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Effect of Different Thermal Processing Methods on Resistant Starch Content of Selected Tubers

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Abstract: Resistant starch is a type of starch that isn't fully broken down and absorbed, but rather turned into short-chain fatty acids by intestinal bacteria. A study conducted to find out the effect of different thermal processing methods on resistant starch content of selected tubers namely potato (*Solanum tuberosum*), cassava (*Manihot esculenta*) and elephant foot yam (*Amorphophallus paeoniifolius*). These tubers processed by different processing methods such as steam, pressure, microwave cooking and conventional cooking and their resistant starch contents estimated by an enzyme method using amyloglucosidase and pancreatic α- amylase enzymes. The mean RS content of raw potato, cassava and elephant foot yam were 26.05 (±0.18), 12.64 (±0.76) and 26.66 (±0.53) g/100 g dry sample respectively. The resistant starch content of processed potato ranges between 5.58 (±0.23) and 7.94 (±0.30) g/100g dry sample, while the processed cassava ranges between 4.13 (±0.27) and 5.95 (±0.01) g/100g dry sample. RS content of elephant foot yam processed by different thermal processing methods such as steam cooking, microwave cooking, pressure cooking and conventional cooking were 7.44 (±0.39), 6.48 (±0.20), 6.27 (±0.15), 5.89 (±0.29) g/100g dry sample respectively. Resistant starch content of steam cooked potato and elephant foot yam samples were significantly ($p>0.05$) higher than the respective samples processed by the other methods.

Key words: Resistant starch · Tubers · Steam · Pressure · Microwave · Conventional

INTRODUCTION

Functional foods are foods that provide health benefits beyond basic nutrition. They typically contain certain physiologically active components which may or may not have been manipulated or modified to enhance their bioactivity. These foods may help prevent disease, reduce the risk of developing disease, or enhance health [1]. Recently, as consumers have become increasingly interested in achieving and maintaining their good health, attention to functional foods has increased.

Resistant Starch (RS) is among the bioactive compounds attracting consumer's interest, especially, those at risk of diabetes and other related diseases. RS, by definition, is a fraction of the starch that is not broken down by enzymes in the small intestine. It then enters the large intestine where it becomes the substrate for bacterial fermentation producing short chain fatty acids-(SCFAs) [2]. RS resists digestion and passes through to the large intestine where it acts like dietary fiber. The consumption of resistant starches may improve glucose and lipid metabolism and can reduce the risk of the above-mentioned disorders. Because RS-rich foods release glucose slowly and lowered the insulin response, it leads to greater access and used of stored fat. It also increase the satiety, inhibits the generation of hunger signals results to reduce the food intake. Not only would these conditions help in the management of clinical conditions, such as diabetes and impaired glucose tolerance, but also possibly in the treatment of obesity and in weight management [3].

Resistant (RS) is a powerful nutrient to our body. When RS is added to food, it can increase fiber content without affecting texture and taste and also increase satiety and decrease hunger along with altering the secretion of hormones related to food digestion [4].

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RS lowers the caloric content of foods when it is used to replace flour. It delivers between 2-3 kilocalories/gram versus 4 kilocalories/gram [5]. It is a valuable tool for formulators of reduced-calorie foods. RS may help increased fat oxidation after a meal; it leads to lower fat accumulation. A possible metabolic effect of resistant starch that may affect body weight [6]. It encourages the growth of healthy bacteria in the bowel and discourages the growth of potentially harmful bacteria and therefore, is called "prebiotic fiber" [7]. Because RS-supplemented diet may significantly increase the populations of *Lactobacilli*, *Bifidobacteria*, *Staphylococci* and S*treptococci* and act as the substrate for growth of the probiotic microorganisms, decrease the *Enterobacteria* population and alter t he microbial enzyme metabolism in the colon [8]. The fermentation of natural resistant starch reduces intestinal pH and the production of potentially harmful secondary bile acids, ammonia and phenols [9].

RS likely been a staple part of our diet for years and it has several benefits, therefore all the people should have access to its benefits and should know about RS rich food materials, which are locally available. Food rich in RS include oats, puffed wheat cereals, raw banana, some legumes, cooked and cooled potatoes and yams, cooked and cooled rice, cooked plantain, rice cereals, barley and millet [10].

Potato, Elephant foot yam and Cassava are most popular tubers consumed by consumers from Northern Sri Lanka. These tubers can consume throughout the year. The potato is the world's fourth most important food crop, after maize, wheat and rice. Potato mainly imports from India, Pakistan and Bangladesh to Sri Lanka. Cassava is the sixth most important crops (after wheat, rice, maize, potato and barley) in the world. It contributes consistently to food security because its mature edible roots can stored in the ground for up to three years. Elephant foot yam is a remunerative and profitable stem tuber crop.

The mature edible tuber can be stored long time. The crop is rich in resistant starch, easiness in cultivation, high productivity, less incidence of pests and diseases. It is a good source of phytoestrogens and is effective alternate of complementary to conventional hormone replacement thereby for symptoms associated with menopause and chronic degenerative diseases in women.

Resistant starches are sensitive for the different heat treatments (cooking, baking) used in the food processing which can cause changes especially in the resistance [11]. Heat treatment of starch to various extents leads to formation of resistant starch. Resistant starch can be obtained by cooking the starch above the gelatinization temperature and simultaneously drying on heated rolls like drum driers or even extruders. Maximum RS yield was obtained at a starch: water ratio of 1: 3.5 (w/w) and a heat treatment at 18% moisture gave increased levels of the degree of crystallinity of normal and waxy starches and thus reduced enzyme susceptibility. However, at 27% moisture, starch degradation to some extent made areas of starch more accessible to enzyme attack. Thus, proper heat treatment could be used as a method of preparation of RS [12].

Now microwave cooking is more popular for fast cooking. Pressure cooking requires much less water than conventional cooking (boiled) and less energy is requires than boiling. Steaming and pressure cooking results in a more nutritious food than boiling because fewer nutrients are leached away into the water. Overcooking or burning food is easily avoided when steaming.

Studies on effect of different thermal processing methods on resistant starch content of tubers under tropical conditions are scanty. In this paper, effect of different thermal processing methods such as pressure cooking, steam cooking, microwave cooking and conventional cooking on resistant starch content of selected tubers namely potato, cassava and elephant foot yam were evaluated and compared with their respective raw tubers.

MATERIALS AND METHODS

In this study thermal processing methods were selected based on common cooking methods practiced in the region. The selected methods were steam cooking, pressure cooking and microwave cooking.

Three tubers namely Potato (*Solanum tuberosum*) (variety-Agria), Cassava (*Manihot esculenta*) (local variety) and Elephant foot yam (*Amorphophallus paeoniifolius*) (local variety) were procured from the Thirunelvely market, Jaffna and brought to the laboratory of the Department of Biochemistry, Faculty of Medicine, University of Jaffna, Jaffna, Sri Lanka to evaluate the effect of different thermal processing methods on resistant starch content of the above tubers. These tubers were selected based on the availability and consumer preference.

Preparation of raw tuber samples and different thermal process samples for analysis.

Preparation of Raw Materials: Raw potato was peeled off and cut into small pieces. Small pieces were minced by mortar and pestle. 0.5 g sample was taken for analysis. The same procedures were repeated with cassava and elephant foot yam samples.

The fresh potato was washed well in to tap water and skin was peeled out clearly. Then, potato was cut in to small pieces and was cooked (conventional) for 15 minutes. After cooking, weight of cooked potato sample and weight of drained water were calculated accurately. Cooked sample was divided into two equal portions and drained water was divided in to two equal portions. One portion of cooked sample and one portion of drained water were mixed and the mixed sample was used to determination of resistant starch and non-resistant starch. Balanced portion of cooked sample was used to determination of moisture content of normal cooking (conventional) potato .The same procedures were repeated with cassava and elephant foot yam samples. In pressure cooking, was cooked in to the pressure cooker for 5 minutes (up to the high pressure gas releasing sound) and steam cooking, on the steam for 10 minutes (without water). In microwave cooking, cooked in to the micro wave oven (as set to potato mode). After cooking, the procedure followed for the preparation of normal cooked (conventional) sample was followed for the preparation of steam, pressure and micro wave cooked samples. The same procedures were repeated with cassava and elephant foot yam samples.

Determination of Resistant Starch and Non- Resistant Starch Contents of Food Samples: The contents of RS, non-resistant starch and total starch were analyzed using the Megazyme resistant starch assay kit (Megazyme International Ireland Ltd, Bray, Ireland).The RS and non- RS contents were estimated according to the procedure developed by the McCleary and Monaghan [13]. It is simple, well explains procedure and accepted by both AOAC and AACC associations [14, 15].

Determination of Non- Resistant Starch: Wet samples, having the moisture contents of (60-80%), 500mg of minced fresh samples were weighed in screw cap tubes. α- amylase and AMG (amyloglucosidase) (3300 U/mL) (4 mL) was added to each tube. The contents of the tubes were mixed and incubated in a shaker water bath (200 strokes/min), at 37°C for 16h. After 16h, tubes were removed and 4.0 mL of ethanol (99%) was added, mixed vigorously on a vortex mixer and centrifuged at 1500 g (approx. 3000 rpm) for 10 min. The supernatants were carefully decanted (supernatant A) and the pellets were re-suspended in 8mL of 50% ethanol, mixed and centrifuged at 1500 ×*g* for 10 min. The supernatants were decanted (supernatant B) and the procedure was repeated again. The supernatants were decanted carefully (supernatant C) and the tubes were inverted on absorbent paper to drain excess liquid and the pellet remained within the tube was used to determine the RS content. The supernatants A, B and C were pooled and the volume was made up to 25 mL with distilled water. Pooled samples (0.5 mL) were taken and analyzed for reducing sugar [16].

Reducing sugar produced was measured using the standard curve for glucose. Standard curve for glucose by 3, 5-dinitrosalicylic acid (DNS acid) method. The method of Miller was used. The absorbance was read with a spectrophotometer at 550 nm.

Determination of Resistant Starch: The pellets remained within the tubes after the determination of non-RS content were re-suspended in 2mL of 2M KOH using magnetic bar for 20 min in an ice bath. Then 8mL of 1.2 M sodium acetate buffer (pH 3.8) and 0.1mL of AMG (3300 U/mL) were added, mixed well and incubated in a water bath at 50°C for 30 min with intermittent mixing. The contents of the tubes were transferred to a 25mL volumetric flask and the volume was made up to 25mL with distilled water.

An aliquot (10mL) of the solution was centrifuged at 1500×*g* for 10 min and 0.5 mL of aliquots of the supernatants (in triplicate) were transferred in to test tubes, mixed with 0.5mL DNS reagent and the contents were heated in a boiling water bath for 5 min and cooled immediately. Then 5mL distilled water was added and mixed well in a vortex mixer. Then the color was read at 550 nm against the reagent blank. Reagent blank was prepared by taking 0.5mL of distilled water instead of the sample. Standard curve for glucose was used to estimate the reducing sugar content.

Statistical Analysis: All the experiments were performed in triplicates. Results were expressed by means of values ±standard deviations of three separate determinations. Comparison of means was performed by CRD (Complete Randomized Design) followed by using in SAS package (Statistical Analysis System) Version 9.1 and MS –Excel 2007. Beside this, significant different were estimated to 95% confidential interval ($P<0.05$).

Table 1: Resistant starch, non-resistant starch, total starch and moisture contents of the selected raw tubers

Raw samples				
Tuber	Moisture (%)	Resistant Starch (%)*	Non- resistant Starch (%)*	Total Starch (%)*
Potato	82.12(±0.08)[a]	26.05(±0.18)[a]	57.32(±3.11)[a]	83.82(±0.35)[a]
Cassava	55.28(±0.87)[c]	12.64(±0.76)[b]	56.48(±1.36)[b]	69.12(±0.60)[c]
Elephant foot yam	80.33(±0.24)[b]	26.66(±0.53)[a]	49.30(±1.19)[c]	75.96(±0.12)[b]

Mean of values ±SD. (n=3). Different letters between treatments show significant difference (P<0.05). * On dry weight basis.

Table 2: Moisture content, resistant starch and non-resistant starch contents of selected tubers processed by different thermal processing.

Heat treatments	Moisture content (%)*	RS (%)*	Non- RS (%)*
Potato			
Normal cooking	85.06	5.58(±0.23)[b]	79.10(±1.08)[a]
Micro wave cooking	79.30	6.73(±0.30)[ba]	75.63(±0.73)[b]
Steam cooking	82.70	7.94(±0.30)[a]	72.86(±0.83)[b]
Pressure cooking	84.58	7.08(±0.03)[a]	71.18(±1.37)[b]
Cassava			
Normal cooking	68.45	4.13(±0.27)[b]	62.07(±0.76)[b]
Micro wave cooking	54.43	5.95(±0.01)[a]	46.42(±0.20)[d]
Steam cooking	59.10	5.03(±0.08)[ab]	54.34(±0.31)[a]
Pressure cooking	70.20	5.53(±0.21)[ba]	64.70(±1.18)[c]
Elephant foot yam			
Normal cooking	82.77	5.89(±0.36)[b]	70.26(±2.46)[a]
Micro wave cooking	72.54	6.48(±0.20)[ba]	65.64(±2.74)[a]
Steam cooking	76.19	7.44(±0.39)[a]	56.62(±0.38)[b]
Pressure cooking	80.35	6.27(±0.17)[ba]	65.31(±1.07)[a]

Mean of values ±SD. (n=3). Different letters between treatments show significantdifference (P<0.05).* On dry weight basis

RESULTS

Starch Contents Selected Raw Tubers: Table 1 shows that resistant starch, non-resistant starch, total starch and moisture contents of the selected raw tubers. The highest resistant starch content was observed in elephant foot yam 26.66±0.53 g/100g dry sample followed by potato 26.05±0.18 g/100g dry sample and cassava 12.64±0.76 g/100g dry sample.

Different Thermal Processing Methods on Resistant Starch Contents of Selected Tubers: Four different thermal processing methods such as conventional cooking (boiling), steam cooking, pressure cooking and microwave cooking were selected for this study. Table 2 shows that moisture content, resistant starch and non-resistant starch contents of selected tubers processed by different thermal processing. In potato, highest resistant starch content was observed in steam cooked potato 7.94±0.30 g/100g dry sample followed by pressure cooked 7.08±0.03 g/100g dry sample, microwave cooked 6.73±0.30 g/100g dry sample and normal cooked (conventional method) 5.58±0.23 g/100g dry sample samples of potatoes respectively.

In cassava, total starch contents of raw cassava are less when compared to potato and elephant foot yam. Among the thermally processed cassava samples the highest resistant starch content was observed in the microwave cooked samples 5.95 ±0.01 g/100 g dry sample followed by pressure cooked 5.53±0.21 g/100g dry sample, steam cooked 5.03±0.08 g/100g dry sample and conventional cooked 4.13±0.27 g/100g dry sample, respectively.

Among the different processing methods the highest resistant starch was observed in the steam cooked elephant foot yam samples 7.44±0.39 g/100g dry sample followed by microwave cooked 6.48±0.20 g/100g dry sample, pressure cooked 6.27±0.15 g/100g dry sample and conventional cooked 5.89±0.29 g/100g dry sample.

DISCUSSION

In this study, determine the resistant starch contents affected by different thermal processing in selected tubers. Highest total starch content was observed in raw potato followed by elephant foot yam and cassava. But the highest resistant starch content was observed in elephant foot yam followed by potato and cassava.

However, there is no significant difference between resistant starch content of potato and elephant foot yam. But these are significantly higher than the RS content of cassava. Even though, highest resistant starch content was observed in raw tubers including potato, elephant foot yam and cassava compare with these tuber involve to any thermal processing. It is due to starch retrogradation of amylose during heating. Starch retrogradation is mostly taken to be an undesirable process that occurs during the storage of starchy foods. Water content, starch source and storage conditions are all well-known factors that can greatly influence starch retrogradation [17]. When the heating, in the presence of water, the amylose in the granule swells; the crystalline structure of the amylopectin disintegrates and the granule ruptures. The polysaccharide chains take up a random configuration, causing swelling of the starch and thickening of the surrounding matrix such as, gelatinization a process that renders the starch easily digestible [18]. Studies on effects of different heat treatments (normal cooking, microwave cooking, pressure cooking and steam cooking) on the rate of hydrolysis, was showed increase in digestible starch and decrease in resistant starch compared with raw tubers. Thermal processing of tubers reduces 21.45% - 30.47% of resistant starch (dry weight basis) content compared with raw samples.

However, based on this study, the steam cooking, pressure cooking and microwave cooking methods produce more resistant starch when compared to conventional cooking method. In potato, better thermal processing method is based on this study steam cooking followed by pressure cooking, microwave and conventional method. Steam cooked potato 29.72 % (dry weight basis) highest compared with conventional method. In cassava, better thermal processing method micro wave method and steam cooked method for elephant foot yam. Even though, microwave cooked cassava and steam cooked elephant foot yam, these are 30.58% (dry weight basis) and 20.83 % (dry weight basis) highest resistant starch contents compared with conventional method respectively.

CONCLUSION

In raw tubers, elephant foot yam and potato have highest resistant starch contents than cassava. The highest resistant starch content observed in steam cooked potato and elephant foot yam and micro wave cooked cassava among the different thermal processing methods. Cassava and elephant foot yam showed higher resistant starch content when compared to normal cooked or boiled tubers.

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