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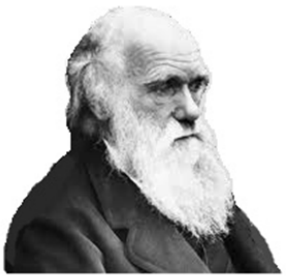

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Enzymatic Hydrolytic Evaluation of Different Cooking Methods on Resistant Starch of Yam and Rice

Chisom Nwokolo[1,*], Bennett Nwanguma[2]

[1] Department of Genetics and Biotechnology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria

[2] Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria

*E-mail address: chisom.nwokolo@unn.edu.ng

ABSTRACT

Food envy is a feeling of isolation experienced by diabetic and obese individuals who have dietary restrictions that prevent them from eating their preferred starchy foods. This can lead to feelings of exclusion and exacerbation in social situations. The psychological strain and emotional toll of avoiding certain foods due to medical restrictions can have negative effects on a person's health, including a higher risk of eating foods to fit in with society and worsen their medical conditions. Rice and yam are popular foods in South Eastern Nigeria, but there is limited information on cooking methods to increase the resistant starch. This lack of information can lead to frustration and feelings of deprivation, making it harder for individuals to adhere to their dietary restrictions. Limited food options may result in nutrient deficiencies, compromising their overall health and well-being. This study aimed to study the effect of different cooking methods, namely conventional and steaming methods, and different cooling methods at room temperature and in the fridge on the resistant starch contents as well as the non-resistant starch contents of rice and yam. The result showed the highest increase in resistant starch of rice (from 1.24 ± 0.42^{a} to 7.41 ± 0.32^{b}) and the highest decrease in the non-resistant starch (from 86.97 ± 2.58^{a} to 36.87 ± 4.87^{b}) when cooked by steaming method and cooled in the fridge at 4 °C for 12 hours. The cooking methods only had a significant increase in the resistant starch of yam only when cooked by conventional boiling method and cooled at room temperature (2.00 ± 0.53^{a} to 5.28 ± 0.18^{b}), though all the cooking methods generally had a decrease in the non-resistant starch contents of yam except the conventional boiling method when cooled at room temperature (84.53 ± 2.60^{a} to 82.55 ± 5.12^{a}).

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***Keywords*:** Resistant starch, Rice resistant starch, Yam resistant starch, Metabolic syndrome, Diabetic food, Low glycemic food

1. INTRODUCTION

Resistant starch (RS) is a type of carbohydrate that is partially or wholly resistant to digestive enzymes [1]. The passage of the undigested starch into the colon, where it serves as a prebiotic and undergoes fermentation to metabolize into short-chain fatty acids (SCFA) such as butyrate, which has great health benefit in the system. Notwithstanding, this definition is largely based on the molecular size of carbohydrate polymer and their level of digestibility by the digestive enzymes, as proposed [2], resistant starch is classified based on the digestive rate of the starch into rapidly digestible starch, slowly digestible starch, and resistant starch. The impact of the influence of the gut microbiota on the fermentation of resistant starch that gives rise to health-beneficial metabolites should be the major focus of the functional properties of resistant starch since the changes that occur in the compositions of fermented metabolites in the gut microbiota can also be a result of the same type of resistant starch [3]. Studies have shown different metabolites of resistant starch fermentation in the colon, indicating a high influence on changes that occur in the human microbiome caused by the metabolic influence of resistant starch activities in the colon [4]. A highly resistant starch diet causes an increase in the ratio of *Firmicutes* to *Bacteroidetes*, an increase in the abundance of some members of *Firmicutes* and concurrent increased enzymatic pathways and metabolites involved in lipid metabolism in the gut [3]. The microbiota family of *Firmicutes* are involved in the breakdown of carbohydrates in the gut that cannot be digested by the body's enzyme through the process of fermentation while *Bacteroidetes* plays the key role in some functions such as production and conversion of energy, transport and metabolism of amino acid and carbohydrates [5]. This functional property of resistant starch is key in energy production, metabolisms, transport of essential SCFA, amelioration of inflammation, oxidative stress, and improved absorption of minerals.

While there is great promise for the enormous health benefits of resistant starch, the consumption pattern is still not sufficient to bring about a significant health outcome in the population that is expected from consuming the right amount of foods high in resistant starch. The majority of the populations still consume foods high in rapidly digestible starch such as bread, cereals, biscuits and much more. This rapidly digestible starch increases glucose absorption, thereby potentiating a hyperglycemic response and triggering insulin secretion and tissue-specific intracellular uptake of glucose that can result in hypoglycemia. This repetition of hypo and hyperglycemia cycles result in insulin resistance to type 2 diabetes and also results in obesity [6].

Rice and yam being one of the major staple foods in Nigeria is considered to have a high glycemic index and low resistant starch [7], [8], although many strategies have been applied to develop a healthier rice [9] none of them have addressed a low-budget easy to be replicated home cooking method. The treatments used in increasing resistant starch in food samples range from amylose level manipulation in plants [10], enzymatic hydrolysis [11], physical treatments [12], chemical modifications [13], exposure to γ-rays [14], and the effect of lipid complexation on the RS content of starches from different botanical sources [15]. The treatments reviewed showed increase in the RS content; but treatments such as, genetic manipulation, enzymatic

debranching, hydrothermal treatments, high hydrostatic pressure, most chemical modifications, γ-irradiation exposure, as well as lipid complexation were shown to be more effective to varying degrees than were extrusion and mineral acid treatments [15]. Optimization of ultrasonic processing parameters applied to develop a highly resistant starch in Rice bran matrixes [16], showed an increased mechanical and thermal stability of RS paste at 40 – 70 °C temperature, indicating a higher tendency to retrogradate. This method is not common in household cooking patterns since whole-grain white rice and yam have more digestible starch than other starchy foods, hence the essence of this study which aims to study low-cost methods that can be applied to improve the resistant starch contents of native cultivated Adani rice and yam while concurrently reducing the non-resistant starch contents as well.

2. MATERIALS AND METHODS

2. 1. Materials

2. 1. 1. Sample Collection

Rice (Adani Rice) and Yam of native origin were obtained from Ogige Market Nsukka, In Nsukka L.G.A, Enugu State, Nigeria

2. 1. 2. Reagents

Alpha Amylase (Termamyl®) (Novozymes A/S), Glucoamylase (AMG®) (Novozyme A/S), Calcium Chloride dehydrate (May and Baker limited, England), Sodium azide (Merck, Germany), Maleic Acid (Merck, Germany), Sodium Hydroxide (Merck, Germany), Glacial Acetic acid (May and Baker limited, England), Potassium Hydroxide (Merck, Germany), Ethanol (Merck, Germany), 3,5-dinitrosalicylic acid (DNS) (Merck, Germany), Sodium potassium tartarate (Merck, Germany), Potassium iodide (Merck, Germany). All other chemicals used were of analytical grade.

2. 1. 3. Equipments

Andrew James Multifunctional food processor (United Kingdom), Bench centrifuge (4000 rpm Abman), Shaking Water bath (Gallenkamp, England), Vortex Mixer (Gallenkamp, England), Magnetic Stirrer (EI 310 Model, Japan), PH Meter (Hanna), Stop Watch/timer (Sunny), Analytical balance (correct to 0.1mg), Spectrophotometer (Jenway 6305, China), Pipettor, Glass test tubes, Glass rod thermometer, Volumetric Flasks. Electric Oven, 4.5 mm screen, Plastic “Lunch Box”, Refrigerator (Haier Thermocool).

2. 2. Methods

2. 2. 1. Grouping of the samples

The food items were cooked by the method of conventional boiling for 10 minutes, and steaming for 15 minutes and was cooled at normal room temperature and in the fridge at 4 °C for 12 hours and were grouped as follows:

Group 1: RCR (Rice Conventional Room) Rice Cooked by boiling and cooled at room temperature for 12 hours

Group 2: RCF (Rice Conventional Fridge) Rice Cooked by boiling and cooled in the fridge at 4 °C

Group 3: RSR (Rice Steam Room) Rice cooked by steaming method and cooled at room temperature for 12 hours

Group 4: RSF (Rice Steam Fridge) Rice cooked by steaming method and cooled in the fridge at 4 °C for 12 hours

Group 5: YCR (Yam Conventional Room) Yam Cooked by boiling and cooled at room temperature for 12 hours

Group 6: YCF (Yam Conventional Fridge) Yam Cooked by boiling and cooled in the fridge at 4 °C for 12 hours

Group 7: YSR (Yam Steam Room) Yam cooked by steaming method and cooled at room temperature for 12 hours

Group 8: YSF (Yam Steam Fridge) Yam cooked by steaming method and cooled in the fridge at 4 °C for 12 hours

2. 2. 2. Cooking of Adani rice by conventional method

The rice sample was sieved after being sorted to remove any potential stones. In conventional cooking method, 100 ± 5 g of rice were washed with warm water and kept in a sieve for the remaining water to drain. After heating a cup of water to boiling point, the rice sample was introduced into the boiling water and cooked for 10 minutes in a closed pot with constant monitoring of the boiling sample to ensure the contents did not dry up. After 10 minutes of counting, the remaining water in the sample were drained and the sample was divided into two halves. Half of it was kept at room temperature in a partially open plate to cool overnight while the other half was kept in the fridge at 4 °C to cool for 12 hours

2. 2. 3. Cooking of Adani rice by steam method

For the steaming method, 100 ± 5 g of rice was washed with warm water and kept in a sieve for the remaining water to drain. A pot containing some water was heated until it began to boil. An iron sieve was fitted on the top of the pot to allow only steam to pass through without direct contact with the boiling water; the washed rice was then introduced into the sieve and allowed to boil for 15 minutes, after which the rice was brought down and divided into two halves. Half of it was kept at room temperature in a partially open plate to cool overnight while the other half was kept in the fridge at 4 °C to cool for 12 hours

2. 2. 4. Cooking of yam by conventional method

After peeling the yam and washing with water, an appreciable quantity of the yam (not more than 60g) was sliced into small chops and introduced into a boiling water and was left to boil for 10 minutes in a closed pot with constant monitoring to avoid burning the sample with dry heat. After the 10 minutes of counting, the boiled yam was kept in a dry container and was divided into two halves. With half kept at room temperature while the other half was in the fridge to cool for 12 hours.

2. 2. 5. Cooking of yam by steam method

From the peeled and washed samples, the yam was put into an iron sieve and placed on top of the pot with boiling water so that steam could easily pass through. This was allowed to boil for 15 minutes, after which the yam was brought down and divided into two halves. Half of it was kept at room temperature in a partially open plate to cool for 12 hours while the other half was kept in the fridge at 4 °C to cool for 12 hours.

2. 2. 6. Cooling and reheating of the samples

After each cooking method, half of the samples were cooled at room temperature while the other half were cooled in the fridge at 4 °C for 12 hours and the same methods of cooking and cooling used for conventional and steaming methods were repeated for each sample after the 12 hours has elapsed.

2. 2. 7. Moisture content determination

The moisture content was determined using the method as described in ISO 1666 method by drying the sample to constant weight. An empty dish was dried for half an hour in an oven at 130 °C and cooled in a desiccators for 40 minutes to room temperature and weighed on weighing balance to the nearest 1 mg. Weight = W_0. 5 g of the samples were distributed in a uniform layer in the dish and the dish lid was closed and weighed to the nearest I mg. Weight = W_1. The empty dish with the sample was placed in the oven preheated to 130 °C. The samples were then dried for 90 minutes after the oven has once again reached 130 °C. The lid was closed and cooled in the desiccator for 30 to 45 minutes and the dish was weighed immediately after removal from dessiccator to the nearest 1 mg. weight = W_2. The percentage moisture content was calculated using the formula

$$100 \times (W_2 - W_0) / (W_1 - W_0) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

2. 2. 8. Assay Procedure

The method as described and accepted by AOAC Official method 2002.02 and AACC method 32-40.01 which allows the measurement of resistant starch, solubilized/non resistant starch and total starch content of samples was used in the assay procedure as outlined in Megazyme resistant starch assay procedure with slight modification.

2. 2. 8. Principle

Samples were incubated in a shaking water bath with α-amylase and amyloglucosidase (AMG) for 16 hr at 37 °C, during which time non-resistant starch was solubilized and hydrolysed to D-glucose by the combined action of the two enzymes. The reaction was terminated by the addition of an equal volume of ethanol and the RS recovered as a pellet on centrifugation. This is then washed twice by suspension in aqueous ethanol (50% v/v), followed by centrifugation. Free liquid was removed by decantation. RS in the pellet was dissolved in 2 M KOH by vigorously stirring in an ice water bath over a magnetic stirrer. This solution is neutralized with acetate buffer and the starch was quantitatively hydrolyzed to glucose with AMG. D-glucose was measured using DNS (3,5–dinitrosalicylic acid). Non-resistant starch

(solubilized starch) was determined by pooling the original supernatant and the washing, adjusting the volume to 100 ml and measuring D-glucose content with DNS.

2. 2. 9. Hydrolysis and solubilization of non-resistant starch

100 mg of the samples were accurately weighed into screw cap tubes, and the tubes were gently tapped to ensure that the samples fall to the bottom. 4.0 ml of dilute α-amylase containing amyloglucosidase were added to each tube. The tubes were tightly capped and mixed on a vortex mixer before arranging them horizontally in a shaking water bath and allowed to incubate at 37 °C for 16 hours with continuous shaking. The tubes were removed from the water bath after 16 hours incubation and excess water on the body of the tubes were drained with paper towel. The tube caps were removed and the contents treated with 4.0 ml of ethanol (99% v/v) with vigorous stirring on a vortex mixer. The tubes were centrifuged at 1,500 g (approx. 3,000 rpm) for 10 minutes (non-capped). The supernatant were carefully decanted and the pellets re-suspended in 2 ml of 50% ethanol with vigorous stirring on a vortex mixer. A further 6.0 ml 50% ethanol was added and the tubes were mixed and centrifuged again at 1,500 g for 10 minutes. The supernatant was decanted and the suspension and centrifugation step was repeated once more. The supernatant were carefully decanted and the tubes inverted on absorbent paper to drain excess liquid.

2. 2. 10. Measurement of resistant starch

A magnetic stirrer bar and 2.0 ml of 2 M KOH were added to each tube and the pellets were re-suspended by stirring the samples for approx. 20 minutes in an ice/water bath over a magnetic stirrer. 8.0 ml of 1.2 M sodium acetate buffer (PH 3.8) was added to each tube with stirring on the magnetic stirrer. 1.1 ml of AMG (300 KNU.ml) was immediately added and well mixed. The tubes were then placed in a water bath at 50 °C for 30 minutes with intermittent mixing on a vortex mixer. After 30 minutes count, the tubes were centrifuged at 1,500 g for 15-20 minutes. 1.0 ml of the supernatant from each tube was treated with 1.0 ml of DNS (3,5 – dinitrosalicyclic acid) and the tubes were boiled for 10 minutes after which sodium potassium tartarate was used to stabilize the colour and the absorbance was read at 571 nm. The Resistant starch was calculated as dry weight of the sample analysed not as its weight that includes the moisture content effect using the following formula.

$\triangle$ E x F x 10.3/0.1 x 1/1000 x 100/W x 162/180
= $\triangle$E x F/W x 9.27
$\triangle$ E = absorbance (reaction) read against the reagent blank
F = conversion from absorbance to microgram
100/0.1 = volume correction
1/1000 = conversion from micrograms to milligrams . . . . . (2)
W = dry weight of sample analysed
= “as is” weight x [(100 – moisture content)/100].
100/W = factor to present RS as a percentage of sample weight.
162/180 = factor to convert from free D-glucose, as determined, to anhydrous –D-glucose as occurs in starch.
10.3/0.1 = volume correction

2. 2. 11. Measurement of non-resistant Starch

The supernatants obtained on centrifugation of the initial incubation were combined together with supernatants obtained from the subsequent two 50% ethanol washings. The volume was adjusted to 100 ml with 100 mM sodium acetate buffer (pH 4.5) in a volumetric flask. 1.0 ml of the aliquot from each tube was treated with 1.0 ml of DNS (3,5 – dinitrosalicyclic acid) and the tubes were boiled for 10 minutes after which sodium potassium tartarate was used to stabilize the colour and the absorbance was read at 571 nm. The non-resistant starch was calculated as dry weight of the sample analysed not as its weight that includes the moisture content effect using the following formula.

△ E x F x 10.3/0.1 x 1/1000 x 100/W x 162/180
= △E x F/W x 90
△ E = absorbance (reaction) read against the reagent blank
F = conversion from absorbance to microgram
100/0.1 = volume correction
1/1000 = conversion from micrograms to milligrams
W = dry weight of sample analysed . . . . . (3)
= "as is" weight x [(100 – moisture content)/100].
100/W = factor to present RS as a percentage of sample weight.
162/180 = factor to convert from free D-glucose, as determined, to anhydrous –D-glucose as occurs in starch.
10.3/0.1 = volume correction

2. 2. 12 Statistical Analysis

Data were analyzed using Statistical Product and Service Solution (SPSS) version 23.0 and the results expressed as Mean ± SD. Significant difference were established by one-way analyses of variance (ANOVA) using Duncan and LSD multiple comparison statistics and the accepted level of significance was $p < 0.05$ for all the result.

3. RESULTS AND DISCUSSION

Conventional cooking method on the resistant and non-resistant starch of Adani rice under different cooling conditions

Table 1 shows a significance effect of conventional method of cooking on the resistant starch contents of Adani rice. When cooled at room temperatures, there was a significant ($p < 0.05$) increase in the resistant starch content of rice by 3.17 mg/ml from 1.85 ± 0.53^{a} to 5.02 ± 0.18^{b} but when cooled in the fridge at 4 °C, there was a significant ($p < 0.05$) decrease in the resistant starch content by 1.32 mg/ml from 4.08 ± 0.53^{a} to 2.76 ± 0.32^{b}.

This initial resistant starch value obtained as 1.85 ± 0.53 after the initial conventional cooking method and cooling at room temperature correlated with the value obtained by Perez *et al*., (2008) [17]. According to Murphy *et al*., (2008) [18], the resistant starch value obtained for white rice was within the range 0 – 3.7 mg/ml per 100g of the sample. In the current study, as shown in table 1, reheating process significantly ($p < 0.05$) increased the resistant starch to 5.02 ± 0.18 when cooled at room temperature and this can be explained by the retrogradation of the starch molecules during cooling at room temperature in such a manner that the branched

chains of amylopectin became double helice thereby making it difficult for digestion by the enzymes. The amylose content that was made more accessible to digestion by the enzymes during gelatinization might have formed crystals during the period of cooling at room temperature thereby increasing its resistance to digestive enzymes. The type and extent of treatments can only affect the digestibility of starch by influencing its gelatinization and retrogradation [17], [19]. Korus *et al*., (2009) [20] studied the effect of cooking time and temperature on the resistant strength of baked foods, and observated that the amylose content that might have leached out during the baking time can quickly retrograde in the first few hours after baking thereby increasing the resistant starch content of the baked food.

When cooled in the fridge at 4 °C, there was a significant ($p < 0.05$) decrease (from 4.08 ± 0.53^{a} to 2.76 ± 0.32^{b}) in the resistant starch content of Adani rice. The reason for this can be explained by the much moisture that penetrated the rice causing forced sheering up of the amylose composition of rice thereby making it more susceptible to digestion [10], and the cooling in the fridge which added more moisture to the starch polymer thereby decreasing the rate of the starch retrogradation and this had a decreasing effect on the resistant features of rice. The more the amylose, the more hard the starch will gelatinize and the more susceptible to retrogradation [21] and gelatinization takes place when there is enough water content [22].

Significant decrease (from 88.17 ± 0.87^{a} to 51.41 ± 3.91^{b}) by 36.76 mg/ml was recorded for the non-resistant starch content of Adani rice when cooled at room temperature. This method shows a reduction in the glycemic index which can also be beneficial to diabetic patients since it has the physiological effect of bringing down the level of glucose in the blood. When cooled in the fridge, a non-significant ($p > 0.05$) change was recorded for the non – resistant starch content of rice.

Table 1. Effect of conventional cooking method on the resistant and non-resistant starch content of Adani rice under different cooling conditions

Cooking / Cooling Methods	**Batches of Resistant Starch Samples (mg /ml)**	
	Initial Cooking	**Repeated Cooking**
Rice Conventional Room (RCR)	1.85 ± 0.53^{a}	5.02 ± 0.18^{b}
Rice Conventional Fridge (RCF)	4.08 ± 0.53^{a}	2.76 ± 0.32^{b}
	Batches of Non-Resistant Starch Samples (mg /ml)	
Rice Conventional Room (RCR)	88.17 ± 0.87^{a}	51.41 ± 3.91^{b}
Rice Conventional Fridge (RCF)	87.09 ± 2.65^{a}	82.65 ± 5.12^{a}

Results are expressed in Means ± SD (n = 3)
Mean values with different letters as superscripts across the row are considered significant ($p < 0.05$)

Steam cooking method on the resistant and non – resistant starch of Adani Rice under different cooling conditions

Table 2 shows a significant effect of steam method of cooking on the resistant starch contents of Adani rice. When steam method was used to cook the rice, both cooling methods had a significant effect on the resistant starch. There was a significant ($p < 0.05$) decrease (from 6.09 ± 0.46^a to 4.05 ± 0.32^b) in the resistant starch of rice when cooled at room temperature by 2.04 mg/ml and this can be explained by the nature of the home rice attributed to the degree of its polymerization and the molecular weight distribution [23]. The high steam treatment had an effect of introducing strong heat without much moisture trapping in the rice molecules. This sharp heat treatment can increase the rate of starch gelatinization, sheering up the molecules of the polymer making them more susceptible to bond with the steam molecules [22]. Apart from the size of the grain, more importance must be attributed to the granule size distribution [24]. This high gelatinization can break up both the amylose and amylopectin molecules into short chains thereby making them more susceptible to digestion. Pin holes, equatorial grooves and small nodules have an impact in the entry of the amylases to digestion [25].

When Adani rice was cooked using steam and cooled in the fridge, there was a significant increase (from 1.24 ± 0.42^a to 7.41 ± 0.32^b) in the resistant starch content by 6.17 mg/ml, and this marks the highest increase of resistant starch in all the cooking methods used in the study. This can be explained by the forced retrogradation of the broken chains of amylose due to cold environment in the fridge as this can cause crystal formation which makes it resistant to digestion [26].

The most significant change in the non-resistant starch content of rice was recorded when it was cooked by steam and cooled in the fridge, a decrease by 50.1 mg/ml (from 86.97 ± 2.58^a to 61.08 ± 7.11^b). A statistically significant decrease by 26.20 mg/ml of the non-resistant starch contents of was also recorded (from 87.28 ± 2.58^a to 61.08 ± 7.11^b) when the rice was cooled at room temperature. This can be of good health benefit for preventing/controlling obesity and mitigating some health implications associated with diabetics

Table 2. Effect of steam cooking method on the resistant and non-resistant starch content of Adani rice under different cooling conditions

Cooking / Cooling Methods	**Batches of Resistant Starch Samples (mg /ml)**	
	Initial Cooking	**Repeated Cooking**
Rice Steam Room (RSR)	6.09 ± 0.46^a	4.05 ± 0.32^b
Rice Steam Fridge (RSF)	1.24 ± 0.42^a	7.41 ± 0.32^b
	Batches of Non-Resistant Starch Samples (mg /ml)	
Rice Steam Room (RSR)	87.28 ± 2.58^a	61.08 ± 7.11^b
Rice Steam Fridge (RSF)	86.97 ± 2.58^a	36.87 ± 4.87^b

Results are expressed in Means ± SD (n = 3)

Mean values with different letters as superscripts across the row are considered significant ($p < 0.05$)

Conventional Cooking method on the resistant and non-resistant starch content of Yam under different cooling Conditions

Table 3 shows a significant effect of conventional method of cooking on the resistant starch contents of yam. When cooled at room temperature, there was a significant ($p < 0.05$) increase in the resistant starch contents of yam by 3.28 mg/ml (from 2.00 ± 0.53^a to 5.28 ± 0.18^b) but when cooled in the fridge at 4 °C, there was no statistical significant ($p>0.05$) change in the resistant starch content of yam. The statistical difference in the resistant starch can be explained by the rate of the retrogradation of the starch molecules which made it feasible for the formation of crystals by amylose creating less area for amylolytic attack [21]. There was no significance change ($p>0.05$) in the non-resistant starch content of yam when cooked by conventional boiling and cooled in the room (from 84.53 ± 2.60^a to 82.55 ± 5.12^a) and this can be explained by the percentage ratio of amylose/amylopectin that might be low and the branch chains of α 1, 6 glycosisdic bonds of amylopectin being higher thereby increasing the surface area for hydrolysis by enzyme, when cooled at room temperature, the rate of retrogradation was low with increased concentration of amylopectin [25], [27].

Yam cooked by conventional boiling and cooled in the fridge showed no significant ($p > 0.05$) change in the content of the resistant starch. This can be explained by high gelatinization that made the starch molecules get saturated with water making retrogradation harder to be achieved before the cold environment in the fridge forced the sample to cool, and this caused little effect on the resistant starch content of yam [26]. Though, there was a significant change ($p < 0.05$) in the non resistant starch content of yam when cooked by conventional boiling and then cooled in the fridge (from 86.65 ± 2.62^a to 39.87 ± 5.13^b). The decrease was by 46.78 mg/ml and this can be explained by the level of amylopectin content of the starch molecule which got gelatinized and broken down into chains of α-1,4-glycosidic bond that was not able to be crystallized efficiently due to the cold condition of the fridge on the sample [25]

Table 3. Effect of conventional cooking method on the resistant and non -resistant starch content of yam under different cooling conditions

Cooking / Cooling Methods	**Batches of Resistant Starch Samples (mg /ml)**	
	Initial Cooking	**Repeated Cooking**
Yam Conventional Room (YCR)	2.00 ± 0.53^a	5.28 ± 0.18^b
Yam Conventional Fridge (YCF)	3.58 ± 0.53^a	4.43 ± 0.32^a
	Batches of Non-Resistant Starch Samples (mg /ml)	
Yam Conventional Room (YCR)	84.53 ± 2.60^a	82.55 ± 5.12^a
Yam Conventional Fridge (YCF)	86.65 ± 2.62^a	39.87 ± 5.13^b

Results are expressed in Means ± SD (n = 3)
Mean values with different letters as superscripts across the row are considered significant ($p < 0.05$)

Steam Cooking method on the resistant and non-resistant starch content of Yam under different cooling Conditions

The steam method of cooking yam as shown in Table 4 shows a significant ($p < 0.05$) increase in the resistant starch (from 1.98 ± 0.53^a to 5.59 ± 0.05^b) by 3.61 mg/ml while yam cooked by steam and cooled in the fridge showed no statistical change ($p > 0.05$) in the resistant starch content of yam. There was a statistically significant ($p < 0.05$) change in the non-resistant starch content of yam when cooled at room temperature (from 86.21 ± 2.79^a to 43.59 ± 5.12^b) by 42.62 mg/ml and a significant decrease in the non-resistant starch content by 23.68 mg/ml was also recorded for yam when cooled in the fridge (from 83.85 ± 2.81^a to 60.17 ± 4.09^b). Yam cooked by steaming had more significant effect in decreasing the solubilized/non-resistant starch of yam than every other method used to cook yam.

Table 4. Effect of steam cooking method on the resistant and non -resistant starch content of yam under different cooling conditions

Cooking / Cooling Methods	**Batches of Resistant Starch Samples (mg /ml)**	
	Initial Cooking	**Repeated Cooking**
Yam Steam Room (YCR)	1.98 ± 0.53^a	5.59 ± 0.18^b
Yam Steam Fridge (YCF)	0.28 ± 0.53^a	0.72 ± 0.32^a
	Batches of Non-Resistant Starch Samples (mg /ml)	
Yam Steam Room (YCR)	86.21 ± 2.79^a	43.59 ± 5.12^b
Yam Steam Fridge (YCF)	83.85 ± 2.81^a	60.17 ± 4.09^b

Results are expressed in Means ± SD (n = 3)
Mean values with different letters as superscripts across the row are considered significant ($p < 0.05$)

Moisture Content of the Food Samples

The moisture content result was calculated as shown in table 5. The results showed the moisture content of the samples with all the cooking method having an increasing effect on the moisture level of the samples when cooled at room temperature and in the fridge as well. There was slight rise in the percentage moisture content for cooking and cooling methods that increased the resistant starch content and decreased the non-resistant starch level. Though the rate of change in the moisture content is inconsistent with the rate of change in the resistant and non-resistant starch content of the samples

Table 5. Change in the Moisture content of the samples with conventional and steaming cooking methods under different cooling conditions

Cooking / Cooling Methods	Batches of moisture content in food samples (%)	
	Initial Cooking	**Repeated Cooking**
Rice Conventional Room (RCR)	66.60	79.48
Rice Conventional Fridge (RCF)	66.19	69.48
Rice Steam Room (RSR)	70.08	71.22
Rice Steam Fridge (RSF)	65.66	66.43
Yam Conventional Room (YCR)	63.36	64.22
Yam Conventional Fridge (YCF)	57.45	65.34
Yam Steam Room (YSR)	60.66	64.66
Rice Steam Fridge (YSF)	55.26	60.68

4. CONCLUSION AND RECOMMENDATION

The study found the effect of different cooking and cooling methods of rice and yam on the digestibility of the starch polymers, though steam cooking method had the highest increase in the resistant starch of rice when cooled in the fridge, but there was no significant change in the resistant starch content of yam when cooked by the same steam method and cooled in the fridge at 4 °C. Steam cooking method with cooling in the fridge also had the highest decrease in the non-resistant starch content of rice while the highest decrease in the non-resistant starch content of yam was recorded when it was also cooked by steam method but cooled at room temperature. The varying degrees of effects of cooking and cooling methods established steam method as the most significant cooking method that improves resistant starch and reduces non-resistant starch levels in rice and yam. Further studies will be suggested on the effect of cooking time and temperature on the resistant starch and crystallization level of starchy foods and evaluation of the levels of amylose/amylopectin present in the samples when cooked by steam method, since this method had the most impactful result in increasing resistant starch and reducing the non-resistant starch contents of rice and yam.

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