Effect of Acetic Anhydride Concentration on the Physical and Functional Properties of Acetylated Cassava Starches

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Abstract:- Native cassava starches isolated from three varieties (TME 419, TMS 98/0505 and TMS 98/0581) were acetylated at different concentrations of acetic anhydride (10, 15, 20, 25 and 30%).Effect of the concentrations was evaluated on properties of the starch like bulk and tapped densities, morphology, degree of whiteness, water absorption and hydration capacities, paste clarity and freeze thaw stability. Bulk density values for native starches ranged between 0.27 and 0.30 g/ml while the values for acetylated starches ranged between 0.37 and 0.48 g/ml. Tapped density values ranged from 0.34 to 0.38 g/ml and 0.53 to 0.63 g/ml (for native and acetylated starch samples respectively). Acetylation did not affect the shape and granular structure of the starches. Degree of whiteness ranged from 93.10 - 95.30% and 93.50 - 96.63% for native and acetylated starches respectively. Water absorption and hydration capacities were significantly reduced by acetylation and paste clarity ranged from 16.78 -26.28% and 20.50 - 30.96% for native and acetylated starches respectively. Degree of acetylation ranged from 0.81 to 3.08%. Acetylation enhanced the functional characteristics of cassava starches and these are good pointers to the possible utilization acetvlated cassava starches in different food applications thus widening its utilization in Nigeria.

Keywords:- Cassava starch, acetic anhydride, acetylated starches, physical and functional properties.

I. INTRODUCTION

The utilization of starch in the food industry for many functions includes serving as thickening, stabilizing, texturing, gelling and encapsulating agents. However, in their native forms, they encounter problems due to their inability to withstand processing conditions and insolubility in cold water, low thermal and shear resistance, high ability to retrograde, loss of viscosity, syneresis tendency and thickening power upon cooking and storage particularly at low pH (Singh et al., 2010). Several physical, chemical and biotechnological modifications have improved the functional properties of starch allowing wide range of applications (Achor et al., 2010). The modified starches generally show better paste clarity, stability, increased resistance to retrogradation and increased freeze-thaw stability (Zenheg et al., 1999).

Starch acetylation is one of the common methods of modifying starch properties by introducing acetate groups to starch granules at low temperature (Xtie *et al.*, 2005). Acetylated starches are used for industrial purposes where they have been tailor made to meet the requirement of end users with added value. They find uses in fast foods, sweets and sausages, baked, dry, and canned and frozen foods (Ayucitra, 2012, Fagbemi *et al.*, 2012).

The utilization of starches in different industries is governed by their physical and functional characteristics like bulk density, tapped density, water absorption capacity, hydration capacity, shape of the granules, retrogradation tendencies and freeze-thaw stability (Singh *et al.*, 2002; Olu-Owolabi *et al.*, 2010; Chiang *et al.*, 2007; Alebiowu and Itiola, 2002). Therefore, this study was carried out to evaluate how the physical and functional properties of cassava starches were affected when modified with different concentration of acetic anhydride.

II. MATERIALS AND METHODS

A. Sources of Materials

Matured, good quality cassava tubers of hybrid TME 419, TMS98/0505 and TMS98/0581 having high starch contents, dried matter contents and high yield per stand, used for the research work were obtained from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

B. Extraction of native starch from cassava

The native starch was extracted from the three varieties according to the method of Sanni *et al.*(2006) with slight modification. Cassava tubers were weighed, peeled, washed and grated in a mechanically driven cassava grater. The pulp was mixed with sufficient water (1:5 w/v) to form slurry which was sieved through muslin cloth. The resulting starch was allowed to sediment for 2 h and the supernatant decanted. The residue washed in clean water three times and the residue (starch) was dried at $55\pm2^{\circ}$ C for 48h in a cabinet dryer. The dried starch was pulverized with a laboratory type blender (Marlex, Ecella model, Kanchan International Limited, Daman, India) and sieved using an aperture size of 125 µm. The product was packaged in high density polyethylene and kept until required for modification and analyses.

C. Acetylation

Acetylation was done according to the method of Iheagwara (2012) with slight modification. One hundred gram of starch was dispersed in 500 ml of distilled water and stirred for 20 min. The pH was adjusted to 8.0 using 3% NaOH. Acetic anhydride (10, 15, 20, 25 and 30 g) was added slowly to the mixture (to obtain 10, 15, 20, 25 and 30% concentration respectively) while maintaining a pH range of 8.0 - 8.5. The reaction was allowed to proceed for 5 min after the addition of the acetic anhydride. The pH of the slurry was adjusted to 4.5 using 10% HCl to terminate acetylation reaction. The starch was filtered and washed four times with water and dried in cabinet dryer at 40 ± 2 °C for 48 h. The acetylated starch was packaged in high density polyethylene bag and kept until required for laboratory analyses.

D. Determination of bulk and tapped densities

Bulk and tapped densities values were determined in a 250ml cylinder according to the method of Picker-Freyer and Brink (2006) with slight modification. Approximately 100g of the sample was gently filled into the cylinder. Bulk volume was read and used to calculate bulk density. The cylinder was tapped for at least 50 times to obtain a constant volume. The tapped volume was read and tapped density was subsequently calculated.

$$Bulk Density (\frac{g}{ml}) = \frac{Weight of Sample}{Loose Volume of Sample}$$
(1)

$$Tapped Density \left(\frac{g}{ml}\right) = \frac{Weight of Sample}{Packed Volume of Sample}$$
(2)

E. Determination of morphological characteristics of starch Morphology of the native starch and the derivatives (up to 20% concentration) was determined using Scanning Electron Microscope (SEM) according to the method of Kunruedee *et al.* (2010). About 1 g of the sample was mounted on the slide and covered with a slip. The size and shape of the starch granules were observed with a scanning electron microscope (ASPEX 3020, Model SIRIUS 50/3.8 Serial No 6918 – 14308. ASPEX Corporation, UK) using 100 X Magnification.

F. Determination of degree of whiteness

The degree of whiteness (%) of the native and acetylated starches was determined according to the method of Fagbemi *et al.* (2012) using photometer (Model C - 300 - 3, Kett Electric Laboratory, China). About 50 g of the starch sample was filled into the cuvette and inserted into the colour comparator and the degree of whiteness was read off and recorded as percentage whiteness Kett scale calibration.

G. Determination of water absorption capacity

Water absorption capacity (WAC) was determined according to the method of Nuwamanya *et al.* (2011). An aqueous suspension was made by dissolving 1 g of starch in 10 ml of water. The suspension was agitated for 3 min on a shaker and allowed to stand for 10 min after which it was centrifuged for 10 min at 3000 rpm. The free water was decanted from the wet starch, drained for 10 min and weighed. The difference in the weight of the water was recorded as water absorbed.

$$Water Absorbtion Capacity (\%) = \frac{Weight of water bound}{Sample Weight} X 100$$
(3)

H. Determination of hydration capacity

Hydration capacity was determined according to the method of Nuwamanya *et al.* (2011) with slight modification. About 1 g of the sample was suspended in 10 ml distilled water. It was agitated for 3 min and allowed to stand for 10 min and centrifuged at 3000 rpm for 10 min. The supernatant was decanted and the wet residue was drained for 10 min and weighed. The hydration capacity was calculated as follows:

$$Hydration Capacity (\%) = \frac{Weight of residue}{weight of sample} X 100$$
(4)

I. Determination of freeze-thaw stability

Freeze thaw stability was determined according to the method of Seidu (2009) with some modifications. Starch slurry (5% w/v db) was prepared with distilled water and the slurry was heated at 90°C for 30min in water bath with constant stirring. The paste was cooled and transferred into centrifuge tube and subjected to alternate freezing and thawing (18 h and 3 hrespectively) for 4 cycles. It wasthen centrifuged at 5000 rpm for 10 min. The percentage water separated wasplotted against number of freeze-thaw cycles.

J. Determination of paste clarity and retrogradation

The paste clarity and retrogradation was measured according to the method of Craig *et al.* (1989). About 1 g of the starch sample was dispersed in 100ml of distilled water and boiled at 100°C for 30min in water bath with constant stirring. It was cooled to 30°C and the percentage transmittance was measured at 640 nm. Samples were stored at 4°C in a refrigerator for a period of five days and the transmittance was measured daily to monitor the tendency for retrogradation.

K. Determination of degree of acetylation of acetylated cassava starches

The degree of acetylation of the acetylated starches was determined according to the method of Golachowski (2003). About 10 g of the sample was mixed with 65 ml of distilled water in a conical flask and neutralized by adding a few drops of 0.1 M NaOH to obtain a faint pink colour with phenolphthalein indicator. Amount of 25 ml of 0.5 M NaOH was added to the mixture and mixed thoroughly for 35 min using an orbital shaker. The resultant mixture was titrated against 0.05 M HCl until the pinkcolour disappeared. The

degree of acetylation was calculated according to equation 5.

$$Acetylation (\%) = \frac{(25 - x) X 0.43 + 0.5 X 100}{g}$$
(5)

volume of 0.05 M HCl used (ml) Х £

$$g = weight of sample (g)$$

L. Statistical analysis

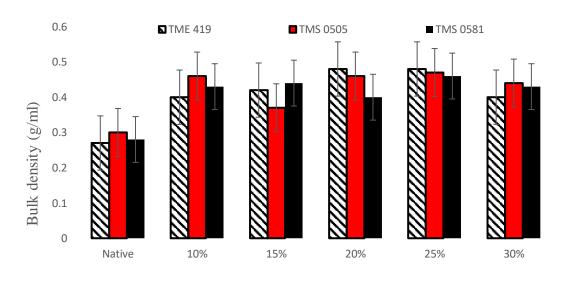
Data generated in three replicates were analyzed statistically with the Statistical Analysis Systems (SAS) package (version 8.2 of SAS institute Inc, 1999). Statistically significant differences (p < 0.05) in all data were determined by Analysis of Variance (ANOVA) procedure while Least Significant Difference (LSD) was used to separate the means.

III. **RESULTS AND DISCUSSION**

A. Bulk density of native and acetylated cassava starches

It was observed that native starches from all the varieties had the lowest bulk density values (Fig 1) which ranged between 0.27 and 0.30g/ml when compared with acetylated samples. Among the native starches, sample from TMS 98/0505 had the highest value (0.30g/ml) while sample from TME 419 had the lowest value. Increase in degree of acetylationsignificantly (p < 0.05) increased the bulk density values. Concentration generally resulted in significant (p < 0.05) increase of 0.37 to 0.48 g/ml in the bulk density values. The observed increase in the bulk density values might be due to formation of slight fusion of granules (Ayucitra, 2012). This result agrees with the report of Fagbemi et al.(2012) who reported an increase in the value of bulk density of acetylated starch obtained from TMS 30572.

Bulk density as the ratio of the mass per unit volume of a substance (Etudaiye et al., 2009) is generally affected by the particle size (Abioye et al., 2011) and the weight of the starch. It is very important in determining the packaging requirement, indicating a lesser package requirement with increase in concentration of material (Adebowale et al., 2008).

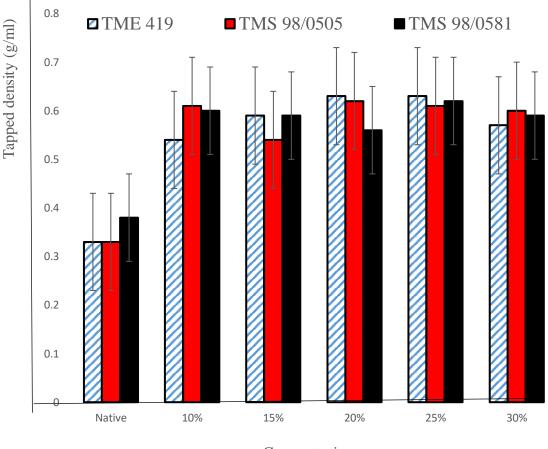


concentration

Fig. 1: Bulk density of native and acetylated cassava starch as affected by concentration and variety

B. Tapped density values of native and acetylated cassava starches

The tapped density values recorded for the native starches (Fig 2) ranged between 0.34and 0.38g/ml with sample from TMS 98/0581 having the highest value. Acetylated starches had values that ranged between 0.53 and 0.63g/ml. Irrespective of the variety, there was significant (p < 0.05) increase in the tapped density values of the acetylated starches when compared with native samples. It was also observed that the tapped density values obtained for each of the varieties were higher than the corresponding bulk density values. This observation is in line with the earlier report of Fagbemi et al. (2012) who reported a higher tapped density value than the bulk density in sulphited acetylated cassava starch from TMS 30572.



Concentration

Fig. 2: Tapped density of native and acetylated cassava starch as affected by concentration

Tapped density, in contrast to bulk density, is related to the packed volume of a substance, it is also dependent on the particle sizes and it provides information about the flowability of powders (Singh *et al.*, 2011).

C. Morphology of native and acetylated starches

The scanning electron micrographs of the starch acetates are shown in Plate 1. The structures of the native starch granules showed no difference in shape with respect to varieties but TMS 98/0505 had larger granule sizes. The native starches were round, small and truncated. The presence of small particles of damaged starch granules indicated that the technology of native starch extraction caused damage to the granules.

Acetylation did not affect the shape and granular structure of the native starches which might be due to relatively small difference in the molecular mass of the acetyl group replacing the hydroxyl group. The starch acetates formed lumps which might be attributed to the introduction of hydrophobic groups. Furthermore, there appeared to be no significant observable changes in shape, size or external appearance of the cassava starch granules after acetylation. Close observation of the photomicrographs shows that acetylation affected the spatial arrangement of the granules. The native starch is more densely packed than acetylated ones. This was obvious in the values obtained for bulk density. This result is a clear assertion of earlier report by Ayucitra (2012) who demonstrated that acetylation did not cause any observable change in the morphology of corn starches. Similar observation of round shape of starch from cassava had been reported by Mbougueng et al. (2012).

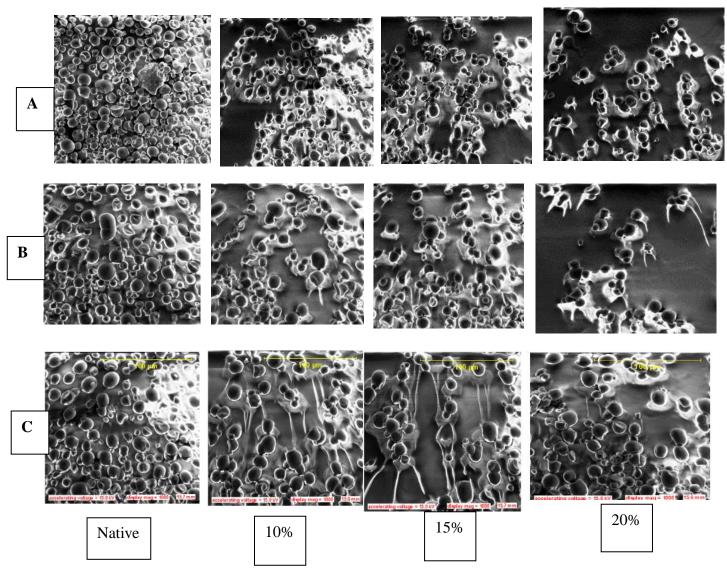


Plate 1: Scanning electron micrograph (X 1000) of native and acetylated cassava starches from (A)TME 419, (B) TMS 98/0505 and (C) TMS 98/0581 as affected by concentration

D. Degree of whiteness of acetylated cassava starches

It was observed from Figure 3 that the degree of whiteness of the native starches ranged from 93.10 to 95.30%. Among the native starches, TMS 98/0505 had the least value while TMS 98/0581 had the highest value. In the acetylated samples, the values ranged between 93.50 and 96.63%. Irrespective of the variety, acetylation, up to 30% acetylation, significantly (p < 0.05) increased the percentage whiteness of cassava starch by about 0.40 to 1.30%... It was also observed among the different varieties that the concentration significantly (p < 0.05) increased the percentage whiteness of cassava starches with samples at

30% treatment level having the highest percent whiteness in all the samples.

The progressive increase in the whiteness with increase in acetylation level might be as a result of bleaching effect of the acetylation which removed impurities and made the starch brighter. Acetylation had been reported to improve the appearance of starch during processing (Fagbemi *et al.* 2012) by preventing chemical reactions that cause coloration (Golachowski 2003). This result agreed with standards (minimum of 90%) whiteness of ISI (2005) set for cassava starches to be useful in food formulation.

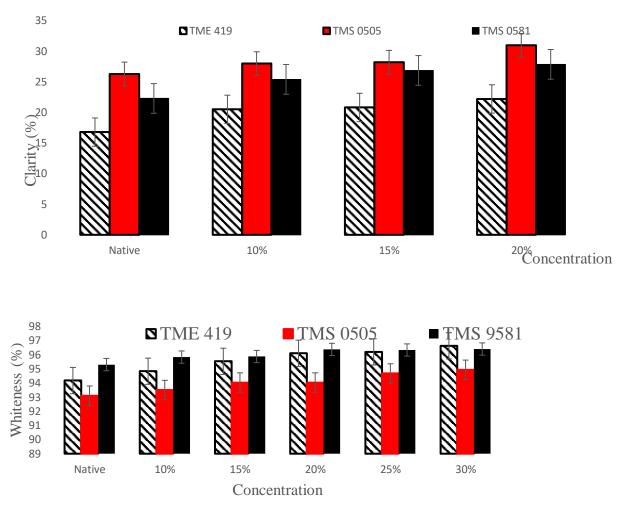


Fig. 3: Whiteness of native and acetylated cassava starches as affected by concentration.

E. Water absorption capacity of native and acetylated cassava starches

The water absorption capacity values (Tables 1a to 1c) for the native starches varied between 13.33% in TME 419 and 16.67% in TMS 98/0505. Values obtained for the acetylated starches were between 6.33 and 16.00%. Irrespective of the variety, acetylation significantly (p<0.05) reduced the water absorption capacities of cassava starches by about 7%. Among the starch acetates, the lowest value of water absorption capacity was obtained for samples from TME 419 at 30% treatment level while the highest value

was obtained for samples from TMS 98/0505 at 25% treatment level.

The decrease in the water absorption capacities of the acetylated starches could be attributed to introduction of hydrophobic (acetyl group) in the place of hydrophilic (OH) group. (Ayucitra, 2012). This result disagrees with the report of Fagbemi *et al.* (2012) who reported increase in water absorption capacity of sulphited acetylated cassava starches. The contrast in the result might be due to absence of sulphite in the present study.

Sample	Water absorption capacity (%)	Hydration capacity (%)
Native	16.67a	136.67a
10%	11.00b	80.00b
15%	13.00ab	83.33b
20%	10.67b	86.67b
25%	16.00a	76.67b
30%	14.00ab	86.67b

Table 1 (a): Functional characteristics of acetylated cassava starch from TME 419 as affected by concentration Mean values with the same letters along the same column are not significantly (p>0.05) different.

Sample	Water absorption capacity (%)	Hydration capacity (%)	
Native	15.00a	193.33a	
10%	14,00b	66.67c	
15%	13.67c	73.33c	
20%	10.67d	76.67c	
25%	10.00d	96.67b	
30%	10.67d	83.33bc	

Table 1 (b): Functional characteristics of acetylated cassava starch from TMS 98/0505 as affected by concentration

Mean values with the same letters along the same column are not significantly (p>0.05) different.

Sample	Water absorption capacity (%)	Hydration capacity (%)
Native	13.33a	203.33a
10%	10.67b	76.67b
15%	9.33c	66.67b
20%	10.67b	70.00b
25%	10.00bc	76.67b
30%	6.33d	63.33c

Table 1 (c): Functional characteristics of acetylated cassava starch from TMS 99/0581 as affected by concentration

Mean values with the same letters along the same column are not significantly (p>0.05) different.

F. Hydration capacity of the native and acetylated cassava starches

It was observed that the hydration capacity values obtained for the native starches (136.67-203.33%, Tables 1a to 1c) were significantly (p<0.05) higher than the values obtained for the acetylated samples (63.33-96.67%). Among the native samples, TMS 98/0505 had the lowest value while TME 419 had the highest value. In the starch acetates, TME 419 had the least value while TMS 98/0581 had the highest value. In general, acetylation resulted in significant (p<0.05) reduction of 73.34 - 106.66% in the hydration capacity values of the cassava starches. This result disagrees with earlier report of Hetti et al. (2009) who reported increased swelling power upon acetylation but with increased temperature. The observed decrease in the hydration capacity could be attributed to the introduction of hydrophobic acetate group. Wurzburg (1986) had also reported that starch acetates are hydrophobic.

G. Paste clarity of the native and acetylated cassava starches

The result of the effect of concentration and variety on the clarity of acetylated cassava starch is shown in Figure 4. The clarity in the native starches variedfrom 16.78 to 26.28 % transmittance. Among the native starches, samples from TME 419 had the lowest value while samples from TMS 98/0505 had the highest value. In the acetylated starch samples, the values ranged between 20.50 % transmittance for samples from TME 419 at 10% acetylation and 30.96% transmittance for samples from TMS 98/0505 at 20% acetylation. Irrespective of the variety considered, there were significant (p < 0.05) improvement in the paste clarity of the starches as shown by increase in % light transmittance with increased concentration.

It was observed that samples from TMS 98/0505 had the highest values at each of the treatment levels while sample from TME 419 consistently recorded the lowest paste clarity values. The increase in the paste clarity values might be attributed to bleaching effect of the acetylation. This effect was also corroborated increase in percentage whiteness of the samples due to acetylation as indicated above. This result agrees with an earlier report by Ayucitra (2012) on acetylated starch. Improved paste clarity is a very useful characteristic in the manufacture of some food items like salad dressings.

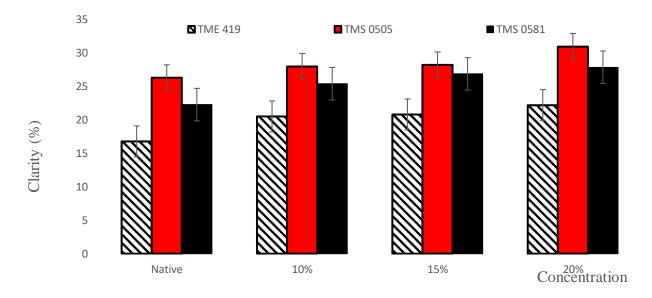


Fig. 4: % Clarity of native and acetylated cassava starches as affected by concentration

This result agrees with the report of Fagbemi *et al.* (2012) who reported improved paste clarity in sulphited starch acetates produced from cassava starches.

H. Freeze thawing stability of acetylated cassava starch.

The results of the effect of concentration and variety on the freeze thawing stability are shown in Figures 5 to 7. The syneresis and the freeze thawing stability of the native and acetylated starches were monitored by determining the percentage water separated during storage at 4 °C for a period of 120 h. It was observed that all the starches started weeping (indication of syneresis) after 24 h as indicated by the increase in percentage water separated.

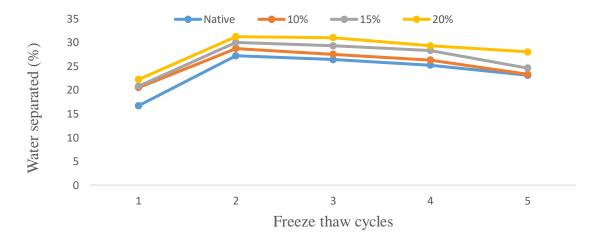


Fig. 5: Freeze thaw stability of native and acetylated cassava starch from TME 419

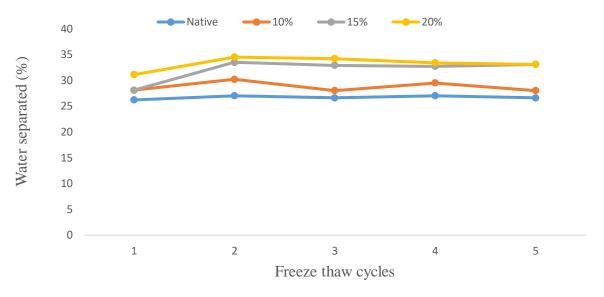


Fig. 6: Freeze thaw stability of native and acetylated cassava starch from TMS 98/0505

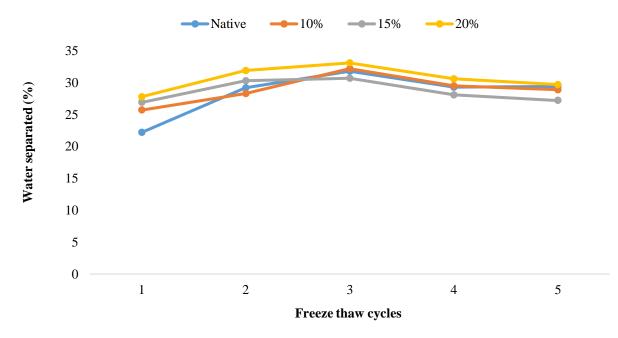


Fig. 7: Freeze thaw stability of native and acetylated cassava starch from TMS 98/0581

There was no much difference in the syneresis pattern of all the starches. In all the varieties considered, the native starches showed the highest resistance to syneresis during freeze thaw cycle (lowest percentage water separation) while the lowest resistance (highest percentage water separation) was observed in starch samples acetylated at 20% level. Weeping (water separation) in freeze thawed gels is due to the rearrangement of amylose in the granules of the starch at low or refrigerated temperature which aids water exudation from the gels (Ayucitra, 2012). An increased percentage separated water during freeze thaw cycles indicates a lack of stability. This trend corroborates the result on the reduced water absorption and hydration capacities of the acetylated cassava starches. The increased syneresis tendency in the acetylated starches might be due to the inability to prevent alignment and association between the macromolecules by the acetyl groups. This result is in contrast to earlier report (Gonzales and Perez, 2002) on the effect of acetylation on rice starch.

I. Degree of acetylation of cassava starches

The results of the effect of acetylation on the degree of acetylation of cassava starches are presented in Table 2. The degree of acetylation ranged between 0.81and 3.08%. Both varietyand concentration of acetic anhydride caused noticeable (p < 0.05) increase in the degree of acetylation. It was also observed that increase in concentration of acetic anhydride significantly (p < 0.05) increased the degree of acetylation.

The difference in the acetyl percentages of cassava starches from different varieties but under similar conditions may be attributed to differences of size and fragility of granules (Singh *et al*, 2002). It may also be due to intergranule packing. This is because the way the amylose chains are packed and the arrangement of amylose and amylopectin chains could affect the chemical substitution in the glucose units (Mbougueng *et al.*, 2012). This result is at variance with the report of Fagbemi *et al.* (2012) on the effect of sulphiting on the physical and functional properties of acetylated cassava starch. The difference in acetyl content reported in this study and the other study may be due to difference in reaction conditions and starch sources used.

Sample	TME 419	TMS 98/0505	TMS 98/0581
Native	0.00f	0.00f	0.00e
10%	0.97e	0.81e	1.29d
15%	1.81d	1.06d	1.70c
20%	2.83c	1.50c	1.74c
25%	2.93b	2.41b	3.00b
30%	3.03a	2.56a	3.07a

Table 2: Degree of acetylation of acetylated cassava starches from TME 419, TMS 98/0505 and TMS 98/0581 as affected by concentration of acetic anhydride

Mean values with the same letters along the same column are not significantly (p>0.05) different.

IV. CONCLUSION

This study reveals that cassava modification with acetic anhydride enhanced bulk and tapped density but reduced water absorption and hydration capacities. Granular shapes were not affected by the modification. There was improvement in the paste clarity, whiteness and freeze thaw stability of the acetylated cassava starches. These characteristics are good pointers to the possible utilization acetylated cassava starches in different food applications thus widening its utilization in Nigeria, the world largest producer of cassava.

AUTHORS' CONTRIBUTIONS

OOL conducted the experiments and analysis, AMA and AFA designed the study and were responsible for the revision of the manuscript. All authors read and approved the final manuscript.

• Authors' declaration on competing interest: The authors wish to state that there is no competing interest whether financially or otherwise.

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