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Production and Characterization of Pre-Gelatinized Cassava (*Manihot Esculenta* Crantz) Starch for Use in Pharmaceutical Tablet Compression

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Abstract:
Pharmaceutical industries in Nigeria rely on the importation of millions of tons of corn starch as their excipients which implies drain on foreign exchange. This study evaluated pre-gelatinized cassava starch as a possible substitute for the corn starches as excipients in pharmaceutical industry.
One of the improved cassava varieties (TME 419) obtained from IITA (name in full) Ibadan was used for this study Native starch from the cassava was modified by pre-gelatinization at 25%, 30 % and 35 % concentrations. Samples were evaluated for physical, flow, functional and tableting properties. Data generated were subjected to analysis of variance (ANOVA) to determine a significant difference among the treatment levels and the means separated using the Duncan multiple ranged test.
The results show that pre-gelatinization significantly ($p<0.05$) increased the bulk density and tapped density values. The rate of water absorption also increased significantly ($p<0.05$) after pre-gelatinization. Notable improvement in the flow characteristics of the pre-gelatinized starches was observed. Pre-gelatinized starches exhibited plastic deformation and tablet compacts produced from the pre-gelatinized starches were harder than those from the native starches.
The information obtained from this study revealed the potential utilization of pre-gelatinized cassava starch as a good alternative in Nigerian pharmaceutical industry.

Keywords: *Characterization, utilization, pre-gelatinized, cassava starch, pharmaceutical tablet*

1. Introduction

Cassava (*Manihot esculenta* Crantz) is an important root crop in several parts of Africa but with few major limitations as a food crop, which include; the presence of toxic cyanoglucosides and rapid perishability of the root tubers (Ikujenlola and Opawale, 2007; Chijindu *et al*., 2008). The rapid rate of perishability can be ascribed to the high moisture content of the roots (80 – 92%) and the conditions such as susceptibility to fungal infection which accelerate rot and decay. Nigeria is the largest producer of cassava in the world, producing about 50 million metric tons annually (FAOSTAT, 2012) with little or no cassava products being exported. Cassava roots contain mainly carbohydrates, of which about 80 – 81% is starch (Etudaiye *et al*., 2009).

Starch, the main plant carbohydrate, is the most important plant derivative used by man and has unlimited significance in the food industry and can be modified to suit various applications using inexpensive methods making it ideal for a number of uses (Satin, 2006). Starch has many industrial applications, such as in food, pharmaceutical, adhesive and other industries. It is widely used in the production of pharmaceuticals because of its relative inertness, wide availability, low cost and high functionality.

These uses are mainly due to its adhesive, thickening, gelling, swelling and film forming properties. Pharmaceutical grade starch can be obtained from various sources depending on the ease of extraction, abundance of the material in any particular location as well as cost. Native starches are however limited in their uses due to various reasons, which include inherent weakness of hydration, swelling and structural organization, weak-bodied, cohesive, rubbery pastes and undesirable gels when cooked (Klanarong *et al*., 2002) and their inability to withstand harsh processing conditions such as extreme temperature, diverse pH.

The seemingly adverse native starch characteristics can be improved upon for different industrial applications through a series of modification techniques to enhance the desired functional properties for many food and industrial applications Whistler, 2009).

Modification rectifies and adjusts the intrinsic properties that limit industrial utilization in a natural or unmodified form (Morikawa and Nishinari, 2000; Fortuna *et al*., 2001). Modified starch products are used in the food, pharmaceutical, paper and textiles industries. To increase the acceptability and palatability of many processed foods to consumers. They are also used to reduce costs of established food and pharmaceutical products as they are now preferred directly as compressible excipients in pharmaceutical industry.

For instance modified rice starch, starch acetate and acid hydrolyzed starch, etc. are well established as multifunctional excipients in pharmaceutical industry (Singh *et al*., 2010). Corn and potato starches are official starches which are extensively used as binders and disintegrants in compressed solid dosage forms. According to Singh *et al*. (2010), starch from moth bean have been evaluated and established as multifunctional tablet excipients. It has also been indicated that pre-gelatinized starch, starch acetate, carboxymethyl starch, cross-linked starch and dextrins are suitable for pharmaceutical use.

At present, most food and pharmaceutical industries in Nigeria rely on importation of millions of tons of corn starch as their major raw material in tablet compression. This implies drain on foreign exchange. To corroborate the efforts of Nigerian Government to maintain its lead as the world's largest cassava producer and improve the livelihood of the smallholder farmers, more studies are needed on value addition to the recently released improved cassava varieties to enhance its adoption among the farmers. There is also cyclic glut in cassava marketing in Nigeria due to limited industrial applications resulting in recurrent wastages and losses. This study therefore explores the potential application of pre-gelatinized starches from a recently released improved cassava variety for possible uses in the Pharmaceutical industry.

2. Materials and Methods

2.1. Source of Materials

Matured, good quality tubers of hybrid cassava TME 419, used for the research work was obtained from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

2.2. Extraction of Native Starch from Cassava

Native starch was extracted from the cassava according to the method of Sanni *et al*. (2006) with slight modification as shown in Figure 1. 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 (starch) was dried at 55±2 ^{o}C for 48 h 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.

2.3. Modification of the Native Starch by Pre-Gelatinization

Pre-gelatinized starch was prepared according to the methods of Herman *et al*. (1989) with slight modification as shown in Fig 2. Starch powder and water were mixed at the ratio of 25:100; 30:100 and 35:100 respectively. The slurry was heated at 80 °C with stirring for 15 min until gelatinized just before the glass transition. The pre-gelatinized starch was dried in a cabinet dryer at 40 °C for 24 h and then powdered using a laboratory blender

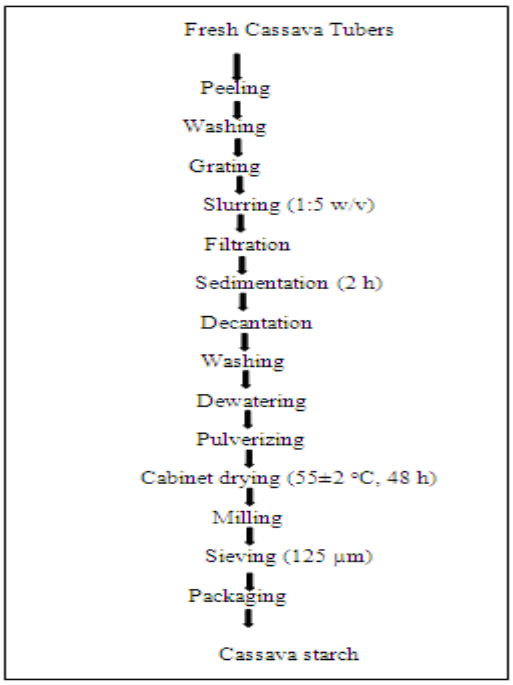

Figure 1: Cassava starch production process (Modified Sanni et al., 2006)

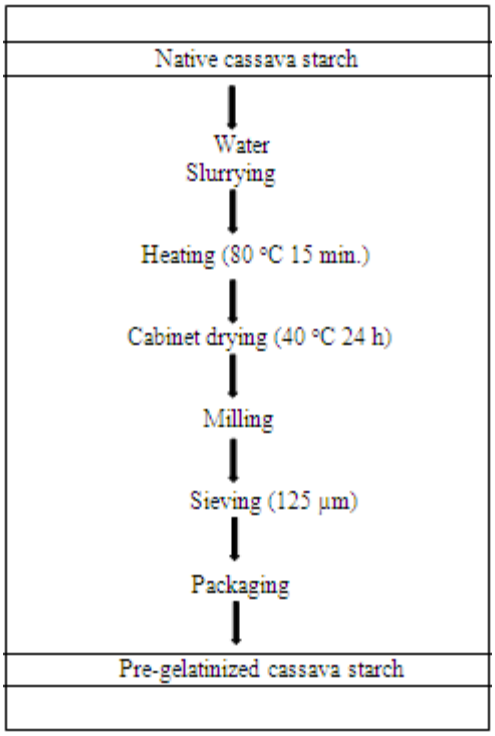

Figure 2: Pre-gelatinized cassava starch production process (Modified Herman et al., 1989)

(Marlex, Ecella model, Kanchan International Limited, Daman, India) and passed through a 125 µm mesh sieve. The pre-gelatinized starch was packaged in high density polyethylene bags and kept until required for laboratory analyses.

2.4. Production of Tablet Compacts from Native and Pre-Gelatinized Starches

Compacts of tablets were produced according to the method described by Olu-Owolabi *et al*. (2010). Approximately 500 mg of each starch sample was compressed using a hand model press (Model C, Serial No 34000 – 297, Carver Laboratory Press Inc. USA) at different loads of 0.8 to 2.4 metric tonnes with 12.5 mm flat faced punches. After ejection, the tablets were stored in high density polyethylene bags before analysis.

2.5. Analyses of the Samples

2.5.1. Determination of Bulk and Tap Densities

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

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

$$Tapped\ Density = \frac{Weight\ of\ Sample}{Packed\ Volume\ of\ Sample} \quad (2)$$

2.5.2. Determination of Sorption and Desorption Characteristics

The sorption and desorption isotherms was determined gravimetrically according to the method of Ohwoavworhua and Osinowo (2010). 2 g of the accurately weighed sample was evenly distributed over the surface of a petri dish. The samples was placed in a large desiccator containing distilled water in its reservoir (RH – 100%) at room temperature and the weight gained by the exposed sampled over a seven-day period was recorded and the amount of water that was sorbed was calculated from the weight difference.

2.5.3. Determination of Morphological Characteristics of Starch

Morphology of the native starch and the derivatives was determined using Light Microscope (LM) according to the method of Kunruedee *et al* (2010). About 1 g of the sample stained with iodine solution was mounted on the slide and covered with a cover slip. The size and shape of the starch granules were observed with a light microscope (Eclipse ci-L S/No 700161, Nickon Corporation, Japan) using 40 X Magnification and the picture taken by the mounted camera (Nickon DS-U3 DS-Fi2-U3).

2.5.4. Determination of Car (Compressibility) Index

The powder flowability was determined according to the method of Picker-Freyer and Brink (2006) by calculating the Carr index on the basis of the bulk and tap densities using the following equations:

$$Carr\ Index = \%\ Compressibility \quad (3)$$

$$Carr\ Index = \frac{Tap\ Density\ -\ Bulk\ Density}{Tap\ Density}\ X\ 100 \quad (4)$$

2.5.5. Determination of Hausner's Ratio

Hausner's (HR) ratio was calculated as the ratio of tap density to the bulk density as follows.

$$HR = \frac{Tap\ Density}{Bulk\ Density} \quad (5)$$

2.5.6. Determination of Angle of Repose

The angle of repose is the angle formed by the horizontal base of the bench surface and the edge of a cone-like pile of the starches and was determined according to the method of Shah *et al*. (2008) with slight modification. About 30 g of the starch sample was passed through a funnel at about 10 cm above the bench surface, after the cone was built, height of the starches forming the cone (h) and the radius of the base were measured. The angle of repose (Θ) was calculated as follows:

$$\Theta = tan^{-1}\frac{h}{r} \quad (6)$$

2.5.7. 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.

$$Water\ Absorbtion\ Capacity = \frac{Weight\ of\ water\ bound}{Sample\ Weight}\ X\ 100 \quad (7)$$

2.5.8. Determination of Hydration Capacity

Hydration capacity was carried out according to Nuwamanya *et al*. (2011) method 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:

$$Hydration\ Capacity = \frac{Weight\ of\ residue}{weight\ of\ sample}\ X\ 100 \quad (8)$$

2.5.9. Determination of Swelling Capacity

Swelling capacity was determined according to the method of van Hung *et al*., (2007) with slight modification. A 50 ml glass measuring cylinder was filled with sample to the 10 ml mark. Distilled water was added at room temperature to give a total volume of 50 ml. The top of the cylinder was tightly covered and the contents mixed by inverting the cylinder. This was repeated after 2 min and left to stand for 3 min. The final volume occupied by the starch was recorded and used for calculation in equation 9

$$Swelling\ Capacity = \frac{Final\ Volume\ of\ starch}{Initial\ Volume\ of\ starch}\ X\ 100 \quad (9)$$

2.5.10. Determination of Compressional Characteristics

2.5.10.1 Heckel Plots

Heckel plots of ln{1/(1-D)} against the applied pressure (P) were plotted for the samples. Values of "k" and "A" were obtained from the slope and intercept of the plots respectively. The values for the total relative density, D_A, was obtained from equation and the values for the relative density D_B were obtained from the difference between D_A and Do for the different samples.

2.6. Statistical Analysis

All data generated were analyzed statistically with the Statistical Analysis System (SAS) package (version 8.2 of SAS Institute Inc. 1999). Level of significance ($p<0.05$) was determined by analysis of variance (ANOVA) and means separated by the Least Significance Difference (LSD).

3. Results and Discussion

3.1. Effect of Pre-Gelatinization on Bulk and Tapped Density Values of Cassava Starch

The result of the effect of pre-gelatinization on the bulk and tapped density values are shown in Fig. 3. Bulk density values ranged between 0.27 and 0.37 g/ml in native starch and 30% pre-gelatinized starch concentration, respectively. It was observed that - concentration significantly ($p<0.05$) increased the bulk density values, however, the increase in samples containing more than 30% concentration was not significant. The progressive increase in the bulk density value caused by increase in concentration might be due to large particle sizes of the starches which might result into less close packing of the starch powder (Singh *et al*., 2011). With less close packing, more space will be required. Bulk density is very important in determining the packaging requirement, indicating a lesser package requirement with increase in concentration of material (Adebowale *et al*., 2008). The value obtained agrees with the range of values recommended for granular excipients (Rowe *et al*., 2012; Singh *et al*., 2011).

For the tapped density, native starch had the lowest value (0.33 g/ml) while the highest value was recorded in pre-gelatinized 30% starch concentration. The trend was similar to that observed for bulk density with pre-gelatinization having significant ($p<0.05$) influence on the tapped density values irrespective of concentration. The increase in the tapped density values due to concentration is an indication that concentration improves the potential of the pre-gelatinized starch to flow and rearrange under compression. The result agrees with the observation of Olu-Owolabi *et al*. (2010) who reported an increase in tapped density values with increase in concentration of heat-moisture treated corn starch.

3.2. Effect of Pre-Gelatinization on Sorption Characteristics of Cassava Starch

The result of the effect of concentration on the sorption characteristics of native and pre-gelatinized cassava starches are shown in Fig. 4. Significant ($p<0.05$) increases were noticed in the sorption characteristics of native and pre-gelatinized starches. On the second day of observation, there were about 50% increase in the weight gain of all the native starches whereas; the pre-gelatinized starches had increased by about 100% due to moisture uptake. The pre-gelatinized starch at concentration of 30 % reached the peak (0.7%) on the second day.

Pre-gelatinization of starches caused significant ($p<0.05$) increase in the rate of water sorption compared to their native starches which is an indication that they are relatively more sensitive to atmospheric moisture and should, therefore, be stored in moisture-tight containers (Ohwoavwohua and Osinowo, 2010). This result agrees with the report of Muazu *et al*. (2011) that potato starch with higher particle size absorbs water faster than maize starch with smaller particle sizes.

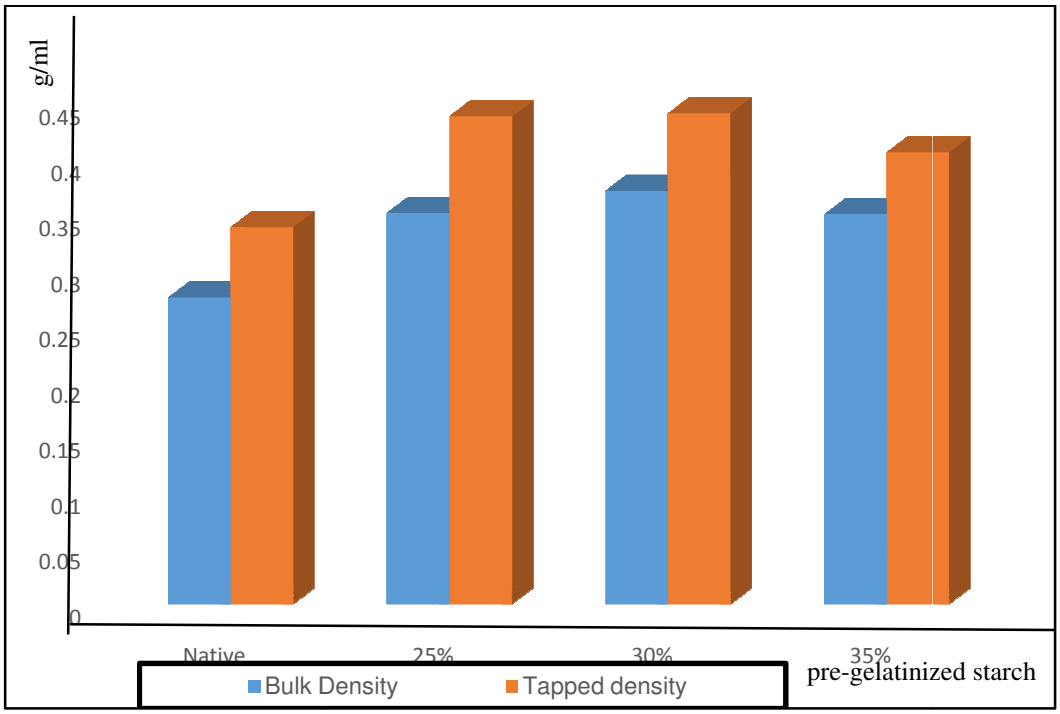

Figure 3: Bulk and tapped densities of native and pre-gelatinized cassava starches as affected by concentration

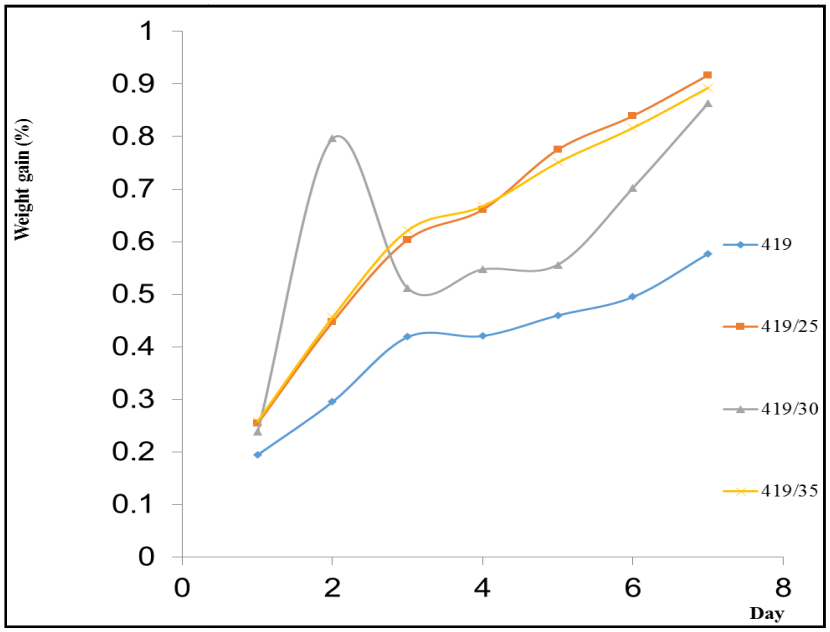

Figure 4: Sorption characteristics of native and pre-gelatinized cassava starches as affected by concentration

3.3. Effect of Pre-Gelatinization on Morphological Characteristics of Cassava Starch

The morphology of the native and pre-gelatinized starches are shown in Figure 6. It was observed that the photomicrographs of the pre-gelatinized starches still possess some similar morphology of the native starches at lower concentrations, but as the concentration increased, the granules showed traces of irregular shapes. At 35 % concentration, for instance, the starch granules had undergone some morphological alterations and enlargement. The particles of native starches were generally oval to spherical with pre-gelatinized starches having bigger particle sizes which agree with the report of Alebiowu and Itiola (2002) on the morphology of native and pre-gelatinized forms of sorghum, plantain and corn starches. The increase in the size of the pre-gelatinized starches might be due to water absorption as a result of amylose leaching during gelatinization which is an indication that pre-gelatinized starch has a higher water absorption capacity than the native starches (Itiola and Odeku, 2005) which is a pointer to better disintegration properties of the pre-gelatinized starches over the native starches (Ohwoavorhua and Osinowo, 2010).

3.4. Effect of Pre-Gelatinization on the Flow Characteristics Cassava Starch

The result of the effect of pre-gelatinization on Carr index is presented in Table 1. The values ranged between 13.73 and 20.30 (u have % in the table) for pre-gelatinized starches. The Carr index was observed to be significantly ($p<0.05$) lower in the pre-gelatinized starches than the native starch (18.61) with the exception of pre-gelatinized 25% starch concentration which may be an indication that the voids present among the particles of native samples were easily filled during compression than those of the pre-gelatinized samples (Abiodun *et al*., 2010). The reduction in the initial compressibility index of the native starch by pre-gelatinization might also be due to increase in the granule sizes. The result agrees with the report of Olu-Owolabi *et al*. (2010).

Irrespective of concentration, pre-gelatinization had no significant ($p>0.05$) influence on the Hausner's ratio of the cassava starches. The Hausner's ratio (HR) describes the ratio of bulk density to the tapped density and it is closely related to the Carr index. It is an indirect measurement of flowability of pharmaceutical excipients by measuring the inter-particulate friction. This behavior may be due to the similarities observed in the shapes of the starch granules (Soares *et al*., 2005). Lower HR of a material indicates better flow properties than higher ones. However, all the Hausner's ratio values fall within a scale of fair to passable flow (>1.12<1.34) as opined by Shah *et al*. (2008).

The values of the angle of repose for the native starch was 36.85^{o} while those of the pre-gelatinized samples ranged between 20.89^{o} and 24.28^{o}. Irrespective of concentration in all the varieties, pre-gelatinization significantly ($p<0.05$) reduced the angles of repose in the samples. The angle of repose, which is a traditional characterization method for pharmaceutical powder flow, is used to characterize solids. It is another indirect measurement of flow characteristics. All the pre-gelatinized starches had excellent flow characteristics as shown by their angle of repose compared to their corresponding native starch that had between good and fair flow characteristics and this agrees with the opinion of Shah *et al*. (2008).

3.5. Effect of Pre-Gelatinization on the Functional Characteristics Cassava Starch

It was observed that the values of the water absorption capacity (Table 2) of the pre-gelatinized starches (48.33 – 58.33%) were significantly ($p<0.05$) higher than the value obtained for the native sample (13.33%). Pre-gelatinization significantly ($p<0.05$) increased the water absorption capacity values of the cassava starch to about four times its original value. The increase in water absorption capacity is an indication that pre-gelatinized starch is capable of absorbing water than the native starch within a short time (Itiola and Odeku, 2005).

The hydration capacity values as affected by pre-gelatinization indicates that the pre-gelatinized samples are capable of absorbing and retaining more water than the native sample and follows similar trend as in water absorption capacity. Pre-gelatinization significantly ($p<0.05$) increased the hydration capacities of cassava starch to about three times the original value. This result suggests that pre-gelatinized starch may exhibit better disintegration property than native starches when incorporated in tablet formulation (Ohwoavorhua and Osinowo, 2010, add other refs. Do same for others having this ref). Pre-gelatinization significantly ($p<0.05$) increased the swelling capacity of the cassava starches to about three folds the original value. The increase in the swelling capacity values may be due to the reduction in the associative forces between adjacent molecules (Sanni *et al*., 2001).

3.6. Heckel Parameters of the Native and Pre-Gelatinized Cassava Starches

The Heckel analysis is routinely performed to study the effect of applied pressure on the relative density of powder during compaction and to determine the deformation mechanism of particles forming compacts (Ohwoavworhua and Osinowo, 2010). The Heckel plots (Figure 7) exhibited an initial curve followed by linear regions because of particle rearrangement and the fragmentation of large aggregates under low compressional pressure but show more linearity of rearrangement process before an appreciable amount of inter-particulate bonding takes place. This shows that pre-gelatinization does not improve the degree of packing due to rearrangement process before extensive inter-particulate bonding takes place. From the data obtained from the Heckel analysis (Table 3) based on the regression analysis of the curve, the slope of the linear portion 'k' can be correlated to the crushing strength of compact as shown by high values of r^2. The native sample had 'k' values of 0.002 which was lower that the values

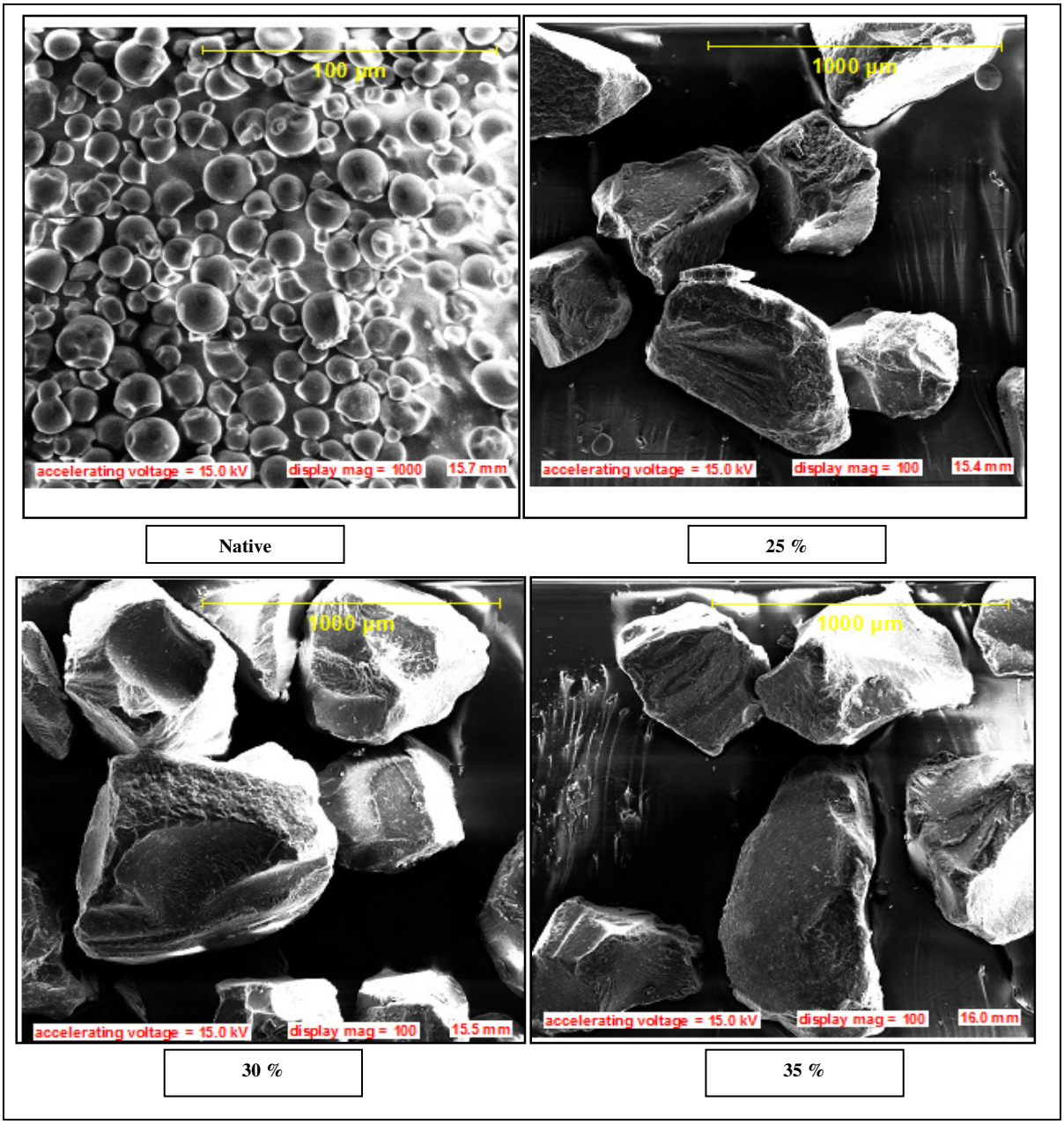

Figure 6: Photomicrograph of native and pre-gelatinized cassava starches as affected by concentration

Sample concentrations (g/100ml)	Carr index (%)	Hausner's ratio	Angle of repose (°)
Native	18.62b	1.23a	36.85a
25	20.31a	1.25a	20.89b
30	15.79c	1.43a	24.28b
35	13.74d	1.16a	24.05b

Table 1: Flow characteristics of pre-gelatinized cassava starch as affected by concentration

Mean with the same letters within columns are not significantly (p>0.05) different

Sample concentration (g/100ml)	Water Absorption Capacity (%)	Hydration Capacity (%)	Swelling Capacity (%)
Native	13.33[b]	203.33[b]	115.00[b]
25	50.00[a]	603.33[a]	321.67[a]
30	48.33[a]	533.33[a]	315.00[a]
35	53.33[a]	580.00[a]	321.67[a]

Table: 2: Functional characteristics of pre-gelatinized cassava starch as affected by concentration

Mean with the same letters within columns are not significantly (p>0.05) different

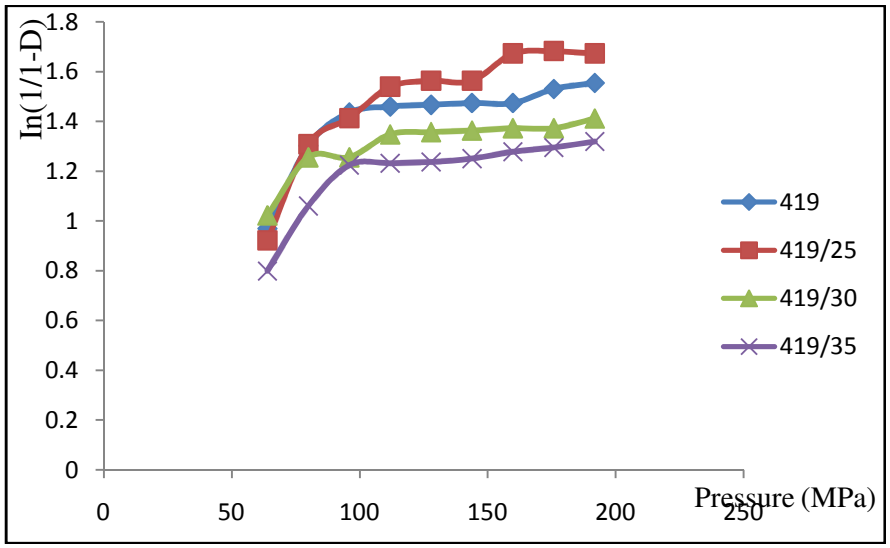

Figure 7: Heckel plots for native and pre-gelatinized cassava starches

Sample	r^2	Do	D_A	D_B	K	A	Pγ (MPa)
Native	0.949	0.1628	0.6594	0.4966	0.002	1.077	500.000
25%	0.997	0.2128	0.5162	0.3033	0.007	0.726	142.857
30%	1.0	0.2124	0.2212	0.0088	0.090	0.250	11.111
35%	0.983	0.1899	0.2882	0.0983	0.013	0.340	76.923

Table 3: Heckel and regression parameters for native and pre-gelatinized starches

recorded for the pre-gelatinized samples (0.007 – 0.09). Pre-gelatinized starches exhibited high values indicating that they produce harder compacts which agrees with the report of Ohvworhvua and Osinowo (2010). There were relative reductions in the mean yield pressure values (Pγ) values after pre-gelatinization of cassava starches from the initial value of 500 MPa in the native starch to final values that ranged from 11.11 to 142.85 MPa. The mean yield pressure (Pγ) is inversely proportional to the characteristics of the material to deform plastically under pressure; hence, pre-gelatinization improved the onset of plastic deformation during compression, and also encourages consolidation and agrees with the reports of earlier researchers (Olu-Owolabi *et al*., 2010; Soares *et al*., 2005).

3.7. Conclusion

It can be concluded from this study that pre-gelatinization improved physical and flow characteristics of cassava starch and enhanced the functional characteristics that could aid its utilization as pharmaceutical excipients. The pre-gelatinized cassava starches deformed plastically during compression, and produced harder tablet compacts that would disintegrate faster than the native starches if ingested.

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