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FUNCTIONAL AND MORPHOLOGICAL PROPERTIES OF ACETYLATED AND ACID-THINNED TZL-COMP3 HYBRID MAIZE STARCHES

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ABSTRACT

This work studied the effect of modification on the functional and morphological properties of TZL-COMP3 hybrid maize starch. Starch isolated from TZL-COMP3 hybrid maize was subjected to acetylation and acid-thinning. The starches obtained – native (NAT), acetylated (ACE) and acid-thinned (ATI), were tested for selected functional properties using established methods. The results of functional analyses showed that swelling power of all hybrid maize starch samples increased with increase in temperature from 55 °C to 95 °C. ACE showed better increase in swelling power. The least gelation concentration (LGC) ranged from 10 - 16 %w/v with NAT and ATI having the least values. Light transmittance was higher in ACE and decreased with increase in the period of storage for all starches. Oil absorption capacities did not significantly improve with modification. Water absorption capacities ranged from 250.00 – 563.67 g/100g with ATI having highest water absorption capacity. It was concluded that modification of TZL-COMP3 hybrid maize starch improved some functional properties with acetylation being the best of the modifications.

KEY WORDS: Hybrid maize starch, acid-thinning, acetylation, properties, SEM.

1.0 INTRODUCTION

Starch, due to its usefulness in food and non-food applications is enjoying increased attention (Olayinka, Adebowale, & Olu-Owolabi, 2013). The worldwide starch production figures continue to grow since there is an increasing demand in food and non-food areas. About 54 % of starch is used for food applications and 46 % for non-food applications. Starches have many useful properties as a food ingredient. It is used as thickening, stabilizing, texturing, gelling, encapsulation, and shelf life extension agents. It plays an important role in determining the quality and texture of many foods; controlling the acceptability and palatability of most food

products (Sandhu, Kaur, Singh, & Lim, 2008). Starch non-food application include its use in the paper industry for surface coating, size press and recycled paper, pharmaceutical industry as binder, disintegrants, release agents, etc (Tharanathan, 2005). Despite the myriad of starch applications, starch in its native form may pose problems with limited applications. These problems include the tendency of its viscosity to increase rapidly and be thickened during heat treatment which may cause difficulties in industrial unit operations such as in pumps and heat exchangers. Some unmodified starches also have drawbacks such as low shear-stress and thermal resistance, thermal decomposition and high degree of retrogradation which limit their use in industrial food application (Kaur, Singh, & Singh, 2007). Native starch deficiencies brought about the use of modified starches as important functional ingredients in processed foods because of their improved functional properties over unmodified starches (Das, Singh, Singh, & Riar, 2010). Starch modifications are of various types. All the modifications are used to improve the properties of starch (Castanha, Divino, Esteves, & Augusto, 2016). Acid-thinning is a type of chemical modification which is conducted with a controlled addition of acid in an aqueous suspension of starch, at a temperature ranging from ambient to a few degrees below the gelatinization temperature, for certain duration. Acid-thinning can change the structural and functional properties of starch. It decreases the molecular weight of the polysaccharide, by increasing the free aldehyde number (Singh, Singh, & Lim, 2007). Starch oxidation is mainly performed through the reaction of starch with an oxidizing agent under controlled pH and temperature and specific reaction time. In commercial conversions, usually sodium hypochlorite is used as the oxidizing agent. Oxidized starches have wide applications in industries such as paper, where it improves the strength and printability of paper. It also has good application in textile, laundry finishing, building material and food industries (Lawal, Adebowale, Ogunsanwo, Barba, & Ilo, 2005).

Currently, important sources of starch are maize, potato, wheat, and cassava. Among these conventional starch sources, maize supplies over 80% of the world starch market. With the growing need for starch, maize remains highly dependable especially if it is draught and pest resistant, high yielding and survives in different environment. These attributes are associated with improved hybrid maize species such as TZL COMP 3. With the dearth of information on this breed, exploring its potential will reduce the problem of over-dependence on the common varieties. The aim of this work therefore was to investigate the effect of acid-thinning and acetylation on the functional properties of hybrid TZL COMP 3 starch.

2.0 MATERIALS AND METHOD

2.1 SAMPLE COLLECTION

Hybrid maize (TZL COMP 3) seeds were obtained from the International Institute for Tropical Agriculture, IITA, Ibadan, Nigeria. Maize seeds were winnowed and extraneous materials removed and stored in air-tight polythene container until required. All other reagents used were of analytical grade.

2.2 ISOLATION OF STARCH

Starch isolation was done by the method of Lawal et al. (2005) with modification. 2 kg of winnowed maize grains were cracked slightly with an electric mill before steeping in 10 L of 0.02 M solution of $NaHSO_3$ for 28 h at 30±2 °C, after which the steeping solution was discarded and the swollen grains were washed with distilled water. The swollen grains were blended for 30 min using a blender (Century Electric Blender). The slurry obtained after blending was re-suspended in 5 L of distilled water. It was screened, using muslin cloth and centrifuged for 30 min at 4500 rpm. Starch obtained after centrifugation was re-slurried in 2 L distilled water and protein was separated from starch by toluene emulsification. Toluene was added (20 mL) to

starch suspension and it was thoroughly mixed for 30 min and allowed to stand for another 2 h. An emulsion layer of denatured protein was formed at the interface as toluene and water separated. The emulsion layer was discarded. The process was repeated for the starch slurry until emulsion layer became negligible. The starch slurry was then washed with acetone and air-dried for 24 h at 30±2 °C. The starch obtained was labelled as native starch (NAT).

2.3 STARCH MODIFICATION

2.3.1 PREPARATION OF ACID-THINNED STARCH (ATI)

Acid thinning of starch was done by the method of Lawal et al. (2005). 100 g of native starch was slurried in 500 mL of 0.15 M HCl. It was stirred magnetically for 8 h, while maintaining a temperature of 50 ^{o}C. The acid-modified starch was filtered and the residue obtained was washed four times with distilled water. It was dried in the air for 48 h at 30±2 ^{o}C.

2.3.2 PREPARATION OF ACETYLATED STARCH (ACE)

Acetylation was carried-out as described by Lawal (2004) with slight modifications. Hundred grams of native starch was dispersed in 500 mL of distilled water; the mixture was stirred for 20 min. The pH of the slurry obtained was adjusted to 8.0 using 1 M NaOH. Acetic anhydride (10.2 g) was added over a period of 1 h, while maintaining a pH range 8.0–8.5. The reaction proceeded for 5 min after the addition of acetic anhydride. The pH of the slurry was adjusted to 4.5 using 0.5 M HCl. It was filtered, washed four times with distilled water and air-dried at 30±2 °C for 48 h.

2.4 FUNCTIONAL PROPERTIES OF STARCH

2.4.1 PERCENTAGE ACETYLATION AND DEGREE OF SUBSTITUTION

The content of acetyl groups (expressed as percentage in dry basis) and the degree of substitution of acetylation were determined according to the method of Lawal et al. (2005). Acetylated starch (5 g) was placed in a 250 mL flask, and distilled water (50 mL) was added upon mixing. A few drops of phenolphthalein indicator were added, and the suspension was titrated with 0.1 M sodium hydroxide to a permanent pink end point. After addition of 0.45 M sodium hydroxide (25 mL), the flask was sealed tightly with a rubber stopper and shaken vigorously for 30 min. After shaking, the stopper was carefully removed and washed down, together with the walls of the flask, with distilled water. The saponified mixture containing excess alkali was then titrated with standard 0.2 M HCl solution until disappearance of the phenolphthalein colour. The native starch was treated in the same manner to obtain a blank value.

$$\% \text{ acetyl (dry basis)} = \frac{(\text{Blank titre} - \text{sample titre})(\text{ml}) \times \text{acid molarity} \times 0.043 \times 100}{\text{sample wieght in gram (Dry basis)}} \quad (1)$$

$$\text{Degree of substitution (DS)} = \frac{162A}{4300 - 42A} \quad (2)$$

2.4.2 AMYLOSE CONTENT (%)

Amylose content of the isolated starches was determined by using the method of (Patil, Gokhale, & Chavan, 2014). 20 mg of starch sample were taken and 10ml of 0.5 N KOH were added to it. The suspension was thoroughly mixed. The dispersed sample was transferred to a 100 mL volumetric flask and diluted to mark with distilled water. An aliquot of test starch solution (10 mL) was pipetted into the 50 mL volumetric flask and 5 mL if 0.1 N HCI were added, followed by 0.5 mL of iodine reagent. The volume was diluted to 50 mL and the absorbance (A) was measured at 625 nm. Amylose content (%) was calculated using the following:

$$\text{Amylose content (\%)} = (85.24 \times A) - 13.19 \quad (3)$$

A = absorbance at 650 nm.

Amylopectin content (%) = 100 – Amylose content (%) (4)

2.4.3 SWELLING POWER AND SOLUBILITY

Effect of temperature on swelling power and solubility determinations were carried out in the temperature range of 55–95 °C, using the method of Lawal (2005). 0.1 g of starch samples were accurately weighed separately and quantitatively transferred into clean dried test tube and weighed (W_1). 10 cm^3 of distilled water was added to the test tube and the mixture was mixed thoroughly for 30 s. The resultant slurries were heated at desired temperatures, varied between 55 and 95 °C for 30 min in a water bath. The mixture was cooled to room temperature and centrifuged at 4500 rpm for 15 min. The residue obtained after centrifugation with the water it retained and the test tube was weighed (W_2).

$$\text{Swelling of starch} = \frac{W_2 - W_1}{\text{weight of starch}} \times 100 \quad (5)$$

Aliquots (5 mL) of the supernatant obtained after centrifugation were dried to a constant weight at 110 °C. The residue obtained after drying the supernatant represented the amount of starch solubilized in water. Solubility was calculated as grams per 100 g of starch on dry weight basis. The effect of pH on solubility and swelling was studied using the method of Lawal (2005). The slurry (1 %, w/v) was prepared with distilled water and the pH was adjusted between 2 and 12 with 0.1 M HCl or 0.1 M NaOH. The slurries were allowed to stand for 1 h at 30±2 °C centrifuged at 4500 rpm for 15 min. The supernatant (5 mL) was dried to constant weight at 110 °C to determine percentage solubility of the starch as stated earlier.

2.4.4 OIL AND WATER ABSORPTION CAPACITY.

The method of Lawal (2005) was used to determine oil and water absorption capacity of the starch. 10 mL of distilled water or oil was added to 1 g of sample. The mixture was mixed thoroughly for 30 s and allowed to stand for 30 min. Then, the volume of the supernatant was recorded.

2.4.5 GELATION STUDIES

The method of Lawal (2005) was used to determine gelation studies. Sample of starch, 2–18 % (w/v), were prepared in test tube with distilled water (10 mL). The starch suspensions were mixed for 5 min. The test tubes were heated for 30 min at 80 °C in a water bath, followed by rapid cooling under running cold tap water. The test tubes were further cooled at 4 °C for 2 h. Least gelation concentration was determined as that concentration when the sample from the inverted test tube did not fall down or slip.

2.4.6 LIGHT TRANSMITTANCE

Paste clarity was studied using the method of Lawal (2005) with modifications. The native and modified starches (50 mg on dry weight basis) were suspended in 5 mL of distilled water and heated in a boiling water bath (with occasional shaking) for 30 min. After cooling to ambient temperature, transmittance (%) was determined at 650 nm against water blank using UV-Visible 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 to 9 days before determining the absorbance.

2.5 STATISTICAL ANALYSIS

Analyses were done in triplicate. Analysis of variance was performed to calculate significant differences in treatment means, and LSD ($P < 0:05$) was used to separate means using SigmaPlot 12 (Systat Software, Inc).

3.0 RESULTS AND DISCUSSION

The results of functional and morphological properties of native (NAT), acetylated (ACE) and acid-thinned (ATI) TZL-COMP3 hybrid maize starches are presented in Tables 1 – 3 and Figures 1 – 4.

The amylose content of NAT was the highest with 24.93%, it however decreased significantly in ACE and ATI starches, which had 11.89% and 6.18%, respectively. The ratio of amylose to amylopectin is crucial in affecting many physicochemical parameters of the starches, since the amorphous component of starches is made up mainly of amylose (Lawal et al. 2005). However, the amylose and amylopectin contents in the sample studied, before and after modification for NAT, ACE and ATI ranged from 6.18 - 24.93% for amylose and 75.11 – 93.83% for amylopectin, but it was highest in the dual modified ATA starch with 93.83% amylopectin content. Percent moisture, ash, protein, fat, fibre, and amylose reduced significantly ($P<0.05$) following oxidation and acid-thinning (Lawal, 2004). The amylose content of sweetsop (19.35%) and soursop (19.31%) was in the range (18–30%) found in normal starches.

Table 1: Chemical compositions of native (NAT), acetylated (ACE) and acid-thinned (ATI) TZL-COMP3 hybrid maize starches.

Parameter	NAT	ACE	ATI
Starch yield (%)	73.25	-	-
Amylose	24.93	11.89	6.18

Amylopectin	75.11	88.11	93.83
Acetyl (%)	-	1.25	-

Swelling power and solubility were temperature dependent, and values increased with an increase in temperature. Swelling power is a measure of hydration capacity and the magnitude of interaction between starch chains within the amorphous and crystalline domains (Mehboob, Ali, Alam, & Hasnain, 2015). The results indicated that the swelling power of the modified starches increased with increase in temperature. ACE showed the highest degree of swelling power. Increase in swelling power with temperature is a result of increase in mobility of starch molecules, which facilitated easy percolation of water and subsequent increase in swelling and solubility. Lawal et al. (2005) reported that their results indicated an increase in swelling power due to an increase in temperature, however, after oxidization and acid thinning, they observed reduction in swelling power of the native starch. Lawal, (2004) reported that acetylation and succinylation improved the swelling power and solubility of the native starch. Mehboob, Ali, Alam, & Hasnain, (2015) after performing dual modification on sorghum starch concluded with the report that addition of succinyl groups increased the swelling power of both native and acid-thinned starches. However, there was only marginal increase in swelling power of sorghum starch. Solubility of native and modified TZL-COMP3 hybrid maize starches increased with increase in temperature. Native starch (NAT) showed the highest solubility with increase in temperature. Lawal (2010) reported that solubility increased as the level of modification increased among hydroxypropylated starches. Singh et al. (2007) reported that acid-thinning increased solubility for both normal and waxy starches and that the effect of acid treatment on solubility was more pronounced in waxy corn starch which may be due to the low

molecular weight linear fraction increment with hydroxyl groups that facilitated solubilisation in warm water. Modified starches showed a higher solubility than native starch.

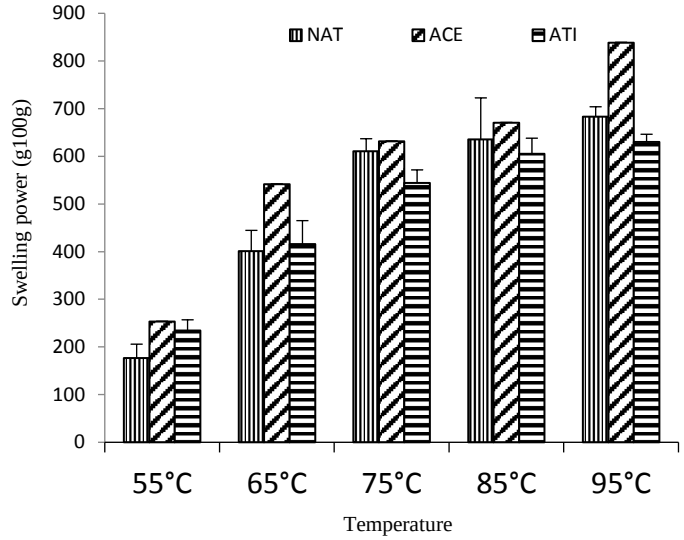

Fig 1: Effect of temperature on swelling power of native (NAT), acetylated (ACE) and acid-thinned (ATI) TZL-COMP3 hybrid maize starches. Error bars are SD of triplicate determinations.

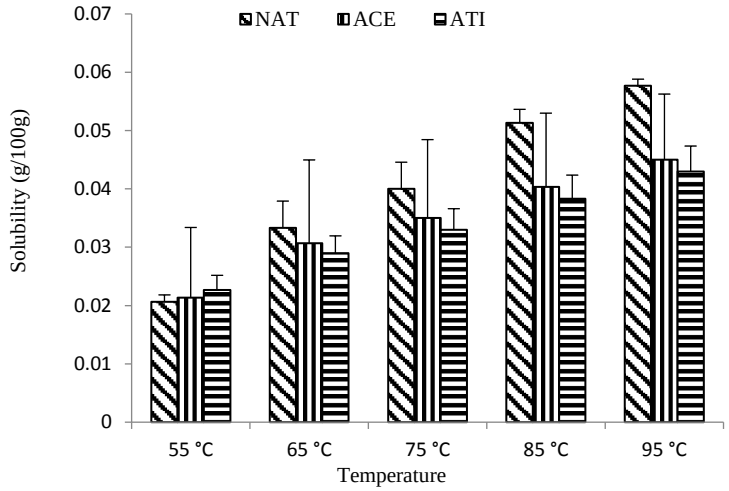

Fig 2: Effect of temperature on solubilities of native (NAT), acetylated (ACE) and acid-thinned (ATI), TZL-COMP3 hybrid maize starches. Error bars are SD of triplicate determinations.

The result of oil and water absorption capacities revealed that the different modifications increased the oil and water absorption, but the rate at which the native and modified starches absorbed water was greater than those of the modified starches. However, acetylated starch

showed the least absorption, while ATI starch had the highest absorption for both water and oil. Lawal et al. (2005) revealed that oxidation increased water absorption capacity and oil absorption capacity of native starch. However, both hydrophilic and hydrophobic properties reduced following acid-thinning. Shah, Masoodi, Gani and Ashwar (2017) explained that water absorption capacity relies upon the molecular structure, crystalline and amorphous regions within the starch and distribution of granular size. The oil absorption capacity involves physical entrapment of oil by capillary action. Increase in water-absorption capacity after succinylation and acetylation has been reported by (Lawal, 2004). In contrast, oil absorption capacity was reduced following succinylation but increased after acetylation. The ability of flours to absorb and retain water and oil may help improve binding of the structure, enhance flavour retention, improve mouth feel and reduce moisture and fat losses of extended meat products (Kaur et al., 2007). Majeed, Wani and Hussain, (2017) reported that water absorption capacity of native, sonicated and dual (sonication and irradiation) treated lentil starches was found lowest in native starch and the highest was observed in dual treated starch. Increase in water absorption with dual treatment may be due to irradiation induced degradation of starch to simpler molecules like dextrins, sucrose and other sugars. These sugars have more affinity for water than native starch.

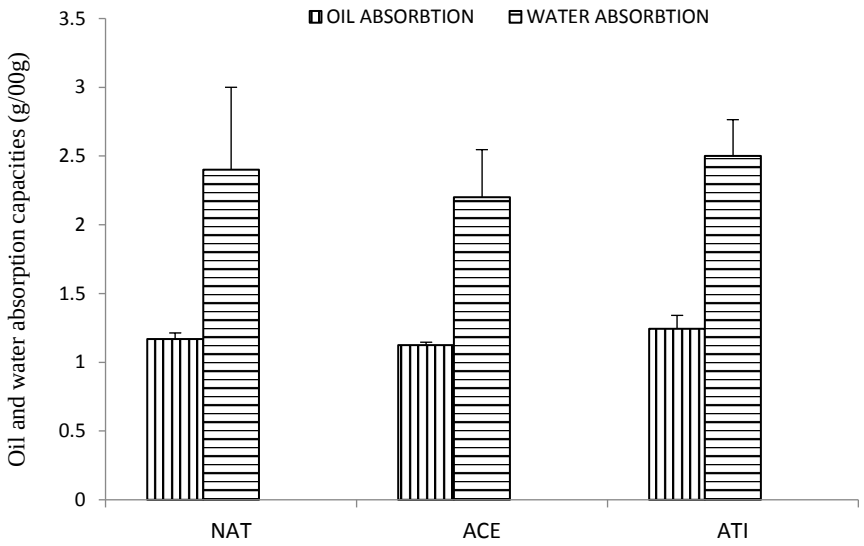

Fig. 3: Oil and water absorption capacities of native (NAT), acetylated (ACE) and acid-thinned (ATI) TZL-COMP3 hybrid maize starches. Error bars are SD of triplicate determinations.

A starch gel is composed of swollen granules, the amorphous region hydrates and swells to a gel phase, during the process of heating (Adebowale & Lawal, 2003). The gelation studies showed that native starch did not form gel until it reached 14 % concentration. ACE however, had an increased least gelation concentration over NAT. The gelation property of ATI starch reduced more than those of NAT and ACE. Lawal et al. (2005) reported that the least gelation concentration increased after oxidation and reduced after acid-thinning. Reduction in least gelation concentration value is an indication of better gelating properties. Starch gelation is a complex process that involves gelatinization, swelling and absorption of water to build a three-dimensional network that offers structural rigidity in various food applications. The building of the structural network involves as well, the bridging of the inter-granular binding forces among the starch molecules, which largely involves hydrogen bonding. The results of Adebowale and Lawal (2003) indicated a reduction in gelation properties of the native starch after succinylation and acetylation. The results of Lawal (2005) indicated that the least gelation concentration of native new cocoyam starch was 8 % (w/v). Following annealing, this value

decreased drastically, to 2 %(w/v), and heat moisture treatment also reduced the LGC of the native starch.

Table Gelation properties of native (NAT), acetylated (ACE), acid-thinned (ATI), acetylated acid-thinned (ACA) and acid-thinned acetylated TZL-COMP3 hybrid maize starches

Starch concentration (%)	NAT	ACE	ATI
2	L	L	L
4	L	L	L
6	L	L	L
8	V	V	V
10	V	G	V
12	V	FG	V
14	G	VFG	V
16	VFG	VFG	G
LGC	14	10	16

LGC = least gelation concentration; L = liquid; V = viscous; G = gel; FG = firm gel; VFG = very firm gel

Light transmittance is required for the starch paste behavior on interaction with light and is affected by remnants of swollen and non-swollen granules (Singh et al., 2007). A decrease in transmittance of pastes with increase in storage period was observed for native and modified starches. Similar trend of decrease in paste clarity with period has been reported by previous workers. Singh et al. (2007), reported that light transmittance of normal and waxy corn starches reduced as the storage days increased from 1st day to the 10th day. Lawal, Adebowale, Ogunsanwo, Barba and Ilo (2005) also reported that the transmittance of pastes from both native and acid-thinned corn starches decreased with increase in storage period. The light transmittance of ACE starch was higher than NAT and ATI. This may be due to the little light

refraction from its swollen granule remnants (Singh et al., 2007). The % transmittance (%T) of all the starches was reduced as the length of storage days increased from 1 to 10. Pronounced reduction in %T was observed in ATI. Similarly, the light transmittance of acid-thinned, and oxidized banana starch were observed to reduce over time as reported by Lawal et al. (2005).

Table 4.3 Influence of storage days on paste clarity of native and modified starches of TL-COMP3 maize breed

	% Transmittance							
Starch sample	DAY 1	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9
NAT	64±0.81	60±0.43	33±0.23	33±0.10	22±0.11	15±0.05	11±0.05	7±0.03
ACE	67±0.05	60±0.26	43±0.15	28±0.14	19±0.08	14±0.05	9±0.05	6±0.02
ATI	50±0.14	47±0.32	30±0.20	27±0.64	18±0.11	12±0.05	9±0.04	6±0.03

4.0 CONCLUSION

The functional and morphological properties of TZLCOMP3 hybrid maize showed that acetylation of starch significantly improved the selected functional properties of hybrid maize starch

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