Effects of Acetylation on the Micromeritics of Yam *(Dioscorea sp.)* Starch Powder for Pharmaceutical Application

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ABSTRACT

Background: Starch constituents contributes to its intrinsic and binding properties. Native starch is insoluble in cold water and tends to undergo uncontrolled viscosity (pasting) on application of heat. These inherent weaknesses of native starch powders have been modified through thermal and chemical processes such as acetylation, acylation, carboxymethylation, oxidation and pregelatinization

Objectives: Effect of acetylation on micromeritics of starch from five yam varieties grown in Jamaica was investigated.

Methods: Native starch pre-treated with pyridine was reacted with acetic anhydride and acetylation process was monitored with Fourier Transformed Infrared Spectroscopy (FTIR) and by volumetric titration of the hydrolyzed starch acetate. Particle size and size distribution were determined microscopically and the mean diameter (dp) was treated to the Edmundson's equation to obtain various derived size parameters.

Results: Yield of starch acetates ranged from 93.18 to 113.96% of different native starch varieties. Presence of a distinct peak in the ester range (1750-1735 cm-1) indicates acetylation and the degree of substitution was between 0.15 ± 0.01 and 0.33 ± 0.03. Starches from different yam varieties differ significantly ($p<0.05$) in specific surface area, particle mass, density, together with particle shape. These parameters could be used to distinguish starch acetates of different botanic sources. Correlation of degree of substitution with the amylose content and the specific surface area suggests that the amylose content contributed more significantly to the degree of the substitution than the effective surface area of the starch.

Conclusion: Acetylation resulted in significant changes of the micromeritics of varieties of native yam *(Dioscorea sp.)* starch powders and may impact formulation compressibility, fluidity, mixing and homogeneity.

Key words: acetylation, yam starch varieties, amylose content, micromeritics.

INTRODUCTION

Starch is composed primarily of amylose and amylopectin, which are formed from glycosidic bonding of α-D-glucose units and, depending on its botanic source, some phosphorous, proteins and fats [1-6]. Each starch constituent contributes to its intrinsic and binding properties. Native starch is insoluble in cold water and tends to undergo uncontrolled viscosity (pasting) on application of heat [1, 3, 7, 8]. These inherent weaknesses of native starch powders have been modified through thermal and chemical processes such as acetylation, acylation, carboxymethylation, oxidationand pregelatinization [9, 10,11]. Modification produces starches with improved thermal stability, predictable swelling characteristics, improved rheology and facilitate process control [12, 13]. Acetylation, one of the most frequently used techniques, involves the esterification of the α-D-glucose units by nucleophilic reactions resulting in the proton of the hydroxyl groups being replaced by acetyl group [14, 15].

Chemically modified potato, tapioca and corn starches, with low degrees of substitution (DS) are approved by the USA Food and Drug Administration [14, 15] for food, paper, biofuel and pharmaceutical application. In the pharmaceutical industry, starch and starch derivatives are used in formulations to improve powder flow, increase bulk of low-dose tablets, high potency drugs, and improve compressibility of tablet mixture. Recent studies [3] have shown high yields of good quality native starch at significantly lower costs of processing than the cereals and other seed sources [1]. However, starches extracted from different varieties of yam differ somewhat in amylose/amylopectin content, intrinsic viscosity, swelling power, and solubility [16]. In addition, they possess sub-optimal intrinsic compressibility, fluidity and consistency of functional performance on a lot-to-lot basis [7, 17]. Although various modification techniques have been applied to several of the conventional and some unconventional starches [12], few of the yam starch species appear to have been modified [10, 18]. Wang et al [18], investigated the morphological, crystalline structure and thermal properties of acetylated Chinese yam (D. opposita) starch. This study was designed to investigate the susceptibility of four yam starch species to acetylation and to assess the effect of acetylation on micromeritics of the derived yam starch powder.

MATERIALS AND METHODS

Materials

Fresh mature tubers of *D. alata* (cv. Sweet yam), *D. rotundata* (cv. Negro yam), *D. cayenensis* (cv. Round leaf yellow yam), *D. polygonoides* (cv. Bitter yam), *and D. esculenta* (cv. Chinese yam) were collected from local farms in Jamaica. The tubers were identified by a botanist at the Botany Department of the University of the West Indies, Mona, Jamaica. The starches were extracted following the procedures presented under methods. Pyridine, acetic anhydride, corn starch reference material and all other chemicals and reagents were obtained from Sigma-Aldrich (USA) while ethanol was a product of PHARMACO-AAPR (USA).

Starch Extraction

Starch extraction was carried out as previously reported 19, 20. Samples were stored in airtight containers until used.

Acetylation

Native starch (5 g) was pre-treated for 60 minutes with pyridine (50 ml) and then reacted with acetic anhydride (5.28 mL) for 30, 45, 60 and 90 minutes respectively under reflux at 100°C. The starch product was then washed with 70% ethanol (200-300 mL), dried by air (24 - 48 hours) at room temperature and then at 60°C in the Labline oven for 24 hours. The starch flakes obtained were pulverized and sifted through a Fisher 250 μm sieve and stored in an airtight container.

Fourier Transform Infrared (FTIR) Spectroscopy

Dried starch samples were milled with KBr (approx. 1:20) and compressed for 5 min at 8 tonnes. After blanking with the empty chamber, the KBr-Starch discs were subjected to the FTIR spectroscopy in the range of 4000-500 cm^{-1} [21] on a Bruker Vector 22 (Bruker Optics, Ettlingen, Germany).

Degrees of substitution

Degree of substitution (DS) was determined as outlined by de Graaf *et al* [22] with minor modifications. Slurries (1:35.7 w/v) of starch acetates were prepared in distilled water, the pH was measured and excess amounts of standardized 0.1N NaOH was added and stirred for 2 minutes. The mixture was left to stand at room temperature for 15 hours and was back-titrated with standardized 0.10 N HCl. The degree of substitution was calculated using Eq. (1):

$$DS = \frac{(C \times v)_{\text{NaOH added}} - (C \times v)_{\text{HCl added}}}{M_{starch}/180.1548}$$

Where C is concentration, v is volume, and Mstarch the mass of starch used. The molecular weight of an anhydroglucose unit is 180.1548 g/mol [22, 23]

Determination of granular density

Granular density of various starch acetates was determined by the liquid displacement pycnometric method [19, 20] using diethyl ether as the displacement liquid. The granular density was calculated using Eq. (2): [24]

$$\rho_s = S \times \frac{(W_2 - W_1)}{Sv - (W_3 - W_2)}$$

Where W1 is the weight of the pycnometer, W2 is the starch plus pycnometer dry mass; and W3 is W2 plus the weight of the pycnometer filled with diethyl ether. S is the specific gravity of diethyl ether (0.714g.cm^{-3}) and v is the volume capacity of the pycnometer.

Optical Microscopy

The starch acetate powder was mounted in Smith's starch reagent 1 and viewed using a light microscope (A.KRUSS Optronic, Japan). Photomicrographs of the particles were taken using a digital camera (Nikon COOLPIXS9, Japan). The projected diameters were measured using an eyepiece graticule previously calibrated with a stage micrometer. The size distribution was analysed and the projected mean diameter (dp) was calculated from Eq. 3.

$$d_p = \frac{\sum df}{\sum n}$$

where dp is the average projected diameter of particles within a specified particle size range; f is the frequency of each mean particle diameter within the range; n is the number of granules measured within the population (n=600). From the mean dp, the derived surface-number mean (dsn) and volume-number mean (dvn) and length-weighted mean diameters (dsl) were calculated by treating the dp to the Edmundson equations Eq. 4 - 625:

The surface-number mean (dsn) was calculated from Eq. 4:

$$d_{sn} = \left[\frac{\sum nd^2}{\sum n}\right]^{\frac{1}{2}}$$

The volume-number mean was calculated using Eq.5 while the d$_{sl}$ was calculated using Eq. 6:

$$d_{vn} = \left[\frac{\sum nd^3}{\sum n}\right]^{\frac{1}{2}}$$

$$d_{sl} = \frac{\sum nd^2}{\sum nd}$$

Other derived parameters including volume-to-surface ratio, surface number mean, volume-number mean, particle volume, the specific surface area per unit weight of starch and number of particles per gram of starch were calculated as previously reported [21, 28]

Results and Discussion

3. Fourier transformed infrared (FTIR) spectroscopy

FTIR spectroscopy was used to confirm starch acetylation and the corresponding structural changes. Comparisons of the samples FTIR (Fig. 1) before and after acetylation showed a new peak within the ester range (1750-1735 cm^{-1}) suggestive of a carbonyl singlet peak [15, 22, 23]. Acetylation led to general decrease in the areas under the starch signature peaks located in the hydroxyl (-OH) stretching (3600-3200 cm^{-1}) and bending (1655-1510 cm^{-1}) regions. The observed decrease is theoretically due to the substitution of nucleophilic acetyl group (CH3CO-) for the proton-(H+) of the hydroxyl group. This was made possible by hydroxyl group activation with

pyridine which induces the nucleophilic attack on the acetic anhydride[27, 29.]

Morphology of starch acetate granules

The photomicrographs of the starch acetates are shown in Fig.2. All samples showed significant dispersion with limited clumping characteristic of native starch powders. The particles of acetylated CR, CY, and BY starches were round, and those of SY, NY and RY were ellipsoidal. Acetylation did not significantly affect the shape and granular structure of native starch possibly due to the relatively small difference in the mass of the substituent acetyl (CH3CO-) replacing the proton (of the hydroxyl group). Similar studies have shown that granular integrity was preserved during acetylation processes at the DS below 0.34[28, 30.]
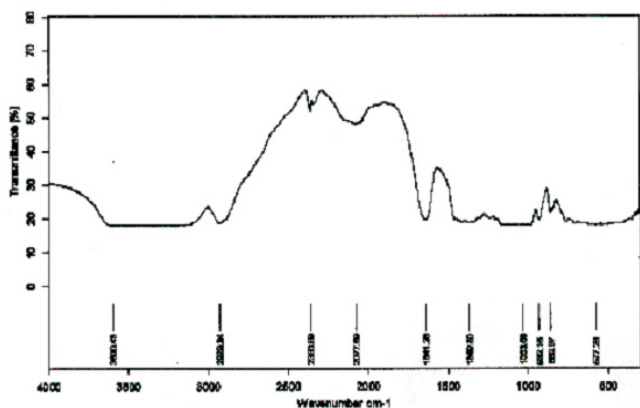
Starch Acetates

Table 1 shows the effect of starch amylose content on the degree of substitution and the corresponding values of projected diameter, granule density and number of particles per gram of powder. Fig. 2 shows the photomicrographs of yam starch acetates at 45 minutes acetylation time.
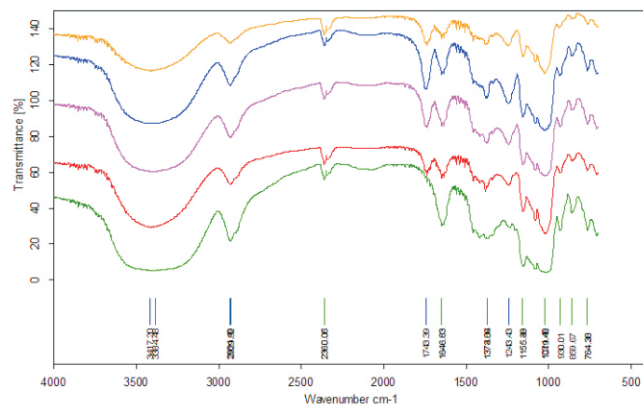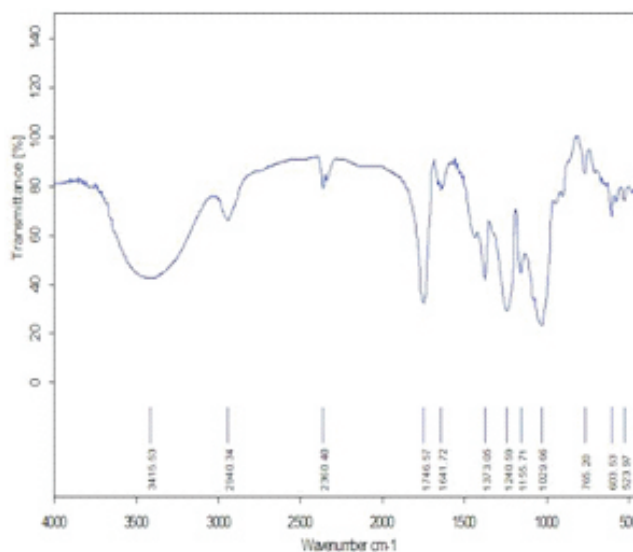

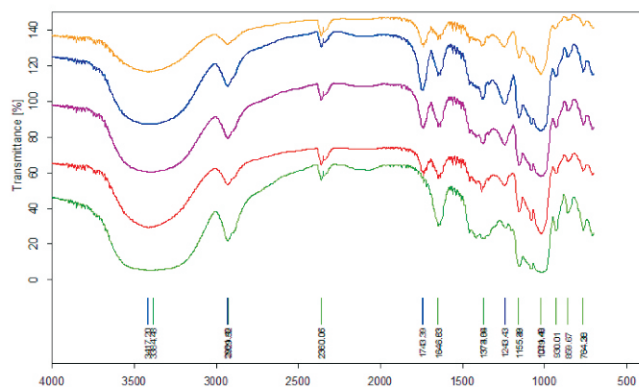**(1b) Corn starch acetate**
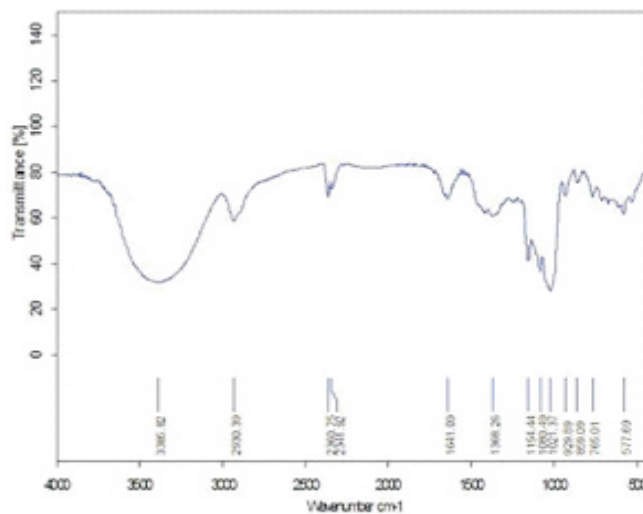
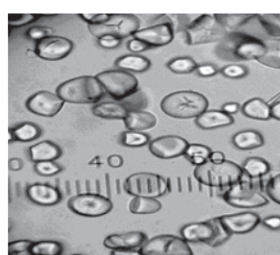**(1c) Native Chinese yam starch**

(1a) native corn starch

(1d) Chinese yam starch acetate

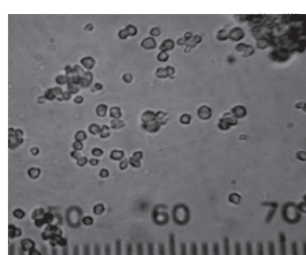**Fig 1 (a-d) Typical infrared spectra of native starch and starch acetates**
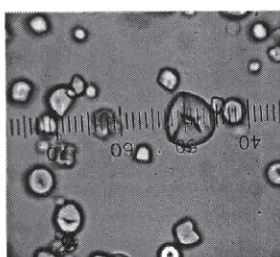
(1e) Bitter yam starch (0) and starch acetates at 15 to 90 min acetylation time

2a (Bitter)   **2b (Chinese)**
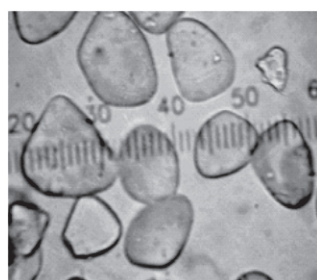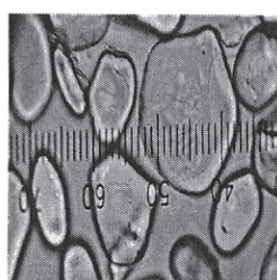

2c (Corn)   **2d (Negro)**

2e (Sweet)   **2f (Yellow)**

Fig .2 Photomicrographs of yam starch acetates at 45 minutes acetylation time

Optimum acetylation occurred at about 30 min with the DS ranging from 0.15 to 0.33 (Table 1).

Efficiency of acetylation and product recovery were generally around 100% for different varieties of native starch and was of the rank order of Chinese, CY (111.68 ± 23.33) > Negro, NY (108.62 ± 0.19) > Sweet, SY (103.04 ± 1.67) > Bitter, BY (101.67 ± 0.17) > Yellow, YY (101.67 ± 2.32) > Corn, CS (97.20 ± 3.14) % w/w respectively (Spearman rank correlation coefficient, R = -0.6). Bello-Pérez et al 30 and Moorthy 28 have reported yields of 87.6% and 88% respectively from plantain and cassava starches.

Amylose straight chain polymer has been suspected of enhancing the efficiency of acetylation reaction by facilitating the access of reactants to the sites for functional group substitution 15, 18. Riley et al 3, 21 reported amylose content of the native yam starch in the order RY > NY > SY > BY > CY and the specific surface area in the order CY > BY > NY > SY > RY. As shown in the present study, the DS of yam starch acetates is of the order of CY > RY > NY   BY > SY.

Regression of the rank order of DS on amylose content of native starch obeyed polynomial model of the fourth order (r^2 = 1.000) according to the equation:
$$y = 0.2083x^4 - 2.75x^3 + 11.792x^2 - 18.25x + 11 \text{ (Eq.7)}$$
Where y = DS, x = amylose content. Similarly, regression of the DS on effective surface area of substrate starch was found to fit the polynomial model of the fourth order (r^2 = 1.0000) according to Eq. 8:
$$y = -0.625x^4 + 7.4167x^3 - 30.875x^2 + 53.083x - 28 \text{ (Eq.8)}$$
Where y = DS and x = specific surface area of starch substrate. The effect of specific surface area of reactants on reaction efficiency has been well documented. [21] However, the polynomial model indicates that the effect of specific surface or amylose content on DS is not a simple linear relationship. Using equations 4 and 5, the DS of a native starch of known specific surface and amylose content subjected to acetic anhydride acetylation could be predicted.

Granule properties

Acetylation produced changes in particle size distribution of substrate starch (Fig 3). Irregular distributions were found for the acetates of BY and CY, while almost mono-sized or homogenous distribution was observed for CY acetate. SY acetate had the largest range of projected mean diameter (dp = 35.66 ± 0.55 to 41.34 ± 3.12 µm) over the range of acetylation time while the CY acetate (dp = 4.71 ± 0.0 to 5.45 ± 0.0 µm) had the smallest (Table 1). Increase in particle size of starch acetates with acetylation time may be due to the time-dependent diffusion of the acetylating agent into the starch matrix (Fig.4).[15]

Changes in particle size distribution may not be a simple function of acetylation but also of the process of particle growth during crystallization.[31] Starch acetates of different crystal habits and sizes could result from variation in substrate starch's unit cell, cell orientation, conditions for crystallization and method of crystal recovery.[32] Paired-sample t-test showed that SS and density of the acetates were

Figure 3 Particle size distribution of scetylated starch powders

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Fig.4 Projected mean granular diameter of the yam starch acetates at different acetylation time.

significantly different from those of the native starch samples (p=0.008 and 0.036 respectively) while the projected mean diameter and its derivatives (number of particles per gram, dsn and dvn) were not significantly affected by acetylation (p > 0.05). On the other hand, paired sample correlation of micromeritics showed very strong relationship between native and starch acetates in mean diameter (r=0.867, p=0.025), SS (r=0.979, p=0.01), particle number/gram (r=0.998, p<0.001), dsn (r=0.892, p=0.017) and dvn (r=0.897, p=0.015). On the other hand, DS was poorly correlated with average diameter (p=0.261) and particle density of acetates was very poorly correlated with that of the native starch (r=0.116, p=0.753).

The specific surface area (SS) of the acetylated starch was inversely correlated with particle size (Table 1). The rank order effect of acetylation time on SS was CY acetate > BY acetate > RY acetate; SY and NY did not show any general pattern. Significant decrease (p = 0.008) in specific surface area was also observed with acetylation, but was time-independent. However, paired sample t-test indicated that only specific surface (p = 0.008) and particle density (p = 0.036) were significantly different among native and acetylated starches. Each parameter has its respective significance on the functional application of the starch acetate in product design, effect on processing and quality of finished products.[30, 32] For instance, high surface-number mean diameter (dsn) would imply high degree of subdivision which could influence mixing and homogeneity as well as content uniformity of the finished product. On the other hand, volume-number mean diameter (dvn) and the specific surface (SS) would influence adsorptive capacity of the powder which may play an important role in liquid adsorption on powder blend. High dsn and dvn may also improve the staying property of topically administered powders.[31]

Granule mean diameters and density of starch acetates increased nominally (Fig. 4) with acetylation time and reached the peak generally
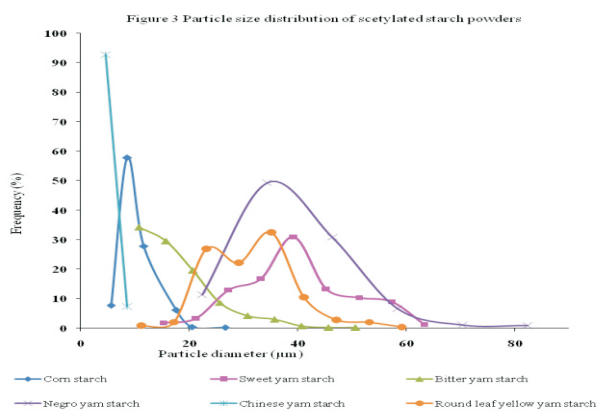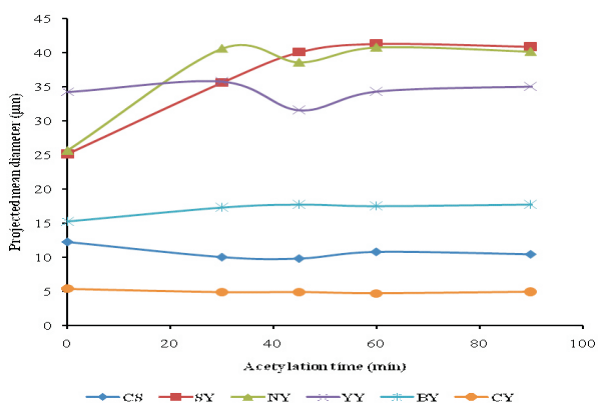
Table 1: Projected mean diameter (d_{ave}), granular density (ρ_g) and number of particles per gram of native and acetylated starches of different yam varieties

Starch sample	Amylose content (% w/w)	Degree of Substitution (30 to 45[†] min reaction)	Projected mean diameter, d_{ave} (µm) (± SEM)		Granular density, ρ_g (± SEM)		Number of particles per gram, N/g ($\times 10^7 g^{-1}$) ±SEM	
			Native	Acetylated	Native	Acetylated	Native*	Acetylated
Bitter	11.97 (0.08)	0.22 (0.01)[†]	15.30 (0.15)	17.78 (0.09)	1.368 (0.001)	1.44 (0.02)	35.291 (3.55)	15.90 (1.99)
Chinese	11.14 (0.03)	0.33 (0.03)	5.40 (0.06)	4.90 (0.04)	1.423 (0.012)	2.47 (0.15)	621.301 37.22)	565.93 (31.51)
Corn (BP) [†]	**27.00 (0.01)**	0.29 (0.01)	**12.30 (0.12)**	**9.83 (0.24)**	**1.485 (0.02)**	**1.96 (0.12)**	**42.5 (4.25)**	**28.84 (2.90)**
Negro	22.15 (0.05)	0.21 (0.01)	25.70 (0.25)	38.63 (1.40)	1.425 (0.002)	2.84 (0.17)	7.317 (1.24)	0.97 (0.14)
Sweet	21.01 (0.06)	0.15 (0.01)	25.16 (0.16)	40.11 (2.84)	1.390 (0.008)	2.17 (0.06	7.143 (1.73)	1.19 (0.21)
Round leaf yellow	26.53 (0.09)	0.29 (0.01)	34.50 (0.14)	31.62 (1.56)	1.525 (0.014)	1.56 (0.02)	2.899 (0.44)	3.19 (0.56)

Standard error of the mean (SEM) = s/√n, where s is the samples standard deviation and n=number of sample (=6). Superscripts of similar letters within a column for each variety, indicate no significant difference (p>0.05). [†]Official corn starch.

Table 2: Surface-number, volume – number mean diameters and specific surface areas of native and acetylated starches of different yam varieties

Derived micromeritics of Starch powder	Surface-number mean diameter, d_{sn} (μm) ± SEM		Volume-number mean diameter, d_{vn} (μm) ± SEM		Specific area, A_{ss} (m^2/kg) ± SEM	
Variety	Native	Acetylated	Native	Acetylated	Native	Acetylated
Bitter (BY)	15.71 (0.02)	19.05 (0.04)	16.11 (1.45)	20.35 (0.85)	258.76 (3.44)	179.19 (2.01)
Chinese (CY)	5.67 (0.07)	5.01 (0.06)	6.00 (0.85)	5.15 (0.07)	626.911 (37.43)	448.00 (26.75)
Corn (CS) BP	**4.24 (0.75)**	**10.21 (0.31)**	**5.06 (1.19)**	**10.65 (0.39)**	**325.501 (2.48)**	**232.01 (1.24)**
Negro (NY)	26.05 (1.53)	39.95 (1.63)	26.45 (2.62)	41.28 (1.95)	154.340 (5.82)	48.24 (2.77)
Sweet (SY)	26.00 (1.59)	41.22 (2.70)	26.83 (3.57)	42.25 (2.55)	151.091 (4.12)	62.31 (1.70)
Round leaf yellow (YY)	34.80 (0.90)	33.05 (2.03)	35.12 (2.97)	33.97 (2.02)	117.401 (5.09)	107.06 (0.20)

Standard error of the mean (SEM) = s/√n, where s is the samples standard deviation; n= number of samples.

Abbreviations: BY=Bitter yam, CS =Corn starch, CY =Chinese yam, DS =Degree of substitution, NY=Negro yam, SY = Sweet yam, YY = Round leaf yellow yam.

at about 30 minutes. Increase in granular diameter and density with acetylation time may be due to the increase in the mass of the anhydroglucose molecules with additional units of acetyl group substituted for protons of the hydroxyl groups. As observed in this study, granular diameter and density provide useful indices of the rate of acetylation and could be used to monitor the progress of acetylation reaction. The effect of acetylation time on the density of yam starch acetates was of the rank order NY > CY >SY > BY   YY (R = 1.00), with more pronounced impact on the densities of NY, CY and SY than BY and YY starch acetates. Significant increase ($p < 0.05$) in granular density of the yam starch granules was observed on acetylation (Table 1). The substitution of acetyl group (CH_3CO+) for the proton ($H+$) would result in increase in mass per unit volume. In addition, the

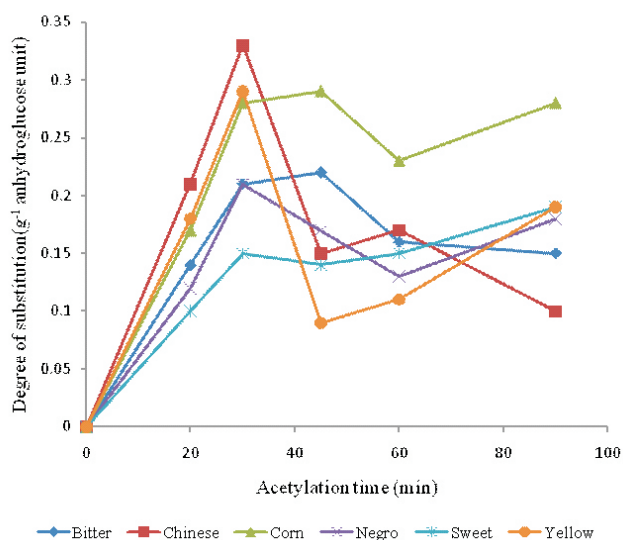Fig. 5 Effect of acetylation time on degree of substitution

Fig. 5 Effect of acetylation time ondegree of substitution

molecules of the individual starch acetate particles appeared to be more tightly packed through stronger hydrogen bonding and van der Waal's forces of attraction. This probably decreased the particle volume with the corresponding increase in particle density.

Particle mass and number of particles per gram of starch varied significantly with acetylation (Table 1). The trend across yam varieties was generally similar to those of the native starches with the

rank order of particle mass being, NY acetate > SY acetate > RY acetate > BY acetate > CY acetate and number of particles per gram of starch of CY acetate > BY acetate > RY acetate > SY acetate > NY acetate. Particle mass is known to increase with particle size with the corresponding decrease in particle number per gram of starch.[29]

CONCLUSION

Pyridine-catalyzed acetylation of yam starch was rapid and efficient. Reactions were complete within 30 to 45 minutes and degree of substitution was generally within the acceptable FDA range of what values (0.01-0.30)15, 23. Acetylation was significantly influenced by the amylose content of substrate starch which, in turn, influenced the specific surface area and density of the starch acetate particles.   FTIR provides a suitable method for monitoring acetylation. Changes in the micromeritics of starch acetates were significant and the physico-mechanical properties of the powders need to be investigated before one can scientifically discuss the pharmaceutical applications of the starch acetates in the formulation of pharmaceutical solid dosage forms.

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