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Isolation, Modification and Characterization of Sweet Potato (*Ipomoea batatas L* (Lam)) Starch

Marcquin Chibuzo Iheagwara*

Department of food science and technology, Federal University of Technology, Owerri, p.m.b. 1526 owerri, Imo state, Nigeria

Abstract

Sweet potato (*Ipomoea batatas L* (Lam)) starch was isolated and subjected to physical, chemical and enzymatic modifications to generate hydrothermally modified (*HMSPS*), acid modified (*AMSPS*) and enzymatically modified (*EMSPS*) sweet potato starches. The proximate, physicochemical, pasting characteristics, light transmittance, freeze-thaw stability of the native and modified starches were characterized. Results obtained revealed that moisture, ash and protein contents were reduced following modifications. Hydrothermal modification (*HMSPS*) caused an increase in swelling power, solubility and water binding capacity while acid and enzymatic modifications reduced them. Also, there was significant reduction (P≤0.05) in sediment volume of all the modified starches with *EMSPS* (1.41 ml) having the least value. Breakdown (BD) and peak viscosity (PV) values declined for all modification with *EMSPS* having the least values of 519cP and 2027cP respectively for BD and PV. However, *EMSPS* and *AMSPS* exhibited improved pasting characteristics, freeze-thaw stability and paste clarity.

Keywords: Sweet potato starch; Hydrothermal; Acid; Enzymatic; Modifications

Introduction

Sweet potato (*Ipomoea batatas L* (Lam) is one of the most economically important species of tropical root and tuber crops, which can grow in great abundance on marginal soils [1]. Sweet potatoes are rich in starch (58-76% on a dry basis) and its starch have properties rather similar to potato starch and has been widely used in starch noodles, bakery foods, snack foods and confectionary products [2]. Sweet potato starch has two major components: amylose and amylopectin. These polymers are very different structurally. Amylose is a relatively long linear polymer α-glucan containing 99% (1→4)-α- and 1% (1→6) linkages while amylopectin is a much larger molecule and a heavily branched structure built from about 95% (1→4)-α-and 5% (1→6)-α-linkages. The structures of these polymers play a critical role in the functionality of native and modified starches [3]. Viscosity, shear resistance, gelatinization, solubility, gel stability and retrogradation are some of the functional properties that depend on the amylose/amylopectin ratio of the starches [4,5]. In foodstuffs, starch is used to influence or control such characteristics as aesthetics, moisture, consistency and shelf stability. It can be used to bind, expand, densify, clarify or opacify, attract or inhibit moisture. Nevertheless, the native starch exhibit some disadvantage certain in industrial applications. The native starch granules hydrate easily, swell rapidly, rupture, loose viscosity and produce weak bodied very stringy and cohesive pastes [6]. Starch modification is often used to circumvent these limitations. In modifications, starch is tailor made to meet the requirements of the end-user, giving rise to a wide range of specialty products. Starch modification is a process of altering the starch structure by affecting the hydrogen bond in a controllable manner. Usually, starch degradation can be done by several methods such as physical alteration, chemical degradation, enzymatic modification or genetic transformation [7]. Hydrothermal treatment as a form of physical alternation involves modification of starch properties through controlled application of heat and moisture which produces physical modification within the starch granules [8]. Acid modification of starch is a granular modification of the native starch achieved through treatment of starch below its gel point in aqueous acid suspension [9]. Enzymatic modifications involves the exposure of starch suspensions to a number of enzymes primarily, including hydrolyzing enzymes that tend to produce highly functional derivatives. The aim of the present study is to isolate starch from the white cultivar of sweet potato tuber, subject it to physical, chemical and enzymatic modifications and investigate the behavior of the native and modified starches with an attempt to broaden what applications it may be used for within the food industry.

Materials and Methods

Materials

Freshly harvested white cultivar of sweet potato (*Ipomoea batatas L* (*Lam*)) tubers were obtained from National Root Crop Research Institute, Umudike, Abia State, Nigeria. All chemical used in the analysis were of analytical grade.

Starch isolation

The method of Sathe and Salunkhe [10] as modified by Adebowale et al. [11] was employed for the starch isolation. Occasional stiring was provided during all extractions.

Starch modification

The isolated native sweet potato starch (NSPS) was subjected to three different types of modifications viz hydrothermal, acidic and enzymatic modification processes.

***Corresponding author:** Marcquin Chibuzo Iheagwara, Department of food science and technology, Federal University of Technology, Owerri, p.m.b. 1526 Owerri, Imo state, Nigeria, Tel: +2348032922630; E-mail: ihesonny@yahoo.com

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Hydrothermal modification

The method described by Collado et al. [12] was employed. The native starch conditioned to 25-28% moisture content (dry basis) was sealed in LDPE bags and kept at 4-6°C for 8h to equilibrate to moisture throughout. Starch sample was taken out of the LDPE bags and placed in a covered baking pan for 3h at 110°C. The baking pan containing the 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.

Acid modification

Acid modification was performed using the method of Wang and Wang [9]. Starch slurry was prepared by dispersing starch (40 g) in 0.14mol equivalent/L (0.14 N) of aqueous hydrochloric acid. The reaction was allowed for 8 h in a water bath at 50ºC and slurry was adjusted to pH 5.5 with 1mol equivalent/L NaOH and the slurry was washed thrice with deionized water and the pH was checked for chloride ions using litmus paper prior to filtration. The starch was dried several nights in a convection oven at 50°C.

Enzymatic modification

The method of Hood and Ameson [13] was used. Crude fungal amylase (0.1%) derived from *Aspergillus oryzae* having enzyme activity 2,000**U**/kg was used. Starch-enzyme suspension was incubated at 37°C for 90 min in 0.04 M acetate buffer at pH 4.7.

Starch characterization

Physicochemical analysis: The moisture, ash and protein were determined using the standard methods of AOAC [14] and Nielsen [15]. Bulk density was determined in accordance to the method described by Balandran-Quintana et al [16].

Solubility and swelling power: The solubility and swelling power were assayed according to the method described by Subramanian et al. [17] and Raina et al [18]. Starch (0.6g) was heated with 40ml of water at 60°C for 30min. Lump formation was prevented by stirring. The dispersion was centrifuged at 3,000rpm for 15min. Supernatant was carefully removed and starch sediment was weighed. An aliquot of supernatant (5ml) was taken in pre-weighed petri dish and evaporated for 2h at 130°C and then weighed. The residue obtained after drying of supernatant represented the amount of starch solubilized in water. The result was expressed as:

$$Solubility(\%) = \frac{W_{ss} \times 100}{W_s}$$

Here, W_{ss} is the weight of soluble starch (g) and W_s is the weight of the sample (g).

$$Swelling\ power(\%) = \frac{W_{sp} \times 100}{W_s \times (100 - \%solubility)}$$

Here, W_{sp} is the weight of sediment paste (g) and W_s is the weight of the sample (g).

Sediment volume: The method of Tessler [19] was employed. Starch (1g) was mixed with 95ml 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 15min. Distilled water was added to make the total weight to 100g. The mixture was transferred to a 100ml graduated cylinder and was sealed.

The starch slurry was kept at room temperature for 24hrs and volume of sediment consisting of starch granules was measured.

Gel consistency: Gel consistency was determined according to the method described by Yadav et al. [20]. Starch samples (0.1 g by dry basis) were wetted in a test tube 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, cooled at room temperature for 5 min followed by cooling in ice water bath for 20 min and then laid down horizontally for 1 h at room temperature. The longer the gel travels within tube implies the lower the consistency.

Water binding capacity: Water binding capacity was determined using the method described by Robertson et al. [21], with some modifications. A suspension of 3 g starch (dry basis) in 60 ml distilled water was agitated for 1 hr and centrifuged at 3,000 rpm for 10 min and excess water was drained for 10 mins and then weighed.

$$Water\ binding\ capacity(\%) = \frac{W_{rs} \times 100}{W_s}$$

Here, W_{rs} is the weight of residual starch (g) and W_s is the weight of the sample (g).

Pasting properties: The pasting characteristics of the starch samples were measured in a standard Brabender viscoamylograph (Brabender Instrument Inc; Duirburg West Germany) according to the procedure described by Lawal et al [22]. The starch suspension (8%) was heated from 30°C to 95°C and kept at this temperature for 30 min before it was cooled to 5°C. A constant rotational velocity of 75 rpm was maintained and the heating as well as cooling rate was 1.5°C /min throughout the process.

Freeze thaw stability: The freeze thaw stability of the starch samples were conducted according to the method described by Kaur et al. [23]. Aqueous suspension of starch (5% w/w) was heated at 95°C under constant agitation for 1 h. The paste was weighed (20 g) into 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°C and thawing for 4h at 30°C. The percent water separated after each freeze thaw cycles was measured in terms of syneresis.

$$Syneresis(\%) = \frac{W_{H_2O} \times 100}{W_s}$$

Here, W_{H_2O} is the water separated (g) and W_s is the weight of the sample (g).

Paste clarity: The paste clarity was studied using the method of Bhandari and Singhal [24] with modifications. Fifty milligrams (dry basis) of the starch samples were suspended in 5 ml of distilled water using 10 ml cotton-plugged test tubes. The test tubes were then heated in a boiling water bath (with occasional shaking) for 30 min. After cooling to ambient temperature, the percentage transmittance (%) was determined at 650 nm against a water blank using a spectrophotometer (Hewlett-Packard spectrophotometer). Also, to monitor tendency for retrogradation, samples were stored for 24 h at 4°C to effect nucleation, after which they were stored at 30 ± 2°C for 1-7 days before determining the absorbance.

Statistical analysis: Data were analyzed in triplicates for analysis of

variance (ANOVA) and Duncan's multiple range test (DMRT) using SPSS version 16.0.

Results and Discussion

Proximate composition

The proximate composition of the native and modified sweet potato starches shown in table 1 indicates that the moisture content of the starch ranged from 9.69%-10.48% with the hydrothermally modified sweet potato starch (HMSPS) having the least value. The moisture content of a powder plays a significant role in the flow and other mechanical properties of the food. However, it depends largely on the method, extent of drying, and the humidity in the surrounding atmosphere [25]. The moisture content values for the native sweet potato starch (NSPS) and enzymatically modified sweet potato starch (EMSPS) were significantly (P≤ 0.05) not different. The 10.48% moisture level of NSPS observed here is higher than the 10.2% and 9.82% moisture levels reported for cassava (*Manihot esculenta*) starch and arrowroot (*Maranta arundinacea*) starch respectively [26,27]. The protein and ash contents of the native sweet potato starch were reduced following modification. However, the ash content of HMSPS and AMSPS were found statistically equivalent (P=0.05) and the protein content of AMSPS were reduced beyond the detection limit. These reductions are due to various degradation that took place during the modification processes and is in agreement with reports by Adebowale et al [11] and Lawal [25].

Functional properties

Swelling power: The swelling power of the native and modified sweet potato starches were significantly different (P≤ 0.05). NSPS had swelling power of 3.49%. The swelling power of HMSPS (4.75%) was higher than AMSPS (3.21%) and EMSPS (3.38%). Swelling power of starch depends on the capacity of starch molecules to hold water through hydrogen bonding and is influenced by a strong micellar network, amylopectin molecular structure and amylose content [28,29]. The increase in swelling power of HMSPS observed can be attributed to increase in long chains of amylopectin and decreasing amylose content and is in agreement with reports by Sasaki and Matsuki [30] and Srichuwong et al. [31]. The reduced swelling power of AMSPS and EMSPS indicates increase in starch crystallinity which restricted the percolation of water within the starch matrices [32].

Solubility: The solubility of the starches exhibited similar pattern to that of swelling power and differed significant (P≤0.05) among the starches. The solubility of ranged from 13.80% (AMSPS) to 20.25% (HMSPS). However, NSPS and EMSPS had intermediate values of 16.13% and 13.80% respectively. Solubility corresponds to hydrophilicity and amylose content and the maximum value of HMSPS showed maximum hydrophilicity and minimum leach out of linear molecules. This is in agreement with findings of Lawal [33] and suggests that HMSPS have weaker inter and intra molecular hydrogen bond. They minimum value of AMSPS obtained showed minimum dissociation corresponding to lesser hydrophilicity and minimum leach out. This result agrees with the findings of Balasubramanian et al. [34], on pearl millet starch and indicates that AMSPS resisted the leaching comparatively to a greater extent and was structurally strong hence the least soluble.

Bulk density: The bulk density of native sweet potato starch (0.59 ± 0.05) was significantly higher than the modified sweet potato starches with AMSPS having the least value (0.41 ± 0.02). Bulk density is a function of particles size, particle size is inversely proportional to bulk density. Bulk density (BD) is the ratio of the mass per unit volume of a substance. It is an indication of the porosity of a product which influences package design.

Sediment volume: Sediment volume is an index of starch gelatinization and provides a clear distinction between various precooked products. It indicates the changes in starch molecular association during the process of modification. Also, it reflects the degree of cross linking in starch [35]. There were significant variation (P≤ 0.05) among the starch samples. The sediment volume of NSPS (1.98 ml) and HMSPS (1.83 ml) were significantly high compared to AMSPS (1.43 ml) and EMSPS (1.41 ml) that showed a significantly (P≤ 0.05) lower sediment volume. This result is in agreement with report by Yadav et al. [35] that acetylated and enzymatic modification lowered the sediment volume of potato and sweet potato flours.

Water binding capacity: Water binding capacity (WBC) is an important parameter that determines starch use in products. It affects functional properties such as viscosity, which is a very important indicator of bulking and consistency of products [36]. The values obtained ranged from 245.93% to 302.85%. The WBC for HMSPS was significantly (P≤ 0.05) higher than the other starch samples. This difference is occasioned by the available of water binding sites which was very predominate in HMSPS and this was in agreement with report by Abraham [37].

Gel consistency: The gel consistency of the starch samples which is proportional to the hydration power and sediment volume was found to be significantly (P≤ 0.05) higher in AMSPS with a travel distance of 70.26 mm HMSPS showed a lower gel consistency with a travel distance of 117.04 mm.

Pasting characteristics: Table 2 shows the pasting characteristics of native and modified sweet potato starches. The peak viscosity for

Sample	Moisture content (%)	Protein (%)	Ash (%)	Swelling power (%)	Solubility (%)	Bulk density (g/m³)	Sediment volume (ml)	Water binding capacity (%)	Gel consistency (mm)
NSPS	$10.48^{a} \pm 0.36$	$0.17^{a} \pm 0.04$	$2.35^{a} \pm 0.02$	$3.49^{b} \pm 0,90$	$16.13^{b} \pm 0.56$	$0.59^{a} \pm 0.05$	$1.98^{a} \pm 0.32$	$278.12^{b} \pm 15.80$	$98.50^{c} \pm 12.61$
HMSPS	$9.69^{c} \pm 0.41$	$0.15^{b} \pm 0.05$	$2.05^{c} \pm 0.38$	$4.78^{a} \pm 0.13$	$20.25^{a} \pm 0.27$	$0.50^{c} \pm 0.03$	$1.83^{b} \pm 0.22$	$302.85^{a} \pm 17.98$	$117.04^{a} \pm 9.68$
AMSPS	$10.37^{b} \pm 0.39$	ND	$2.05^{c} \pm 0.17$	$3.21^{d} \pm 0.22$	$13.80^{d} \pm 0.32$	$0.41^{d} \pm 0.02$	$1.43^{c} \pm 0.21$	$245.93^{d} \pm 13.79$	$70.26^{d} \pm 10.42$
EMSPS	$10.48^{a} \pm 0.15$	$0.13^{a} \pm 0.03$	$2.19^{b} \pm 0.25$	$3.38^{c} \pm 0.28$	$15.19^{c} \pm 0.12$	$0.54^{b} \pm 0.02$	$1.41^{d} \pm 0.24$	$249.04^{c} \pm 18.83$	$101.21^{b} \pm 13.79$

NSPS – Native sweet potato starch, *HMSPS* – Hydrothermally modified sweet potato starch, *AMSPS* – Acid modified sweet potato starch, *EMSPS* – Enzyme modified sweet potato starch.
ND – Not determined.
Values with different superscripts differ significantly at P0.05.

Table 1: Physicochemical characteristics of native and modified sweet potato starches.

Starch	RVA Parameters (cP)						
	FV	T	BD	PV	SB	$P_{time(min)}$	$P_{temp(^{o}C)}$
NSPS	4153^{b}	985^{c}	1593^{a}	2578^{a}	3168^{b}	5.32^{c}	85.34^{c}
HMSPS	5240^{a}	786^{d}	1350^{b}	2136^{c}	4454^{a}	5.16^{d}	83.26^{d}
AMSPS	3609^{c}	1583^{a}	649^{c}	2232^{b}	2026^{c}	5.53^{b}	87.11^{b}
EMSPS	3204^{d}	1508^{b}	519^{d}	2027^{d}	1696^{d}	5.81^{a}	88.25^{a}

NSPS, HMSPS, AMSPS and *EMSPS* are as defined in table 1.
FV – Final viscosity, T – Trough, BD – Breakdown, PV – Peak viscosity, SB – set back viscosity, $P_{time(min)}$ – Peak time, $P_{temp(^{o}C)}$ – Peak temperature.
Values with different superscripts differ significantly at P≤0.05.

Table 2: Pasting characteristics of native and modified sweet potato starches.

NSPS was 2578cP and this was reduced following the modification processes with EMSPS having the least value of 2027cP. The reduction in peak viscosity is caused by partial cleavage of the glycosidic linkages of the starch thereby resulting in the decrease of the molecular weight of the starch molecules. This partially degraded network was not resistant to shear and could not maintain the integrity of the starch granule thereby producing lower peak viscosity. This is in agreement with the findings of Lawal [25], and Adebowale et al. [11], and Ocloo et al. [38]. The breakdown viscosity which estimates the paste resistance to disintegration in response to heat and shear decreased following the modification. The NSPS has the highest value (1593cP) and EMSPS the least value (519cP). The differences in the breakdown values of the starches may be attributed to the granule rigidity tipid content, the temperature and the degree of mixing and shear applied to the mixture [39]. The setback value which reflects the degree of retrogradation of starch pastes was reduced in AMSPS and EMSPS but increased in HMSPS. The reduction indicates that new substituent groups have been introduced into the modified derivatives and this restricted the tendency of the starch molecules to realign after cooling, thereby encouraging the lower setback values observed in AMSPS and EMSPS [22]. However, the high retrogradation property of HMSPS can be ascribed to a high degree of association of starch molecules caused by a strong tendency for hydrogen bond formation between hydroxyl groups on adjacent starch molecules due to the inability of the constituent amylases to hydrolyze the starch molecules [40]. The final viscosity which indicates the ability of starch to form various paste or gel after cooling was reduced in AMSPS (3609cP) and EMSPS (3204cP) but increased in HMSPS (5204cP). The results indicates that there was a great re-association tendency for HMSPS whereas the conformational reordering and rearrangement occasioned by the acid and enzymatic modification processes restrained the affinity of the hydroxyl groups of one molecule for another with the introduction of other functional groups. The introduction of functional groups to replace the hydroxyl groups limits formation of such binding forces and hence accounted for the reduction in final viscosity of AMSPS and EMSPS compared to NSPS and this is in agreement with reports by Lawal [25] and Iheagwara [41]. The trough (holding viscosity) reduced in HMSPS and increased in AMSPS and EMSPS. This indicates that the hydrothermal treatment greatly affected shear thinning during the holding period. The pasting temperature at use which is the temperature at which a perceptible increase in viscosity occurs and is always higher than the gelatinization temperature [42] was reduced in HMSPS (8526c) and increased in AMSPS (8711°C) and EMSPS (88.25°C). This result suggest that EMSPS would take longer time to gelatinize during processing while HMSPS with relatively lower pasting temperature would be easier to cook and would require less heat for gelatinization to start [40]. The peak time observed to attain peak viscosity was shorter in HMSPS (5.16 min) and longer in EMSPS (5.18 min). This indicates that HMSPS would have low resistance to swelling and as such would be expected to swell rapidly and become susceptible concurrent shear induced disintegration than in other starches [43].

Freeze-thaw stability: The freeze thaw stability an indicator of the tendency of starch to retrograde [44,45], measure by degree of syneresis, describes release of water by gels that have been kept for longer periods or refrigerated or frozen. This is an important factor to be considered when formulating refrigerated and frozen foods [46]. Results of eight cycles of freeze-thawing are shown in figure 1 in terms of syneresis. The syneresis as a result of the eight freeze thaw cycles measured for the excluded water by centrifugation [47] was found to increase as the number of freeze thaw cycles increase. The amount of water released by the frozen starches differ significantly (P≤ 0.05). Maximum syneresis (15.2%) was observed in HMSPS and this is in agreement with report by Adebowale et al. [48] for millet starch which showed increase in hydrophilic and hydrophobic tendencies with increasing level of hydrothermal treatment. The acid and enzymatic modifications resulted in decrease in syneresis. Considering the amount of water release in all the cycles, gels from EMSPS exhibited lowest tendency to syneresis compared to other starches. This result indicates that EMSPS is more stable to freeze-thawing than others and hence would be better suited for use in freeze products than others [3]. The fall in syneresis for AMSPS and EMSPS can be attributed to reduction in the inter chain bonding between the starch molecules and this supports the findings by Lawal [33]. Among all the starches, AMSPS and EMSPS would be more suitable as they presented the highest free-thaw stability.

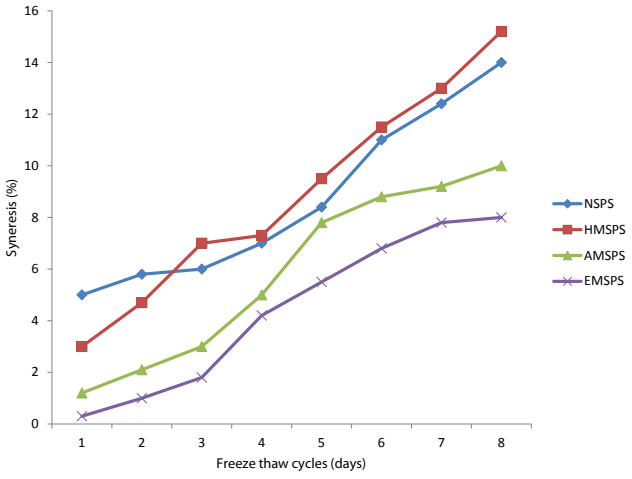

Figure 1: Effect of freeze-thaw cycles on syneresis in native and modified sweet potato starches.

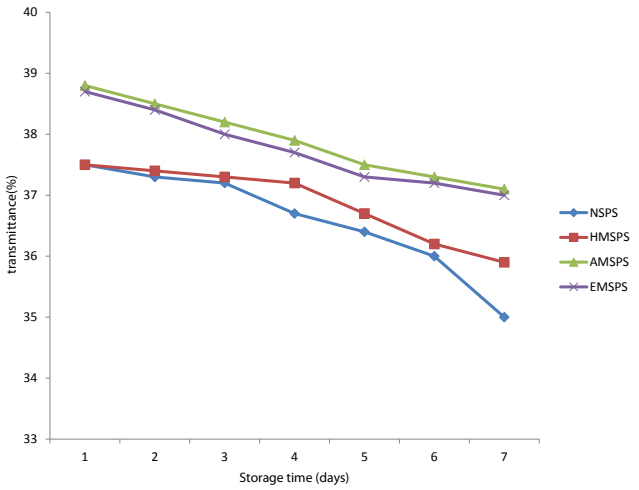

Figure 2: Effect of storage time on transmittance of native and modified sweet potato starches.

Paste clarity: Paste clarity is a much desirable functionality of starches for its utilization in food industries since it directly influences brightness and opacity in foods that contain it as thickners [3]. The influence of storage days on paste clarity of the sweet potato starches presented in figure 2 was found to decrease for all the samples. Similar time dependent reduction in % transmittance has been reported by Bello-Perez et al. [49], for banana starch. Lawal [25] got cocoyam starch and for starch media. However, percentage transmittance (650nm) increased after modification. EMSPS and AMSPS exhibited comparatively better transmittance than HMSPS and NSPS throughout the storage period though AMSPS produced the most remarkable increase in percentage transmittance. This remarkable increase in % transmittance of AMSPS can be attributed to chemical substitution of the hydroxyl group in the starch molecule with carboxyl functional group and this causes repulsion between adjacent starch molecules and apparently reduces interchain association which facilitates improved percentage transmittance [25]. The marked reduction of percentage transmittance of the native starch (NSPS) is a result of retrogradation tendency and this agrees with reports by Lawal [25] and Iheagwara [41].

Conclusion

On the basis of the results from this investigation, it is evident that the modification processes were effective in altering the characteristics of the sweet potato starch. The acid modified sweet potato starch (AMSPS) and enzyme modified sweet potato starch (EMSPS) exhibited improved pasting characteristics, paste clarity and freeze thaw stability. The hydrothermally modified sweet potato starch had high swelling power, solubility and water binding capacity. From these results, there is inferential evidence that AMSPS, EMSPS and HMSPS can be used as a strategic working tool to manipulate the sweet potato starch to meet various specific needs. Therefore, this study will add to the area of starch modification with a view to attempt to broaden what application they may be used for within the food industry.

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