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Synthesis and physico-chemical characterization of modified starches from banana (*Musa* AAB) and its biological activities in diabetic rats

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ABSTRACT

This study describes a simple method of preparation and physico-chemical properties of modified starches (type-3 resistant starches) from banana (*Musa* AAB), and the modified starches investigated as functional food with a beneficial effect on type-2 diabetes. RS3 was prepared using a method combined with debranching modification and physical modification; native and modifies starches were characterized by scanning electron microscope (SEM), powder X-ray diffraction (XRD), differential scanning calorimetry (DSC) and rapid visco analyzer (RVA). Use of the enzymatic and physical modification methodology, improved the yield of RS (26.62%) from *Musa* AAB. A reduced viscosity and swelling power; increased transition temperatures, water absorption capacity and solubility index with B-type crystalline pattern and loss of granular appearance were observed during the debranching modification and physical modification. The modified starches exhibited beneficial health effects in diabetic and HFD rats who consumed it. These results recommend that dietary feeding of RS3 was effective in the regulation of glucose and lipid profile in serum and suppressing the oxidative stress in rats under diabetic and HFD condition. This current study provides new bioactive starches, with potential applications in the food and non-food industries.

1. Introduction

Now-a-days, the occurrence of obesity related problems are on the rise due to the modern life style, consumption of excess dietary fat and reduction in physical activities [1,2]. Obesity related issues also lead to complications like hyperlipidemia [3], non-alcoholic fatty liver disease [4], various cardiovascular diseases [2], and diabetes [5] in human beings. In general, diabetes is a form of metabolic disorder, which occurs due to the dietary intake of excess carbohydrates and lipids. In particular, type 2 diabetes mellitus (T2DM) is a common endocrine and metabolic disease [6]. T2DM is caused by an absolute or relative lack of insulin in the blood [7], resulting in metabolic abnormalities such as obesity, hypertension, low levels of high-density lipoprotein (HDL-C), elevated triglyceride (TG) levels, hyperglycemia and resistance to insulin [4]. In diabetes, major health problems are oxidative damage [8], dysfunction and eventual organ failure [9,10].

With improvement in social and economic environment in developed and developing countries, the occurrence of diabetes (T2DM) has rapidly increased over the years. Apart from genetic reasons, the dietary pattern of a person plays a key role in the occurrence of metabolic syndrome. A major reason could be the increased influence of western diet consumption which has an excess fat content in addition to poor minerals and fibre [11]. Due to the increased occurrence of diabetes in humans, current research is focused on the development of drugs for treatment and control of T2DM. Various drugs have been developed for the treatment and control of T2DM; however, the long-term usage of anti-diabetic drugs results into considerable side effects with the symptoms of hypoglycemia and malfunction of kidney and liver [7,10,12,13]. As medication cannot possibly have an alternative for treatment of T2DM, the focus should towards the prevention or delayed onset of diabetes by exploring the functional adjuncts responsible for it.

Resistant starch (RS) is a new functional ingredient with a low glycemic index which is often employed in the development of functional food products. RS could be defined as the fraction of starch, which escapes digestion in the small intestine and undergoes fermentation in the large intestine in the presence of

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microflora resulting into formation of short chain fatty acids, especially butyric acid [14,15]. RS can act as a dietary fiber with similar physiological effects [16]. Several reports suggested that the RS exhibits significant effect on physiological functions such as body weight management [17], prevention of gastrointestinal diseases [18], treatment of hyperglycemia and hypercholesterolemia, and enhancement of mineral absorption [19,20].

RS obtained various sources such as cereals (corn, rice and wheat), tubers (yam and potato) and pulses are widely used in the production of functional food products; and also various methods are involved in the preparation of RS from various sources including physical [7], chemical [2] and enzymatic [21] modification. Augmented the utility of RS with wider applications in food and non-food industries has increased the focus of research for the development of the RS from various food sources. Banana, is a common edible fruit of the genus *Musa* encompassed in the family *Musaceae*. Currently, worldwide production of banana is around 140 million tons, because of its extensive cultivation in tropical and sub-tropical regions of World. India ranks first in banana production and 'Poovan' (*Musa* AAB) cultivar is abundantly grown in Southern regions due to its higher adaptability to climatic conditions and cropping patterns [22,23]. The unripe bananas are highly consumed because of their high starch content. Due to the presence of high amount of starch, bananas are of great concern in the field of starch research. However, limited studies are available for the production of RS from *Musa* AAB using pullulanase enzyme and followed by its applications.

From this basis, the objective of the present study was to the synthesis of resistant starch (RS3) from *Musa* AAB using a method of debranching modification followed by autoclaving and retrogradation; and evaluate their physico-chemical characteristics of native and modified starches, including morphological, physico-chemical, crystalline and thermal properties. Their biological activities (hypoglycemic and lipid lowering effects) of modified starches were also analysed using diabetes (T2DM) and obese rats fed with HFD.

2. Materials and methods

2.1. Materials

Unripe poovan banana (*Musa* AAB) cultivar used in the study was obtained from the local harvest in the State of Tamil Nadu, India. Only unripe bananas were used in the study which did not have even small traces of yellow colour on its peel. Pullulanase from *Bacillus acidopullulyticus* (Promozyme 400 L) and Streptozotocin (STZ) were procured from Sigma-Aldrich Company, USA. Resistant starch assay kit was obtained from Megazyme International Ireland Limited, Ireland. Reagent kits of glucose, total cholesterol, TG, HDL-C, LDL-C, glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) were purchased from AGGAPE-diagnostics Pvt. Ltd, Kerala, India. All other chemicals and reagents were analytical grade.

2.2. Preparation of native starch

Native starches from unripe poovan banana were extracted by Reddy et al. [22] with slight modifications as described. Raw poovan banana was peeled, washed and macerated for 2 to 3 min with sodium sulphite solution (1.22 g/L) using a waring hand blender (Prestige Hand Blender PHB 5.0, Bangalore, India). Homogenate was passed through 60 and 100 U.S mesh sieves using slurry and water (1:4 ratio). The filtrate was allowed to stand for 2 h at room temperature. After that supernatant was decanted off and then starch slurry was centrifuged (Eppendorf centrifuge, Model 5804, Mumbai, India) at 3000 rpm for 30 min. The white starch sediments were dried in a hot air oven at 40 °C for overnight, and ground to pass a sieve (100 U.S) for further analyses.

2.3. Preparation of resistant starch

2.3.1. Debranching of starch

Debranching of native banana starch was performed based on the method described in Reddy et al. [21] with a slight modification. Starch sample (10 g) and 100 mL of sodium acetate buffer (0.1 M and pH 5.3) was mixed with pullulanase (40 U/g dry starch) and incubated in shaking water bath (Remi RSB-12, Mumbai, India) at 60 °C for 10 h. The sample was heated in boiling water bath for 10 min to inactivate the enzyme. The starch gelatinization prior to enzymatic hydrolysis was performed with the sample in boiling water bath for 10 min, before adding the enzyme.

2.3.2. Preparation of resistant starch

The starch samples, retrograded enzymatically hydrolysed native starch (REHNS) and retrograded enzymatically hydrolysed gelatinized starch (REHGS) in suspensions (10%, w/w dwb) were autoclaved at 121 °C for 30 min, cooled to 4 °C and stored at this temperature for 24 h. The samples were then freeze dried. In the samples, the RS content was determined using a Megazyme resistant starch assay kit with the description of Association of Official Analytical Chemists (AOAC) 2002.02.

2.4. Morphological characteristics

The structure of the native and modified starch granules was observed by scanning electron microscope (SEM, HITACHI, S-3400N, Tokyo, Japan). The powdered sample was sprinkled on double sided sticky tape placed on aluminium stubs and covered with carbon coating layer, observed, and photographed.

2.5. Colour and amylose content

Colour of the native and modified starches was measured using Hunter Lab Colorimeter (D-25, Hunter Lab Associates Inc.) after standardisation using Hunter Lab colour standards. L^* (lightness), a^* (redness to greenness) and b^* (yellowness to blueness) values of native and modified starches were analysed. Amylose content of native and modified starches was determined according to the method of Williams et al. [24].

2.6. Pasting properties

The pasting properties of native and modified starches were investigated with a Rapid Visco-Analyzer (RVA starch master 2, Newport Scientific, Warriewood, NSW, Australia) according to method of Reddy et al. [25].

2.7. Water absorption capacity, solubility index and swelling power

The water absorption capacity (WAC), water solubility index (WSI) and swelling power (SP) of native and modified starches were analysed respectively, according to the methods described by Reddy et al. [21].

2.8. X-ray diffraction

Powder X-ray diffraction (XRD) analysis of the native and the modified starches was carried out on powder X-ray diffractometer (Shimadzu XRD 7000) with Cu Kα value of 1.54 radiation at step count of 2°/min, with a 2θ range of 10° to 50° using a voltage of 40 kV and filament current 30 mA.