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Preparation and characterization of resistant starch III from elephant foot yam (*Amorphophallus paeonifolius*) starch

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ABSTRACT

The purpose of this study was to assess the properties of resistant starch (RS) III prepared from elephant foot yam starch using pullulanase enzyme. Native and gelatinized starches were subjected to enzymatic hydrolysis (pullulanase, 40 U/g per 10 h), autoclaved (121 °C/30 min), stored under refrigeration (4 °C/24 h) and then lyophilized. After preparation of resistant starch III, the morphological, physical, chemical and functional properties were assessed. The enzymatic and retrogradation process increased the yield of resistant starch III from starch with a concomitant increase increase in its water absorption capacity and water solubility index. A decrease in swelling power was observed due to the hydrolysis and thermal process. Te reduced pasting properties and hardness of resistant starch III were associated with the disintegration of starch granules due to the thermal process. The viscosity was found to be inversely proportional to the RS content in the sample. The thermal properties of RS increased due to retrogradation and recrystallization ($P < 0.05$).

1. Introduction

In the food processing industries, starch contributes to important characteristics including thickening, gelling, consistency and shelf stability in a diverse range of diverse applications. Potato, rice and wheat starch have been exclusively employed for this purpose in transforming the microstructure and functionality of products in the food industries (Wheatley, Liping, & Bofu, 1996). Though starch contributes significantly to the quality and consistency of commercial products, it has a high glycemic index which often makes it unfavourable in terms of its effects to the consumer.

In order to meet the growing demands of the consumers for functional foods, carbohydrates which can act as functional ingredients and have a beneficial effect to human health are favoured over carbohydrates with a high glycemic index. Resistant starch (RS) is one of the naturally occurring carbohydrate which is defined as the sum of starch and starch degradation products which cannot be digested in the small intestine of humans and when reached in the large intestine, it undergoes fermentation by the commencal intestinal microorganisms production, resulting in the production of short chain fatty acids (Annison & Topping, 1994). These short chain fatty acids can be partially absorbed in the small intestine and be a source of energy to the mucosal cells or can support the growth and metabolism of the colonic microbiota, with the undigested mass being excreted in the stool (Xue, Newman, & Newman, 1996). Besides its vital physiological role as a functional ingredient in lowering the risk of diet-related diseases, RS when compared with traditional insoluble fibres also has many favourable features for thea food industry. More specifically, RS is a natural white colour powder with bland taste and has acceptable appearance and texture (Sajilata, Singhal, & Kulkarni, 2006).

RS is classified on the basis of its botanical source and processing methods (Englyst, Kingman, & Cummings 1992). RS can be classified in four types including RS1, physically inaccessible starch; RS2, native starch granules; RS3, retrograded starch and RS4, chemically modified starch (Sajilata et al., 2006). Sources of cereal grains, roots, tubers and legumes produce resistant starch through the process of cyclic heating, autoclaving and extrusion methods. Gelatinization and cooling processes which are generally referred to as annealing procedures, are the common methods used to enhance the formation of RS3 (retrograded starch) (Thompson, 2000). Ample studies are available on the RS of the cereals, pulses, and tubers, especially cassava and potato starch. One such starch source which is not explored commercially is *Amorphophallus paeonifolius*, which is also known as elephant foot yam, an herbaceous, perennial C3 crop. It is a tropical tuber which has originated

Abbreviations: ΔHgel, enthalpy of gelatinization; DSC, differential scanning calorimetry; PHI, peak height index ; *R*, gelatinization range; RC, relative crystallinity; RS, resistant starch; SEM, scanning electron microscopy; SP, swelling power; T_0, onset temperature; T_C, conclusion temperature; T_P, peak temperature; TPA, texture profile analyzer; WAC, water absorption capacity; WSI, water solubility index; XRD, X-ray diffractometer.

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from the south eastern Asian region and is extensively used in Indian cuisines (Ravi, Ravichandran, & Suja, 2009).

Though several researchers have studied the flour and starch obtained from yam tubers in order to find new food applications (Okaka, Okorie, & Ozo, 1991) a limited number of studies on starch and resistant starch fr *A. paeonifolius* are available. Taking into account the need for RS3, as this would constitute a functional food ingredient, the study was designed to elucidate the preparation of starch and resistant starch (RS3) from elephant foot yam. The objectives of the study were to prepare RS3 retrograded starch from isolated starch of elephant foot yam (*A. paeonifolius*) and characterise its physico-chemical and functional properties.

2. Materials and methods

2.1. Materials

The tuber of elephant foot yam (*A. paeonifolius*) was purchased from a local market. The resistant starch assay kit was purchased from Megazyme International (Ireland), whereas the enzymes used in the study included pullulanase from *Bacillus acidopullulyticus* (Promozyme 400L) and heat stable α-amylase from *Bacillus licheniformis* (Termamyl 120L), obtained from Sigma (USA).

2.2. Isolation of starch

The starch was isolated from elephant foot yam (*A. paeonifolius*) according to the method of Amani, Buleon, Kamenan, and Colonna (2004). The tuber was peeled, cut into small pieces and immediately suspended in 0.1% (w/v) sodium metabisulphite solution. Then, the sample was homogenised with warring blender and suspended in a large amount of 4% NaCl. The slurry was filtered through a 100 μm sieve and the filtrate was centrifuged at 2660g for 15 min. This procedure was repeated for four times and the recovered white coloured starch was then oven dried at 48 h at 45 °C.

2.3. Preparation of resistant starch

2.3.1. Enzymatic hydrolysis of elephant foot yam starch

Enzymatic hydrolysis of elephant foot yam starch was performed by using the procedure used by Polesi and Sarmento (2011) with a slight modification. The elephant foot yam starch (10% w/w db) was suspended in sodium acetate buffer (0.1 M and pH 5.3) and mixed with pullulanase enzyme (40 U/g dry starch), and the mixture incubated in a shaking water bath at 60 °C for 10 h. The sample was heated in a boiling water bath for 10 min to inactivate the enzyme. Starch gelatinization, prior to adding the enzyme, was performed by boiling the sample in a water bath for 10 min.

2.3.2. Preparation of resistant starch

Starch samples, namely elephant foot yam starch (S1), native hydrolyzed by enzyme (S2) and gelatinized hydrolyzed by enzyme (S3) in suspensions (10% w/w db) were autoclaved at 121 °C for 30 min, cooled and kept at 4 °C for 24 h. The samples (S1, S2 and S3) were then lyophilized.

2.3.3. Determination of resistant starch content

The amount of RS in the samples was analysed by using a Megazyme resistant starch assay kit, which is based on the Association of Official Analytical Chemists (AOAC) methods (2002.02).

2.4. Scanning electron microscopy (SEM)

The morphological characteristics of S1, S2 and S3 were evaluated using scanning electron microscope (HITACHI Model S-3000H) with a magnification 500× to 1500×. The powdered samples were sprinkled on double-sided stick tape placed on aluminium stubs and were covered with a gold–palladium layer.

2.5. Physicochemical characteristics

2.5.1. Chemical composition

The moisture content of S1 was recorded in terms of weight loss after heating at 130 ± 2 °C for 2 h using 2 g of sample. The amount of ash, protein, and fat were analysed according to AACC methods 08–01, 46–13 and 30–25, respectively. The amount of starch was analysed using the AOAC method 8.020.

2.5.2. Amylose content

The total amylose content of the starch samples (S1, S2and S3) was analsyed according to the method described by Williams, Kuzina, and Hlynka (1970). Starch samples (20 mg) were added to 10 mL of 0.5 N KOH and the resultant suspension was thoroughly mixed. Subsequently, the dispersed sample was transferred into a 100 mL volumetric flask and diluted to the mark with distilled water. An aliquot (10 mL) was pipetted into a 50 mL volumetric flask and 5 mL of 0.1 N HCl were added followed by 0.5 mL of iodine reagent, and the solution made up to 50 mL with water; the absorbance was then measured at 625 nm. The measurement of amylose was determined from a standard curve developed using amylose as the standard.

2.5.3. Water absorption capacity (WAC) and water solubility index (WSI)

The WAC and WSI of the samples (S1, S2 and S3) were analysed according to the procedure described by Anderson, Conway, Pfeifer, and Griffin (1969). Briefly, a sample of 0.5 g was mixed with 6 mL of distilled water, and centrifuged. The supernatant was heated at 30 °C with continuously stirring for 30 min in a water bath. The suspension was placed in a petridish and dried at 105 °C for 4 h to obtain the dry solids weight, and the wet sediment was weighed. The WSI and WAC were determined as: WSI = (weight of dry solids in supernatant/weight of dry sample) × 100; WAC = weight of wet sediment/(weight of the dry sample-weight of the dry solids).

2.5.4. Swelling power (SP)

The swelling power of the samples (S1, S2and S3) was anasyed using the method described by Nattapulwat, Purkkao, and Suwithayapan (2009). A sample (0.2 g) was dispersed in water (20 mL) to form a suspension. The suspension was heated to 85 °C in a water bath for 30 min with vigorous shaking every 5 min. The starch gel was then centrifuged at 2200 rpm for 15 min. The weight of the sediment was used to calculate the swelling power. The supernatant was dried and weighed to measure the amount of dissolved starch in the supernatant. The swelling power was determined as: swelling power = weight of sediment/(weight of dry starch − weight of dissolved starch).

2.5.5. Pasting properties

The viscoamylographic property of the samples (S1, S2 and S3) were performed with a Rapid Visco Analyser (RVA starch master 2, Newport Scientific, Warriewood, NSW, Australia) using 2 g of sample in 25 mL of water. The following parameters: paste temperature, peak viscosity, breakdown viscosity, final viscosity and setback viscosity were measured from the viscoamylographs.