



EFFECT OF CHEMICAL TREATMENT AS A GUIDE FOR *IN PLANTA* ACETYLATION OF STARCH
REQUIRED FOR THE PRODUCTION OF BIODEGRADABLE PLASTICS

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ARTICLE INFO

Article History:

Received 04th September, 2011
Received in revised form
09th October, 2011
Accepted 07th November, 2011
Published online 31th December, 2011

Key words:

Bioplastics,
Pollution control,
Starch acetylation,
Starch quality.

ABSTRACT

The problems associated with the indiscriminate disposal of plastic products derived from fossil sources and the need to highlight production of alternatives using plants as factories formed the basis of the current research, which is aimed at evaluating the effect of chemical treatment as a pointer to the need for *in planta* acetylation of starch that would be useful in the production of biodegradable plastics. Native starches from cassava, sweet potato and maize were acetylated using acetic anhydride at 68 and 89% (w/v), respectively. After acetylation, the degree of substitution (DS) of each starch acetate was evaluated and seen to range between 1.21 and 1.92. Our results equally demonstrated that acetylating the starch enhanced the tensile strength, percentage elongation and thermostability of the bioplastics produced therefrom, which was observed as a function of the DS of the starch acetates and the level of plasticization. Implicit in these results, therefore, is the conclusion that starch acetylation can enhance its qualities for the production of biodegradable plastics.

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INTRODUCTION

Lately, interest in finding environmentally friendly alternatives to non-degradable plastic materials made from carbon fossils has placed a major emphasis on natural macromolecular polymers such as starch from agricultural sources (Xu *et al.*, 2004) though its strong hydrophilicity and poor mechanical properties have limited its use, especially as a raw material in the nascent biodegradable plastics industry. The development of new starch-based packaging materials implies that following their modification, either through genetic transformation or with chemicals that involve the incorporation of hydrophobic components in the formulation (Derradji-Serghat *et al.*, 1999) that will enhance product quality, the overall quality of starch can be improved. An example of such chemical modification processes is acetylation, which has been demonstrated (Moorthy, 1985) to improve significantly the physicochemical properties of starch. However, one major constraint with this approach lies in the use of chemicals (Firouzabadi *et al.*, 2007) which are considered to be environmentally unfriendly. Moreover, there remains a significant portion of society, especially in Europe, that is less accepting of plant genetic manipulation methods geared towards the production of raw materials for bioplastics manufacture and passionately argues against using such products for packaging purposes. Many among this sector view the use of transgenic plant-derived products as the first step towards ecological destruction (Teli and Timko, 2004). This problem notwithstanding, the manipulation of starch biosynthesis pathways through metabolic engineering holds an

enormous potential for the production of tailor-made low molecular weight polymers with new functionality for applications in bioplastics production. Using this strategy, Firouzabadi *et al.* (2007) has reported the expression of an amyloplast-targeted *Escherichia coli* maltose acetyltransferase (MAT) gene in tubers of wild-type and mutant amylose-free (amf) potato plants. They demonstrated that the acetyl content of starches from different transformants showed low values in their degree of substitution (DS). The DS value of the transgenic starch with the highest acetylation was 10 times lower than that of the industrial sample. Based on this the authors suggested that the acetyl groups may either have been attached to amylopectin, amylose and/or maltooligosaccharides. They also concluded that there may be opportunities in the *in planta* acetylation of starch if the issue of availability of acetyl-coenzyme A and the improvement of maltose acetyltransferase activity towards longer acceptors can be addressed satisfactorily. Though the focus of the current research was the chemical acetylation of starch, our ultimate objective is to transfer and introduce a gene with the ability of attaching acetyl groups to α -glucan into any of the common tropical starch-producing plants in order to enhance *in situ* synthesis of starch acetates. However, before this can be accomplished and demonstrated it is important to assess the impact of the process on the production of biodegradable plastics, which will then serve as a guide for the *in planta* acetylation process.

MATERIALS AND METHODS

Chemicals and plant materials

Acetic anhydride, glacial acetic acid, and tetraoxosulphate (vi) acid (H₂SO₄) of technical grade were purchased from BDH

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Reagents Limited, Poole, England. Cast iron fillings were obtained from the Nigerian Metallurgical Institute, Onitsha, Anambra State, Nigeria. Fresh tubers of cassava (var. TMS 30555) were obtained courtesy of the Cross River Agricultural Development Programme (CRADP), Calabar, Nigeria, while maize grains and potato tubers were purchased from some local stores in Watt market, Calabar Municipality, Cross River State, Nigeria.

Starch processing

The protocol of Udensi et al. (2009) was adopted with slight modifications. Cassava and potato tubers were washed, peeled and grated while grains of maize were soaked in water and milled to a fine mash. The different mashes were mixed with a high volume of distilled water (10 times the mash volume). Fibres in the mash were removed by sieving the watery mash through a fine muslin cloth while the fine starch particles were allowed to sediment. Immediately, thereafter, the water was decanted and the sediments washed again to obtain a white, odourless and tasteless starch. The starch was dried under sunlight, sieved and stored in airtight containers.

Preparation of starch acetates (acetylation of starch)

Native starch derived from each of the plants listed above was processed according to Udensi et al. (2009). Six thousand five hundred (6,500) grams of glacial acetic acid was mixed with 400g of acetic anhydride in a fume cupboard. Two hundred (200) grams of concentrated H₂SO₄ was then added to the mixture before transferring into a glass-lined cast iron agitated acetylator. The mixture was cooled to 70°C and about 1,400g of each starch sample was added slowly. The whole mixture was allowed to stand for 8 hours while keeping the temperature at 45°C. The resulting viscous fluid was diluted with equal parts of concentrated acetic acid and 10% H₂SO₄, and allowed to stand for 15 hours at a temperature of 38°C. During this process, hydration of some of the acetic groups occurred. This was controlled by pouring the resulting mixture in large volume of water and the precipitate obtained was centrifuged at 3,000 rpm for 15 minutes to separate the liquid further. The precipitate was washed, dried and kept safely for further analyses. The same protocol was followed for the starch derived from each of the three different plants. The acetylation process was carried out using acetic anhydride at 68% and 89%, respectively. To ascertain whether there was complete acetylation, about 20g of the different starches was added to 100ml of acetone separately. Complete dissolution indicated complete acetylation (Aziz et al., 2004).

Determination of degree of substitution (DS)

The degree of substitution (DS) of the different starch samples was evaluated according to the method of Miladinov and Hanna (2000) with minor modifications. About 0.5g of acetylated starch powder was transferred to a 250ml conical flask. Fifty milliliters of distilled water was added and the pH of the mixture adjusted to 7.0 with 0.5N NaOH. However, excess NaOH was titrated with HCl back to pH 7.0. Titration was done in triplicates and the mean value taken. Thereafter, the samples were heated on a hot plate until a transparent solution was obtained. The DS was

computed using the following formula:

$$DS = \frac{162 \times [(N \text{ NaOH} \times V \text{ NaOH}) - (N \text{ HCl} \times V \text{ HCl})]}{1000 \times W - 42 \times [(N \text{ NaOH} \times V \text{ NaOH}) - (N \text{ HCl} \times V \text{ HCl})]}$$

Where

N NaOH	=	Normality of NaOH
NHCl	=	Normality of HCl
V HCl	=	Volume of HCl
W	=	Weight of starch sample

Protocol for bioplastics production

The formulations and protocols for the production of biodegradable plastics were according to Udensi et al. (2009) with a few modifications. The formulations contained 45, 60, 75 and 90%, respectively, of native starch and starch acetates from the different sources. Gelatin, agar and sorbitol were dissolved in 70ml of distilled water. One hundred milliliters (100ml) and 150ml of glycerol from a 1% stock solution was added into the mixture and stirred thoroughly for complete dissolution for each round of production. The mixture was loaded into an oven (Continent, MG800G) at a regulated temperature of 80°C. At intervals of about 30 seconds, the process was interrupted while the contents were stirred to forestall solidification at the bottom. It was removed from the oven when it began to simmer. The plastics then were poured into a non-stick baking pan and allowed to dry in an oven (Multioven, Japan). This protocol was followed for all the compositions and different starch samples used in the current study (Table 1).

Evaluation of the rate of degradation of the bioplastics (soil burial test)

The biodegradable plastics produced were divided into tiny squares of 1mm² each. A rectangular hole of about 1.4cm x 0.5cm was dug in the soil where the bioplastics were placed and covered with soil. Data on the rate of biodegradation were recorded at weekly interval for the different levels of starch compositions for a total duration of 6 weeks

Estimation of tensile strength and percentage elongation of bioplastics

The tensile strength and percentage elongation of the bioplastics produced were evaluated according to Stevens(2002). Templates were designed from different products. The width and thickness of each template was measured using a meter rule and micrometer screw gauge, respectively, and all values were recorded. Each sample was clamped in place and weights were added slowly and gently to the balance pan with short intervals between each addition. The weights at the point of breakage were accurately recorded for all the samples. The tensile strength was calculated thus:

$$\frac{\text{Weight at break} + \text{weight of pan}}{\text{Cross sectional area of bioplastics}}$$

For elongation, the lengths of the different templates were measured using a meter rule. The apparatus was set up with a meter rule hung beside the clamp. This was to enable readings to be recorded accurately. At the point of break of the hung bioplastics, the final readings were taken. Thus the

percentage elongation was computed using the formula:

$$\frac{\text{Final length} - \text{initial length} \times 100}{\text{Initial length}} \quad \frac{100}{1}$$

Thermo-stability estimation

Strips of bioplastics measuring 10cm x 1cm x 0.0253cm were placed on a metal tray in an oven. The oven temperature was raised to 30°C for 8 minutes. The appearance of the bioplastics was observed in order to check if there were distortions on their shapes. These bioplastics were then exposed to 40, 50, 60 and 70°C, respectively, in the oven. The temperature at which the bioplastics started to melt was recorded for each of those produced from the various starch acetates (Paula, 2004).

Experimental design and statistical method

Data were fitted into a 3x4x2 factorial layout using completely randomized design and were subjected to the analysis of variance (ANOVA) while Least Significant Difference (LSD) was adopted for mean separation where significant.

RESULTS

Effect of acetic anhydride concentration on the degree of substitution (DS)

The DS of the different starch acetates was significantly affected ($P < 0.05$) by the concentration of acetic anhydride used. An increase in acetic anhydride concentration resulted in an increase in the DS value. As shown in Figure 1, maize acetate had the highest DS values while cassava and potato acetates were slightly different from each other at 68% and 89% concentrations, respectively, of acetic anhydride.

Effect of source of starch on the tensile strength and percentage elongation of bioplastics

Bioplastics produced from native cassava, potato and maize starches showed significant differences ($P < 0.05$) in their tensile strength and this was observed to be dependent on the source and percentage composition of starch and glycerol level used. Table 2 shows that while the bioplastics produced from native maize starch had the highest tensile strength, there was no significant difference ($P > 0.05$) between those produced from native cassava and potato starches. However, bioplastics produced from the starch acetates differed ($P < 0.05$) considerably in their tensile strength (Table 3) with those produced from acetylated maize starch (AMS) having the highest tensile strength and percentage elongation values followed by those produced from cassava and potato acetates, respectively. Expectedly, bioplastics produced from the native starches and starch acetates showed a starch composition-dependent effect on the tensile strength and percentage elongation even though those produced from 90% starch content deviated from this trend. There were no significant differences ($P > 0.05$) between the tensile strengths of bioplastics produced from formulations containing 60% and 90% starch levels and those containing the starch acetates. Tensile strength and elongation of bioplastics produced from native and acetylated starch under different acetic anhydride treatments. There were significant differences ($P < 0.05$)

between the tensile strengths of bioplastics produced from the native starches and starches acetylated using different acetic anhydride concentrations. Interestingly, an increase in the acetic anhydride concentration used led to an increased tensile strength of the products (Tables 4 & 5). The effect of starch (native or acetylated) on elongation was similar to that observed for tensile strength (Tables 6, 7 & 8). Our results also indicated that an increased level of plasticizer used caused a decrease in the tensile strength of the bioplastics and their percentage elongation (% E).

Thermo-stability of bioplastics produced from un-acetylated and acetylated starches from cassava, potato and maize

Results on the thermo-stability of bioplastics produced from native and acetylated starches showed that there were significant ($P < 0.05$) effects of starch composition and the level of glycerol in the bioplastics formulations used, acetylation notwithstanding. The starch acetates and the native starches did not affect thermo-stability of the bioplastics. Our results revealed that a higher quantity of starch in the bioplastics formulation led to a lower thermo-stability and faster rate at which they brittle. Increasing the level of glycerol enhances the thermo-stability of the product (Figure 2). Results obtained showed that the bioplastics did not melt at an oven temperature of 65°C for 8 minutes but rather became deformed. These brittle bioplastics when removed from the oven became normal without deformation.

DISCUSSION

Starch acetylation and tensile strength of bioplastics

Though the focus of the current study was starch acetylation using acetic anhydride to improve its quality, our ultimate aim is to introduce genes into starch producing plants that would confer on them the capacity of attaching acetyl groups to α -glucan in order to synthesize starch acetates in situ. Leggio *et al.* (2003) had reported earlier that acetyl-coenzyme A can be cleaved such that it can transfer its acetyl group to the C6 position of a glucosyl residue of an acceptor molecule, which in our view is reminiscent of the same mechanism employed by acetic anhydride when acting on starch molecules during chemical acetylation. Acetylating starch before use for the production of bioplastics is aimed at enhancing the mechanical properties of starch-based bioplastics, which undoubtedly will be a panacea for the lingering problems of contamination with synthetic plastics. The increase in the degree of substitution (DS) observed of the different starch acetates as the concentration of acetic anhydride used increases (Figure 1) can be attributed to the fact that more acetyl groups became available for bonding. It is equally possible that there was an increased displacement of hydroxyl groups in the glucose monomers of the starch and their subsequent substitution with acetyl groups. This may have led to the increased carbon chain lengths of the starch acetates, resulting in an increased hydrophobicity (Thiebaud *et al.*, 1997; Zhang *et al.*, 1997). Figure 1 shows that maize acetate had a higher DS at the different acetic anhydride concentrations used, indicating that the starch may have had higher carbon chains substitution, suggesting higher hydrophobicity than cassava and potato acetates.

Table 1: Bioplastics recipes

Bioplastics	Starch composition (%)			
	45	60	75	90
Additives	45	60	75	90
Starch (g)	8.0	14.6	29.2	83.0
Gelatin (g)	3.0	3.0	3.0	3.0
Agar (g)	3.2	3.2	3.2	3.2
Sorbitol (g)	3.5	3.5	3.5	3.5
Glycerol (ml)	100;150	100;150	100;150	100;150
Water (ml)	70	70	70	70

Table 2: Tensile strength (TS Kg/cm²) of bioplastics produced from native cassava, potato and maize starches

Starch source	Glycerol level	Percentage starch composition (%)			
		45	60	75	90
UACS	100	6.81±0.58d	6.38±0.97c	8.62±0.44f	7.44±1.08de
	150	5.44±1.19b	4.36±0.05a	7.64±0.98e	6.70±0.18cd
UAPS	100	5.43±0.03b	7.30±0.34d	7.66±0.68e	9.07±0.55g
	50	5.08±0.10b	6.14±0.46c	6.08±0.54c	8.29±0.52ef
UAMS	100	6.14±0.25c	9.53±0.29g	17.45±0.64i	10.25±0.40h
	150	5.91±1.0bc	7.62±0.67e	10.37±0.29h	8.85±0.32f

UACS = Un-acetylated cassava starch; UAPS = un-acetylated potato starch; UAMS = un-acetylated maize starch; LSD (P = 0.05) = Least significant difference.

Table 3: Tensile strength (TS Kg/cm²) of bioplastics produced from acetylated cassava, potato and maize starches

Starch source	Glycerol level	Percentage starch composition (%)			
		45	60	75	90
ACS	100	8.54±0.48b	13.11±1.02f	12.13±0.67ef	9.78±1.19c
	150	6.04±1.93a	11.52±3.53de	9.35±1.36c	7.66±0.56b
APS	100	6.92±0.19a	7.51±0.96b	10.44±0.30cd	9.37±0.53c
	150	5.81±0.90a	5.87±0.28a	8.32±0.77b	7.29±0.71ab
AMS	100	8.47±1.3b	11.98±0.78de	19.88±1.36g	13.88±0.19f
	150	6.60±1.00a	8.59±0.37bc	13.80±0.37f	10.77±0.27cd

ACS = acetylated cassava starch; APS = acetylated potato starch; AMS = acetylated maize starch; LSD (P = 0.05) = Least significant difference.

Table 4: Tensile strength (TS Kg/cm²) of bioplastics produced from un-acetylated cassava starch and cassava acetates at different concentrations of acetic anhydride

Starch source	Glycerol level	Percentage starch composition (%)			
		45	60	75	90
UACS	100	6.81±0.58ab	6.38±0.97ab	8.62±0.44cd	7.44±1.08bc
	150	5.44±1.19a	4.36±0.05a	7.64±0.98bc	6.70±0.18ab
ACS ₆₈	100	8.57±0.29cd	12.13±0.39fg	13.11±0.59fg	9.78±0.69de
	150	6.34±0.85ab	9.35±0.79de	11.52±0.98ef	7.66±0.33bc
ACS ₈₉	100	9.31±0.29cd	13.57±0.41gh	14.10±0.31gh	9.79±0.18de
	150	6.43±0.65ab	10.35±0.60de	11.35±1.22ef	9.19±0.61cd

UACS = Un-acetylated cassava starch; ACS₆₈ = acetylated cassava starch at 68% acetic anhydride; ACS₈₉ = acetylated cassava starch at 89% acetic anhydride; LSD (P = 0.05) = Least significant difference.

Table 5: Tensile strength (TS Kg/cm²) of bioplastics produced from un-acetylated maize starch and maize acetates at different concentrations of acetic anhydride

Starch source	Glycerol level	Percentage starch composition (%)			
		45	60	75	90
UAMS	100	6.14±0.25a	9.53±0.29cd	17.45±0.64k	10.25±0.40de
	150	5.91±1.00a	7.62±0.67b	10.37±0.29de	8.85±0.32cd
AMS ₆₈	100	8.47±0.75c	11.98±0.45g	19.88±0.79i	13.81±0.10hi
	150	6.57±0.28a	8.59±0.21c	13.80±0.21hi	10.77±0.04ef
AMS ₈₉	100	10.52±0.26ef	13.10±0.05gh	21.75±0.41m	15.97±0.10j
	150	7.93±0.13c	10.04±0.06de	16.32±0.43j	12.28±0.28g

UAMS = Un-acetylated maize starch; AMS₆₈ = acetylated maize starch at 68% acetic anhydride; AMS₈₉ = acetylated maize starch 89% acetic anhydride; LSD (P = 0.05) = Least significant difference.

The tensile strength of bioplastics produced from native cassava, potato and maize starches showed significant differences ($P < 0.05$) with the maize starch-based bioplastics having the highest tensile strength at 75% starch composition. Niba *et al.* (2005) reported varying physicochemical properties of different starch sources. Though Udensi *et al.* (2009) reported that amylopectin part of

the starch affected tensile strength of the bioplastics they produced, it may have played an insignificant role in the tensile strengths of the bioplastics produced from the starches due to synergistic interactions between all the physicochemical properties inherent in the starch and bioplastics additives used. It has been reported that there is a negative correlation between the percentage starch

Table 6: Percentage elongation (% E) of bioplastics produced from modified cassava, potato and maize starches at 68% acetic anhydride concentration

Starch source	Glycerol level	Percentage starch composition (%)			
		45	60	75	90
ACS	100	3.58±0.23bc	4.25±0.14c	5.00±0.63d	3.96±0.15bc
	150	2.25±0.52a	3.75±0.26bc	4.50±0.90c	3.21±0.04ab
APS	100	2.79±0.23a	2.50±0.38a	4.08±0.04c	3.58±0.23bc
	150	2.08±0.29a	2.04±0.08a	3.13±0.98ab	2.79±0.33a
AMS	100	3.46±0.30ab	4.33±0.18c	8.42±0.46f	6.42±0.17e
	150	2.50±0.19a	3.42±0.04ab	6.33±1.22e	5.79±0.04e

ACS = acetylated cassava starch; APS = acetylated potato starch; AMS = acetylated maize starch; LSD (P = 0.05) = Least significant difference.

Table 7: Percentage elongation (% E) of bioplastics produced from modified cassava, potato and maize starches at 89% acetic anhydride concentration

Starch source	Glycerol level	Percentage starch composition (%)			
		45	60	75	90
ACS	100	3.98±0.2cd	4.68±0.18e	5.35±0.13ef	3.87±0.15cd
	150	2.43±0.15a	4.02±0.28cd	4.67±0.50e	2.99±0.06ab
APS	100	3.21±0.3bc	2.75±0.32ab	3.99±0.04cd	3.23±0.23bc
	150	2.18±0.22a	2.12±0.05a	3.22±0.48bc	2.88±0.33ab
AMS	100	4.53±0.34e	4.97±0.35e	9.56±0.36h	6.98±0.14g
	150	3.10±0.15bc	3.53±0.04bc	6.74±1.12g	5.23±0.04ef

ACS = acetylated cassava starch; APS = acetylated potato starch; AMS = acetylated maize starch; LSD (P = 0.05) = Least significant difference

Table 8: Percentage elongation (% E) of bioplastics produced from un-acetylated cassava starch and cassava acetates at different acetic anhydride concentrations

Starch source	Glycerol level	Percentage starch composition (%)			
		45	60	75	90
UACS	100	2.63±0.19ab	2.38±0.13a	3.46±0.18bc	2.92±0.15b
	150	1.92±0.34a	1.71±0.23a	2.75±0.51b	2.21±0.08a
ACS ₆₈	100	3.58±0.23c	4.25±0.14c	5.00±0.63d	3.96±0.15c
	150	2.25±0.52a	3.75±0.26c	4.50±0.90d	3.21±0.04b
ACS ₈₉	100	3.21±0.15b	4.38±0.19d	4.88±0.07d	4.17±0.18c
	150	2.79±0.30b	4.29±0.33d	4.25±0.19c	4.00±0.38c

UACS = Un-acetylated cassava starch; ACS₆₈ = acetylated cassava starch at 68% acetic anhydride; ACS₈₉ = acetylated cassava starch 89% acetic anhydride; LSD (P = 0.05) = Least significant difference

Table 9: Rate of biodegradability of native and acetylated starches after a 6 weeks period

Starch source	Glycerol level	Percentage starch composition (%)			
		45	60	75	90
Cassava	NS	71.05±0.43e	76.24±0.18h	83.75±0.18l	86.48±0.35n
	AS	61.66±0.34b	66.32±0.33d	80.65±0.33k	78.45±0.08i
	NS	72.32±0.32f	76.35±0.41h	84.22±0.83l	91.00±0.65o
Potato	AS	62.34±0.63b	65.09±0.22c	74.43±0.45	79.89±0.39j
	NS	72.38±0.12f	77.03±0.19h	85.00±0.07lm	92.21±0.18p
Maize	AS	59.68±0.30a	64.37±0.27c	71.17±0.08e	79.65±0.38j

ACS = acetylated cassava starch; APS = acetylated potato starch; AMS = acetylated maize starch; LSD (P = 0.05) = Least significant difference.

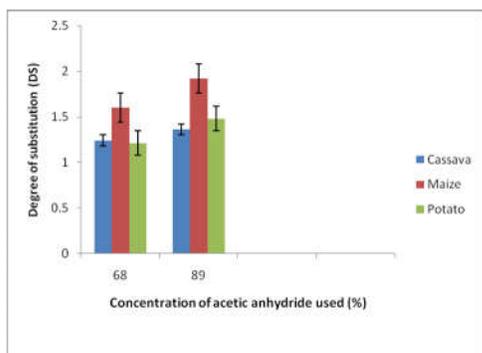


Fig. 1: Effect of acetic anhydride concentration on the degree of substitution of cassava, potato and maize acetates

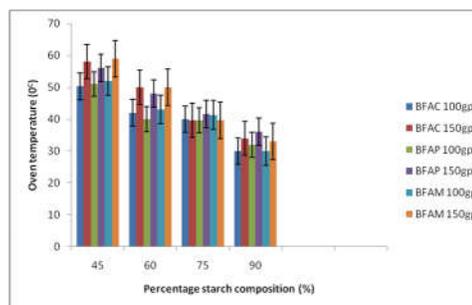


FIG.2: Effect of percentage starch composition and glycerol level on thermo-stability of bioplastics produced from starch acetates (68% acetic anhydride). {B = bioplastics; F = from; A = Acetylated; C = cassava; P = Potato; M = Maize; 100gp = 100ml of glycerol plasticization; 150gp = 150ml of glycerol plasticization

composition in the bioplastics and their tensile strengths due to moisture absorption tendency (Shah, 1985; Stevens, 2002). This was not the case with the present results as bioplastics produced from the materials containing 75% starch had the highest tensile strength, the starch source notwithstanding. The mechanisms underlying this condition are, however, not currently understood. Aside from this, it was also observed that the level of plasticization significantly ($P < 0.05$) affected the tensile strength of bioplastic materials; the higher the plasticization level, the lower the tensile strength. The present reports thus confirmed the reports of Larotonda *et al.* (2004) and Morais *et al.* (2001).

The tensile strength of bioplastics produced from starch acetates was significantly ($P < 0.05$) higher than that produced from the native starches (Tables 4 & 5). Niba *et al.* (2005) reported that different physicochemical properties of starch reflect the different properties of starch-producing plants. However, after acetylation, these properties were significantly improved (Rutenberg and Solarek, 1984), which depends on the kind of starch, pretreatment and the acetylation process employed (Agboola *et al.*, 1991). By implication, this means that starch acetylation must have caused these differentials in the tensile strength of the bioplastics materials. Since the bioplastics produced from maize acetate showed a higher tensile strength, it could be speculated that the higher DS values of maize acetate both at 68% and 89%, respectively, might not be unconnected to this effect (Fig. 1). These results seem to suggest that acetylation of starch modifies its physicochemical properties, which ultimately influences their tensile strengths. It is equally likely that the acetyl groups from the acetic anhydride may have been attached to amylopectin component of the starch, which possibly may have led to the significant impact on physicochemical properties of starch (Firouzabadi *et al.*, 2007), quite in line with the reports of Parendosh and Hudson (1993) and Stevens (2002). The improvement of products' binding, thickening and stability due to starch acetylation according to Graaf (2000) might have also had an additive effect on the tensile strength. This could be likened to genetic manipulation though particular traits are often the target in this process. It then implies that genetic manipulation geared towards increasing the amylopectin level in starch as recommended by Udensi *et al.* (2009) for the production of starch-based plastics might not achieve the desired objective singly, especially as it concerns tensile property, which is an important quality of synthetic plastics. Tables 6 and 7 provide a comparison of the elongation of bioplastics produced from cassava and maize acetates at varying acetic anhydride concentrations while Table 8 compares percentage elongation of cassava at varying concentration of acetic anhydride. It is obvious that bioplastics produced using 89% acetic anhydride concentration had a higher elongation, especially at 75% starch inclusion in both conditions. This is obviously the trend followed by the effect of starch acetates on tensile strength. This effect could be attributed to an increased DS, which may have also increased the length of the carbon chain (Thiebaud *et al.*, 1997; Zhang *et al.*, 1997). This may have led to a loss of starch granules as a result of increased substitution of acetyl groups due to acetylation, leading to the formation of the beehive and fibrous-like structure (Xu *et al.*, 2004) observed and an

alteration in the crystalline structure of the acetates, thus causing the smooth surface of the starch granules to become rough. Though this result seems to suggest that increasing the DS of starch acetates, which is a function of acetic anhydride concentration, will lead to bioplastics products with high tensile strength and material elongation, the process should be meticulously monitored to prevent distorting the starch properties.

Acetylation of starch and the thermo-stability of bioplastics

It is known that starch in its native state is unstable and the degree of instability varies with the source of material. However, starch source did not play any significant role in the thermo-stability of the bioplastics products produced in the current study. At higher percentage starch inclusion, thermo-stability of the bioplastics became lower, native or starch acetates notwithstanding. This result is contrary to that of Graaf (2000). It was observed that increased plasticization increases thermo-stability, which might be attributed to the impartation of flexibility and pliability on the bioplastics by plasticizers (Stevens, 2002). One of the major properties of synthetic plastics is their ability to melt when heated. This enhances the sealability of the materials. The reverse was the case with the results obtained in the current study as the tested bioplastics instead of melting embrittled between 50 - 650C, which was more obvious for the 90% starch composed bioplastics. When the bioplastics were removed from the oven, they become normal without deformation and deterioration. Haapanen (2004) observed that bioplastics produced from glycerol gelatin mix melt rather than embrittle. This can be attributed to the base material (that is, the starch) that was used for the production of the bioplastics. This implies that the overall quality of the bioplastics produced in the current research need to be improved so that they can become sealable and be used for retaining fluid substances like water. This research was aimed at evaluating the effect of acetylating starch on the production of biodegradable plastics (bioplastics) as a pointer to in planta acetylation. This became imperative following the menace caused by the contamination of synthetic plastics in the ecosystem and the need to scale up production using plants as factories. The results reported in this report are an indication that acetylating of starch will be of immense importance to the nascent starch-based bioplastics industry. Implicit in this lays the fact that in planta acetylation researches should be prioritized in a bid to modify starch in situ in order to forestall the unforeseen problem of chemical pollution of the environment.

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