Blending of Acetylated Cassava Starch, Cassava Flour and Wheat Flour for Composite in Bread Making

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Authors’ contributions
This work was carried out in collaboration between all authors. Author CIO designed the study, performed the statistical analysis, wrote the first protocol, and wrote the first draft of the manuscript and managed literature searches. Authors CCO, NCI, GCO and COO managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT
Chemical modification of cassava starch was carried out by acetylation and the resultant modified cassava starch was blended with high quality cassava flour and wheat flour as composites for bread production. High quality cassava flour (HQCF) and cassava starch were produced from TMS 30575 variety. The starch was treated chemically with acetic anhydride. Wheat flour, cassava flour and acetylated cassava starch were blended at varying ratios of 90:7:3, 80:11:9, 70:18:12, 60:25:15, 50:32:18 respectively and 100% wheat flour served as the control. The various composites were subjected to proximate and functional analyses; and thereafter used in bread production, following the straight dough method. The composite breads produced were subjected to physical analysis and sensory evaluation. The data were analyzed statistical and the means compared at 5% probability. Values obtained from the functional analysis ranged from 1.09-1.99 ml/g; 0.61-0.73 g/ml; 7.90-8.60 ml/g; 7.99-8.60 ml/g and 46.67-51.67°C for the swelling index, bulk density, oil absorption capacity, etc.
1. INTRODUCTION

Bread is an important staple food world over because it furnishes superior nutritional, sensorial and textural characteristics, ready-to-eat convenience as well as cost competitiveness Ade-omowayye et al. [1]. In most countries of the world bread has become an increasingly important food- it provides more nutrients than any single food source Rychnovsky [2]. All breads are nutritious, contributing valuable quantities of proteins, iron, calcium, and some of the B-vitamins to our diet, which meets the daily dietary food requirement Amendola [3]. Eddy et al. [4] reported that fibre in bread increases intestinal transit time for bile salt derivatives such as deoxycholate, which are effective chemical carcinogen, hence reducing incidence of carcinoma of the colon. The idea of substituting part of wheat flour with other starchy crops, during bread making in non wheat producing countries is not new Ade-omowayye et al. [1]. The reason is because wheat bread is relatively expensive due to cost of importation and cannot be grown locally as a result of climatic limitation Olaoye et al. [5]. Organizations like Food and Agriculture Organization FAO [6], has acknowledged the need for strategic development and use of popular foods like bread to meet nutritional needs of malnourished people. This led to the initiation of the composite flour program Edema et al. [7]. Previous works have shown that tropical root and tuber crops: cocoyam, cassava, taro and others can serve as alternative sources of major raw material for bread making Edward [8]. The use of composite flour (CF) (partial substitute to wheat flour) has currently received the support of policy makers and for example: the Federal Government of Nigeria has mandated the flour mills to add a minimum of 10% high quality cassava flour (HQCF) to wheat flour for making composite flour meant for baking purposes Shittu et al. [9].

Although, there is now a substantial amount of available composite bread technology, such breads still require at least 70% wheat flour to be able to rise Eggleston et al. [10]. Cassava flour is deficient in gluten (gliadin and glutenin) which contributes to the elasticity and cohesiveness of the dough; as such Cassava flour and some other non-wheat flours cannot alone be used for bread making. Gluten essentially forms a network that traps the CO₂ produced during fermentation from escaping, thus bringing about rising of the dough. Application of functional but expensive emulsifiers, mono and diglycerides are known to strengthen the dough and enhance the production of non-wheat bread Pomeranz [11]. Acetylation, esterifies the starch molecules and improves its functional ability by increasing its viscosity and swelling power Bentancur et al. [12]. Adebowale [13] reported that acetylation of starch retards retrogradation or recrystallization of starch in baked goods. However these attributed of modified starch have not been tried in leavening dough when non wheat flour is incorporated as composite in bread production. The present study investigates the influence of added acetylated starch in strengthening the dough when cassava flour is used as component in bread making. The study is justified by the need to increase the cassava component in composite bread making.

2. MATERIALS AND METHODS

2.1 Procurement and Production of Unfermented High Quality Cassava Flour

The fresh cassava tubers used for this study were harverst purchased from the farm centre of Imo State Polytechnic, Umuagwo, Imo State Nigeria and identified as TMS 30575. Acetic anhydride (food grade) was purchased from Gatelab Laboratories, No 88 Royce Road and baking ingredients were purchased from Ekeonuwa market all in Owerri, Imo State Nigeria. The processing of cassava starch, unfermented high quality cassava flour and...
baking of bread were carried out in the Pilot Plant of Department of Food Science and Technology, Federal University of Technology Owerri, Nigeria.

### 2.2 Production of Cassava Flour

The method reported by IITA [14] was followed for the production of unfermented cassava flour. Ten kilograms (10 kg) weight of cassava roots were peeled manually using a stainless steel knife. The peeled tubers were washed, grated to obtain cassava mash. The mash was de-watered, sieved, oven dried, milled into fine particle size to yield unfermented cassava flour and packaged in air tight bag. This process was completed within 24 h from harvesting to avoid fermentation in accordance with IITA specification.

### 2.3 Production of Cassava Starch

Five kilograms (5 kg) of the freshly harvested cassava tubers were peeled with stainless steel knife washed with clean water and grated to obtain smooth slurry. The slurry was further mixed with water to form free flowing slurry which was then filtered using a muslin cloth into a plastic bucket. The filtration continued until all the starch was extracted and the woody mass discarded. The filtrate was allowed to settle in the plastic bucket before the supernatant was decanted leaving the starch at the base of the bucket. After decanting the supernatant, the surface of the starch was washed several times with clean water to obtain a white- odourless starch. The thick starch paste was scooped into a clean calico bag and pressed to dewater, crumbled within the palm, sun-dried to obtain cassava starch and packaged in air tight container.

### 2.4 Acetylation of Cassava Starch

Acetylated starch was prepared following the method described by Van Hung and Morita [15] but with slight modification of process conditions. One kilogram of high quality cassava starch was weighed in 5 L acrylic vessel equipped with baffles and mixed with 2.5 L of distilled water. The vessel was equipped with a stirrer, which provided mechanical agitation to ensure homogenous mixing during the process. The starch slurry was mixed for 1.0 h at room temperature to fully suspend the starch granules. The pH was adjusted to 8.0 with 1.0 M aqueous sodium hydroxide, acetic anhydride (8%, w/w, on a starch dry basis) was added drop wise. Sodium hydroxide (1.0 M) was added simultaneously at a rate a 1.0mL/min. The pH of the suspension was maintained at a range of 7.8 to 8.5 during the reaction. The reaction was allowed to proceed for 60 min after final addition of acetic anhydride. Thereafter, the slurry was adjusted to pH 5.5 with 1.0 M hydrochloric acid to neutralize the reaction and followed by vacuum- filtration, and the resulting cake mixed with 2.0 L of distilled water. The vacuum- filtration was repeated and followed by washing thrice with distilled water to remove residual acid. Then the slurry was oven dried overnight at 40°C and packed in air proof bag. A duplicate experiment was performed.

### 2.5 Validation of Acetylation (Determination of Acetyl Content and Degree of Substitution (DS))

The titrimetric method of Wurzburg [16] as reported by Ayucitra [17] was followed in determining the percent acetylation (% acetyl) and degree of substitution (DS). One gram of the acetylated cassava starch sample was suspended in 50 mL of 75% ethanolic solution and was kept in a water bath at 50°C for 30 min with constant stirring. The slurry was then cooled to room temperature and 40 mL of 0.5 M potassium hydroxide added to the suspension. The slurry was allowed to stand for 72 h at room temperature with occasional swirling. The excess alkali was titrated with 0.5 M hydrochloric acid using phenolphthalein as an indicator. The solution was allowed to stand for another 2 h. Any additional alkali that might leach from the sample was titrated. Blanks with native cassava starch were analysed concurrently. The sample volume, the hydrochloric acid normality and the volume of hydrochloric acid required to titrate the blank and sample were recorded. Duplicate measurements were performed for each starch sample.

### 2.6 Formulation of the Composite Flour

The composite flour was formulated from wheat, cassava flour and acetylated cassava starch using the following ratios:

- 100% wheat flour (serving as the control)
- 90% wheat flour, 7% cassava flour and 3% cassava starch
- 80% wheat flour, 11% cassava flour and 9% cassava starch
- 70% wheat flour, 18% cassava flour and 12% cassava starch
60% wheat flour, 25% cassava flour and 15% cassava starch
50% wheat flour, 32% cassava flour and 18% cassava starch.

2.7 Proximate Analysis of the Composite Flour for Bread Making

The protein, moisture, crude fibre, fat, ash and carbohydrate contents were determined as described by AOAC [18].

2.8 Functional Properties of the Composite Flour for Bread Making

2.8.1 Bulk density

Five grams (5 g) of the flour was weighed into a graduated cylinder and its volume was recorded. Then, the cylinder was tapped against a table for 15 min until there was no further change in volume. Bulk density (g/mL) = weight of sample (g)/volume of the sample (mL)

2.8.2 Water/oil absorption capacity

The method described by Onwuka [19] was used for determination of oil/ water absorption capacity. One gram (1 g) of the flour was weighed into 15 mL centrifuge tube and 10 mL distilled water or the oil was added. The sample was mixed thoroughly and allowed to stand for 3 min at room temperature and centrifuged at 2000 rpm for 30 min. The volume of free water or oil (the supernatant) was read directly from the graduated centrifuge tube.

2.8.3 Wettability

The method described by Onwuka [19] was used. One gram (1 g) of flour was weighed into a 25 mL graduated cylinder with a diameter of 1.0 cm. A finger was placed over the open end of the cylinder before it was inverted and clamped at a height of 10 cm from the surface of a 600 mL beaker containing 500 mL of distilled water. The finger is removed to allow the test material to be damped. The time for complete dampening of the sample was recorded.

2.8.4 Swelling capacity

This was determined using the method described by Okezie and Bello [20]. Swelling capacity was determined as the ratio of swollen volume of a unit weight of the sample when left in contact with excess water. One gram (1 g) of the flour was dispensed into a calibrated 10 mL measuring cylinder. Ten millimetres (10 mL) of distilled water was added to the sample and the initial volume (V_i) was recorded. The suspension in the cylinder was left to stand for 1 h and the final volume (V_f) obtained.

Swelling capacity (mL/mL) = Final volume (V_f) – Initial volume (V_i) ................................... (1)

2.8.5 Wet gluten

The gluten content of the composites was determined by weighing 20 g of the flour into a beaker 50 mL of water added and mixed thoroughly. The dough formed was allowed to stand at room temperature for 30 min after which it was washed in running water until cloudy colour disappeared. The resultant mass was weighed in order to derive the percent wet gluten.

2.8.6 Bread Production

The bread loaves were produced using a modified bread recipe (see Table 1) and straight dough method. All the ingredients were mixed in bowl and transferred to the bowl containing composite flour; these were further mixed together for about 10 min. The dough was kneaded for 5 min and subsequently shaped and transferred into previously greased pans; and allowed to proof for about 45 min. The proofed dough was baked in an oven at 200°C for 25 min. The bread was allowed to cool at room temperature, weighed and the volumes determined.

Table 1. Recipe for 100% wheat flour bread and composite bread

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour</td>
<td>200</td>
</tr>
<tr>
<td>Yeast</td>
<td>2.0</td>
</tr>
<tr>
<td>Sugar</td>
<td>20</td>
</tr>
<tr>
<td>Fat</td>
<td>10</td>
</tr>
<tr>
<td>Salt</td>
<td>2.0</td>
</tr>
<tr>
<td>Water</td>
<td>Variable</td>
</tr>
</tbody>
</table>

2.8.7 Determination of bread volume

The loaf volume was measured according to seed displacement method described by Onwuka [19]. The loaf samples were weighed using Triple beam balance (OHAUS, 700/800 series). Soya bean seeds were used to fill the volumeter to a level marked X, poured out and the weight determined (W_i). Thereafter the bread sample
was placed in the volumeter and the available space was filled with the seeds and the weight of seeds displaced by the bread was obtained \(W_2\), the difference obtained is the weight of the loaf. The volume of the volumeter is measured by the length \(x\) width \(x\) height \(V_1\).

\[
\text{Loaf volume} = \frac{W_1 \times V_1}{W_2}
\]

\(W_1\) = weight of seed filled in the volumeter  
\(W_2\) = weight of seed displaced by the loaf of bread  
\(V_1\) = capacity of the volumeter.

2.8.8 Sensory attributes of the bread

Sensory evaluation was carried out on the bread samples using 20-man semi-trained panelists selected from the University Community. A 9-point Hedonic scale where: 1.0 is dislike extremely, 5.0 represents neither like nor dislike and 9.0 is like extremely was used. The samples were sliced and a slice was presented in a coded manner to the panelists, who were required to evaluate following attributes: crust colour, crumb colour, texture, aroma, taste and overall acceptability.

2.8.9 Statistical analysis

The data obtained were analyzed using the SAS software (SAS [21]) for ANOVA and separation means at 5% level of probability was tested using least significance difference (LSD)

3. RESULTS AND DISCUSSION

The percent acetyl content and degree of substitution (DS) of acetylated cassava starch obtained from process validation is presented in Table 2. The values showed a gradual increase in both percent acetyl content and degree of substitution as the treatment is replicated. A similarity in trend was reported by Singh et al. [22], who attributed the variation to differences in starch structure, reaction velocity and other process conditions.

3.1 Proximate Composition of Wheat Flour, Cassava Flour and Acetylated Cassava Starch Composite

Table 3, shows the result of the proximate analysis of the composite flour from wheat, cassava flour and acetylated cassava starch including 100% wheat flour which served as a control. The moisture content of the composite samples ranged from 12.13 to 12.94%. The extent of substitution of wheat flour with cassava flour and acetylated starch did not influence values obtained for the moisture.

Table 2. The %acetyl content and degree of substitution (DS) of acetylated cassava starches obtained from 1st and 2nd treatments

<table>
<thead>
<tr>
<th>Starches</th>
<th>Acetyl content (%)</th>
<th>DS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS-1</td>
<td>3.87</td>
<td>0.17</td>
</tr>
<tr>
<td>ACS-2</td>
<td>4.56</td>
<td>0.19</td>
</tr>
<tr>
<td>NCS</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

ACS-1: acetylated cassava starch (1st treatment);  
ACS-2: acetylated cassava starch (2nd treatment);  
NCS: native cassava starch

The protein content of the flours varied significantly at P<0.05 with YW2 (100% wheat) having the highest of 15.50% while PL6 had the lowest of 11.46%. There appears to be gradual decrease in protein content with increasing inclusion of acetylated starch and high quality cassava starch. This could be attributed to decrease in gluten content as the wheat flour is gradually substituted.

The ash content of the flours showed that PL6 had the highest (1.87%) while YW2 had the least 0.25%. The ash content of the various blends increased with increased level of substitution of wheat flour with cassava flour and acetylated cassava starch, which implies that the composite flours contain more inorganic nutrients than the wheat flour.

The carbohydrate content of the flour samples ranged from 72.17% in PL6 to 68.66% in the reference flour (100% wheat). It is also observed that the carbohydrate content increased with increased inclusion of cassava flour and acetylated cassava starch. This could be as a result of high content of carbohydrate in cassava flour and the acetylated cassava starch. According to Enwere [23], carbohydrate is the predominant food nutrient in roots and tuber crops. It is also observed that the carbohydrate values differed significantly, with the exception of UW1 and XX9, which did not differ at p>0.05.
3.3 Bread volumes ranged from 842.6 to 1060.5 cm$^3$, with sample Q11 (90:7:3) having the highest volume (1060.5 cm$^3$). This might be attributed to the influence of acetyl cassava starch and gluten reaction, which networked the dough structure and trapped escaping gasses thereby increasing the loaf volume. However, sample PL6 (50:32:18) had the lowest loaf volume (842.6 cm$^3$). The highest loaf volume obtained for the control (YW2, 100% wheat flour) and sample 5V2 (80:11:9; wheat flour: cassava flour: acetylated cassava starch) makes technical sense that acetyl starch inclusion gave desired result when added in small amount. Another noteworthy inference is that the loaf volume gradually decreased from the peak (1060.5 cm$^3$) as the amount of cassava flour increased, but statistical difference ($p>0.05$) was obtained for the control (YW2, 100% wheat flour) and sample 5V2 (80:11:9; wheat flour: cassava flour: acetylated cassava starch). The gradual decrease in loaf volume might be attributed to the increase in the amount of cassava flour which is poor in gluten and does not have physical strength to retain gas during proofing.

The loaf weight also had a significant increase ($p<0.05$) as the blends increased in the amount of cassava flour and acetylated starch. The observed increase in loaf weight corresponds to the increase in bulk density as the substitution ratio of non wheat materials increased. See Table 5.

The specific loaf volume showed similar trend with that of the loaf volume, however sample Q11 had the highest loaf volume (4.06 cm$^3$/g) while PL6 had the least 3.06 cm$^3$/g.

### 3.2 Functional Properties of a Composite of Wheat Flour, Cassava Flour and Acetylated Cassava Starch

The result in Table 4 shows that the functional properties of the composite flour differed significantly ($P<0.05$) for the swelling index, wetability, bulk density and wet gluten. It is observed that the oil absorption and water absorption capacities did not differ significantly ($p>0.05$).

The swelling index had the least value at QII (1.49 ml/ml), which gradually increased as the substitution of wheat flour increased and the highest value was obtained for PL9 (1.99 ml/ml). According to Bentancur et al. [12], acetylation has been reported to increase paste and gel clarity, solubility and swelling power. Similarly, a gradual and significant increase in bulk density was observed as the cassava flour and acetylated starch increased, with PL9 having the highest value of 0.73 g/ml.

The highest wet gluten was reported for YW2 (100% wheat flour (28.97%), which gradually declined as the portions of cassava flour acetylated starch increased. This trend is expected since the cassava flour and treated starch are known to be poor in gluten.

### 3.3 The Physical Characteristics of the Bread

The physical properties of bread fabricated from wheat flour/cassava flour/acetylated cassava starch blends are represented in Table 5. The bread volumes ranged from 842.6 to 1060.5 cm$^3$, with sample Q11 (90:7:3) having the highest volume (1060.5 cm$^3$).
3.4 Sensory Attributes of the Breads from Wheat Flour, Cassava Flour and Acetylated Cassava Starch Composite

The result presented in Table 6 is the sensory attributes of breads produced from various composite flour. There were no statistical difference in taste, aroma and the crumb colour of the breads, but other attributes: texture, crust colour and overall acceptability differed significantly at $p<0.05$. The statistical similarity in the rating for taste, aroma and crumb colour might be attributed to the use of high quality cassava flour and acetylated starch which were odourless, colourless and tasteless. The difference in rating of the breads’ texture followed a pattern of increasing substitution ratio of non wheat flour.

The breads from samples Q11 and 5V2, did not differ in their acceptability scores among the control sample, YW2. However, the poor score for the sensory attributes goes to show that inclusion of acetylated starch might not play any sensory role but contributes to improvement of the bread structure and shelf stability. Adebowale et al. [13] reported that acetylation of starch retards starch retrogradation or recrystallization. Acetylation reduces syneresis in freeze-thawed gels, which principally is caused by the rearrangement of amylose molecules in starch granules at reduced temperature which acts to exclude water from the gel structure (Liu [24]).
Table 6. Sensory evaluation of bread from wheat flour/cassava flour/acetylated cassava starch composite

<table>
<thead>
<tr>
<th>Samples</th>
<th>Taste</th>
<th>Aroma</th>
<th>Crumb colour</th>
<th>Texture</th>
<th>Crust colour</th>
<th>Overall Acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>YW2</td>
<td>6.9±0.28a</td>
<td>6.6±0.1a</td>
<td>7.3±0.3a</td>
<td>7.7±0.4a</td>
<td>7.1±0.1a</td>
<td>7.4±0.3a</td>
</tr>
<tr>
<td>Q1I</td>
<td>6.5±0.14a</td>
<td>6.7±0.3a</td>
<td>7.0±0.1a</td>
<td>7.4±0.28a</td>
<td>6.9±0.28a</td>
<td>6.8±0.28a</td>
</tr>
<tr>
<td>5V2</td>
<td>6.3±0.14a</td>
<td>6.1±0.3a</td>
<td>6.9±0.28a</td>
<td>6.7±0.28a</td>
<td>6.7±0.3a</td>
<td>6.6±0.1a</td>
</tr>
<tr>
<td>XX9</td>
<td>6.8±0.11a</td>
<td>6.5±0.1a</td>
<td>7.0±0.2a</td>
<td>6.8±0.1ab</td>
<td>6.7±0.28a</td>
<td>6.4±0.1b</td>
</tr>
<tr>
<td>UW1</td>
<td>5.7±0.14a</td>
<td>6.1±0.2a</td>
<td>6.4±0.1a</td>
<td>5.8±0.3bc</td>
<td>5.0±0.33c</td>
<td>5.5±0.4bc</td>
</tr>
<tr>
<td>PL6</td>
<td>5.4±0.28a</td>
<td>5.5±0.2a</td>
<td>6.3±0.4a</td>
<td>5.5±0.1c</td>
<td>5.1±0.1bc</td>
<td>5.1±0.28c</td>
</tr>
<tr>
<td>LSD</td>
<td>1.0800</td>
<td>1.280</td>
<td></td>
<td></td>
<td></td>
<td>0.990</td>
</tr>
</tbody>
</table>

*Means are triplicate determination. Means with different letters of alphabets differ significantly at P<0.05. YW2 is 100% wheat flour; Q1I is 90:7.3 (wheat flour:cassava flour:acetylated cassava starch); 5V2 is 80:11:9 (wheat flour:cassava flour:acetylated cassava starch); XX9 is 70:18:12 (wheat flour:cassava flour:acetylated cassava starch); PL6 is 50:32:18 (wheat flour:cassava flour:acetylated cassava starch).*

4. CONCLUSION

The study revealed that adding low amount of acetylated starch as component of flour for bread making at substitution ratio of 90.7%:7.3% (wheat flour: cassava flour: acetylated cassava starch) enhanced the loaf volume and the specific volume. Improvement was also obtained in the bread texture and crust colour. These findings are of technical benefit to the bread maker because they lead to high profit.

Incorporation of acetylated starch at high amount in bread making does not improve the physical and sensory attributes of bread. The increasing component amount of cassava flour in the blends was found to mask expected influence the acetylated starch might have had on the functional and physical properties of bread.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


