Rheological Evaluation of Cocoyam-Bambara Groundnut-Xanthan Gum Composite Flour Obtained from the Optimization of Its Chemical Composition and Functional Properties

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Abstract

Composite flour consisting cocoyam, Bambara groundnut and xanthan gum was produced. The effect of the addition of Bambara groundnut and xanthan gum on the nutritional composition and rheological properties of the composite flour were evaluated. The rheological properties of the composite flour were compared to that of wheat-cocoyam-Bambara groundnut flour (60%, 30%, 10% and 60%, 30%, 10%, 50%, 10%, 35%). The addition of Bambara groundnut enhanced the protein content and minerals composition of the composite flour blend while the addition of xanthan gum enhanced the functional and rheological characteristics. The dough mixing properties of xanthan gum-based sample, CBX2 (77.916% cocoyam flour 21.834% Bambara groundnut flour 0.250% xanthan gum) compared favourably with that of wheat-based sample, CBW1 (60% wheat/30%, cocoyam/10%, bambara groundnut flour) the water absorption were 64 and 57% dough development were 6 and 6.3 min dough stability were 7 and 7.7 min and the mixing tolerance index were 11 and 11.3 BU for CBX2 and CBW1 respectively. The pasting profile of xanthan gum-based composite flour was better than wheat-based composite flour in peak viscosity, final viscosity, breakdown viscosity and setback viscosity. Therefore, cocoyam could effectively be utilized in the production of baked product and in particular bread production when the composite flour is formulated as obtained in CBX2 blends.

Keywords: Bambara groundnut flour; Cocoyam flour; Farinograph; Pasting; Xanthan gum; Rheology

Introduction

Rheology studies materials flow and deformation, and it is measured by controlling a well-defined deformation or strain applied to a material in a given time, and the response measured in terms of stiffness, modulus (loss or dynamic), viscosity and strength of the material [1]. Rheology is being used to define product functionality such as mixing behaviour, sheeting and baking performance [1]. The main rheological techniques for measuring dough properties are divided into descriptive empirical techniques and fundamental measurements [2]. Instruments for descriptive empirical measurements of rheological properties include farinograph, amylograph, mixograph, extensograph [1] while instruments for fundamental measurements include the rheometer. Farinograph is a widely used rheological instrument. It provides information that is vital to the subsequent baking process, such as apparent viscosity, water absorption, mixing time and intensity, stability and consistency [3].

Another rheological instrument used in conjunction with farinograph is the amylograph. Amylograph is a descriptive empirical rheological measuring instrument that measures the apparent viscosity and gelatinization temperature [1].

Recently, researchers are working on composite flours made from cereals, tubers and sometimes in combination with legumes to serve as viable alternative to wheat flour [4-6]. Main focuses are on several underutilized crops like cocoyam, Bambara groundnut, acha, millet. Cocoyam taro (Colocasia esculenta) is a well-known food crop its corms are an important source of starch. Cocoyam ranks third in importance after cassava and yam among the root and tuber crops that are cultivated and consumed in rural areas in Nigeria. The nutritional and chemical compositions as reported by FAO [7] shows that cocoyam, if fully exploited would enhance the food security of people living in the tropics [8]. Bambara groundnut (Vigna subterranea) is a pulse with subterranean fruit-set and is cultivated by smallholders over much of semi-arid Africa [9]. The protein of Bambara groundnut is of good quality and has surplus lysine which complements cereals. Gums are hydrocolloids polysaccharides which are carbohydrates, and made up of a bunch of sugar molecules bonded together. In gluten-free baking, gums are added to recipes to mimic the elasticity, chewy texture and fluffiness the protein gluten provides in traditional baked goods. Different gums behave in different ways; xanthan gum is by far the most frequently used gum in gluten free baking [10]. This study was meant to enhance the nutritional composition and rheological characteristics of cocoyam flour by adding Bambara groundnut and xanthan gum, respectively. The proximate and minerals composition as well as the functional properties of the composite flour was optimized using response surface methodology. The pasting characteristics and farinograph properties of the optimum blends were evaluated.

Materials and Methods

Materials

Cocoyam tubers, Bambara groundnuts and wheat flour were sourced from Oba market, Akure, Nigeria. Xanthan gum was obtained from Lagos, Nigeria. All reagents were of analytical grade.

Cocoyam flour preparation

Cocoyam tubers were thoroughly washed and cleaned to remove dirt’s manually peeled, thinly sliced, soaked in water containing sodium mathabisulphite (to prevent enzymatic browning) for 20 min, blanched at 70°C for 5 min and oven dried at 65°C for 10 h. Milling was carried out using an attrition mill, made to pass through 0.037 mm sieve and the fine cocoyam flour obtained was stored inside a sealed polythene bag for further processing [11].
Preparation of Bambara groundnut flour

Bambara groundnuts (1 kg) were manually sorted to remove extraneous materials. It was subsequently soaked in boiled water for 30 min, manually dehulled and oven-dried at 65°C for 24 h. It was milled using a hammer mill, made to pass through 250 mm sieve and stored in polythene back at room temperature for further processing.

Experimental design, development of flour combination

The optimal mixture design of response surface methodology (design expert 8.0.3.1. trial version) was used for the optimization of proximate composition and minerals compositions and functional properties. The independent variables were cocoyam flour (60-85 g/100 g), Bambara groundnut flour (15-30 g/100 g) and xanthan gum (0.1-0.3 g/100 g).

Determination of proximate composition

Moisture content: The method of AOAC [12] was used for the determination of the moisture content of the composite flour. The sample (5.0 g) weighed into a petri dish of known weight was oven-dried at 105°C for 4 h. After the 1 h, the petri dish with sample was cooled in a desiccator and the reweighed. The moisture content was calculated (Eq. 1).

\[
\text{Percentage moisture content} = \frac{\text{changein weight}}{\text{weight of food before drying}} \times 100
\]

Ash content: The sample (5.0 g) was weighed into crucibles in duplicate ashes in a muffle furnace at 550°C until a light grey ash was obtained and a constant weight obtained. The sample was cooled in the desiccator. After cooling, new weights were obtained and the ash content calculated (Eq. 2).

\[
\% \text{Ash} = \frac{\text{Weight of ash}}{\text{Weight of original food}} \times 100
\]

Fat content: About 2 g of the composite flour sample wrapped in a filter paper placed in an extraction thimble and extracted using N-hexane at its boiling point for 3 h. After the extraction period, the solvent was recovered the fat content was placed inside a desiccator and then weighed. The percentage fat content was calculated using Eq. (3).

\[
\text{Total fat content (g)} = \frac{\text{Weight of fat extracted}}{\text{Weight of food sample}} \times 100
\]

Crude fibre content: Method of AOAC [12] was used. About 5g of the sample was added into a 500 ml Erlenmeyer flask and 100 ml of TCA digestion reagent was subsequently added. It was boiled and refluxed for exactly 40 min counting from the start of boiling. The sample was subsequently cooled, filtered through a 15.0 cm What-man paper; the residue was washed with hot water, transferred to a porcelain dish and dried at 105°C for 12 h. The dried sample was transferred to a desiccator, weighed (Wf) and ashed in a muffle furnace at 500°C for 6 h and cooled. The weight after ash (Wf) was observed. The percentage crude fibre was calculated (Eq. 4).

\[
\% \text{crude fibre} = \frac{W_f - W_a}{W_f} \times 100
\]

Where, \( W_f \) = Weight of crucible+fiber+ash

\( W_a \) = Weight of crucible+ash

\( W_f \) = Dry weight of food sample

Protein content: Kjeldahl method was used in determining the protein content. Exactly 0.25 g of sample was weighed into a boiling tube and digested using 4 ml each of \( \text{H}_2\text{SO}_4 \) and \( \text{H}_2\text{O}_2 \) at 375°C, thoroughly mixed for about 2 h until digestion was completed (indicated by a blue-green coloration). About 1 ml of the digest was pipette into 25 ml volumetric flask, 3 drops each of polyvinyl alcohol solution and mineral stabilizer were added and made up to the 25 ml mark with distil water. Exactly 1 ml of Nessler reagent was added till a pink coloration appeared. Nitrogen concentration was spectrophotometrically obtained at 460nm [12]. Percentage nitrogen was calculated using Eq. (5) while the crude protein was calculated using Eq. (6).

\[
\% \text{Nitrogen} = \frac{\text{concentration} \times 0.0075}{\text{weight of sample (g)}}
\]

\[
\% \text{crude protein} = \% \text{Nitrogen} \times \text{conversion factor of 6.25}
\]

Total carbohydrate content: The method of Egounlety [13] was used. Total carbohydrate was determined by difference between 100 and the total sum of the percentage of fat, moisture, ash, crude fibre and protein content.

Functional properties

Water/oil absorption capacity: 1 g of sample was weighed into a clean conical graduated centrifuge tube and was mixed thoroughly with 10 ml distilled water/oil using a warring mixer for 30 s. The sample was allowed to stand for 30 min at room temperature and centrifuged at 5000 rpm for 30 min. The volume of the free water (supernatant) or oil was read directly from the graduated centrifuge tube. The absorbed or water was converted to weight (in grams, g) by multiplying by the density of oil (0.894 g/ml) and water (1 g/ml), respectively. The oil and water absorption capacities were express in grams of oil/water absorbed per gram of flour sample [12].

Bulk density: The sample (20 g) was put in a calibrated 20 ml measuring cylinder; the bottom of the cylinder was tapped repeatedly onto a firm pad on a laboratory bench until a constant volume was observed. The packed volume was recorded. The bulk density was calculated as the ratio of the sample weight to the volume occupied by the sample after tapping [12].

Swelling index: 3 g of the sample was weighed into a clean dry measuring cylinder. The volume occupied by the sample was recorded before 5 ml of distilled water was added. This was left to stand undisturbed for an hour, after which the volume was observed and recorded again. The index of swelling ability of the sample was determined using Eq. (7) [12].

\[
\text{Swelling index} = \frac{\text{volume occupied by sample after swelling}}{\text{volume occupied by sample before swelling}}
\]

Foaming capacity: 2 g of the sample was blended (using Kenwood blender) with 80 ml distilled water for 5 min and poured into the measuring cylinder. The height of the foam was determined by taking the reading [12].

Mineral analysis: Flame photometry method was used in the determination of potassium while Calcium, Magnesium, Zinc and Iron were determined using Atomic Absorption Spectrophotometer [12].

Determination of the composite flour dough rheology

Pasting properties: The pasting characteristics of the composite flour were evaluated using Rapid Visco Analyser (RVA). The amount of sample to be used and water to be added was determined from the instrument by inserting the moisture content (14% in this case). The mixture were vigorously but carefully stirred until there was no more lumps. The solution was carefully transferred into the canister, inserted into paddle coupling properly. The measurement cycle was initiated...
by depressing the motor tower of the instrument. The test was then allowed to proceed and terminate automatically according to the pre-set time and temperature regime. The pasting properties were recorded at the end of the experiment (RVA Operation Manual, 1995).

Farinograph analysis: Farinograph Testing was carried out with the use of a Brabender Farinograph. The flour sample of around 48g on a 14% moisture basis was weighed and placed into the corresponding farinograph mixing bowl. Distilled water (calculated by the instrument using the water content of the sample) was added from a burette until dough was formed. As the dough is mixed the farinograph records a curve on the graph paper depicting the farinograph parameters.

Anti-nutritional analyses: Exactly 10 ml of 70% aqueous acetone was added to 0.2 g of finely ground sample inside a 50 ml glass bottle. The bottle were put in an ice bath shaker and shaken for 2 h at 30°C, centrifuged and the supernatant stored in ice. About 0.2 ml of the supernatant was later pipetted into the test tube and 0.8 ml of distilled water was added. Standard protein solutions were prepared from a 0.5 mg/ml of the stock and the solution made up to 1 ml with distilled water. Folin ciocalteau reagent (0.5 ml) was added to both the sample and the standard followed by 2.5 ml of 20% Na2CO3. The solution was then vortexed and allows incubating for 40 min at room temperature; its absorbance was read at 725 nm against a reagent blank concentration of the same solution from a standard tannic acid curve was prepared [14].

Determination of phytate

The sample (4 g) was soaked in 100 ml of 2% HCl for 3 h then filtered through a Whattman filter paper. About 25 ml of the filtrate was put inside a conical flask and 5 ml of 0.3% of ammonium thiocyanate solution added as an indicator. Thereafter, 53.5 ml of distilled water was added to give it the proper acidity and this was titrated against 0.00566 g/ml of standard iron (iii) chloride solution containing about 0.00195g of iron per ml until a brownish yellow colouration persist for 5 min [15].

Determination of trypsin inhibitor

Trypsin inhibitor was determined by mixing 1g of the composite flour with 50 ml of 0.01 N NaOH (pH 8.4-10.00) and allowed to stand for 3 h while stirring at intervals. About 2 ml diluted extract was dispensed into test tubes and 2 ml of cold trypsin solution (4 mg in 200 ml of 0.001 M HCL) was added. The tubes were placed in water bath at 37°C and 5 ml of Benzoyl-DL-arginine-P-nitro anilide hydrochloride was added to each tubes. The reaction was terminated after 10 min by the addition of 30% acetic acid and the content thoroughly mixed, centrifuged at 3000 rpm and the absorbance of the filtrate was measured at 410 nm against reagent blank [16].

Statistical analysis

Data were analysed statistically using response surface methodology (Design Expert software version 8.3.0.1 trial version). In order to correlate the response variable to the independent variables, multiple regressions was used to fit the coefficient of the polynomial model of the response. The quality of the fit of the model was evaluated using analysis of variance (ANOVA). Results were also analysed using SPSS statistical package (Version 17.0) analysis of variance (ANOVA), Duncan multiple range test and mean ± standard deviation was chosen to determine any significant difference among the samples.

Results and Discussion

Proximate composition of cocoyam-based composite flour

The results of the proximate composition are presented in Table 1. The moisture content of the composite flour ranged from 3.95 to 5.57 g/100 g. The range is acceptable for shelf stability of flours, which should be less than or equal to 10.00 g/100 g [4,5,17]. The ANOVA result in Table 2 indicated that moisture content had no significant (p>0.05) model terms. In fact, the R2 and adj R2 values were very low (0.4041 and 0.1062, respectively) which showed that the regression had very low fitting. For moisture content, the R2 and adj R2 values are acceptable for shelf-stable flour. In addition, it could be inferred that the presence of xanthan gum actually promote the low moisture content observed in the study. A similar work using cassava starch instead of xanthan gum had high R2 and adj R2 values of 0.9974 and 0.9934, respectively [6] as against low values obtained in the present work (Table 1).

The ash content varied from 0.84 to 2.54 g/100 g. The ANOVA result in Table 2 showed that only the model terms AB (A-B) that was significant (p ≤ 0.05). Ash content in the flour is an indication of the presence in the principal mineral contents which could be of vital importance to the body. The values of R2 and adjusted R2 indicated that the raw materials in the composite flour favoured ash content (Table 2).

Xanthan gum enhanced the fat content. The R2 and adj R2 values

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<th>M</th>
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<td>1.61</td>
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</table>

Table 1: Proximate properties of flour from cocoyam, bambara and xanthan gum.

Cy: Cocoyam; B: Bambara groundnut; X: Xanthan gum; P: Protein; C: Carbohydrate; A: Ash; CF: Crude fiber; F: Fat; M: Moisture
were very high. Likewise the model (cubic) was highly significant \((p \leq 0.05)\). An implication of the results is that the composite flour may have challenges of being rancid with time. The use of another binder (starch) instead of xanthan gum for cocoyam-Bambara groundnut composite flour showed that the starch would not support fat content like xanthan gum \([6]\). Xanthan gum may therefore function as retainers of oil in the composite flour.

The crude fiber ranged from 0.76-1.12% for sample CBX. The report indicated that cocoyam and Bambara groundnut had an appreciable amount of crude fiber. The ANOVA result showed that the linear mixture component and AB model terms were significant \((P \leq 0.05)\). The R2 and adjusted R2 values are 0.6100 and 0.4149 respectively, which was moderate. Hence the incorporation of xanthan gum might hinder the expression of crude fibre in the composite flour.

The crude protein ranged from 11.11-20.35% in the composite flour. The R2 and the adjusted R2 value are 0.8753 and 0.6883, respectively. The protein content of 100% wheat flour was about 12%. Bambara groundnut definitely enhanced the protein content to a level of 20.35%. Legumes have also been found to positively influence rheological properties so incorporation of bambara groundnut is expected to enhance the rheological characteristics of the composite flour in addition to the addition of xanthan gum.

### Functional properties of optimized cocoyam (Colocasia esculenta) based flour

The functional properties of the composite flour were shown in Table 3. The foaming capacity ranged from 4.10 to 10.15. The addition of Bambara groundnut (a protein source) enhanced the whipping ability of the flour blends \([18]\). The foaming capacity could be an advantage in the solubility of products and also in the ability of dough to incorporate air resulting in the raising, consequently giving the end product a honeycomb structure as desired in bread, cake and ice cream. The ANOVA (Table 4) indicated that the linear mixture components, AB, AC, BC, ABC, AB (A-B), AC (A-C), BC (B-C) were significant \((P \leq 0.05)\) model terms while the R2 and adjusted R2 were 0.9969 and 0.9922, respectively (Table 3).

Foaming stability for sample the sample ranged from 1.90 to 5.60. The ANOVA (Table 4) showed that Model and model terms (AB, AC, BC, ABC, AB (A-B), AC (A-C), BC (B-C)) were significant \((P \leq 0.05)\). The addition to the addition of xanthan gum.
The water absorption capacity (WAC) of the sample ranged from 2.2 to 2.8 g/100 g. Okezie and Bello [19] stated that water absorption capacity is important in bulking and consistency of products as well as baking applications. Better water absorption and retention suggests better performance in texture of baked products [19].

The oil absorption capacity (OAC) ranged from 1.40 to 1.80 for CBX. The ANOVA in Table 4 showed that the composite flour had acceptable ANOVA parameters which indicated that the composite flour is good flavour retainers. The least gelation value ranged from 2.1 to 2.8% for the composite flour. In general, the composite flour had good functional properties judging from their ANOVA (Table 4) except for the swelling ability.

Mineral properties of optimized cocoyam (Colocasia esculenta) based flours

The results of the minerals composition are presented in Table 5. The results of the ANOVA showed that the R2 and adjusted R2 for calcium and potassium very above 98%. The R2 for iron was 80.5% while the adjusted R2 values above 84% for all the elements [6]. This implies that cassava starch is a better binder when mineral elements are to be considered in composite flour production. However, xanthan gum had good performances in calcium, potassium and iron, average performance in zinc and below average performance in magnesium from statistical perspective.

Selected optimal flour blends

Two samples, CBX1 (75.575% cocoyam flour/23.875% bambara groundnut flour/0.250% xanthan gum) and CBX2 (77.916% cocoyam flour/21.834% bambara groundnut flour/0.250% xanthan gum) were selected based on their high protein, crude fibre and ash contents for rheological evaluation (pasting characteristics and farinograph). The rheological properties were compared with composite flour containing wheat (wheat-cocoyam-bambara groundnut composite flour), which were CBW1 (60% wheat, 30% cocoyam, 10% bambara groundnut flour) and CBW2 (72% wheat, 19% cocoyam, 9% bambara groundnut flour). The reason for the choice of the blends for wheat-based composite flour wheat content between 60% and 81% had been reported to be appropriate for composite flour that is wheat-based [20].

Farinograph properties of optimized flour blends

The farinograph properties of the flour blends were presented in Figure 1 and Table 6. Dough development time (DDT) is the time...
from the first addition of the water to the development of the dough's maximum consistency. It is an indication of the mixing time of the flour. Stronger flour had higher DDT, but higher DDT is not an indication of better dough mixing. DDT of around 3 min had been reported for 100% whole wheat flour. Samples CBX2 and CBW1 had better DDT when compared to 100% wheat flour.

Samples CBX2 and CBW1 also had better dough stability. Dough stability indicates the strength of the dough, with higher values suggesting stronger dough. Flours with long stability times are generally more suited for variety bread production and often require longer mixing times. According to Mohamed et al. most commercial bread flours have a stability value of up to 10 min. The mixing tolerance index (MTI) is the difference in Farinograph Units between the top of the curve and the top of the curve measured 5 min after the peak is reached [21]. Flour with a MTI greater than 50 B.U. indicates less tolerance and often indicates more difficulties during mechanical handling and makeup of the dough. Samples CBX2, CBW1 and CBW2 had good mixing intolerance. MTI influences baking quality [21] (Table 7).

Pasting properties of the selected optimized cocoyam (Colocasia esculenta) based flours

The rapid visco analyzer indicates starch behaviour to temperature and shearing forces. The results of the pasting properties are shown in Figure 2. Pasting properties and behaviour of flour is the characteristic of starch and reflects its properties like swelling and ability to absorb water [22]. The blends had peak viscosity and final viscosity that ranged from 3290.50 RVU to 5232.50, which showed that the samples may be suitable for product requiring high gel strength and elasticity [23]. High peak viscosity also indicates high starch content [24]. The pasting temperature is the minimum temperature required to cook the flour [25]. The pasting temperature for the samples ranged from 84.75 to 86.30°C. Breakdown is a parameter that measures the ease with which the swollen granules can be disintegrated [25]. Sample with xanthan gum incorporation had lower setback and breakdown viscosities. Lower setback is an indication of starch stability. Overall, sample with xanthan gum incorporation had better rheological characteristics than the composite flour with wheat and cocoyam.

<table>
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<tr>
<th>Sample</th>
<th>Water absorption (%)</th>
<th>Dough development time, DDT (min)</th>
<th>Dough stability (min)</th>
<th>Mixing tolerance index (BU)</th>
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<td>Not good</td>
<td>Not good</td>
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<td>11</td>
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Table 6: Result of farinograph property of the selected optimized flour blends.

<table>
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<tr>
<th>Sample</th>
<th>Tannin (mg/g)</th>
<th>Trypsin inhibitor (%)</th>
<th>Phytate (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBX1</td>
<td>1.79 ± 0.10c</td>
<td>54.56 ± 2.12a</td>
<td>29.16 ± 0.71b</td>
</tr>
<tr>
<td>CBX2</td>
<td>1.76 ± 0.00c</td>
<td>52.27 ± 1.17a</td>
<td>31.11 ± 0.22b</td>
</tr>
<tr>
<td>CBW1</td>
<td>3.19 ± 0.5b</td>
<td>25.25 ± 1.02c</td>
<td>11.12 ± 1.24c</td>
</tr>
<tr>
<td>CBW2</td>
<td>4.33 ± 0.16c</td>
<td>30.51 ± 1.69c</td>
<td>12.36 ± 0.00c</td>
</tr>
</tbody>
</table>

Table 7: Result of the antinutritional properties of the selected optimized flour blends.

Values are mean ± standard deviation of triplicate samples

Values on vertical row with the same superscript are not significantly different at P=0.05

CBX=Cocoyam Flour, Bambara groundnut flour and Xanthan gum CBW=Cocoyam Flour, Bambara groundnut flour and Wheat flour

![Figure 2a: Pasting profile of sample CBW1.](image)
Figure 2b: Pasting profile of sample CBW2.

Figure 2c: Pasting profile of sample CBX1.

Figure 2d: Pasting profile of sample CBX2.
Anti-nutritional properties of the selected optimized flour blends

Samples CBX (cocoyam, Bambara groundnut and xanthan gum composite flours) had lower tannin content than wheat-based composite flour (CBW). Both values were lower than 27.69-42.82 mg/g and 25-58 mg/g reported for cereal brans [26]. Samples CBX had higher trypsin and phytate contents than samples CBW. Trypsin and phytate contents of 58% and 25.7% had been reported for whole soy bean flour [27] and wheat flour [28], respectively.

Conclusion

The potential utilization of cocoyam-based composite flour was evaluated in terms of nutritional suitability and rheological characteristics. The research showed that cocoyam could be used effectively as composite flour meant for baked products with adequate protein, ash, fibre and minerals composition. The functional characteristics and rheological properties were enhanced by addition of xanthan gum at 0.25%. The composite flour also compared favourably with the rheological characteristics of wheat-cocoyam based composite flour at 60% wheat inclusion.

References