Physicochemical properties of low-amylose yam (Dioscorea spp.) starches and its impact on α-amylase degradation in vitro

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Abstract: Starches from four low-amylose yam cultivars, cv.Chinese yam, Bitter yam, Yampie and Akam cultivated in Jamaica were extracted and the relationship between physicochemical properties and in vitro digestibility investigated. A direct correlation between starch physicochemical properties and digestibility of the low-amylose starches was observed. Chinese and Bitter yam starches with the lowest amylose content were found to have the highest digestibility in vitro (21.27 ± 0.01 % and 18.11 ± 0.02 % respectively), while Akam and Yampie starches with higher amylose content had significantly lower percentage digestibility (p<0.05). The mean granular diameter of the starches ranged from 5.4 µm for Chinese yam to 29.58 µm for Yampie. The variations observed in the granular size may have influenced the surface properties of the starches, as Chinese yam was found to have the largest specific surface area (625.91 m²/kg) while Yampie had the lowest (117.4 m²/kg). The digestibility of the starches was also influenced by granule diameter, specific surface area, crystalline pattern and surface-no. mean of the starches studied.

Keywords: α-Amylase, Low-Amylose, Crystallinity, Specific Surface Area, Starch, Yam (Dioscorea Spp)

1. Introduction

Yam tubers (Dioscorea spp) are consumed as a source of digestible carbohydrate by millions of people in the tropical and subtropical regions and in some European Countries [1]. Yam tubers are classified as either edible or non-edible, where non-edible cultivars are used primarily for their medicinal properties. Research shows that wild/non-edible yams may be used in the treatment of hypercholesteremia, menopausal symptoms [2], lipid metabolism and cardiovascular disease [3]. According to Bahado-Singh et al. [4] some edible yams may be beneficial to persons living with diabetes as the glycemic index of the cooked tubers are usually low to medium.

The major nutritional component of yam is starch, accounting for approximately 70-90% w/w of the tuber [1]. Due to the high starch content, extensive research have been done which have revealed intra-varietal variations in their physicochemical and functional properties, among different cultivars [5-10]. In particular, variations in the amylose/amylopectin content of yam starches and their effect on starch properties and functionality were previously reported [9, 11-12]. Studies have also shown that the differences in amylose content may illicit variations in functional characteristics such as digestibility, crystallinity, physical properties, functionality and glycemic indices [7, 13-14]. Such variations can impact on the resulting metabolic effects and susceptibility of the native starch to α-amylase digestion [15, 16]. Starch digestibility is of primary significance to health conscious, diabetic and hyperlipidemic individuals as starches that are highly degraded tend to illicit higher insulin demand than those that are less digestible [16]. Jenkins et al. [17] reported that easily digested starches have a higher insulin demand than the slower degrading starches. This can affect the
sensitivity of insulin, and lead to or reduce the risk of developing type II diabetes [18].

The amylose content of yam starches has been shown to vary with cultivar, geographical location and planting season. Yam starches with amylose contents ranging between 11 % and 30 % have been reported (Table 1). Studies show that differences in enzymatic degradation of starches can be linked to the amylose content along with crystallinity, particle size distribution and surface area of the granules [15, 19, 20]. Other studies have shown that starch digestibility is directly affected by the physiochemical properties of the individual starches [11, 15].

Table 1. Amylose content of yam starches.

<table>
<thead>
<tr>
<th>Starch Species</th>
<th>Amylose content (%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. rotundata</td>
<td>21-23, 20.9-24.6</td>
<td>Farhat et al [9]</td>
</tr>
<tr>
<td></td>
<td>21.5-23.5</td>
<td>Moorthy and Nair [12]</td>
</tr>
<tr>
<td></td>
<td>21-23</td>
<td>Riley et al [7]</td>
</tr>
<tr>
<td></td>
<td>21.6</td>
<td></td>
</tr>
<tr>
<td>D. cayenensis</td>
<td>25-29</td>
<td>Rollande-Sabate et al [6]</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>Gallant et al [27]</td>
</tr>
<tr>
<td></td>
<td>21, 25</td>
<td>Farhat et al [9]</td>
</tr>
<tr>
<td></td>
<td>26-27</td>
<td>Rollande-Sabate et al [6]</td>
</tr>
<tr>
<td>D. alata</td>
<td>21.1</td>
<td>Rasper and Coursey [10]</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Gallant et al [27]</td>
</tr>
<tr>
<td></td>
<td>20.1-23</td>
<td>Riley et al [21]</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>Rollande-Sabate et al [6]</td>
</tr>
<tr>
<td>D. dumentorum</td>
<td>14.2</td>
<td>Rasper and Coursey [10]</td>
</tr>
<tr>
<td></td>
<td>14.8</td>
<td>Sibanda et al [13]</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>Rollande-Sabate et al [6]</td>
</tr>
<tr>
<td>D. esculenta</td>
<td>15</td>
<td>Rasper and Coursey [10]</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Riley et al [7]</td>
</tr>
<tr>
<td>D. schimperiana</td>
<td>24.5</td>
<td>Sibanda et al [13]</td>
</tr>
</tbody>
</table>

This study was therefore designed to investigate the relationship between the physicochemical properties of low-amylose yam starches and their digestibility in vitro.

2. Materials and Methods

2.1. Materials

Freshly harvested matured tubers (harvested 9 months after planting) of Dioscorea trifida cv. Yampie, Dioscorea bulbifera cv. Akam, Dioscorea polygonoides cv. Bitter yam, and Dioscorea esculenta cv. Chinese yam were collected from a local farm in Jamaica. The tubers were washed, peeled and diced for immediate starch extraction. All reagents used were analytical grade and sourced from Sigma-Aldrich, USA.

2.2. Methods

2.2.1. Starch Isolation

Starch was extracted following previously reported technique [21]. Batches of freshly harvested tubers (1000 g) were peeled, diced and homogenized in 1 % NaCl solution (9000 mL) using a Waring commercial blender. The mixture was filtered through a No. 140 Fisher analytical sieve (pore diameter, 106 µm) and washed through with water. Starch granules in the combined washings were allowed to settle overnight (12 hours) at room temperature, the supernatant was decanted and the slurry centrifuged (Beckman centrifuge) at 3000 x g for 10 minutes. The brown top layer was scraped off and the starch re-suspended in 1 % w/v Sodium Chloride solution and de-ionized water respectively and centrifuged after each washing. Starch was then dried at 60 °C until constant weight in a Gravity Compression oven (Precision Scientific, GCA Corporation, USA), milled and stored in glass containers until used.

2.2.2. Determination of Apparent Amylose Content

Apparent Amylose content was determined as outlined by Farhat et al. [9] with modifications [21]. The defatted Starch (100 mg) was dispersed in ethanol (1 ml) and 1 M NaOH (9 ml). The volume was made up to 100 ml with distilled water and a 5 ml aliquot transferred to a volumetric flask containing water (25 ml). Acetic acid (0.5 ml) and Iodine solution (1 ml) were added and the volume made up to 50 ml with water and optical density recorded at 620 nm.

2.2.3. Scanning Electron Microscopic Studies of Starch Granules

Starch samples were sieved using a number 60 (250 µm) Fisher Scientific sieve, mounted and coated with gold (1 nm) using a Polaron sputter coater and analyzed using a Philips 505 Scanning Electron Microscope (Phillips, Holland) at a magnification of 3.26 x 10^2 for Akam, Yampie and Bitter yam starches and 1.32 x 10^3 for Chinese yam Starch.

2.2.4. Determination of Micromeritic Properties

Starch samples were passed through a 250 µm sieve. A small quantity of the powder was dispersed in liquid paraffin and a slide of the dispersion was examined on a Leica DMRME light microscope (Leica, Germany). Particle size of the starches samples were studied using an eye piece.
graticle previously calibrated with a stage micrometer [22, 23]. At least 500 granules were measured from each sample. The projected mean granule diameter was calculated using the statistical equation:

\[ \bar{d} = \frac{\sum nd}{\sum n} \]  

(1)

Where \( d \) is granule diameter falling within a defined size range and \( n \) is the frequency number in the respective size range.

2.2.5. Determination of Specific Surface Area

The specific surface area of the starch granules was calculated using the equation of a sphere:

\[ S_w = \frac{6}{\rho d_{av}} \]  

(2)

Where \( \rho \) is the density of the granules and \( d_{av} \) the volume to surface ratio.

2.2.6. Determination of Starch Crystalline Form

Crystalline pattern of yam (Dioscorea spp.) starches were determined by the method of Farhat et al. [9]. X-ray spectra of starch samples were recorded at 2 \( \theta \) angles from 4 ° to 38 ° with a step size of 0.005 ° at 25 °C using a Bruker D5005 X-ray diffractometer. Potato, corn and pea starches were used as reference standards.

2.2.7. Determination of Percentage in Vitro Digestion

Percentage digestion was determined as outlined by Hassan and West [24] with slight modifications. Starches (dry weight) used as reference standards. X-ray diffractometer. Potato, corn and pea starches were recorded at 2 \( \theta \) angles from 4 ° to 38 ° with a step size of 0.005 ° at 25 °C using a Bruker D5005 X-ray diffractometer. Potato, corn and pea starches were used as reference standards.

2.2.8. Determination of Yam Starch Caloric Value

The yam starches studied had amylose contents ranging from 11.14 % to 19.74 % (Table 2). Previous studies have shown that the amylose content of yam starches can be as high as 30 % [9, 27]. Of the four low-amylose yam starches studied, Chinese yam was found to have the lowest amylose content (11.14 %) while Akam, an aerial tuber, the highest (19.74 %). It is unclear as to what factors influence intra-varietal variations in amylose content. However, studies have shown that such disparities in the amylose/amyllopectin ratio may be due to genetic variations and environmental conditions [9, 28]. It has been further postulated that amylose content may be affected by the expression of the amylose extender gene, where starches from sources with high expression of the gene would have higher amylose contents than those with lower gene expression [28, 29]. It was also reported that a genetic variation in amylose content is due to allelic difference at the Wx-B1 locus on Chromosome 4A in sorghum starches [29].

A correlation between the amylose content, starch granule size distribution, crystalline structure and percentage digestibility in vitro was observed. Starches with low amylose content such as Chinese yam and Bitter yam were found to display type C or Type-A crystalline structures and were more susceptible to \( \alpha \)-amylase digestion, while those with higher amylose content conformed to the type B structure and were less susceptible to \( \alpha \)-amylase digestion in vitro. Padmanabhan and Lasome [30] reported similar correlation between amylose content and starch crystallinity. Low-amylose cassava starches were found to be more crystalline thus conforming to type A or type C structure while high amylose varieties were more amorphous and exhibited the type-B structure. Previous studies have shown that starches with high amylose content tend to be more resistant to enzymatic degradation resulting in lower degrees of digestion in vitro [19]. Mir et al. [31] reported that starches with lower amylose content were more accessible by digestive enzymes and generally had lower quantities of resistant starch. As such, low amylose starches were more digestible than their counterparts with higher amylose.

2.2.9. Statistical Analysis

Samples were analyzed in replicates of 6 and evaluated using the One-Way ANOVA Duncan’s t-test (p<0.05).

3. Results and Discussion

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Table 2. Percentage in vitro digestibility and amylose content of yam starches (dry weight).

<table>
<thead>
<tr>
<th>Starch Source</th>
<th>Amylose Content (%)</th>
<th>In vitro Digestibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yampie</td>
<td>12.58 ± 0.70</td>
<td>17.45 ± 0.01</td>
</tr>
<tr>
<td>Akam</td>
<td>19.74 ± 0.70</td>
<td>15.78 ± 0.03</td>
</tr>
<tr>
<td>Bitter Yam</td>
<td>11.97 ± 0.80</td>
<td>18.11 ± 0.02</td>
</tr>
<tr>
<td>Chinese Yam</td>
<td>11.14 ± 0.30</td>
<td>21.27 ± 0.01</td>
</tr>
</tbody>
</table>

Mean ± SEM (n = 6). Superscripts sharing different letters are significantly different (p<0.05).

2.2.7. Determination of Percentage in Vitro Digestion

Percentage digestion was determined in vitro as outlined by Hassan and West [24] with slight modifications. Starch (10 mg) was suspended in porcine pancreatic \( \alpha \)-amylase solution (5 ml of a 60 mg/ml solution), buffered with 0.05 M citric acid – sodium acetate buffer and 0.02 % CaCl\(_2\) at pH 5.5. Samples were incubated for 24 hrs at 40 °C and the reaction stopped by addition of (1 ml of a 1M NaOH solution), followed by centrifugation at 2000 rpm for 10 minutes. Reducing sugars were determined by the method of Nelson [25].

2.2.8. Determination of Yam Starch Caloric Value

The caloric values of the yam starches were determined in a calibrated bomb calorimeter (calibrated with benzoic acid). Fuse wire (10 mm) was threaded through the electrodes and configured to a point directly above and resting on the starch pellet in the bomb head. One milliliter (1 mL) of water was then added to the bottom of the bomb, and the bomb head lowered into the bomb. The sealed bomb was then pressurized with pure oxygen to 30 atmosphere followed by equilibration (bomb and calorimeter) and ignition. After combustion the bomb was slowly depressurized, washed with distilled water and titrated against 0.07 N sodium carbonate.

2.2.9. Statistical Analysis

Samples were analyzed in replicates of 6 and evaluated using the One-Way ANOVA Duncan’s t-test (p<0.05).
content. The relationship between amylose content, post-prandial glucose concentrations and emptying of human gastrointestinal tract were also reported [32, 19]. It has been reported that starches with low amylose content illicit higher blood glucose concentrations and slower gastric emptying rates. It is important to note however that other fundamental properties such as granule size and size distribution, degree of crystallinity, granule porosity, specific surface area, polymerisation, starch form (native vs. modified) and non-starch components such as lipids also influence starch digestibility [33, 34].

Table 3. Granule shape, crystalline type and caloric value of yam (Dioscorea spp) starches.

<table>
<thead>
<tr>
<th>Starch Source</th>
<th>Granule Shape</th>
<th>Crystalline Type</th>
<th>Caloric Value (kcal/100 gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yampie</td>
<td>Polyhedral</td>
<td>B</td>
<td>347.71 ± 5.01</td>
</tr>
<tr>
<td>Akam</td>
<td>Triangular</td>
<td>B</td>
<td>351.13 ± 8.18</td>
</tr>
<tr>
<td>Bitter Yam</td>
<td>Round</td>
<td>A</td>
<td>363.15 ± 9.01</td>
</tr>
<tr>
<td>Chinese Yam</td>
<td>Ellipsoid</td>
<td>C</td>
<td>349.82 ± 9.29</td>
</tr>
</tbody>
</table>

Mean ± SEM (n = 6). Superscripts sharing different letters are significantly different (p<0.05)

The low-amylose yam starches studied displayed three distinct crystalline patterns/structures (Table 3, Fig. 1). X-ray diffraction analysis revealed that both Yampie and Akam exhibited the open hydrated hexagonal crystallite (type-B), while Bitter yam displayed the staggered monoclinic crystallite (type-A) and Chinese yam an intermediate crystalline form (type-C). It has been reported that tuber starches usually exhibit the Type – B structure as a result of high amylose content or low amylose/amylopectin ratio [9]. On the other hand starches with low amylose/ high amylopectin contents are of either the type-A or the intermediate type-C form [35]. Studies have shown that the amylose/amylopectin ratio can impact on the percentage starch crystallinity thereby influencing the crystalline pattern [30, 35]. The crystalline patterns of the starches studied may correlate with the percentage amylose digestion obtained as that the type-C and type-A starches were found to be more digestible than the type-B forms under in vitro conditions. This is could be due to the granular packing of low amylose/high amyllopectin starch as the high number of branch chains provides more access points for degradation. Similar findings were reported by Noda et al. [34].

The caloric value of the starches studied ranged from 347.71 kcal/100 g – 363.15 kcal/100 g starch (Table 3). The results obtained were within the expected range for starches (300 kcal/100 g- 400 kcal/100 g starch). Yampie starch was found to have the lowest caloric value (347.71 kcal/100 g) while Bitter yam was found to have the highest (363.15 kcal/100 g). No direct correlation between physicochemical properties of the low-amylose starches and caloric value was observed.

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**Figure 1.** X-Ray Diffraction Pattern of (I) Akam, (II) Chinese yam starches, (III) Bitter yam, and (IV) Yampie.
Table 4. Micromeritic properties of yam (Dioscorea spp.) starches.

<table>
<thead>
<tr>
<th>Yam cultivar</th>
<th>Projected mean diameter (µm)</th>
<th>Geometric mean Diameter (µm)</th>
<th>Surface –no. mean (µm²)</th>
<th>Specific surface area (m²/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yampie</td>
<td>29.58 d</td>
<td>12.87 d</td>
<td>30.13 d</td>
<td>124.11 c</td>
</tr>
<tr>
<td>Akam</td>
<td>27.06 c</td>
<td>10.37 c</td>
<td>27.71 c</td>
<td>136.78 c</td>
</tr>
<tr>
<td>Bitter Yam</td>
<td>15.30 b</td>
<td>11.58 b</td>
<td>15.71 b</td>
<td>258.76 b</td>
</tr>
<tr>
<td>Chinese Yam</td>
<td>5.41 a</td>
<td>7.789 a</td>
<td>5.67 a</td>
<td>626.91 a</td>
</tr>
</tbody>
</table>

Mean ± SEM (n = 6). Superscripts sharing different superscripts are significantly different (p<0.05)

Figure 2. Scanning Electron micrographs of (I) Yampie, (II) Akam, (III) Bitter yam, and (IV) Chinese yam starches.

Microscopic and micromeritic analyses of the starch granules highlighted the differences in granular size, size distribution, shape, surface area and surface-no. mean (Table 4 and Fig. 2). Chinese yam starch had the smallest mean granule diameter, geometric diameter, surface-no. mean and highest specific surface area while Akam was the inverse. The percentage enzymatic degradation was found to correlate with starch particle size and specific surface area. Chinese Yam, Bitter Yam and Yampie starches were found to be the most susceptible to α-amylase digestion under in vitro conditions while Akam was the least susceptible (Table 2). The degree of α-amylase digestion increased with decrease in mean and geometric granular diameter, and increased specific surface area. Similar correlations between granular size and digestibility have been reported [34, 36, 37].

The results from the study imply significant variations in the physicochemical properties among the four low-amylose yam cultivars studied. The in vitro digestibility of the starches was found to correlate with the starch crystalline form, mean and geometric granule diameter, specific surface area and amylose content. This further implies that care should be taken when consuming or utilising these low-amylose yam starches in food preparations as they are digested at a faster rate when compared to high amylose types. This could result in rapid increases in the postprandial blood glucose leading to greater insulin demand and other endocrine responses when consumed [38]. Additionally, one must also consider the caloric value of the starches when formulating nutritional plans.

4. Conclusions

The physicochemical properties of the low-amylose yam starches studied varied significantly (p<0.05). A correlation between amylose content, crystalline form, granule size and specific surface area was observed. In addition to this, starches with small granule diameters, of type-C or type-A crystalline form and high specific surface area were the most susceptible to α-amylase digestion in vitro. No direct correlation between amylose content and caloric value was observed.

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References


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