Application of Multivariate Techniques for Characterizing Composition of Starches and Sugars in Six High Yielding CMD Resistant Cassava (Manihot esculenta Crantz) Varieties

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Abstract

High yielding and cassava mosaic disease (CMD) resistant cassava varieties have been developed by the Crop Research Institute of Ghana with varying compositions and concentrations of starches and sugars. This study characterized four of these improved cassava varieties (Ampong, Broni bankye, Sika and Otuhia) together with two traditional varieties (Amakuma and Bankye flita) for their composition of starches and sugars using principal component and cluster analyses. The concentration of total sugars, reducing and non-reducing sugars, sucrose, starches, amylose and amylopectin were determined using standard analytical methods. Results obtained were total sugar (4.04-18.47%), non-reducing sugar (2.08-16.21%), sucrose (1.98-15.40%), starch (15.39-31.07%) and amylose (30.57-40.33%) and these were significantly different (p < 0.05) amongst the studied cassava varieties. The improved varieties (Ampong, Broni bankye, Sika and Otuhia) had high total sugar levels ranging from 7.19 to 18.47%. With the exception of Broni bankye (improved variety) all the improved and traditional varieties were high starch and amylose containing varieties. These differences in the biochemical composition of the traditional and improved cassava varieties could be used in their selection for specific food and industrial processing applications.

Keywords: Cassava; Starches; Sugars; Multivariate techniques; Principal component analysis; Cluster analysis

Introduction

Cassava (Manihot esculenta, Crantz) is a major staple root crop in many tropical and subtropical developing countries, especially in West Africa. Grown in more than 90 countries, it ranks as the 6th most important source of energy in human diets on a worldwide basis and as the third staple food after rice and corn/maize [1-4]. Cassava root is an energy-dense food. In this regard, cassava shows very efficient carbohydrate production per hectare. It produces about 250,000 calories/hectare/day, which ranks it before maize, rice, sorghum, and wheat [5]. The root is a physiological energy reserve with high carbohydrate content, which ranges from 32% to 35% on a fresh weight (FW) basis, and from 80% to 90% on a dry matter (DM) basis. Eighty percent of the carbohydrates produced is starch [6] 83% is in the form of amylopectin and 17% is amylose [7,8]. Roots contain small quantities of sucrose, glucose, fructose, and maltose [9]. Cassava has both sweet and bitter varieties. The root of the sweet cassava varieties is made up of about 17% sucrose with small amounts of dextrose and fructose [3-5,10,11].

Starch is the major caloric source in a variety of diets of people worldwide. Thus, starches from various plant species have received very extensive attention in food research. Starch has a unique chemical and physical characteristics and nutritional quality, which set it apart from all other carbohydrates. It occurs naturally as discrete particles called granules, which are composed of a mixture of two polymers - amylose and amylopectin [1,2]. Aryee [12] reported that the starch content of fresh cassava tuber is between 25% and 34% while that of cassava flour ranges from 67.92% to 88.11%. Starch plays an important role in developing food products, either as a raw material or as a food additive such as a thickener, gelling agent, stabilizer, or texture enhancer. Corn, potato, and cassava are the most common sources of starch for such industries [4,13,14].

Amylose is an essentially linear polymer of (1→4)-linked α-D-glucopyranosyl residues and has molecular weight of about 106 [15]. Several workers have previously reported wide variations in amylose content in different cassava varieties and products. Rickard and his colleagues [16] in their work on cassava starch from fresh roots reported an amylose range of 13.6–23.8% while Moorthy and Matthew [1] reported amylose content ranging from 22.6% to 26.2%. Barimah [17] in his work on starch from dry cassava chips also reported amylose levels of 23.3–24.6% while Aryee [12] reported high amylose content of 44.3% on cassava flour. Amylose content determines the stability of a viscous solution formed when heat is applied. Dakubu and Bruce-Smith [18] established that starches from fully matured cassava varieties had normal amylose content which varied from the range of 13.6 – 19.1%. Amylopectin is a very large, highly branched molecule consisting of much shorter chains of (1→4)-α-D-glucose residues connected by (1→6)-α-D-glucosidic linkages [15]. Starches that contain amylopectin molecules with a large proportion of long-branched chains display higher gelatinisation temperature and enthalpy changes [2,13,19,21].

Aryee and his colleagues [12] reported that cassava varieties with high starch content could be used as thickeners, for starch

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production and for many other food products that require starch or flour. Invariably, the starch content largely influences the functional and physico-chemical properties of food systems, and these properties provides information on how a food ingredient will behave in a food system. These properties include swelling power, swelling, volume, solubility, water binding capacity and pasting properties. Tester and Morrison [13] noted that root and tuber starches are characterised by low lipid content (<1%), which does not have a pronounced effect on the functional properties compared to those from cereal starch. Proteins and ashes that are in low quantities in starches also do not have pronounced influence on their functional properties [22].

Mégnanou and others [23] reported that cassava varieties with high moisture and reducing sugar contents would be suitable for fermentation, and could be successfully used as raw material in ethanol, organic acids, lactic bacteria and biofuel industries. In addition, starch is used to improve the moisture retention, to control the water mobility, and also to maintain the quality of food products during storage [24]. Since most foods are aqueous systems, the quality of final food products depends strongly on interactions between water and other ingredients as well as the contribution from individual ingredients [25]. Many polysaccharides have strong capabilities of associating with water and thus can perform as effective agents to control the behaviour of water in complex food systems [26].

As variations in starch and sugar concentrations in different cassava varieties would influence to varying levels their organoleptic properties, functionality and physico-chemical properties during their applications in food and industrial processing, characterization of the new and improved varieties for their starch and sugars content would be beneficial in selecting cassava varieties for specific food processing and industrial applications. Thus, the objective of this work was to apply multivariate techniques to characterize the concentrations of starches and sugars in some traditional and improved high yielding and cassava mosaic disease (CMD) resistant cassava varieties grown in Ghana.

Materials and Methods

Materials

Six varieties of cassava comprising four improved varieties and two traditional varieties obtained from the experimental fields of the Crop Research Institute of the Council for Scientific and Industrial Research (CSIR) situated at Pokuase in the Greater Accra region of Ghana were used in the study. Table 1 gives the description of these traditional and improved varieties.

Sample preparation

Cassava samples were harvested from the fields of Crop Research Institute, Pokuase in the Greater Accra Region of Ghana and transported immediately to the laboratory. At the laboratory, the samples were cleaned, peeled and washed with potable water. Samples from the distal, middle and apical sections of peeled tubers were cut into cube and oven dried at 60°C for 48 h. The oven dried samples were ground in a Hammer mill (Christy and Norris Ltd., Chelmsford, Surrey, UK) into flour to pass through a 250-mm sieve. The flour samples obtained were then packaged into polypropylene bags and kept at room temperature (25°C) for analyses.

Analytical methods

Starch determination: The starch was extracted from the six varieties by sedimentation method [27]. The tubers were sorted out and washed with clean water. The tubers were peeled, washed, with clean water to remove all the dirt and grated into chunks of about 0.5 - 1 mm thick. The 400 g of the samples were weighed and milled with 800 ml of water using Waring Blender (Philips 8010G, Springfield, MA, USA). The slurry sample was filtered through a clean cheese cloth. The solids retained by the cloth were washed with 4000 ml of water and filtered through cheese cloth until there was little or no starch in the residue. The filtrate was allowed to sediment overnight and the liquid decanted and discarded. The starch was dried under room temperature until completely dry. The dried starch was weighed and expressed as a percentage of fresh weight.

Determination of amylose and amylopectin content: The amylose content of the samples was determined by standard methods [28]. One gram iodine and 10 g KI was dissolved in water and made up to 500 ml. About 100 mg amylose was dissolved in 10 ml 1N NaOH and made up to 100 ml with water. One (1) ml of distilled ethanol and 10 ml of 1N NaOH was added to 100 mg of the flour sample which was heated in a water bath for 10 minutes. The extract was diluted to 100 ml and 2.5 ml of it was added to 20 ml distilled water with three drops of phenolphthalein. 0.1 N HCl was added drop by drop until the pink colour just disappeared. 1 ml of iodine reagent was added and made up to the volume 50 ml. The absorbance was read at 590 nm. A serial dilution of 0.2, 0.4, 0.6, 0.8 and 1 ml of the standard amylose solution was done with the development of colour as in case of the sample. The amount of amylose present in the sample was calculated using the standard curve. For the blank 1ml of iodine reagent diluted to 50 ml with distilled water. The amount of amylopectin was obtained by subtracting the amylose content from that of starch.

Determination of total sugars: Total sugars were determined by methods described by AOAC [27]. Ten grams of the sample was dissolved in 100 ml of distilled water. 10 ml of concentrated HCl was added to the solution and heated in a water bath for 10 minutes. The solution was then neutralized with a base preferably NaOH. The solution was made up to 200 or 300 ml with distilled water and filtered. 10 or 25 ml of mixed Fehling’s solution was pipetted into a conical flask followed by the addition of 15 ml of the solution from the burette. The solution was heated and on boiling three drops of methylene blue was added. Further quantities of the solution were added from the burette (1 ml at a time) at 10 – 15 seconds interval to the boiling liquid until the indicator is completely decolourised. The titre values obtained correspond to mg of invert sugar per 100 ml.

Determination of reducing sugars, non-reducing sugars and sucrose: Reducing sugars were determined by methods described by AOAC [27]. About 50 g of the sample was dissolved in 150 ml of distilled water. The solution was made up to 200 ml with distilled water and filtered. About 25 ml of mixed Fehling solution was pipetted into a conical flask followed by the addition of 15 ml of the solution from the burette. The solution was heated and on boiling three drops of methylene blue was added. Further quantities of the solution were added from the burette (1 ml at a time) at 10 – 15 seconds interval to the boiling liquid until the indicator is completely decolourised. The titre values obtained correspond to mg of invert sugar per 100 ml. The amount of non-reducing sugars was obtained by subtracting the reducing sugars from that of the total sugars. The amount of sucrose in the sample was also obtained by multiplying the amount of non-reducing sugars by the factor 0.95.

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Statistical analysis

Statistical analysis and graphical presentation were done with Minitab version 14 and Microsoft Excel version 2007. Analysis of variance for the cassava varieties was conducted at a level of significance of p<0.05. Cluster Analysis (cluster observation) was carried out to group cassava varieties with similar characteristics. Principal component analysis (PCA) was used to ascertain patterns and explore the relationships between the various parameters and the cassava samples.

Results and Discussion

Concentrations of sugars of the cassava varieties

Table 2 shows the sugar content of the cassava varieties studied. Wide variations were observed in the total sugar content of the cassava varieties which ranged between 4.04 % (Bankye fitaa) to 18.47 % (Otuhia). The traditional varieties (Amakuma and Bankye fitaa) had low sugar contents as compared to the improved varieties (Ampong, Broni Bankye, Sika and Otuhia). Padonou and his colleagues [29] and Aryee and his colleagues [12] reported values from 1.57% to 7.50% for study on cassava genotypes. The total sugars for Amakuma and Bankye fitaa were within the range reported while the improved varieties Ampong, Broni bankye, Sika and Otuhia were relatively higher signifying higher sweetness levels. It was observed that the reducing sugar content of the cassava varieties varied with ranges from 1.55% (Ampong) to 2.42% (Broni Bankye). The traditional varieties had appreciable reducing sugar contents at 1.82% and 1.95% for Amakuma and Bankye fitaa respectively. Except for Ampong (1.55%), all the other varieties had their reducing sugar content above value of 1.67 g/100g by Mégannou et al. [23].

Non-reducing sugars formed the major component of the total sugars of the cassava varieties with the improved varieties having high values. Non-reducing sugars varied with ranges from 2.08% to 16.21% with Bankye fitaa being the highest and Otuhia the lowest. There were variations amongst the varieties for sucrose which ranged from 1.98% (Bankye fitaa) to 15.40% (Amakuma). Okigbo [5] and Charles et al. [10] reported that 17% of the cassava root is made up of sucrose with small amounts of dextrose and fructose. One way analysis of variance of the total sugars, reducing sugar, non-reducing sugar and sucrose data showed that there were significant differences (p<0.05) amongst the cassava varieties (improved and traditional) except for reducing sugar content.

Certain varieties of cassava have traditionally been designated as sweet or bitter, purportedly in relation to their sugar content. The sweet varieties are supposedly much lower in hydrogen cyanogen (HCN) content than the bitter varieties [10]. Both the improved and local varieties showed high values for total sugar but had low hydrogen cyanogen content this was contrary to what Padonou et al. [29] observed in his studies on the quality of boiled cassava roots. He observed that bitter cultivars had higher total sugar and protein contents but lower fibre content. Instead the improved varieties having high levels of total sugars had high protein and crude fibre whilst the traditional varieties were vice versa.

Starch content of the cassava varieties

There were variations in the starch content of the cassava varieties which ranged from 15.39% to 31.07% (Table 3). Broni Bankye (improved variety) had the lowest while Amakuma (traditional variety) had the highest starch content. Previous studies reported starch values ranging between 25% and 35% [3,4]. Mégannou et al. [23] also reported values between 3.32% and 23.24% when studying improved cassava varieties in Cote d’Ivoire. The starch content of both the improved and traditional cassava varieties were within the reported range from literature. The local varieties had considerable high starch content than the improved varieties but low content for free sugars. Statistical analysis conducted on the data showed significant difference (p<0.05) amongst the cassava varieties.

Amlose content of the cassava varieties varied significantly (p<0.05) giving a range of 30.57% to 40.33% (Table 3). Amakuma (traditional variety) had the highest value with Broni Bankye (improved variety) having the lowest. The variation in amlose content may be attributed to varietal effect. Amlose content determines to a large extent the nutritional value and storage stability of the roots.

Table 1: Variety, Type, Age and Source of Samples.

| Sample          | Type       | Age at harvest (months) | Source                                |
|-----------------|------------|-------------------------|---------------------------------------|
| Ampong-CSIR     | Improved   | 12                      | Crops Research Institute, Fumesua, Ghana |
| Broni bankye-CSIR | Improved    | 12                      | Crops Research Institute, Fumesua, Ghana |
| Sika-CSIR       | Improved   | 12                      | Crops Research Institute, Fumesua, Ghana |
| Otuhia-CSIR     | Improved   | 12                      | Crops Research Institute, Fumesua, Ghana |
| Amakuma         | Traditional| 12                      | Crops Research Institute, Fumesua, Ghana |
| Bankye fitaa    | Traditional| 12                      | Crops Research Institute, Fumesua, Ghana |

Table 2: Sugars concentrations in the traditional and improved cassava varieties.

| Variety       | Total Sugars (%) | Reducing Sugars (%) | Non-Reducing Sugars (%) | Sucrose (%) |
|---------------|------------------|---------------------|-------------------------|-------------|
| Ampong 1      | 7.19±3.66  a      | 1.55±0.59           | 5.64±3.22               | 5.36±0.05   |
| Broni Bankye 2 | 13.81±1.95  a      | 2.42±0.62           | 11.39±2.18              | 10.82±2.07  |
| Sika 1        | 6.27±1.40  c,d     | 1.96±0.31           | 4.33±1.65               | 13.61±1.57  |
| Otuhia 1      | 18.47±0.81  b      | 2.26±0.40           | 16.21±0.52              | 15.40±0.49  |
| Amakuma 2     | 4.51±2.50  c,d     | 1.82±0.16           | 2.68±2.52               | 2.55±2.40   |
| Bankye fitaa 2 | 4.04±1.03  c,d     | 1.95±0.91           | 2.08±0.39               | 1.98±0.37   |

In each column means followed by different letters (a, b, c, etc.) are significantly different at p < 0.05.

Table 3: Starch content of traditional and improved cassava varieties.

| Variety       | Starch (%) | Amylose (% starch) | Amylopectin (% starch) |
|---------------|------------|--------------------|------------------------|
| Ampong 1      | 21.40±2.50  a | 31.25±4.56         | 68.75±4.56             |
| Broni Bankye 2 | 15.39±1.87  a | 30.57±4.98         | 69.43±4.98             |
| Sika 1        | 23.76±1.02  a | 32.80±1.42         | 67.20±1.42             |
| Otuhia 1      | 24.07±2.44  a | 36.73±3.96         | 63.30±3.92             |
| Amakuma 2     | 31.07±1.91  a | 40.33±3.09         | 59.70±3.08             |
| Bankye fitaa 2 | 25.27±1.05  a | 35.53±3.75         | 64.47±3.75             |

In each column means followed by different letters (a, b, c, etc.) are significantly different at p < 0.05.

1 improved variety and 2 traditional variety.
Cluster and principal component analysis for sugars and starches concentrations of cassava varieties

Cluster and principal component analysis was applied to the biochemical characteristics of the cassava samples. This was done to group cassava varieties with similar characteristics and to display pattern and interrelationship between samples and their biochemical characteristics. Figure 1 shows the cluster observations dendrogram for biochemical characteristics of the cassava varieties. This partitioned the samples into two clusters based on similarity of characteristics. One improved variety (Ampong) formed the first cluster with traditional varieties Amakuma and Bankye fitaa, whilst the other improved varieties Broni bankye, Sika and Otuhia formed the third cluster. In this case similarity between varieties was not divided along improved and traditional lines. Among the new varieties Ampong was similar to the local varieties Amakuma and Bankye fitaa leaving the other improved varieties Broni bankye, Sika and Otuhia formed the third cluster. In this case similarity between varieties was not divided along improved and traditional lines. Among the new varieties Ampong was similar to the local varieties Amakuma and Bankye fitaa leaving the other improved varieties in the second cluster. The principal component analysis applied to the biochemical characteristics of the cassava varieties shows that two components explained a total of 61.9% of the total variability in the data. PC1 accounted for 61.9% of the variation in the biochemical characteristics while PC2 explained 28.8% (Figures 2 and 3).

The sample score plot (Figure 2) confirms the portioning observed in the cluster analysis. All the improved varieties were found on the negative side of the PC1 except Ampong which was found loaded on the positive side with the traditional varieties. The improved varieties plot (Figure 3) showed a loading of free sugars and amylopectin to the negative side of the x-axis (PC 1) which is related to the loadings of the new varieties on the sample score plot. The positive side of the x-axis (PC 1) of the variable weights plot shows the loading of starch and amylose content which is related to the loadings of traditional varieties on the sample score plot. This suggests that Ampong and the traditional varieties are different from the improved varieties – on the biochemical level – based predominantly on starch and free sugars. Ampong having low total sugar content was characteristic of the traditional varieties. Broni bankye on the other hand had lower starch content than Sika and Otuhia, but they were related in terms of similarity by their high content of free sugars. The high content of free sugars in the improved varieties may be due to the increased activities of plant enzyme, amylases which break down or hydrolyze starch into the constituent sugars.

There were significant differences at p<0.05 among the cassava varieties. The improved varieties had high content of free sugars except Ampong. The traditional varieties (Amakuma and Bankye fitaa) had high starch and amylose content. The improved varieties because of their higher free sugars contents would be suitable for fermentation, and could be successfully used as raw material for industrial production.
of ethanol, organic acids and lactic bacteria. The traditional varieties because of their high starch content could be used as thickeners, for starch production which can be used to improve the moisture retention, control the water mobility, and also maintain the quality of food products during storage. The traditional varieties with their high amylose contents could also be used for industrial production of ethanol, as well as glucose and high fructose syrups.

Conclusion

The improved and traditional cassava varieties studied had appreciable quantities of sugars and starches with total sugar (4.04-18.47%), non-reducing sugar (2.08-16.21%), sucrose (1.98-15.40%), starch (15.39-31.07%) and amylose (30.57-40.33%) and these values were significantly different amongst the traditional and improved cassava varieties. The improved varieties (Ampong, Broni bankye, Sika and Oтуhua) had higher sugar levels as compared to the traditional varieties (Amakuma and Bankye fitaa) which had relatively lower sugar concentrations. With the exception of Broni bankye (improved variety) all the improved and traditional varieties were high starch and amylose containing varieties. The improved varieties because of their higher free sugars contents would be suitable for fermentation, and could be successfully used as raw material for industrial production of ethanol, organic acids and lactic bacteria. On the other hand, the traditional varieties because of their high starch content could be used as thickeners, and for starch production. These products can be used to improve the moisture retention, control the water mobility, and also maintain the quality of food products during storage. The traditional varieties with their high amylose contents could also be used for industrial production of ethanol, as well as glucose and high fructose syrups.

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