setback, and final viscosities (in Pa.s), peak time (min), and pasting temperature (°C) were recorded from the RVA curves.

2.4.5 Freeze-Thaw stability

Freeze-thaw stability (syneresis) process was determined following the method of Krishnakumar and Sajeev ^[8], Cahyana *et al.* (2019) ^[13]. A 5 % (w/v) aqueous starch suspension was heated at 95 °C for 30 min with gentle agitation in a shaking water bath, and then cooled to room temperature. A 10 mL aliquot of the paste was transferred into a pre-weighed centrifuge tube and subjected to a freeze-thaw cycle: frozen at -16 °C for 24 h, followed by thawing at 25 °C for 2 h. The sample was then centrifuged at 3000 rpm for 20 min. After centrifugation, the supernatant was carefully decanted and weighed. The percentage of syneresis was calculated using the following equation.

$$\text{Syneresis (\%)} = \frac{\text{weight of liquid decanted}}{\text{Total weight of gel before centrifugation}} \quad (5)$$

3. Statistical analysis

Each functional property was analyzed in triplicate to ensure accuracy and reproducibility. Statistical analyses were performed using Design-Expert software (version 25.0) based on Response Surface Methodology (RSM) to evaluate the effects of the different treatments. Graphs were generated using Microsoft Excel and Google Colab. The significance of model terms and treatment effects was assessed by analysis of variance (ANOVA) at $p < 0.05$.

4. Results and Discussion

4.1. Effect of HMT, ANN, and APPJ on the swelling power and solubility of cassava starch

The effect of the different treatments on the solubility and swelling power of cassava starch at 95 °C is presented in Table 1 and Fig. 5. Solubility increased after HMT and APPJ, with values of $20.5 \pm 2.34\%$ ($p < 0.05$) for HMT-25% and $22.91 \pm 3.6\%$ ($p < 0.05$) for HMT-30%, 23.4 ± 1.55 ($p < 0.05$) for APPJ, compared with the control $19.1 \pm 2.59\%$. This increase may be attributed to partial molecular disintegration and structural loosening of the starch granules during modification [5, 6, 14]. The increased solubility of the modified starch enhances its dispersibility in aqueous systems, which can improve texture and stability in food formulations [15, 16]. In contrast, annealing significantly reduced solubility to $15.08 \pm 0.25\%$ ($p < 0.05$), indicating development of a more ordered granular structure that resists solubilization. The lower solubility in annealed starch is attributed to strengthened interactions between amylase-amylopectin chains and among amylopectin molecules, which restrict molecular leaching and limit dissolution from the granules. These findings are consistent with previous reports on the effect of annealing on starch solubility [4, 5, 14, 17, 18]. The reduced solubility observed after annealing indicates improved starch stability, which is advantageous for thickening applications in canned foods, sauces, and bakery fillings. The various treatments significantly influenced the swelling power of cassava starch.

The control sample displayed a significantly higher swelling power (7.07 ± 0.57 g/g, $p < 0.01$), reflecting its enhanced ability to absorb water at elevated temperatures.

HMT treatment reduced the swelling power, with values declining to 6.51 ± 0.96 g/g ($p < 0.05$) for HMT-25% and further to 6.35 ± 1.64 g/g ($p < 0.01$) for HMT-30%. This reduction may be attributed to structural reorganization within the crystalline regions of starch, resulting in

increased crystallinity, formation of amylase-lipid complexes, stronger interactions between amylose and amylopectin molecules, and reinforced intramolecular bonding, which collectively restrict water penetration during HMT. These observations are consistent with findings reported for common buckwheat [9], maize [10], potato and cassava [19], rice [20], sorghum [21], and corn starches [22]. Conversely, starch treated by ANN exhibited a reduction in swelling power compared to the control, as shown in Table 1. This decrease can be attributed to enhanced molecular organization and minimal disruption of the granular structure. Similar trends have been reported for barley [23], wheat starch [24], and groundnut [25]. Reduced swelling power contributes to improved starch stability, making it advantageous for high temperature applications such as pasta, noodles, and baked products, where excessive thickening or gelatinization is undesirable. In the APPJ-treated sample, solubility increased significantly from 19.1% to 23.4% ($p < 0.01$), likely due to the action of reactive plasma species that partially degraded or depolymerized starch granules into smaller fragments.

It was observed that cold plasma treatment enhanced the solubility of cassava starch under non-pasting conditions. This increase in solubility suggests partial disruption or loss of the starch granule surface structure following APPJ treatment. Similar effects have been reported for wheat starch [18], corn starch [14, 26], banana starch [27], and red adzuki bean starch [28]. The enhanced solubility of cassava starch after cold plasma treatment has practical relevance in the food industry, improving its functionality in instant and processed foods. Increased solubility facilitates better dispersion in cold water, making it suitable for applications in instant soups, sauces, beverages, ready to eat meals, and thickening systems. Conversely, a marked reduction in swelling power was observed following APPJ treatment of cassava starch. As shown in Table 1, the swelling degree significantly decreased from 7.07 g/g to 2.65 g/g ($p < 0.01$) compared to the native starch. This decline could be attributed to partial destruction of starch chains induced by the APPJ process, causing the swollen granules to lose their ability to retain water. Similar findings have been reported for wheat starch [18], banana starch [27], and maize starch [29]. The reduced swelling power of starch offers advantages in specific food applications such as instant noodles, soups, confectionery, and meat products, where controlled thickening and textural stability are desirable.

Table 1: Effect of HMT, ANN and APPJ treatments on solubility and swelling power of native and modified cassava starches at 95°C.

Parameter	Samples					
	Native	HMT 25%	HMT 30%	ANN (1:3)	ANN (1:4)	APPJ
Solubility (%)	19.10 ± 2.59^b	20.50 ± 2.34^b	22.91 ± 3.60^a	15.08 ± 0.25^c	10.44 ± 2.45^d	23.40 ± 1.55^a
Swelling power (g/g)	7.07 ± 0.57^a	6.51 ± 0.96^b	6.35 ± 1.64^b	7.00 ± 1.13^a	4.15 ± 0.07^c	2.65 ± 0.03^d

Values are expressed as mean $\pm$ standard deviation ($n = 3$). Means followed by different superscript letters within the same row indicate significant differences ($p \leq 0.05$)

Table 2: Effect of HMT, ANN and APPJ treatments on water and oil absorption capacities of native and modified cassava starches

Parameter	Native	HMT 25%	HMT 30%	ANN (1:3)	ANN (1:4)	APPJ
WAC (g/g)	2.21 ± 0.49^d	2.55 ± 0.38^c	2.58 ± 0.31^c	3.30 ± 0.62^b	4.85 ± 0.20^a	3.70 ± 1.15^b
OAC (g/g)	2.61 ± 0.45^a	1.75 ± 0.27^d	1.73 ± 0.33^d	2.22 ± 0.57^c	2.13 ± 0.69^c	2.44 ± 0.40^b
Syneresis (%)	25.57 ± 1.97^a	16.72 ± 1.26^c	14.80 ± 0.19^d	15.58 ± 0.34^d	18.05 ± 0.05^b	17.48 ± 0.35^b

Values are expressed as mean $\pm$ standard deviation ($n = 3$). Means followed by different superscript letters within the same row indicate significant differences ($p \leq 0.05$)

4.2 Effect of HMT, ANN, and APPJ on the pasting properties of cassava starch

The pasting properties of cassava starch subjected to different treatments are presented in Table 3, with corresponding pasting curves illustrated in Fig. 4. The treatments significantly affected the pasting parameters, including peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV), setback viscosity (SV), peak time (PTi), and pasting temperature (PTE). The control starch exhibited a peak viscosity of 3.812 Pa·s, which decreased following HMT-25%, HMT-30%, and APPJ treatments, showing values of 3.267 Pa·s, 3.150 Pa·s, and 3.024 Pa·s ($p < 0.01$), respectively. The reduction in peak viscosity can be attributed to structural alterations induced by HMT and APPJ, particularly amylose leaching and rearrangement within the granules. In contrast, ANN-treated starch displayed the highest peak viscosity relative to the native starch, indicating that annealing enhanced the swelling ability of starch granules. Trough viscosity, representing the minimum viscosity during heating, increased after treatments, suggesting improved structural stability of the starch granules under heat and shear conditions [30].

Breakdown and setback viscosities decreased significantly following starch modification, indicating improved paste stability. The ANN-treated starch exhibited a high trough viscosity of 1.779 Pa·s and a moderate breakdown viscosity of 2.235 Pa·s, reflecting enhanced swelling and partial structural stability. Final viscosity, which represents the ability of starch to form a gel upon cooling, increased in all treatments, with values exceeding that of the control (1.939 Pa·s). All treatments displayed comparable peak times. However, the pasting temperature decreased slightly in HMT-treated starch (71.80 °C for HMT-25% and 70.95 °C for HMT-30%) compared to the control (72.65 °C), indicating improved gelatinization efficiency due to thermal modification. In contrast, ANN and APPJ treated starches exhibited higher pasting temperatures, suggesting increased resistance to gelatinization, likely resulting from the formation of a more ordered starch structure and strengthened molecular interactions.

After plasma treatment, a noticeable change in viscosity was observed. During plasma exposure, both depolymerization and cross-linking reactions occur simultaneously and competitively. Reactive plasma species can weaken molecular bonds within starch, leading to depolymerization

and granule disruption, which in turn reduces viscosity [32-34]. These findings are consistent with previous reports on cassava [4, 5], potato starches [35], banana starch [36], corn starch [14]. Reduced viscosity enhances processability and allows better texture control in batters, soups, beverages, and gluten-free formulations by preventing excessive thickening. Conversely, increased viscosity improves thickening, gelling, and stability, making starch suitable for use in sauces, soups, dairy, and bakery fillings. The freeze-thaw stability (syneresis) of cassava starch samples, expressed as the amount of water released after repeated freeze-thaw cycles, is presented in Table 2 and Fig. 5. The results indicate that the stability of cassava starch improved significantly after HMT, ANN, and APPJ treatments compared with the native starch. The control sample exhibited the highest syneresis among all treatments, showing a progressive decrease in stability due to the pronounced retrogradation tendency of native starch, where re-association of starch chains during freezing results in water expulsion upon thawing. HMT markedly improved freeze-thaw stability, with HMT-30% displaying the lowest syneresis value ($14.8 \pm 0.19\%$, $p < 0.01$). This improvement can be attributed to structural changes induced by HMT, including enhanced interchain interactions and reduced amylopectin mobility, which help suppress retrogradation. HMT-25% also exhibited improved stability, though to a lesser extent than HMT-30%. These findings align with previous reports on potato [35] and legume starches [37]. Annealing resulted in intermediate freeze-thaw stability, with syneresis values lower than the control but slightly higher than those of the HMT-treated samples. ANN-treated starch exhibited syneresis values of $15.58 \pm 0.34\%$ ($p < 0.05$) and $18.05 \pm 0.05\%$ ($p < 0.01$) for ANN 1:3 and 1:4, respectively. The improvement in stability can be attributed to increased molecular order and reduced amylose leaching induced by the annealing process. Similar observations have been reported for barley starch. In the case of APPJ treatment, starch showed a significant reduction in syneresis to $17.48 \pm 0.35\%$ ($p < 0.05$) compared with the untreated control [6]. Overall, HMT particularly HMT 30% proved most effective in enhancing the freeze-thaw stability of cassava starch, while annealing provided moderate improvements. These findings underscore the potential of these modification techniques to reduce syneresis in starch-based products, thereby extending their applicability in frozen and refrigerated food formulations.

Table 3: Effect of HMT, ANN and APPJ treatments on pasting properties of native and modified cassava starches

Parameter	Native	HMT 25%	HMT 30%	ANN (1:3)	ANN (1:4)	APPJ
Peak Viscosity (Pa·s)	3.82±0.00 ^b	3.267±0.00 ^c	3.150±0.00 ^c	4.014±0.00 ^a	3.955±0.04 ^a	3.024±0.00 ^d
Trough Viscosity (Pa·s)	1.35±0.00 ^d	1.681±0.00 ^b	1.693±0.00 ^b	1.779±0.00 ^a	1.698±0.00 ^b	1.465±0.01 ^c
Breakdown Viscosity (Pa·s)	2.45±0.00 ^a	1.586±0.00 ^c	1.457±0.00 ^d	2.235±0.00 ^b	1.606±0.04 ^c	1.552±0.02 ^c
Final Viscosity (Pa·s)	1.93±0.00 ^c	2.231±0.00 ^a	2.262±0.00 ^a	2.191±0.00 ^b	2.202±0.01 ^b	1.968±0.04 ^c
Setback Viscosity (Pa·s)	0.58±0.00 ^a	0.550±0.00 ^b	0.569±0.00 ^a	0.412±0.00 ^c	0.504±0.01 ^b	0.503±0.03 ^b
Peak Time (min)	3.73±0.01 ^b	3.73±0.02 ^b	3.73±0.06 ^b	3.73±0.08 ^b	3.67±0.01 ^c	3.83±0.03 ^a
Pasting Temperature (°C)	72.65±0.31 ^c	71.8±0.24 ^d	70.95±0.41 ^d	76.7±0.25 ^a	75.5±0.40 ^b	73.1±0.50 ^c

Values are expressed as mean ± standard deviation (n = 3). Means followed by different superscript letters within the same row are significantly different ($p \leq 0.05$)

4.3 Effect of HMT, ANN, and APPJ on the absorption capacities of cassava starch

The water absorption capacity (WAC) and water activity of starch samples under different modification methods are presented in Table 2 and Fig. 5. WAC increased

significantly after treatments. Native starch exhibited a WAC of 2.21 ± 0.49 g/g, which rose to 2.55 ± 0.38 g/g ($p < 0.01$) for HMT 25%, 2.58 ± 0.31 g/g ($p < 0.01$) for HMT 30%, and 3.70 ± 1.15 g/g ($p < 0.01$) for APPJ. Notably, ANN treated

starch showed the highest WAC (4.85 ± 0.20 g/g) in the ANN 1:4 treatments, attributable to enhanced starch granule hydration resulting from improved molecular alignment. Modified starch samples demonstrated stronger interactions between water and hydroxyl groups compared with the unmodified starch. During modification, hydrogen bonds within the starch were disrupted, causing slight expansion of the amorphous regions and increased hydrophilicity, which facilitated greater water absorption [39]. These findings are consistent with reports on maize [10], red sorghum [39], potato and wheat [40] starches.

Oil absorption capacity (OAC) decreased significantly after HMT, ANN, and APPJ treatments, with values declining to 1.75 ± 0.27 g/g for HMT-25%, 1.73 ± 0.33 g/g for HMT-30%, and 2.44 ± 0.40 g/g for APPJ, compared with 2.61 ± 0.45 g/g in the control. This reduction may be attributed to decreased surface hydrophobicity and a lower number of non-polar binding sites on the granule surface following HMT and APPJ treatments. Similar findings have been reported for red sorghum starch [39] and rice starch [41].

In contrast, ANN treated starch showed only a moderate decrease in OAC, suggesting partial retention of oil-binding capacity. Previous studies have reported comparable results for pea [11] and maize starches [10].

Oil absorption capacity (OAC) decreased significantly after HMT, ANN, and APPJ with values dropping to 1.75 ± 0.27 g/g (HMT-25%), 1.73 ± 0.33 g/g (HMT-30%), and 2.44 ± 0.40 g/g (APPJ) 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 and APPJ previous results agreed in case of red sorghum starch [39] and rice starch [41]. 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 [10]. Higher water absorption capacity improves moisture retention in baked goods and meat products, enhancing texture and shelf life, while reduced oil absorption capacity benefits low fat dressings and fried snacks.

Table 4: Key findings of the research

Parameters	Solubility (%)	Swelling power (g/g)	Water Absorption Capacity (g/g)	Oil Absorption Capacity (g/g)	Pasting Properties (Peak Viscosity Pa. s)	Freeze-Thaw stability (%)
Control	↓	↑	↓	↓	↑	↑
HMT - 25%	↑	↓	↑	↓	↓	↓
HMT - 30%	↑	↓	↑	↓	↓	↓
ANN (1:3)	↓	↓	↑	↓	↑	↓
ANN (1:4)	↓	↓	↑	↓	↑	↓
APPJ	↑	↓	↑	↓	↓	↓

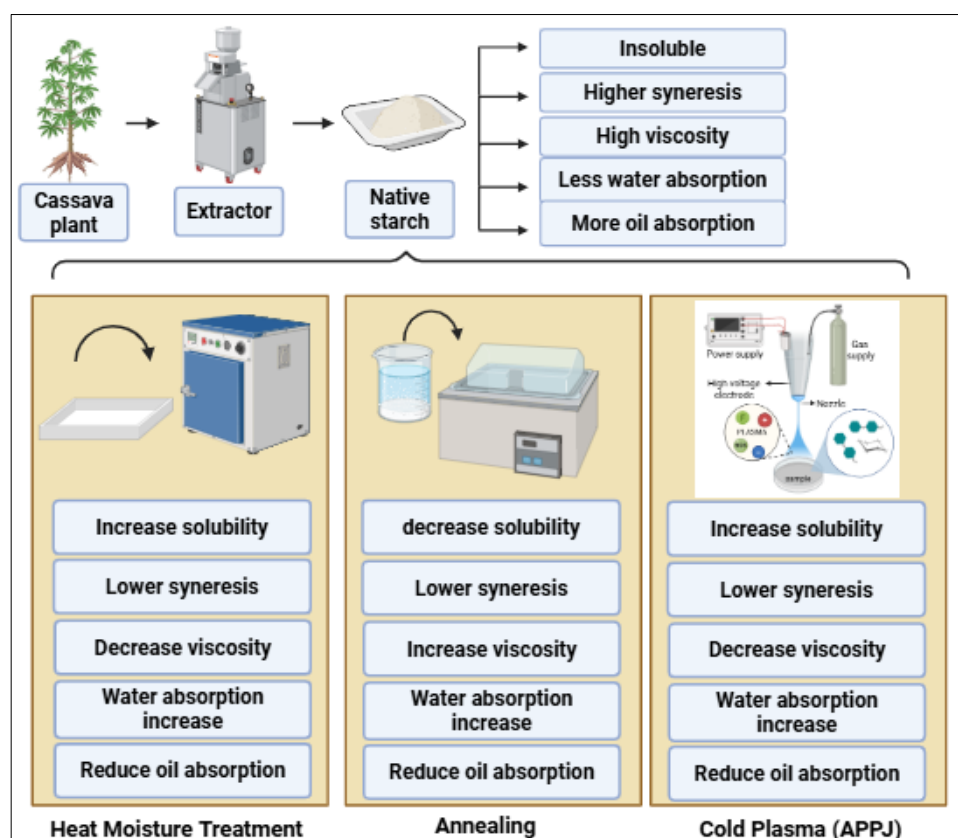


Fig 1: Graphical Abstract illustrating starch extraction and modification of cassava starch using HMT, ANN, and APPJ techniques

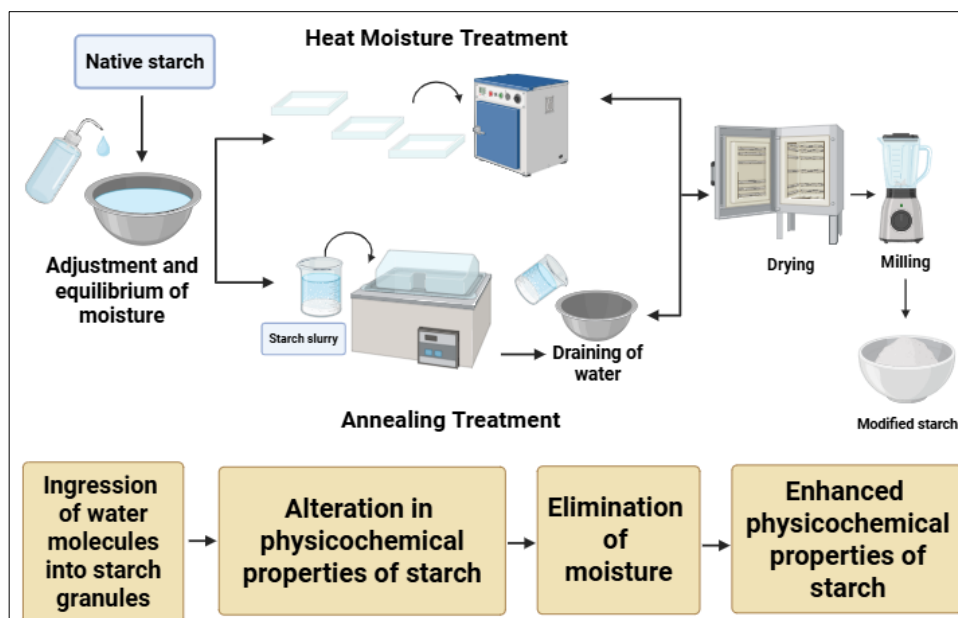


Fig 2: Effect of heat moisture treatment (HMT) and Annealing (ANN) on cassava properties

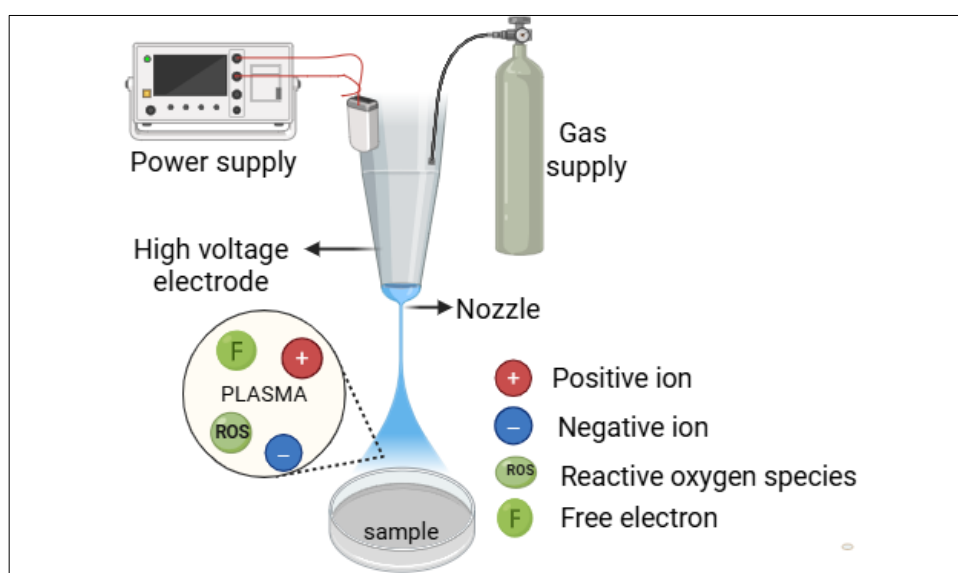


Fig 3: Modification of cassava starch by atmospheric pressure plasma jet

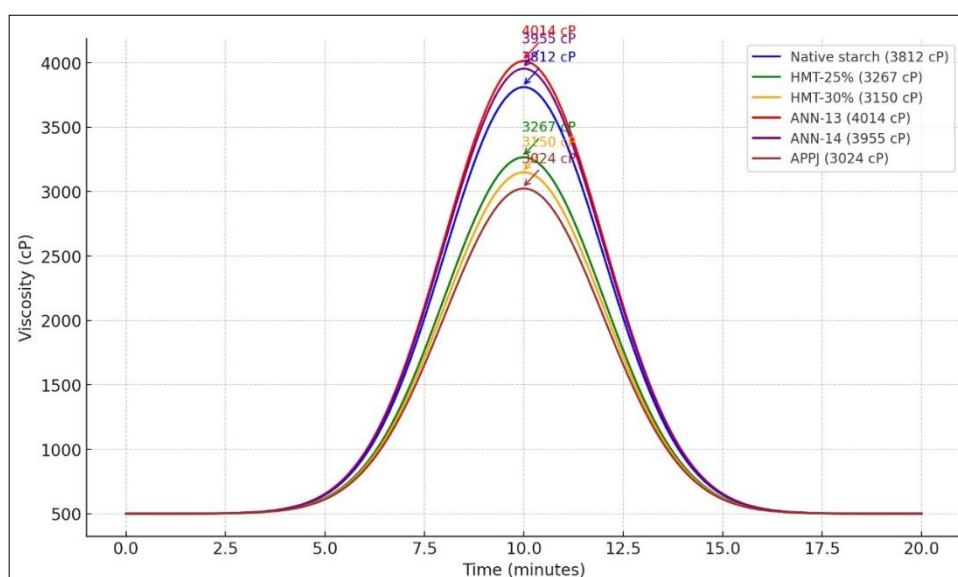


Fig 4: Pasting properties of cassava starch under different modification treatments

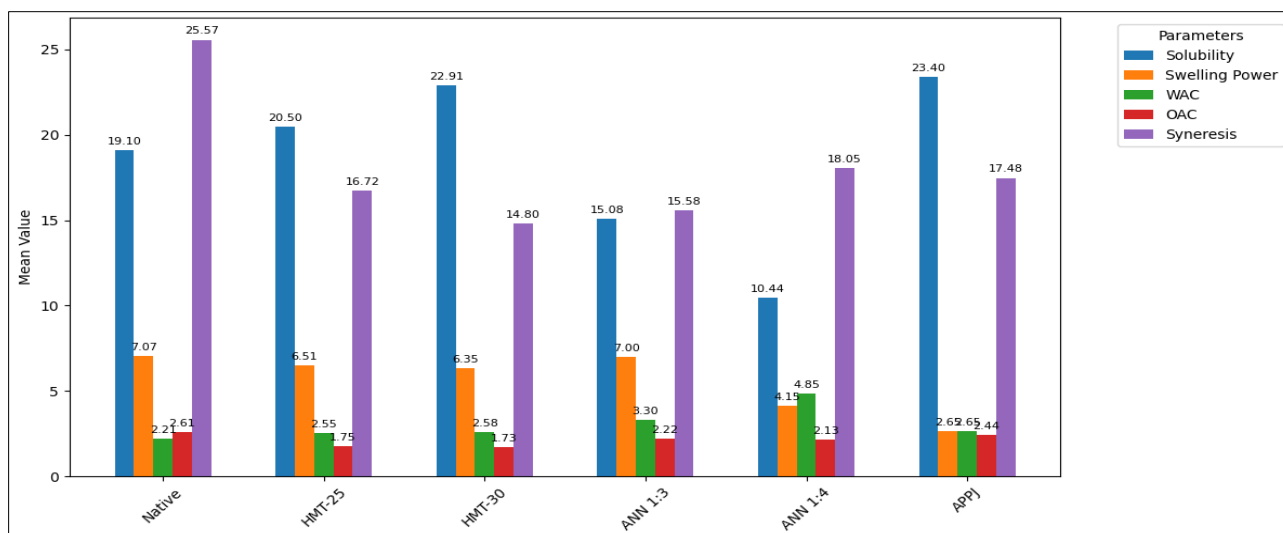


Fig 5: Effect of different modification treatments (HMT, ANN, APPJ) on functional properties of cassava starch

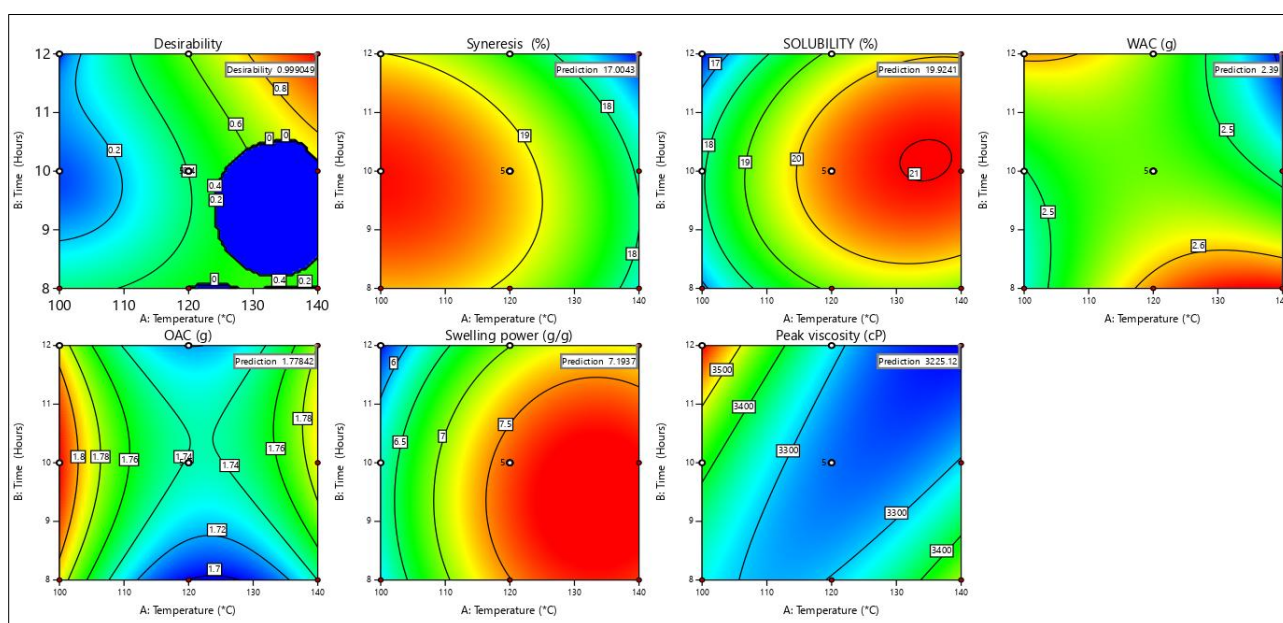


Fig 6: Response surface plot for HMT optimization (25% moisture level) (A: Temperature, B: Time)

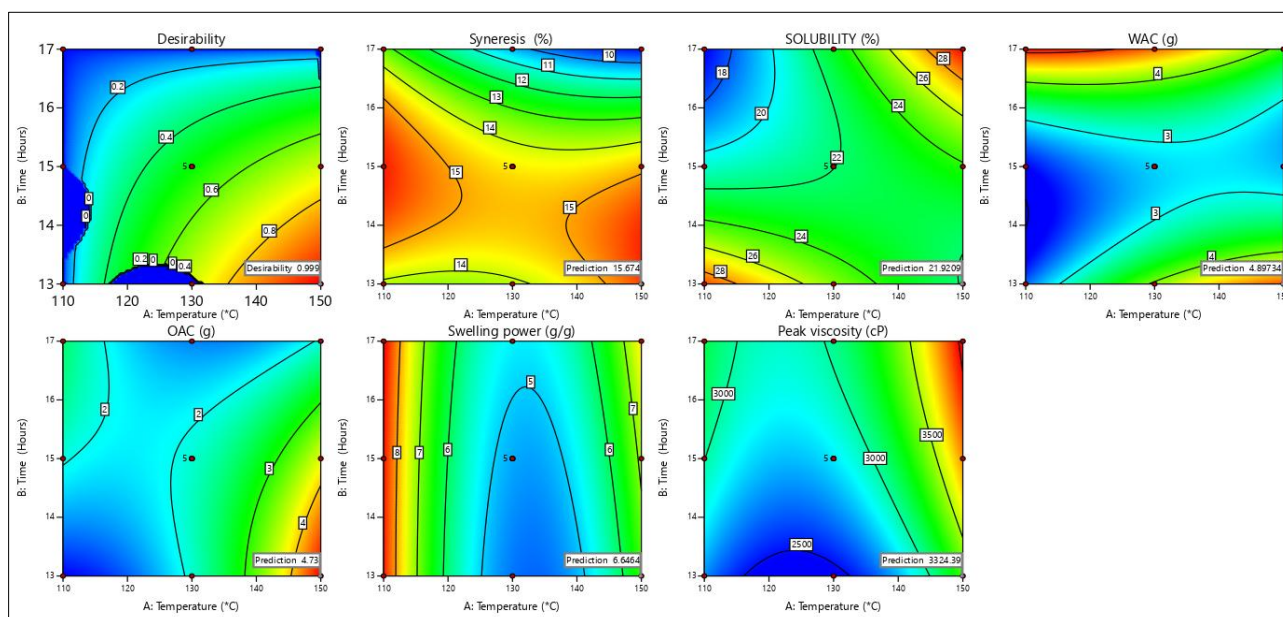


Fig 7: Response surface plot for HMT optimization (30% moisture level) (A: Temperature, B: Time)

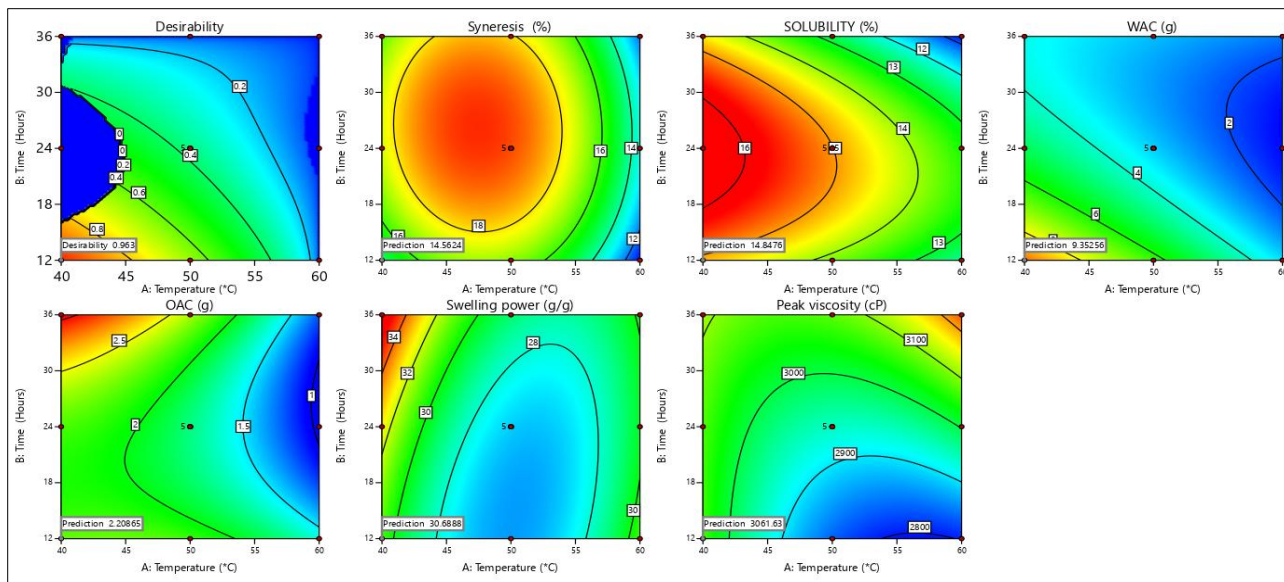


Fig 8: Response surface plot for ANN optimization (1:3 starch to water ratio) (A: Temperature, B: Time)

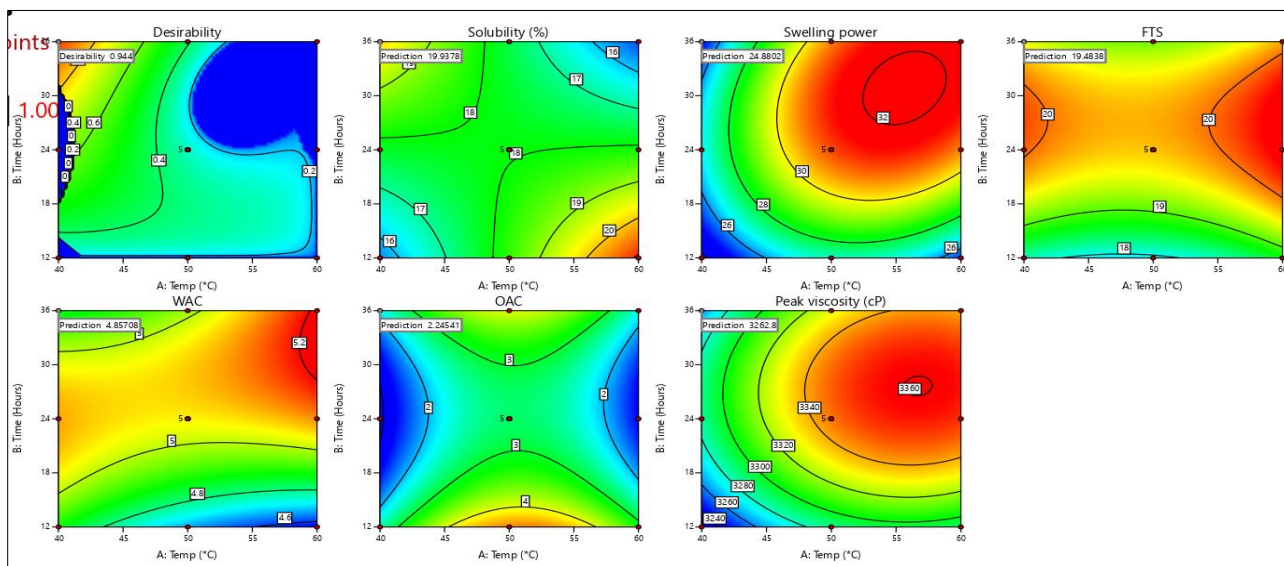


Fig 9: Response surface plot for ANN optimization (1:4 starch to water ratio) (A: Temperature, B: Time)

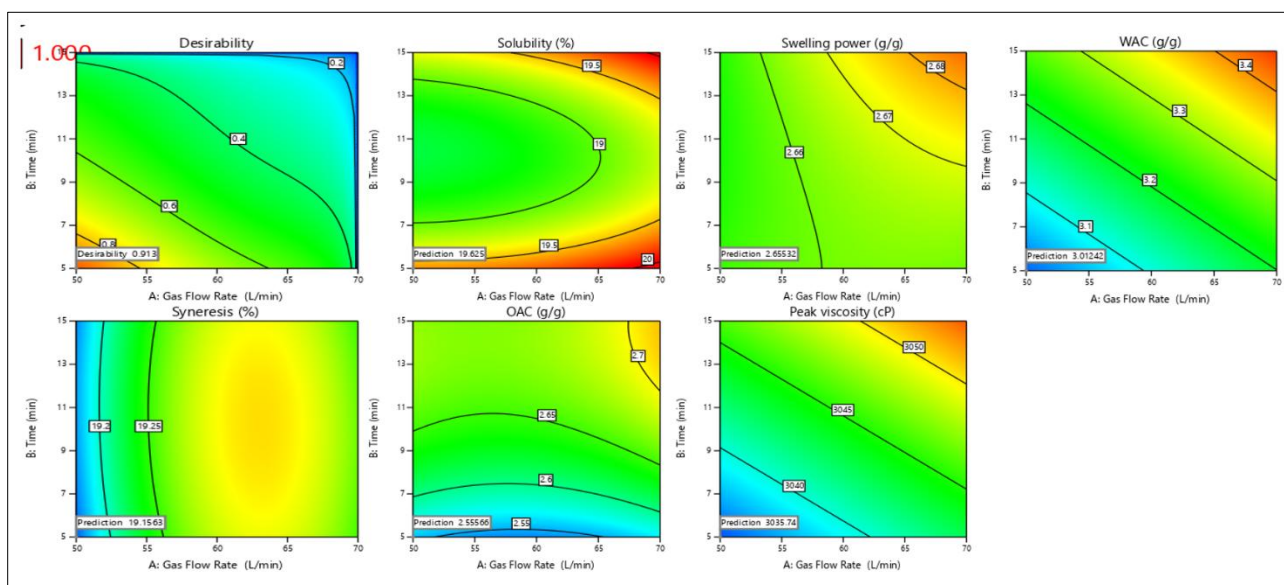


Fig 10: Response surface plot for APPJ optimization (A: Gas flow rate, B: Time)

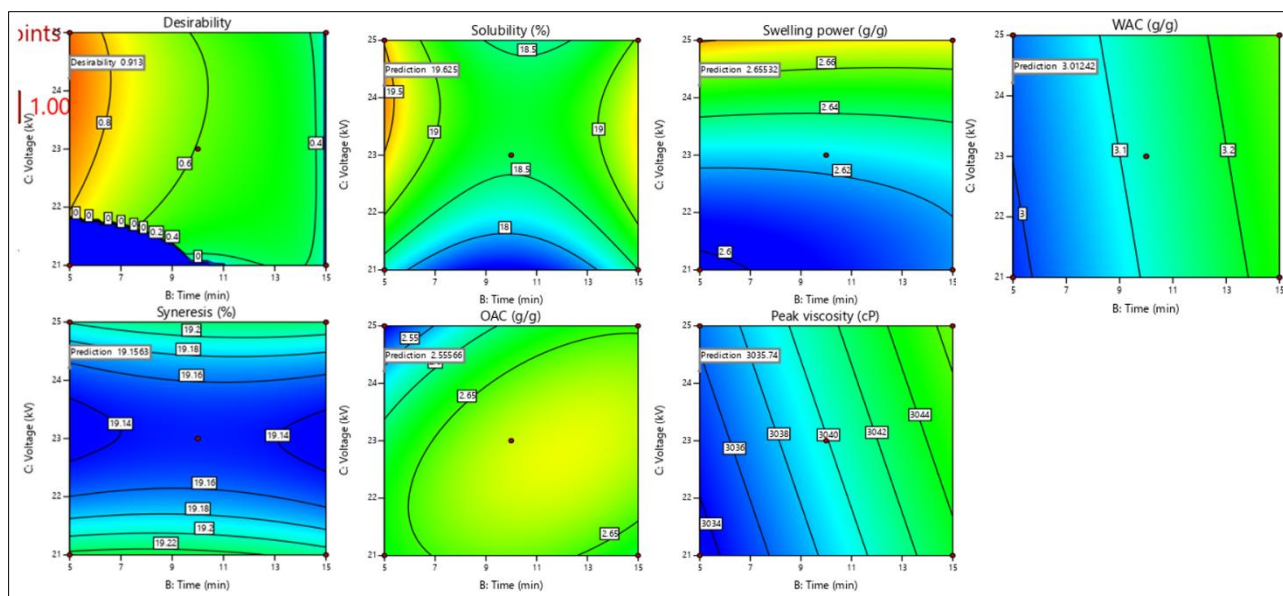


Fig 11: Response surface plot for APPJ optimization (B: Time, C: Voltage)

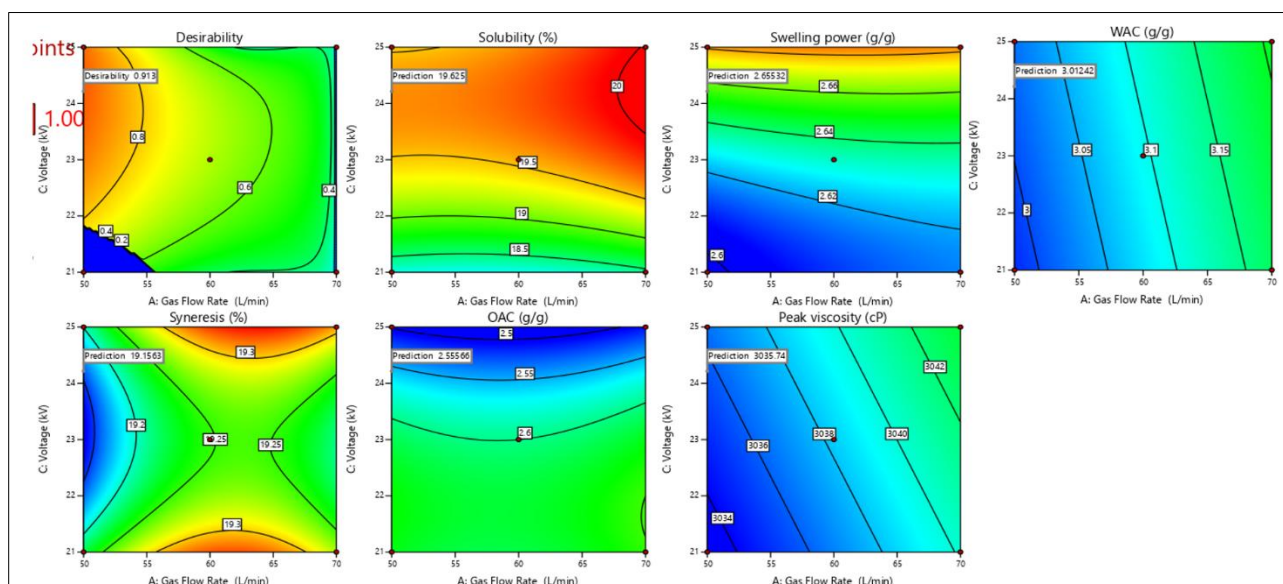


Fig 12: Response surface plot for APPJ optimization (B: Time, C: Voltage)

5. Conclusion

This study demonstrates that heat moisture treatment (HMT), annealing (ANN), and atmospheric pressure plasma jet (APPJ) significantly modify the physicochemical properties of cassava starch. HMT and APPJ treatments increased solubility and reduced swelling power, while peak viscosity decreased under HMT and APPJ but increased following ANN. The treatments enhanced water absorption capacity while reducing oil absorption capacity, indicating altered molecular interactions within the starch matrix. Numerical optimization revealed optimal conditions of HMT-25% at 140 °C for 12 h (desirability = 0.999) and HMT-30% at 150 °C for 17 h (desirability = 0.999). For ANN treatments, the optimal conditions were ANN 1:3 at 40 °C for 12 h (desirability = 0.963) and ANN 1:4 at 40 °C for 36 h (desirability = 0.944). The APPJ treatment was optimized at 50 L/min gas flow rate, 25 kV voltage, and 5 min treatment time (desirability = 0.913). The regression models exhibited high significance ($p < 0.01$) and excellent fit, with R^2 values ranging from 0.85 to 0.99 and low prediction errors (<2%), confirming the models' strong predictability, validity, and reliability. These findings

highlight the effectiveness of HMT, ANN, and APPJ in enhancing the functional properties of cassava starch for diverse food industry applications. As physical modification techniques, these methods eliminate the need for chemical agents, offering a safe and sustainable approach to tailoring starch functionality for specific food requirements. Future research should explore molecular and structural characterization, advanced physical modification techniques (e.g., extrusion, radiation, mechanical treatment, and pulsed electric fields), and practical food industry applications to further enhance cassava starch performance.

6. Novelty of the research

The primary aim of this research was to modify the physicochemical properties of cassava starch using HMT, ANN, and APPJ to develop enhanced functional ingredients for food industry applications.

1. Systematic evaluation of HMT, ANN, and APPJ modifications on starch from the high starch yielding cassava cultivar Sree Reksha. Unlike previous studies, it integrates multiple modification techniques and provides a comprehensive analysis of extraction

efficiency, physicochemical properties, and functional applications, offering new insights into structure-function relationships.

2. The modifications systematically increased solubility and water absorption capacity while reducing syneresis, pasting viscosity, oil absorption capacity, and swelling power. These tailored properties enable targeted applications in diverse food systems based on specific functional requirements.
3. The study validates clean label physical modification methods for customizing starch functionality, providing valuable insights for future research and commercial starch processing.

Authors contribution:

Yedla Nikhil Krishna: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Software, Writing - original draft. S. Parveen: Experimental design, Supervision, Project administration, Writing - review & editing, Investigation, Software. T. Krishnakumar: Validation, Writing - review & editing. M. Anand: Resources, Visualization, Data Analysis. K. Gurusamy: Technical support, Laboratory setup, Resources.

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Conflicts of Interest

The authors declare no conflicts of interest.

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