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Isolation, Modification and Characterization of Finger Millet (*Eleucine coracana*) Starch

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Abstract: Isolated finger millet (*Eleucine coracana*) starch was subjected to different modifications (hydrothermal, acidic and enzymatic) and characterized in terms of yield, moisture, protein, ash, bulk density, swelling power, solubility, sediment volume, colour, gel consistency, water binding capacity (WBC), pasting properties, freeze thaw stability and paste clarity, and compared with native starch. Moisture content ranged from 4%-5%. Protein and ash content were lowest in case of acid modified starch (AMS). Hydrothermally modified starches (HTMS) showed maximum water binding, peak viscosity and syneresis. Swelling power was decreased for all modifications. Solubility and color (a and b values) decreased for AMS and EMS. However, L values increased with all modifications. EMS showed maximum bulk density, swelling power, solubility, and sediment volume and gel consistency. Paste clarity decreased with storage period and found maximum for EMS.

Key words: Finger millet starch, isolation, modification, hydrothermal treatment, acidic, enzymatic.

1. Introduction

Starch is often used as a thickener, water binder and emulsion stabilizer and gelling agent. It has a unique property to absorb water, yielding gel, if its suspension is heated. However, the native starches are functionally restricted for food applications because of their structural weakness. Native starch pastes and gels may revert or retrograde back to insoluble form. Hence, modification or processing of starch is necessary to engender their range of functionality. Modification is a process of altering starch structure by affecting the hydrogen bond in a controllable manner to change the form/shape of granule and composition of amylose and amylopectin molecules. Usually, starch modification can be done by several methods such as physical, chemical, enzymatic or genetic transformation [1]. Acid modification changes the physicochemical properties of starch without destroying its granule structure [2]. During hydrothermal modification, starch properties are modified through controlled application of heat and moisture.

Finger millet is a highly nutritious staple food crop cultivated in many parts of India and Africa [3]. Finger millet is nutritionally superior to all other cereals [4-5]. It is rich in dietary fibre and calcium [6-7]. In African countries, it is used as an infant and a refreshment food for adults and convalescents [8]. Finger millet is a potential source of commercial native starch exhibiting functional synergism with tapioca. Present investigation was undertaken with an objective to optimize the process conditions for starch isolation from finger millet and also to study the characteristics of native and modified finger millet starch.

2. Materials and Methods

2.1 Starch Isolation

Finger millet grains were cleaned manually and ground in a commercial hammer mill (Bells India Instrumentation, New Delhi). The ground material was

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passed through 100 µm sieve to be used for starch isolation. The protein fraction was removed using centrifugation technique as described by Qian et al. [9] with slight modification. The flour was steeped in 0.1%, 0.2%, 0.3% NaOH (1:6 w/v) containing 0.2% $NaHSO_3$ and kept at 45 ºC in a water bath for 90 min with continuous stirring followed by centrifugation (3,000 rpm) using Eltek MP 400 R Centrifuge (Electrocraft, Mumbai, India) for 15 min. Top brown protein layer was removed and the white starch was resuspended in distilled water and centrifuged until there was no longer any visible protein present. Final starch was resuspended in distilled water and adjusted to a pH range of 6.5-7.0 using 1 M HCl and filtered through Buchner funnel under vacuum with subsequent drying at 50 °C for 12 h. The dried starch cake was ground, passed through 75 µm sieve and stored in LDPE bags (90 µm) at ambient temperature (30 ± 2 ºC) till further use.

2.2 Starch Modification

Isolated native starch (NS) was subjected to three different types of modifications *viz*., hydrothermal, acidic and enzymatic treatments.

2.2.1 Hydrothermal Modification

Starch was conditioned to 25%-28% moisture content (dry basis) in LDPE bags were kept at 4-6 ºC for 8 h to equilibrate the moisture throughout. Conditioned starch sample was placed in a covered baking pan for 3 h at 110 °C. Sample was shaken occasionally for even distribution of heat and then cooled to room temperature followed by drying at 50 °C and sealed in polyethylene bags [10].

2.2.2 Acid Modification

Starch slurry was prepared by dispersing starch (40 g) in 0.14 mol equivalent/L of aqueous hydrochloric acid (60 g). The reaction was allowed for 8 h in a water bath at 50 ºC. Thereafter, pH of the slurry was adjusted to 5.5 with 1 mol equivalent/L NaOH and the slurry was washed three times with deionized water prior to filtration. Starch was dried overnight in a convection oven at 50 ºC [11].

2.2.3 Enzymatic Modification

Crude fungal amylase (0.1%) derived from *Aspergillus oryzae* was used [12]. Starch-enzyme suspension was incubated at 37 °C for 90 min in 0.04 M acetate buffer at pH 4.7.

2.3 Proximate Analysis

Starch recovery was expressed as percent starch yield based on finger millet flour. The moisture, ash and protein were determined using standard procedures [13]. Also bulk density was determined as per the method described by Balandrán-Quintana et al. [14].

2.4 Swelling Power and Solubility

Starch (0.6 g) was heated with 40 mL of water at 60 °C for 30 min. Lump formation was prevented by stirring. The mixture was centrifuged at 3,000 rpm for 15 min. Supernatant was carefully removed and starch sediment was weighed. An aliquot of supernatant (5 mL) was taken in pre-weighed petridish and evaporated overnight at 130 °C and then weighed. The residue obtained after drying of supernatant represented the amount of starch solubilized in water [15]. The result was expressed as:

$$\text{Swelling power}(\%) = \frac{\text{Weight of sediment paste (g)} \times 100}{\text{Weight of sample (g) (db)} \times (100 - \%\,\text{solubility})}$$

$$\text{Solubility}(\%) = \frac{\text{Weight of soluble starch (g)} \times 100}{\text{Weight of sample (g)}}$$

2.5 Sediment Volume

Starch (1 g) was mixed with 95 mL of distilled water. The pH of starch slurry was adjusted to pH 7.0 using 5% NaOH/HCl followed by heating in a boiling water bath for 15 min. Distilled water was added to make the total weight to 100 g. The mixture was transferred to a 100 ml graduated cylinder and was sealed. The starch slurry was kept at room temperature for 24 h and volume of sediment consisting of starch granules was measured.

2.6 Colour Determination

Colour was measured by CR-300 chroma meter (MINOLTA, Japan), as L, a, and b values. L value represents lightness, a value represents redness/greenness, and b value represents yellowness/blueness.

2.7 Gel Consistency

Starch samples (0.1 g, dry basis) were wetted in a test tube (16 × 150 mm) with 0.2 mL of 95% ethanol containing 0.025% bromothymol blue and dispersed in 2 mL of 0.2 N KOH. The tubes were heated in a vigorously boiling water bath for 8 min. There it was cooled at room temperature for 5 min followed by cooling in an ice water bath for 20 min and laid down horizontally for 1 h at room temperature. Longer the gel travel within tube, lower is its consistency [16].

2.8 Water Binding Capacity

Water binding capacity was determined using the method described by [17], as modified by Medcalf [18]. A suspension of 5 g starch (dry basis) in 75 mL distilled water was agitated for 1 h and centrifuged at 3,000 rpm for 10 min and excess water was drained for 10 min and then weighed.

$$\text{Water binding capacity (\%)} = \frac{\text{Weight of residual starch (g)} \times 100}{\text{Weight of sample (g)}}$$

2.9 Pasting Properties

Rapid Visco-Analyzer (Newport Scientific Pvt. Ltd., Warriewood, Australia) was used to determine pasting properties of starch. A suspension of 3 g starch (14% moisture, wet basis) in 25 mL of distilled water underwent a controlled heating and cooling cycle under constant shear. It was held at 50 °C up to 1 min. Samples was heated (50 °C-95 °C, 12 °C/min) and held at 95 °C for 5 min. Subsequently, sample was cooled (50 °C, 12 °C/min) and held at 50 °C for 5 min. A RVA plot of viscosity (cP) versus time (s) was used to determine peak viscosity (PV), trough (T), breakdown (BD), final viscosity (FV), set back (SB), peak time (P_{time}) and peak temperature (P_{temp}).

2.10 Freeze Thaw Stability

Aqueous suspension of starch (5%, w/w) was heated at 95 ºC under constant agitation for 1 h. The paste was weighed (20 g) in to previously weighed centrifuge tubes and capped tightly. It was centrifuged (1,000 rpm, 10 min) to remove free water. The supernatant was decanted and tubes containing starch paste were subjected to eight freeze thaw cycles followed by centrifugation (4,000 rpm, 30 min). Alternate freezing and thawing was performed by freezing for 24 h at -18 ºC and thawing for 4 h at 30 ºC. The percent water separated after each freeze thaw cycles was measured [19] in terms of syneresis.

$$\text{Syneresis(\%)} = \frac{\text{Water separated (g)} \times 100}{\text{Total weight of sample (g)}}$$

2.11 Paste Clarity

Light transmittance (%) of paste from starch was measured as per method illustrated by Craig et al. [20] and Perera [21]. A 2% (dry basis) aqueous suspension of starch was heated in boiling water bath for 30 min with constant stirring. The suspension was cooled to room temperature. Sample was stored for 7 days at 4 °C, and transmittance was measured at an interval of 24 h for 5 days at 640 nm against a water blank using Spectrophotometer (Spectroscan 80-DV UV-VIS).

2.12 Statistical Analysis

The analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were performed for comparing differences among mean values using SPSS version 16.0.

3. Results and Discussion

3.1 Proximate Analysis

Starch yield of finger millet was found to be 60.37% (dry basis) (Table 1). Starch yield of 52.4% [22] and

Table 1 Proximate composition of native and modified finger millet starches.

Sample	Yield (%)	Moisture (%)*	Protein (%)*	Ash (%)*
NS	60.37	$4.1^{a} \pm 0.14$	$2.71^{c} \pm 0.10$	$2.70^{c} \pm 0.36$
HTMS	-	$4.9^{c} \pm 0.14$	$2.63^{b} \pm 0.21$	$2.77^{d} \pm 0.22$
AMS	-	$4.4^{b} \pm 0.28$	$2.50^{a} \pm 0.01$	$1.28^{a} \pm 0.44$
EMS	-	$5.0^{d} \pm 0.28$	$2.89^{d} \pm 0.10$	$2.42^{b} \pm 0.22$

*Values are means ± S.D's of duplicate determinations.

NS, Native finger millet starch; HTMS, hydrothermally modified finger millet starch; AMS, acid modified finger millet starch; EMS, enzymatically modified finger millet starch.

56.1% [23] were reported for finger millet starches. Also, about 58.7% and 30.9%-32.3% starch yield were reported for maize [24] and oat [2], respectively. Modifications caused a slight increase in moisture content. Hydrothermal and acidic modification caused a slight decrease in protein content. However, higher protein content (2.89%) was observed for enzymatically modified starch (EMS). Acid modified starch (AMS) and EMS had lower ash content of 1.28% and 2.42%, respectively. However, slightly raised ash content was observed for hydrothermally modified starch (HTMS).

3.2 Bulk Density

EMS showed a significantly ($P < 0.05$) higher bulk density (0.502 g/mm³) as compared to other samples (Table 2). An increase in bulk density was observed with corresponding decrease in swelling power for EMS. This could be due to improvement of gel structure that limits expansion in starch. An increase in bulk density was observed in acid modified corn starch [25].

3.3 Swelling Power

NS showed a swelling power of 3.53%. Enzymatic modification caused maximum reduction in swelling power (3.18%) as compared to HTMS (3.49%) and AMS (3.45%). Starch molecules integrated with water as temperature was raised. The insoluble starch granules started to swell because of hydration. The amylose and amylopectin dissociated in suspension, resulting in increase of starch solubility. The starch modification through hydrothermal treatment has been found to reduce swelling power of finger millet starch [26]. This might be due to the decreased viscosity during heating and an increase in onset temperature of swelling and increased stability during cooking [27, 28]. Increase in starch crystallinity after starch modifications may have restricted the percolation of water within starch matrices [27, 29], causing reduced swelling power of modified starches.

3.4 Solubility

Solubility corresponds to hydrophilicity and amylose content, i.e., more dissociation of inter and intra molecular hydrogen bonds, leading to higher amylose leaching exerting increased solubility [22]. Solubility of finger millet starches ranged from 10.70% (AMS) to 16.94% (EMS) with intermediate values for NS (14.85%) and HTMS (13.4%). Higher solubility values for EMS may be due to more dissociation of starch molecules. These results showed that the modified finger millet starches have weaker inter and

Table 2 Physical characteristics of native and modified finger millet starches.

Sample	Bulk density (g/mm³)	Swelling power (%)	Solubility (%)	Sediment volume (mL)	Hunter colour value			Gel consistency (mm)	Water binding capacity (%)
					L	a	b		
NS	$0.435^{a} \pm 0.04$	$3.53^{d} \pm 0.14$	$14.85^{c} \pm 0.21$	$1.0^{b} \pm 0.0$	$76.30^{a} \pm 0.60$	$2.75^{c} \pm 0.07$	$8.87^{c} \pm 0.02$	$92.5^{a} \pm 3.6$	$256.9^{b} \pm 1.34$
HTMS	$0.435^{a} \pm 0.02$	$3.49^{c} \pm 0.09$	$13.40^{b} \pm 0.00$	$0.5^{a} \pm 0.0$	$76.43^{b} \pm 0.19$	$2.96^{d} \pm 0.05$	$10.77^{d} \pm 0.11$	$92.5^{a} \pm 3.53$	$290.8^{d} \pm 13.71$
AMS	$0.435^{a} \pm 0.01$	$3.45^{b} \pm 0.42$	$10.70^{a} \pm 0.42$	$0.5^{a} \pm 0.0$	$77.66^{d} \pm 0.31$	$2.55^{b} \pm 0.05$	$8.50^{b} \pm 0.06$	$97.5^{b} \pm 2.12$	$282.6^{c} \pm 23.41$
EMS	$0.502^{b} \pm 0.01$	$3.18^{a} \pm 0.25$	$16.94^{d} \pm 3.16$	$1.5^{c} \pm 0.0$	$76.60^{c} \pm 3.74$	$2.23^{a} \pm 0.12$	$6.75^{a} \pm 0.23$	$120.0^{c} \pm 0.70$	$199.9^{a} \pm 0.42$

[a] Values are means ± S.D's of duplicate determinations are reported.

NS, Native finger millet starch; HTMS, hydrothermally modified finger millet starch; AMS, acid modified finger millet starch; EMS, enzymatically modified finger millet starch.

intra molecular hydrogen bond. The NS, AMS and HTMS underwent dissociation to lesser extent. Heat moisture treatment of waxy maize starch showed an increase in solubility [30]. HTMS solubilised at faster rate as compared to NS [26]. It was reported that the native sago starch showed lower solubility at temperatures between 60 °C and 80 °C [1].

3.5 Paste Clarity

Paste clarity (% transmittance) for finger millet starch increased with modification (Fig. 1). EMS and AMS exhibited comparatively better transmittance than HTMS and NS. Increase in paste clarity after hydroxypropylation for finger millet and maize starch was reported by Lawal [22] (finger millet starch) and Liu et al. [31] respectively. This may be attributed to greater stability of starch structure after modification due to the improvement in inter and intra molecular bonding. This characteristic is particularly useful in foods such as jellies and fruit pastes to get desired consistency. The transmittance was found to decrease for all the samples with storage (Fig. 1). EMS and AMS showed comparatively better transmittance than the NS and HTMS samples throughout the storage period. Similarly, decrease in the light transmittance with storage time was reported by Achille et al. [32].

3.6 Sediment Volume

Sediment volume indicates the changes in starch molecular association during the process of modification [33] and it indicates the degree of cross-linking in starch. EMS showed higher sediment volume (1.5 mL) than NS (1.0 mL). Increase in sediment volume for EMS may be due to subsequent grouping and formation of enzyme starch complex. Both HTS and AMS showed a sediment volume (0.5 mL) as compared to NS. Similarly it was reported that the acetylation and enzyme modification lowered the sediment volume in potato and sweet potato flours [33].

3.7 Colour

Not much variation was observed for colour values among starch samples. L values increased slightly with modification viz. HTMS (76.43), EMS (76.60) and AMS (77.66) as compared to NS (76.30). Higher values for AMS may be due to the effect of acid that

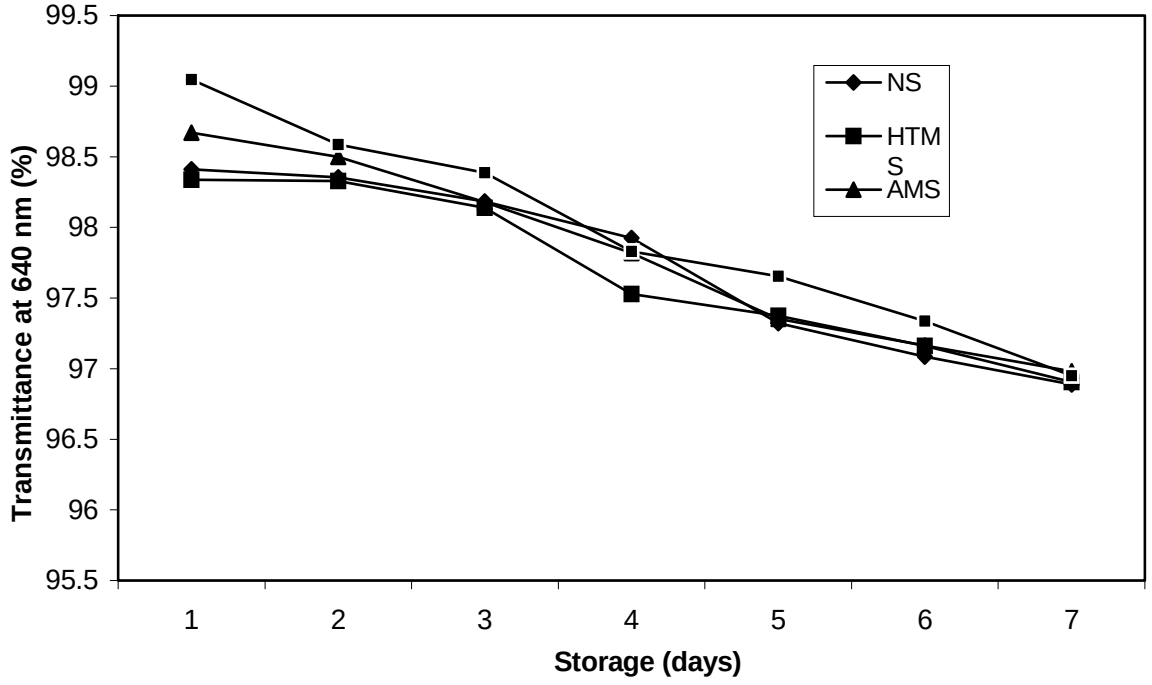

Fig. 1 Paste clarity of native and modified finger millet starches.

lead to smaller particle size and higher refractive index. A fall in colour a and b values as compared to NS was observed with AMS and EMS. Colour a and b values were maximum for HTMS (Table 2).

3.8 Gel Consistency

EMS traveled a longer distance (120 mm) as compared to NS (92.5 mm) which signifies lower gel consistency. AMS (97.5 mm) showed similar trend, but to lesser extent. Gel consistency of HTMS (92.5) was same as NS. It was reported that the acid catalyzed hydrolysis of starch as opposed to enzymatic (α-amylase) hydrolysis can take place at branch points as well as at linear segments [34]. Thus the properties of greater gelling power or retrogradation of AMS could be attributed to reducing the degree of branching and increased percentage of linear segments. Thus, gel consistency was proportional to the sediment volume of aqueous starch dispersion [35].

3.9 Water Binding Capacity

Water binding capacity of HTMS (290.8%) was higher than NS (256.9%). Acidic and hydrothermal modification increased water binding capacity. However, enzymatic modification decreased the water binding capacity of finger millet starch. The difference in availability of water binding sites in modified starch among the various samples leads to variation in water binding capacity [36, 37].

3.10 Pasting Properties

Secondary increase in viscosity (setback) during cooling phase was observed to be minimum for NS (2319 cP). This retrogradation or setback is influenced by various factors viz., amylose content, length of amylose molecules and state of dispersion of amylose chains [38]. The process of gel formation and setback depends on polymer association especially the linear amylose fraction presented in starch molecule [39]. A tendency was noted for waxy-textured potato starch to exhibit low peak paste viscosity and a high degree of setback [40]. EMS showed lower values for pasting characteristics than other starch samples (Table 3). AMS showed a slightly higher PV (2626 cP) and T (1910 cP) than the NS, yet other pasting characteristics were lower [BD of 715.5 cP, FV of 3405 cP and TSB of 1495 cP] than NS. HTMS showed higher values for PV of 3449 cP, T of 1496 cP, BD of 1953 cP, PV 4467 cP) and SB of 2971 all signifying lower extent of modification. From these observations it may be concluded that the enzymatic and acidic modification are more effective. It was reported that the low peak viscosity indicates lower water holding capacity for starch [41]. Similarly, EMS showed comparatively lower water holding capacity.

3.11 Freeze Thaw Stability

The percentage syneresis was found to increase with freeze thaw cycles (Fig. 2). Low temperature or repeated freeze thaw cycle treatment of concentrated pastes gives rise to cryotropic gel formation and final product of sponge like textures [42]. Repeated FT cycle enforces phase separation and ice growth [43], because starch is syneresised and water is separated from gel. Maximum syneresis was observed in HTMS. An increase was observed in hydrophilic and hydrophobic tendency of finger millet starch with increasing level of hydrothermal conditioning [26]. Acid and enzymatic modification resulted in decrease in syneresis (Fig. 2) and an increase in freeze thaw stability. Least syneresis was exhibited by EMS at the end of eight freeze thaw cycle. Lawal [22] explained that reduction in inter chain bonding between starch molecules in modified starch resulted in fall in syneresis. This was a result of addition of a new functional group. Similar results were reported for hydroxypropyl potato starches [19].

4. Conclusion

The investigation was carried out with an aim of standardizing the isolation and characterization of starch modification by different treatments. The results indicate that modification of finger millet starch change

Table 3 Pasting characteristics of native and modified finger millet starches.

Sample	RVA Parameters (cP)						
	PV	T	BD	FV	SB	P_{time}	P_{temp}
NS	$2598^{b} \pm 13.89$	$1297^{b} \pm 10.00$	$1301.5^{c} \pm 2.51$	$3615^{c} \pm 14.01$	$2319^{b} \pm 18.56$	$4.865^{b} \pm 0.44$	$80.80^{b} \pm 0.75$
HTMS	$3449^{d} \pm 12.98$	$1496^{c} \pm 5.65$	$1953.0^{d} \pm 4.24$	$4467^{d} \pm 77.78$	$2971^{b} \pm 8.49$	$4.365^{a} \pm 0.21$	$60.43^{a} \pm 1.37$
AMS	$2626^{c} \pm 22.63$	$1910^{d} \pm 14.14$	$715.5^{b} \pm 3.54$	$3405^{b} \pm 16.97$	$1495^{a} \pm 32.53$	$5.570^{d} \pm 0.23$	$87.65^{d} \pm 0.07$
EMS	$1450^{a} \pm 35.36$	$1032^{a} \pm 14.14$	$417.5^{a} \pm 10.61$	$2364^{a} \pm 94.75$	$1332^{a} \pm 46.67$	$5.330^{c} \pm 0.00$	$84.95^{c} \pm 1.34$

PV = Peak Viscosity, T = Trough, BD = Breakdown, FV = Final Viscosity, SB = Set Back,
Ptime = Peak Time and Ptemp = Peak Temperature.

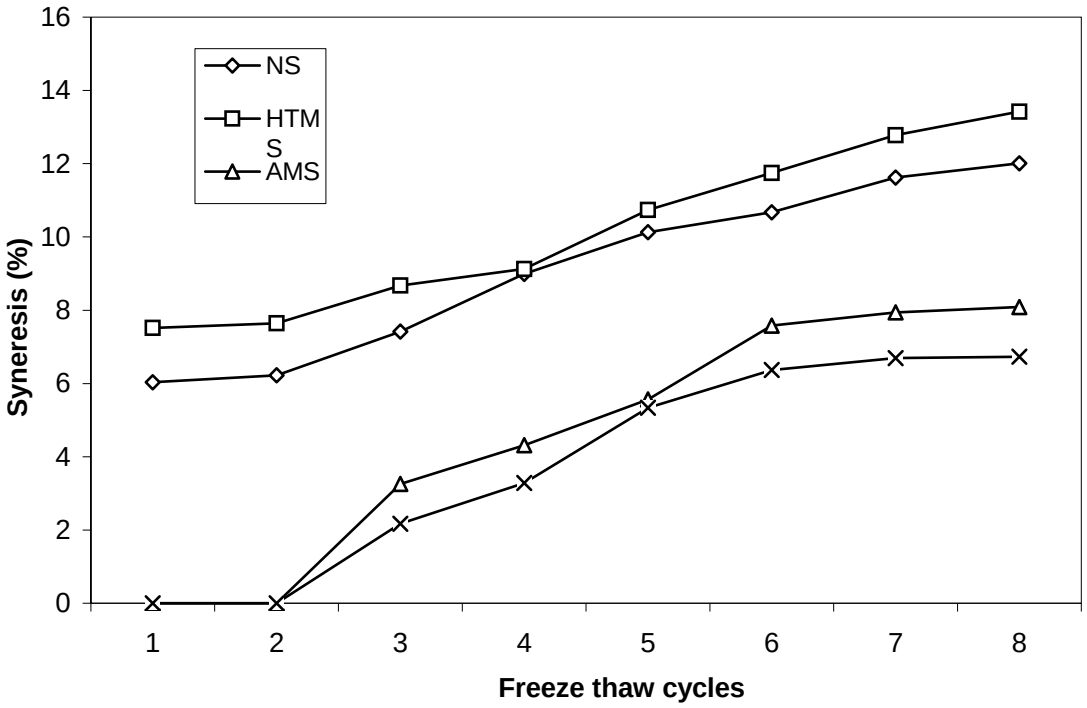

Fig. 2 Effect of modification on the freeze-thaw stability of native finger millet starch.

the characteristics of starch. EMS had a higher bulk density and sediment volume. Swelling power of finger millet decreased with modifications. EMS also showed better solubility. AMS had higher L colour value. AMS and EMS had slightly lower a and b colour values. HTMS showed maximum WBC. Both EMS and AMS showed comparatively lower breakdown, final viscosity and setback while HTMS showed higher values of breakdown, final viscosity and setback. The paste clarity values of finger millet starches for EMS and AMS. Finger millet, a low cost, easily available and rich source of starch could thus serve as a source of modified starches, especially AMS and EMS. These could be exploited not only from the commercial angle but also to understand the behaviour of starch for the future.

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