Effect of steam cooking and storage time on the formation of resistant starch and functional properties of cassava starch

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Effect of steam cooking and storage time on the formation of resistant starch and functional properties of cassava starch

V.F. Abioye1*, I.A. Adeyemi1, B.A. Akinwande1, P. Kulakow2 and B. Maziya-Dixon2

Abstract: In this study, resistant starch (RS), type 3, was prepared by debranching, steam cooking and storage of cassava native starch obtained from two Nigeria varieties (TMS 30572 and 98/0581). The native starch samples were debranched with isoamylase enzyme, steam cooked both at atmospheric and high pressure (15 psi) and stored under refrigeration (5–7°C) and freezing condition (−18°C) for 48 h. The effects of these processing conditions on the formation of RS were determined. The debranching process increased the resistant starch contents of the cassava starch to about 70% over the undebranched samples. The RS contents from the steam cooking process ranged between 5.99 and 19.55 g/100 g. The highest value was obtained in TMS 30572 debranched and steam cooked at atmospheric pressure while the least RS was obtained from TMS 98/0581 undebranched but steam cooked at atmospheric pressure. The RS contents increased with increase in storage time with refrigeration having a higher resistant starch contents compared with freezing. Formation of RS decreased the Swelling Power, Water Absorption Index and Water Solubility Index of the Starch while the Syneresis Value increased. This study showed that cassava starch is suitable for isoamylase debranching, and RS formation.

ABOUT THE AUTHORS

V.F. Abioye research focus has been on reduction of post-harvest losses and value addition of indigenous crops through processing. Abioye have worked on processing of some indigenous crops such processing of fonio, sweet potato, African yam bean and Finger millet into breakfast meal to reduce post harvest losses and to increase their economic values. Abioye have also studied the effects of processing conditions of the locally produced foods such as Agidi, Ogi (corn), yam flour and how these conditions could be improved to enhance food nutrition and security. Production of resistant starch from cassava is also a project aimed at reducing the post harvest losses and maximizing the economic potential of indigenous crops such as cassava. The team consist of a cassava breeder, who worked on different varieties of cassava, crop utilization specialist who supervised the processing procedure and two professors who are experts in food nutrition, processing and post harvest.

PUBLIC INTEREST STATEMENT

There is increasing awareness on the part of the consumers on the health benefits of food in addition to the nutritional value. Resistant starch has the same physiological properties in the body as fibers and a better texture, they help in digestion, prevention of some diseases such as colon cancer and related diseases. It can also address the problem of low intake of fibre due to poor texture. Resistant starch can be produced from crops with high amylose content, and cassava has been reported to be suitable for resistant starch production. Steam cooking method could be used in the production of resistant starch from Nigeria cassava varieties. Nigeria being the largest producer of cassava could venture into production of resistant starch as functional foods in enhancing the health status of the people and also increase the economy potential of the crop.
1. Introduction

There is a growing demand for functional foods which reveals the awareness of the consumers on the additional health benefit of food beyond its basic nutrition (Annunziata & Pascale, 2009; Li & Gao, 2010). Starch is a polymer of glucose with two distinct structural forms, amylose and amylopectin. During gelatinization of starch, the structure relapses to a structure that could be highly resistant to hydrolysis by amylase which is referred to as resistant starch (RS) (Deepa, Singh, & Naidu, 2010). Resistant starch (RS) has been defined as the total amount of starch and the products of starch that is not digested in the small intestine of healthy people and passes into the colon where it can be fermented by natural microflora to short-chain fatty acids (Bird, Brown, & Topping, 2000; Birt et al., 2013; Yadav, Sharma, & Yadav, 2007).

The positive effect of RS in nutrition is as a result of the fermentation process during which short chain fatty acids (primarily acetates, propionates and butyrates) are produced in the large intestine (den Besten et al., 2013; Macfarlane & Macfarlane, 2011; Mikuliková, Benková, & Kraic, 2006). These reduce the pH in the large intestine which prevents the growth of pathogenic micro-organisms. In this, it has physiological effects in the human body that are similar to that of dietary fiber (Mikuliková et al., 2006; Zhongkai, Xiaohong, & Julia, 2013). It has been shown to reduce risks of some diseases, including colon cancer, coronary heart disease, and glycemia (Kendall, Emam, Augustin, & Jenkins, 2004). In addition to these physiological benefits in humans, RS has potential as a unique ingredient that can yield high-quality foods (Baixauli, Salvador, Martinez-Cervera, & Fiszman, 2008; Yue & Wang, 1998). Application of RS in certain products showed improved crispness, expansion, better mouth feel, colour and flavour compared to products produced with traditional insoluble fibres (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010; Milasinovic, Milica, & Ljubica, 2009).

RS is classified into four categories which are: (1) physically inaccessible to digestion by entrapment in a non-digestible matrix (RS1); ungelatinized starch (RS2); retrograded starch RS3, and chemically modified starch RS4 (Haralampu, 2000; Malshick, Kyungsoo, & Paul, 2003; Pongjanta, Utaipattan, Naivikul, & Piyachomkw, 2009). RS3 seems to be particularly interesting widely, because it can retain its functional characteristics when RS is used as a nutritional ingredient in cooked foods (Gao, Li, Jian, & Liang, 2011). Processing raw food materials in most cases destroys RS1 and RS2, but it can produce RS3. RS3 is produced by gelatinization, which is a disruption of granular structure by heating starch with excess water, and then retrogradation. It is generally believed that RS3 fraction mainly consists of retrograded amylose, the quantity formed being directly proportional to the amylose content of the starch and could be influenced by some factors such as amylose content, chain length, heating temperature, storage time and temperature (Ozturk, Koksel, Kahraman, & Ng, 2009). Storage and processing techniques may have effects both on the gelatinization and retrogradation processes (García-Alonso, Jimenez-escrig, Martincarron, Bravo, & Sauro-calixto, 1999; Noda et al., 2008), which are critical factors in formation of RS3.

Studies have been carried out on different methods and materials for the production of resistant starch (Mohamed, Jamiliah, Abbas, Abdul Rahman, & Roselina, 2008; Nednapsis, Boonma, & Karuna, 2009; Pongjanta, Utaipattanaseep, Naivikul, & Piyachomkwan, 2008). Researchers have worked on the suitability of cassava for RS production and reported its suitability after debranching (Mutungi, Onyango, Jaros, Henle, & Rohm, 2009; Nednapsis, Patcharee, Karuna, & Onanong, 2010; Worawikunya, 2007). Steam cooking of legumes has been reported to increase the indigestible RS from 19 to 31% (Tovar & Melito, 1996). Nigeria has been ranked as the world’s largest producer of cassava since 2005 (FAOSTAT, 2012). Production of cassava in Nigeria in 2010 was estimated to reach 37.5 million tonnes (FAOSTAT, 2012). Thus, making cassava a good choice for RS formation with potentials as a food ingredient for manufacturing health food.
There are, however, limited information on the influence of steam cooking on the formation of resistant starch produced from cassava starch and the effects on the functional properties of starch. This research was conducted to investigate the effects of steam cooking as a processing method and storage on the formation of RS and the functional properties of the starch produced from two local varieties of cassava in Nigeria.

2. Materials and methods

2.1. Materials
The varieties of cassava used for this research work were TMS 30572 and 98/0581 which were obtained from International institute of Tropical Agriculture (IITA) Ibadan. This selection was based on the percentage yield and amylose content of CMD resistant cassava clones as reported by Sanni, Adebowale, Maziya-Dixon, and Dixon (2008). Other materials used were commercial isoamylase obtained from *Pseudomonas* sp. and were purchased from Sigma–Aldrich, Steinheim, Germany; Amyloglucosidase (EC. 3.2.1.3 from *Aspergillus niger*, 11,500 U/mL) and pancreatic-α-amylase which were obtained from SIGMA US All the chemicals and equipment used were of analytical standard.

2.2. Methods

2.2.1. Starch extraction
Starch was extracted from these two varieties of cassava using the standard methods of starch extraction (IITA, 2005). About 1 kg of fresh cassava tubers from each variety was used. The tubers were peeled, washed, grated with the grating machine (DANDREAs agrimport, model: 59911) and filtered through a muslin cloth. The filtrate was stirred with a stirring rod for 2 min and allowed to stand for 1 h to facilitate starch sedimentation. The top liquid was decanted and discarded. The water was changed several times to avoid fermentation. The remaining moist starch was then stirred up with water and washed several times to obtain a reasonably clean starch paste. The starch paste was thinly spread on trays and dried in the cabinet dryer at temperature of 50°C for about 10 h. The dried cassava starch samples were milled to a very fine particle size on a micro mill, and kept in zip-lock bags for further analyses.

2.2.1.1. Enzymatic debranching of cassava starch. Prior to debranching, the optimal concentration of isoamylase enzyme was determined. Cassava starch samples was debranched with enzyme isoamylase; an aqueous starch slurry (20% w/w) was cooked in a pan on an electric element at temperature of 85°C with conitnuous stirring for 15 min and autoclaved at 121°C for 15 min (pressure of 1.94 atm). The starch gel was suspended with 50 mmol/L sodium acetate buffer pH 3.5 to obtain the gel of 7.5% w/w. The gel was cooled to 50°C and 90 mU/g starch of isoamylase enzyme was added. The suspension was incubated in a shaking water bath at 50°C for 12 h. Enzyme activity was terminated by heating at 85°C for 30 min. The sample was then cooled to room temperature. Both the debranched and undebranched samples were then freeze-dried and packaged until further analyses.

2.2.1.2. Production of resistant starch by Steam cooking. The debranched and undebranched starch samples were subjected to steam cooking both at atmospheric pressure and high pressure as described by Alejandra, Fulgencio, and Delcour (1998). About 5 g of each of the samples both debranched and undebranched were weighed in a centrifuge tube and mixed with 40 ml of distilled water. The starch was steam cooked at atmospheric pressure for 30 min and at high pressure for 15 min, then cooled to room temperature with subsequent storage for 0, 24 and 48 h under refrigeration temperature (5–7°C) and freezing temperature (−28°C). The samples were dried in a commercial oven dryer at 45°C for a maximum of 12 h, pulverized to a fine particle size by a micro mill and kept in Zip-lock bags for further analyses.
2.2.2. Assessment of the properties of the native starch

The moisture content was determined by drying in an oven at 103°C until constant weight, the ash content by incineration in a muffle furnace at 600°C, the crude protein by Macro Kjeldahl method using 6.25 as the nitrogen to protein conversion factor, the fat content by soxhlet extraction and the crude fibre by acid-base digestion as described by AOAC (2005). Amylose/amylopectin contents of starch were determined using the total starch assay procedure (AACC, 2005).

2.2.2.1. Resistant starch determination. Resistant starch yield was determined as described by McCleary and Monaghan (2002). About 100 mg of the sample was weighed into a 50 ml centrifuge tube and 4 ml of 1.0 M sodium maleate buffer (pH 6.0) containing pancreatic α-amylase (10 mg/ml) and amyloglucosidase (3 U/ml) was added, the tube was covered with paraffin film, mixed and placed horizontally in a shaking water bath. The solution was incubated at 37°C with continuous shaking for 16 h. To the solution was added 4 ml of 99% ethanol to precipitate the starch and mixed vigorously on a vortex mixer. It was centrifuged at 1,500 rpm for 10 min. The supernatant was decanted and the residue rinsed twice with 8 ml 50% ethanol, followed by centrifugation at 3,000 rpm for 10 min.

The residue was re-suspended with 2 ml of 2 M potassium hydroxide in an ice bath with stirring for 20 min and 8 ml of 1.2 M sodium acetate buffer (pH 3.8) was added with 0.1 ml of amyloglucosidase (3,300 U/ml). The sample was mixed and incubated at 50°C with continuous shaking for 30 min. The sample was then diluted with water and centrifuged at 3,000 rpm for 10 min. The liberated glucose was determined which gave the measure of the resistant starch.

2.2.3. Functional property

The bulk density of the starch samples were determined by the method of Onwuka (2005). A 50 g flour sample was put into a 100 ml measuring cylinder and tapped to a constant volume. The bulk density (g/ml) was calculated as weight of starch (g) divided by flour volume (ml). Water absorption index (WAC) was determined using the method of Mbofung, Abubakar, Njintang, Abduo, and Balaam (2006). One gram of the flour was mixed with 10 ml of water in a centrifuge tube and allowed to stand at room temperature (30 ± 2°C) for 1 h. It was then centrifuged at 3,000×g for 10 min. The volume of water on the sediment water measured. Water absorption capacities were calculated as ml of water absorbed per gram of flour. Pasting characteristics were determined with a Rapid Visco Analyzer (RVA) (ModelRVA 3D+, Newport Scientific Australia) as described by Ikegwu, Nwobasi, Odoh, and Oledinma (2009). The pH was determined using a pH meter (cheaker 3 model).

2.2.4. Statistical analysis

All data were subjected to analysis of variance (ANOVA) using Statistical Analysis System Institute version 9.2 package. Means were separated using LSD Test at 5% level of probability (SAS Institute, 2002, 2003).

3. Result and discussion

3.1. Proximate composition

The composition of the cassava starch is as presented in Table 1. The composition of the starch obtained was within the range reported by Adebowale, Sanni, and Lawal (2010), Nuwamanya, Boguma, Emmambux, Taylor, and Patrick (2010) and Ajala, Otutu, and Bamgbose (2012) for cassava starches. The total starch obtained from the cassava roots were 89.23 and 88.9% and the total minor components were 1.13 and 1.07% for TMS 30572 and TMS 98/0581, respectively which is very close to the values recommended for high purity index (TTSA, 2007). The values obtained for the amylose content of the starch samples were within the range reported by Sanni et al. (2008) for improved cassava varieties.
3.1.1. Effect of steam cooking on resistant starch formation

The resistant starch contents of the two varieties of cassava starch subjected to steam cooking are as presented in Table 2. Steam cooking at atmospheric pressure gave higher RS contents than the samples steam cooked at higher pressure indicating that there may not be need to increase pressure in steam cooking process targeted at increasing RS contents. TMS 30572 had higher RS contents in the two steam cooking methods. This could be traced to the amylose content of the cassava starch. Enzymatic and physicochemical investigation has shown that, amylose crystallization during starch retrogradation is responsible for RS\textsubscript{3} formation. Amylose is reported to be more resistant to digestion than other starch molecules because of its tightly packed structure and it is usually referred to as more important factor in production of RS, indicating that the variety of cassava used in production of RS is very important especially the amylose content of the starch. Steaming cooking method generally increased the yield of resistant starch which is in line with the previous researchers; Sajilata, Singhal, and Kulkarni (2006) who reported that, resistant starch measured directly in conventionally and high-pressure steamed beans were 3–5 times higher than in the raw pulses and that prolonged steaming as well as short dry pressure.

3.2. Effect of debranching on formation of RS

The effect of the debranching process is as shown in Table 2. The debranching process was able to produce about 70% increase in RS contents over the undebranched starch samples as shown in Table 2. This is because the isoamylase enzyme catalyzes the hydrolysis of the $\alpha$-1,6 glycosidic bonds which would produce more free linear chains in the hydrolysate. Upon isoamylase debranching, the linear chains produced, similar to amylose, could participate in crystal formation by chain elongation and folding. These newly formed crystals are more perfect and firmer than the crystals of native starch granules and resist digestion in the small intestine hence increase the RS contents (Gao et al., 2011; Nednapis et al., 2010). The result is in line with other researchers that debranching of starch increases the RS content (González-Soto, Agama-Acevedo, Solorza- Feria, Rendón-Villalobos, & Bello-Pérez, 2004; Ozturk et al., 2009). Worawikunya (2007) reported that the more the debranching, process the higher the RS contents. Milasinovic et al. (2009) also reported that the resistant starch obtained from normal maize starch increased from 10.2 to 25.5% after debranching depending on degree of hydrolysis. Thus, debranching was a possible factor to improve RS content.

### Table 1. Composition of the two varieties of cassava starch

<table>
<thead>
<tr>
<th>Composition</th>
<th>TMS 30572</th>
<th>TMS 98/0581</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>89.23 ± 0.15</td>
<td>88.9 ± 0.17</td>
</tr>
<tr>
<td>Amylose</td>
<td>22.5 ± 0.50</td>
<td>21.0 ± 0.29</td>
</tr>
<tr>
<td>Amylopectin</td>
<td>77.5</td>
<td>79</td>
</tr>
<tr>
<td>Moisture content</td>
<td>12.30 ± 0.01</td>
<td>11.44 ± 0.01</td>
</tr>
<tr>
<td>Protein</td>
<td>0.17 ± 0.06</td>
<td>0.13 ± 0.12</td>
</tr>
<tr>
<td>Fat</td>
<td>0.37 ± 0.06</td>
<td>0.37 ± 0.15</td>
</tr>
<tr>
<td>Fibre</td>
<td>0.23 ± 0.12</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Ash</td>
<td>0.36 ± 0.02</td>
<td>0.37 ± 0.02</td>
</tr>
</tbody>
</table>

### Table 2. Resistant starch contents (g/100 g) of the two varieties of cassava starch obtained by steam cooking

<table>
<thead>
<tr>
<th>Variety</th>
<th>Atmospheric pressure</th>
<th>High pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Undebranched</td>
<td>Debranched</td>
</tr>
<tr>
<td>TMS 30572</td>
<td>6.01</td>
<td>19.55</td>
</tr>
<tr>
<td>TMS 98/0581</td>
<td>5.99</td>
<td>19.25</td>
</tr>
</tbody>
</table>
3.3. Effect of storage on RS formation

The RS content of cassava starch steam cooked at atmospheric pressure and stored under refrigeration and freezing condition for 48 h is as shown in Figure 1(a) and (b), respectively. RS contents of the cassava starch increased on storage in both refrigeration and freezing condition with increase in time of storage. Under a refrigerated condition, the resistant starch content of the variety 30572 increased from 6.01 to 6.190 and 19.55 to 21.25 g/100 g, for undebranched and debranched samples, respectively while for freezing condition it increased from 6.01 to 6.13 and 19.55 to 20.55 g/100 g, for undebranched and debranched samples, respectively. The same trend of increase was also observed in the variety 98/0581. The RS contents of cassava starch steam cooked at High pressure and stored under refrigeration and freezing condition is as shown in Figure 2(a) and (b). There were increases in the RS contents with increase in storage time. This is in line with previous researchers reports (Dundar & Gocmen, 2013; Kavita, Varghese, Chitra, & Jamuna, 1998; Namratha, Asna, & Prasad, 2002), that storage has beneficial impacts on RS formation. The lower values obtained in the samples stored under freezing condition could be attributed to lower availability of water compared to the refrigerated samples.

4. Effect of steam coking on the functional properties of cassava starch

The effects of steam cooking process on the functional properties of both undebranched and debranched starch of TMS 30572 and TMS 98/0581 are as shown in Table 3. The bulk density for both undebranched starch and debranched starch for the two varieties was 0.67 g/cm³. The results indicate that the steam cooking process does not have effect on the bulk density of the starch and formation of RS in foods may not require extra packaging. Bulk density is generally affected by the particle size and the density of the flour and it is very important in determining the packaging requirement, material handling and application in wet processing in the food industry (Abioye, Ademowoaye, Babarinde, & Adesigbin, 2011). The swelling power of the starch samples ranged between
10.78 and 20.09%. Swelling power is an important parameter especially in characterization of starches from different botanical origins which display different swelling powers at a given temperature. High swelling power results into high digestibility and ability to use starch in solution suggesting improved dietary properties and the use of starch in a range of dietary applications (Nuwamanya et al., 2010). Swelling power of starch is inhibited by amylose and lipids and that it is also a function of amylopectin rather than amylose (Sasaki & Matsuki, 1998). Akanbi, Nazamid, and Adebowale (2009) also reported that the swelling power has been related to the negative correlation between associative binding within the starch granules and apparently, the strength and character of the micellar network. Lower values were recorded with samples debranched and steam cooked than the undebranched starch samples which could be as a result of process which involves decreasing the amylopectin and increasing more of amylose that resulted in decrease in swelling power.

Water absorption index (WAI) values were between 2.99 and 8.48 g/g. Higher values were obtained in undebranched starch samples. WAI reflects the ability of starch to absorb water and is an indirect measure of the amount of intact and fully gelatinized starch granules. This implies that the steam cooking process had effects on the water absorption index of the starch sample and the result is in agreement with the result of Worawikunya (2007). The water solubility index (WSI) is used as a measure for starch degradation which depends on several factors such as starch origin, amylose and amylopectin content, isolation procedure and thermal history (Narbutaite, Makaravicius, Juodeikiene, & Basinskiene, 2008; Singh & Smith, 1997). The WSI decreased with increase in the formation of RS indicating that the lower the RS contents, the higher the water solubility index.

The degree of syneresis increased with increase in the formation of RS. Syneresis characterizes the starch stability during storage and is also an index of starch retrogradation at low temperature. It increased with increase in the steam cooking and hence in the formation of RS; this is in line with the previous studies that resistant starch is as a result of retrograded starch. This shows that the starch with high contents of resistant starch may not be stable on storage especially at low temperatures.

### 5. Conclusion

Based on the research carried out, it could be inferred that the variety, debranching process, processing method and storage condition and time had effect on formation of resistant starch. Indicating that in the choice of cassava variety for the production of resistant starch, the percentage amylose content of the cassava starch is of great importance. A debranching process with isoamylase is suitable for partially debranching amylopectin molecules of the cassava starch. Steam cooking at atmospheric pressure had higher yield of resistant starch than the samples steam cooked at higher pressure. Indicating that there may not be need to spend extra cost in increasing the pressure. Steam Cooking process in formation of RS resulted in a decrease in the swelling power, water absorption and water solubility of the starch while the syresis value increased.
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Competing Interest
The authors declare no competing interests.

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