

## Effect of autoclaving on the formation of resistant starch from two Nigeria Cassava (*Manihot esculenta*) varieties

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### Abstract

This study evaluated the effect of autoclaving process on the production of resistant starch (RS) from cassava starch. RS was prepared by debranching, autoclaving and storage of cassava starch from two Nigeria varieties (TMS 30572 and TMS 98/0581). Starch suspensions were prepared with different starch water ratios (1:1; 1:3; and 1:5), debranched with isoamylase, autoclaved at 110 and 121°C for four heat and cool cycles, stored under refrigeration and freezing condition for 48 h. Debranching process increased RS by about 73-78%. Higher RS was obtained at higher temperature and with significant difference ( $p < 0.05$ ) from each other. RS increased with storage time from 6.23 to 9.60 and 22.40 to 25.77 g/100 g for unbranched and debranched samples, respectively, after 48 h. This study indicated the potentials of these Nigeria cassava varieties in the production of RS which could serve as functional food.

## 1. Introduction

There is a greater awareness on the part of consumers of the relationship between nutrition and health which has led to the popularity of novel foods with good nutritional and health potentials (Azzurra and Paola, 2009). There is growing interest in novel foods with substances that promote health such as resistant starch. Resistant starch (RS) has been defined as the sum of starch and products of starch breakdown that is not absorbed in the small intestine of healthy individuals (Englyst *et al.*, 1992; Muir *et al.*, 1993; Öztürk and Koksel, 2014). It reaches to the colon and then fermented by beneficial microorganisms in the colon, resulting in the production of short chain fatty acids mainly acetic, propionic and butyric acids (Baghurst *et al.*, 1996). They directly affect the large intestine by decreasing the pH value, which prevents the growth of pathogenic microorganisms and increases the potential for mineral absorption. Fatty acids stimulate colonic blood flow and increase nutrient flow (Haralampu, 2000; Topping and Clifton, 2001; Schwiertz *et al.*, 2002; Champ, 2004; Chun-Ho *et al.*, 2013).

The slow hydrolysis of RS makes it useful for the slow release of glucose, which can be especially useful in controlling glycemic plasma responses (Raben *et al.*, 1994). RS is a non-caloric food component that does not contribute to the increase in blood glucose. In this, it has physiological effects in the human body that are similar

to that of dietary fiber, which has been shown to reduce risks for some diseases, including colon cancer, coronary heart disease and glycemia (Ranhotra *et al.*, 1996; Champ *et al.*, 1999). Some other benefits include increased faecal bulk and increased excretion of butyrate and acetate. Besides physiological benefits in human, RS has been reported to have potential as a unique ingredient that can yield high-quality foods. For example, application tests of RS showed improved crispness and expansion in certain products and better mouthfeel, colour, and flavour as compared with products produced with traditional, insoluble fibres (Yue and Waring, 1998; Milasinovic *et al.*, 2010; Sharma *et al.*, 2016).

Classification of RS is generally made into four categories (RS<sub>1</sub>–RS<sub>4</sub>) based on the mechanism that contributes to their resistance to digestion (Sajilata *et al.*, 2006). RS<sub>1</sub> is the starch that escapes digestion because it is physically inaccessible by entrapment in a non-digestible matrix, they are found in partly milled grains, seeds, and legumes. RS<sub>2</sub> consists of raw starch granules (ungelatinized) which have retained their crystal structure; therefore, they are not attacked by digestive enzymes, they are found in raw potato, banana, and high-amylose corn starches. RS<sub>3</sub> consists mainly of retrograded or recrystallized amylose (Garcia-Alonso *et al.*, 1999), this can be found in bread, corn flakes, or potatoes. RS<sub>4</sub> can be produced by chemical

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modifications, such as conversion, substitution, or cross-linking. Such modifications prevent digestion of RS<sub>4</sub> by blocking access to enzymes and by forming typical linkages, examples are starch phosphates, hydroxypropyl starches, starch acetates and citrate (Wepner *et al.*, 1999; Dundar and Gocmen, 2013).

Among different resistant starches, retrograded resistant starch (RS<sub>3</sub>) has great commercial importance since its crystalline polymorphs exhibit an endothermic transition from 120 to 165°C that typically survives most, but not all, food processing conditions (Milasinovic *et al.*, 2009). The degree of formation of RS in foods depends on the type of starch, processing condition adopted and is also influenced by the duration and storage conditions (Chou *et al.*, 2014). Processing techniques include baking, pasta production, extrusion cooking, steam cooking, autoclaving and others (Sajilata *et al.*, 2006). Autoclaving has been reported in the formation of resistant starch from maize starch, high amylose corn starch, pulses and in cassava (Sajilata *et al.*, 2006).

Commercially, the starches used in preparing RS<sub>3</sub> are derived from high amylose corn starch containing greater than 40% amylose. The current trend in this research area is the investigation of alternative sources for RS production. Resistant starch had been produced from different local crops like sago, maize, banana, rice and cassava (Mohamed *et al.*, 2008; Pongjanta *et al.*, 2008; Vatanasuchart *et al.*, 2009). Native cassava starch contains amylose which ranges from 19.6 to 24.1%. It has been reported to be suitable after amylopectin debranching (Worawikunya, 2007; Mutungi *et al.*, 2009 and Vatanasuchart *et al.*, 2010). Nigeria is the world's largest producer of cassava. It has been estimated that Nigeria's production of cassava reached 45 million tonnes annually. The country has consistently been ranked as the world's largest producer of cassava since 2005 (FAOSTAT, 2012). Thus, making cassava starch a good choice for RS formation, with potentials as a food ingredient for manufacturing health food.

However, there is scanty information on the influence of autoclaving on the formation of resistant starch from cassava starch especially from Nigerian Cassava varieties. The main objective of this research work was to explore the availability of improved varieties of cassava in Nigeria, in the production of resistant starch to enhance the use of cassava starch as a functional food and an industrial product. Thus, this study evaluated the effects of isoamylase debranching, different autoclaving condition and storage on the formation of resistant starch.

## 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 the International Institute of Tropical Agriculture (IITA) Ibadan. This selection was based on the percentage yield and amylose content of cassava mosaic disease-resistant cassava clones as reported by Sanni *et al.* (2008). The enzymes used were commercial isoamylase obtained from *Pseudomonas sp.* and was purchased from Sigma-Aldrich, Steinheim, Germany; Amyloglucosidase (EC. 3.2.1.3 from *Aspergillus niger*, 11, 500 U/mL) and pancreatic- $\alpha$ -amylase which were obtained from SIGMA U.S. All the chemicals used were of analytical grade.

### 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, 1990). About 1 kg of fresh cassava tubers from each variety was used. The tubers were peeled, washed, grated with the grating machine (DANDREA agrimport, model: 59911) and in excess of water, 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 a cabinet dryer (Model LEEC F2). The cabinet dryer consists of an insulated chamber fitted with perforated trays. The drying process was achieved at a temperature of 50°C for about 10 hrs. The dried cassava starch samples were milled on a micro mill, sifted through 212  $\mu$ m sieve and kept in zip-lock bags for further analyses.

#### 2.2.2 Enzymatic debranching of cassava starch

The debranching of the cassava starch was carried out as described by Mutungi *et al.* (2009). Prior to debranching, the optimal concentration of isoamylase enzyme was determined. Cassava starch samples were debranched with enzyme isoamylase; an aqueous starch slurry (20% w/v) was cooked in a pan on an electric element at a temperature of 85°C with continuous stirring for 15 mins and autoclaved at 121°C for 15 mins (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/v. 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 hrs. Enzyme activity was terminated by heating

at 85°C for 30 mins. The sample was then cooled to room temperature. Both the debranched and undebranched samples were then freeze-dried (Labconco FreeZone Plus 4.5 Liter Cascade Console Freeze Dry Systems (Kansas City, MO) and packaged until further analyses.

### 2.2.3 Production of resistant starch by autoclaving

The debranched and undebranched starch samples were subjected to autoclaving using the method of Milasinovic *et al.* (2009). Starch suspension was made at starch-to-water ratio of 1:1, 1:3, and 1:5 with distilled water. The suspensions were autoclaved at temperatures of 110°C and 121°C for 15 mins. The autoclaving and cooling of samples were done in four cycles (Sangick *et al.*, 2004). Samples were then subsequently cooled to room temperature with subsequent storage for 0 hrs, 24 hrs and 48 hrs 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 hrs, pulverized to a fine particle size by a micro mill, sifted through 212 µm sieve and kept in Zip-lock bags for further analyses.

### 2.3 Resistant starch determination

Resistant starch content was determined as described by McCleary *et al.* (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 hrs. 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 1500 rpm for 10 mins. The supernatant was decanted and the residue rinsed twice with 8 mL 50% ethanol, followed by centrifugation at 3,000 rpm for 10 mins. The residue was re-suspended with 2 mL of 2 M potassium hydroxide in an ice bath with stirring for 20 mins and 8 mL of 1.2 M sodium acetate buffer (pH 3.8) was added with 0.1 mL of amyloglucosidase (3300 U/ml). The sample was mixed and incubated at 50°C with continuous shaking for 30 mins. The sample was then diluted with water and centrifuged at 3,000 rpm for 10 mins. The glucose was quantified with glucose oxidase/peroxidase reagent (GOPOD), which gave a measure of the RS content of the sample.

### 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 (DMRT, 1955) at 5% level of probability.

## 3. Results and discussion

### 3.1 Effect of debranching on formation of resistant starch

RS obtained from debranched cassava starch had more than 70% increase in resistant starch contents as shown in Tables 1 and 2. The debranching process partially debranched amylopectin molecules of the cassava starch and consequently providing small linear fragments and small clusters of the amylopectin molecules for retrogradation/recrystallization and hence the formation of more resistant starch. This is in line with reports of Berry (1986), Vatanasuchart *et al.* (2010) and Babu and Parimalavalli (2018) who reported that debranching of amylopectin with pullulanase before

Table 1. Effect of autoclaving at 110°C on formation of resistant starch content (g/100 g)

Variety	Cooling cycle	Undebranched			Debranched		
		1:1	1:3	1:5	1:1	1:3	1:5
30572	1	6.21 <sup>d</sup>	6.07 <sup>f</sup>	6.24 <sup>d</sup>	22.63 <sup>c</sup>	22.47 <sup>c</sup>	22.51 <sup>c</sup>
	2	7.52 <sup>c</sup>	7.39 <sup>d</sup>	7.54 <sup>b</sup>	24.31 <sup>c</sup>	23.79 <sup>b</sup>	23.95 <sup>c</sup>
	4	9.07 <sup>a</sup>	9.01 <sup>b</sup>	9.14 <sup>a</sup>	25.93 <sup>a</sup>	25.31 <sup>a</sup>	23.43 <sup>a</sup>
98/0581	1	6.18 <sup>d</sup>	6.19 <sup>c</sup>	6.11 <sup>d</sup>	22.41 <sup>f</sup>	22.52 <sup>c</sup>	22.30 <sup>c</sup>
	2	7.51 <sup>c</sup>	7.52 <sup>c</sup>	7.45 <sup>c</sup>	23.74 <sup>d</sup>	23.84 <sup>b</sup>	23.63 <sup>d</sup>
	4	8.98 <sup>b</sup>	9.17 <sup>a</sup>	8.96 <sup>a</sup>	25.27 <sup>b</sup>	25.30 <sup>a</sup>	25.12 <sup>b</sup>

Means with the same alphabet in the same column are not significantly different ( $p>0.05$ )

Table 2. Effect of autoclaving at 121°C on formation of resistant starch content (g/100 g)

Variety	Cooling cycle	Control			Debranched		
		1:1	1:3	1:5	1:1	1:3	1:5
30572	1	6.70 <sup>d</sup>	6.52 <sup>c</sup>	6.15 <sup>c</sup>	22.90 <sup>c</sup>	22.70 <sup>d</sup>	22.41 <sup>c</sup>
	2	7.90 <sup>c</sup>	7.87 <sup>b</sup>	7.35 <sup>d</sup>	24.34 <sup>b</sup>	23.92 <sup>c</sup>	23.63 <sup>b</sup>
	4	9.21 <sup>b</sup>	9.19 <sup>a</sup>	8.82 <sup>b</sup>	25.72 <sup>a</sup>	25.40 <sup>a</sup>	25.12 <sup>a</sup>
98/0581	1	6.79 <sup>c</sup>	6.50 <sup>c</sup>	6.21 <sup>c</sup>	23.01 <sup>c</sup>	22.71 <sup>d</sup>	22.43 <sup>c</sup>
	2	8.07 <sup>b</sup>	7.75 <sup>b</sup>	7.90 <sup>c</sup>	24.33 <sup>b</sup>	23.92 <sup>c</sup>	23.86 <sup>b</sup>
	4	9.64 <sup>a</sup>	9.21 <sup>a</sup>	9.13 <sup>a</sup>	25.97 <sup>a</sup>	24.80 <sup>a</sup>	25.40 <sup>a</sup>

Means with the same alphabet in the same column are not significantly different ( $p>0.05$ )

subjecting it to heating and cooling cycles substantially increased the RS content and this was attributed to an increase in the content of linear starch chains as a result of debranching.

### 3.2 Effect of autoclaving on formation of resistant starch

The result of the resistant starch content obtained from autoclaving at 110°C is as shown in Tables 1. RS content obtained with starch/water ratio of 1:1 for the undebranched starch sample was 6.21 and 6.18 g/100 g, respectively, for TMS 30572 and TMS 98/0581. The RS contents increased to 9.07 and 8.98 g/100 g after four heat and cool cycles. RS of debranched samples increased from 22.63 and 22.41 to 25.93 and 25.27 g/100 g in TMS 30572 and TMS 98/0581, respectively. The same trend was observed in the samples with starch/water ratio of 1:3 and 1:5. RS content increased significantly ( $p < 0.05$ ) with heat and cool cycles, while there were differences in the resistant starch content with effect of starch/water ratio but not all, were significant ( $p > 0.05$ ). Highest RS obtained for the undebranched and the debranched sample was 9.17 g/100 g and 25.93 g/100 g, respectively.

The RS contents increased more than 18% for all the starch/water ratios after four autoclaving heat and cool cycles. This is in line with the other reports that the formation of RS in maize starch was affected by a number of autoclaving heat and cool cycles (Sajilata et al., 2006; Koxsel et al., 2007, Ozturk et al., 2011; Dundar and Gocmen, 2013). Milašinović et al. (2009) also reported that the starch/water ratio did not significantly affect the RS yields but the number of autoclaving heat and cool cycles did. Repeated heat/moisture treatments have been reported to have effects on the hydrolysis limit of pancreatic  $\alpha$ -amylase and hence increase in RS (Haralampu, 2000).

The values of the resistant starch obtained from autoclaving at 121°C are as shown in Table 2. The values of resistant starch with starch/water ratio of 1:1 for the debranched starch sample ranged between 6.70 and 9.21 and 6.79 and 9.64 g/100 g, in TMS 30572 and TMS 98/0581, respectively. There were increases in the resistant starch content with the increase in the number of autoclaving heat and cool cycles and these increases were significant ( $p < 0.05$ ). Higher values of resistant starch contents were recorded for samples autoclaved at 121°C than those autoclaved at 110°C and the differences were significant ( $p < 0.05$ ). The mean effects of autoclaving temperatures on the resistant starch contents confirmed that higher autoclaving temperature has a beneficial impact on resistant starch formation as reported by Dundar and Gocmen (2013) who studied the effects of autoclaving temperatures on the formation of

resistant starch.

### 3.3 Effect of storage on formation of RS

The effect of storage on formation of RS was determined using the starch sample steam cooked at 121°C with starch water ratio 1:1 heated and cooled after four times stored under refrigeration and freezing conditions for 48 hrs based on the resistant starch contents. The result is as shown in Figure 1. Storage both at refrigeration and freezing conditions increased the formation of RS. The longer the storage time the higher the RS contents, indicating that storage condition and time had effects on the formation of resistant contents. This is in line with previous findings that low storage temperature increased the resistant starch content while the major changes had been attributed to retrogradation of starch (Kavita et al., 1998; Namratha et al., 2002; Agama Acevedo et al., 2004; Ramakrishnan, 2009; Jagannadham et al., 2017). The highest RS value obtained was 9.72 g/100 g and 26.52 g/100 g for both undebranched and debranched samples, respectively which were obtained under refrigeration after 48 hrs of storage.

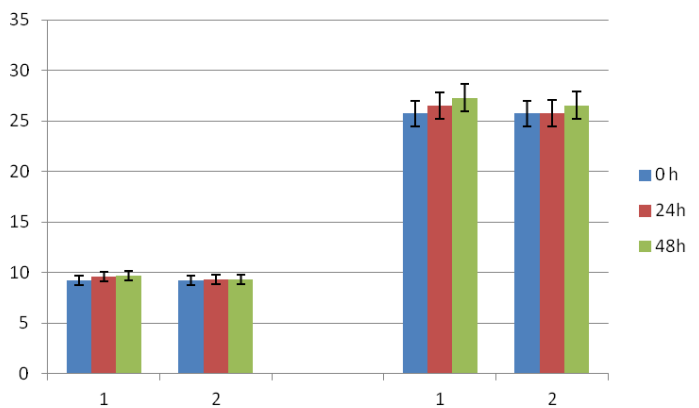


Figure 1. Effect of storage condition on formation of RS in undebranched and debranched starch samples. 1 - Refrigerated samples. 2 - Frozen samples.

## 4. Conclusion

Based on the research carried out, it could be inferred that the variety, debranching process, autoclaving temperature and storage conditions and time had effects on the formation of resistant starch. A debranching process with isoamylase is suitable for partially debranching amylopectin molecules of the cassava starch. Autoclaving at the temperature of 121°C had higher yield of resistant starch contents than the samples autoclaved at 110°C. Heat and cool cycles and storage condition had effects on the formation of RS.

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