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Physico-chemical and functional properties of Resistant starch prepared from red kidney beans (*Phaseolus vulgaris.L*) starch by enzymatic method

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ABSTRACT

The objective of this study was to evaluate the production, physico-chemical and functional properties of Resistant starch (RS) from red kidney bean starch by enzymatic method. Native and gelatinized starch were subjected to enzymatic hydrolysis (pullulanase, 40 U/g/10 h), autoclaved (121 °C/30 min), stored under refrigeration (4 °C/24 h), and lyophilized. The enzymatic hydrolysis and thermal treatment of starch increased the formation of RS which showed an increase in water absorption and water solubility indexes and a decrease in swelling power due to hydrolytic and thermal process. The process for obtaining RS changed the crystallinity pattern from C to B and increased the crystallinity due to the retrogradation process. RS obtained from hydrolysis showed a reduction in viscosity, indicating the rupture of starch molecules. The viscosity was found to be inversely proportional to the RS content in the sample. The thermal properties of RS increased due to the retrogradation and recrystallization ($P < 0.05$).

1. Introduction

The growing demand for functional foods reveals the awareness of the consumers on the health benefits and the choice of functional foods. With starch being the predominant important component in human diet contributing 60–70% of the total energy consumed mainly derived from cereals and pulses. Starch being a polymer of glucose has two distinct structural forms, amylose and amylopectin (Tharanathan & Tharanathan, 2001). On gelatinization starch may relapse to a structure that could be highly resistant to hydrolysis by α-amylase which is termed as resistant starch (RS) (Annison & Topping, 1994). This RS is a form of starch or fractions of starch that is not hydrolysed by enzymes in human digestive system because of its crystalline nature and the ratio of amylose and amylopectin (Englyst et al., 1992). The functional properties of resistant starch has been acknowledged for the control of obesity, diabetes and subsequently, for reducing the risk of cardiovascular diseases (Asp, 1997; Morita et al., 2005) and in prevention of colon cancer by decreasing the concentration of secondary bile acids, ammonia and phenol content (Birkett, Muir, Phillips, Jones, & Deak, 1996; Hylla et al., 1998) due to the increased short chain fatty acid (SCFA) levels, especially butyric acid produced by the fermentation of RS by the gut microflora in the colon (Cummings, Beatty, Kingman, Bingham, & Englyst, 1996; Scheppach, Fabian, Sachs, & Kasper, 1998).

RS can be defined as the sum of starch and its degradation products that are not absorbed in the small intestine of healthy humans, and it reaches the large intestine through digestive tract, where it undergoes fermentation in the presence of intestinal micro flora and produces short chain fatty acids (Annison & Topping, 1994) such as acetic acid, propionic acid and butyric acid (Scheppach et al., 1998) and gases in the large bowel (Muir et al., 1995; Thompson, 2000).

The RS can be divided into four major groups such as RS1, physically inaccessible to digestion entrapment in a non-digestive matrix (Haralampu, 2001); RS2, native and ungelatinized starch granules as in raw banana and potato; RS3, retrograded starch (Bird, Lopez-Rubio, Shrestha, & Gidley, 2009) and RS4, chemically modified starch (Englyst et al., 1992; Sajilata, Singhal, & Kulkarn, (2006)) Resistant starch is generally prepared from starch with high amylose content in carbohydrate rich foods through autoclave, baking and extrusion methods. After these processes, to enhance the formation of RS3 the starch is subjected to cooking and cooling. Unbranched, lower molecular mass can raise the level retrograded starch after gelatinization and cooling. So, molecular mass reduction techniques or debranching of amylopectin by the action of acids or enzymes have been evaluated to improve the RS production

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

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(Leong, Karrim, & Norziah, 2007; Polesi & Sarmento, 2011; Mutungi, Rost, Onyango, Jaros, & Rohm, 2009).

Legume starches have reduced bioavailability, which is attributed to several factors such as high amylose contents (30–65%), C- type crystallinity and strong interactions between amylose chains (Hoover & Zhou, 2003). Among all legumes, Red kidney beans (*Phaseolus vulgaris* L.) commonly known as Rajmah, constitutes a high amount of proteins (20–15%), complex carbohydrates (50–60%) and a better source for vitamins, minerals and poly unsaturated free fatty acids (Rehman, Salariya, & Zafar, 2001; Reyes-Moreno & Paredes-Lopez, 1993). Hence the present study was attempted to elucidate the preparation of starch and resistant starch from red kidney beans focusing its rich quantity of starch.

The specific objective of the present study was to prepare resistant starch from the isolated starch of red kidney beans (*P. vulgaris* L.) and characterize their physico-chemical and functional properties.

2. Materials and methods

2.1. Materials

Red kidney beans vernacular name being Indian Rajmah (*P. vulgaris*) was purchased from local market. The seeds were cleaned manually from the dirt, foreign materials etc. and stored at room temperature for further use. Pullulanase enzymes from *Bacillus acidopullulyticus* (Promozyme 400 L), heat stable α-amylase from *Bacillus licheniformis* (Termamyl 120 L), Sigma chemical company, USA and Pepsin and Pancreatin 3× (from porcine pancreas) from HiMedia labs, Mumbai, India were purchased. The Resistant starch assay kit was purchased from Megazyme International Ireland Limited, Ireland.

2.2. Isolation of starch

The starch was obtained from red kidney beans using the methodology described by Wani, Sogi, Wani, Gill, & Shivhare (2010).

2.3. Preparation of resistant starch

2.3.1. Enzymatic hydrolysis

Enzymatic hydrolysis of red kidney beans starch was carried out by the method of Polesi and Sarmento (2011) with a slight modification. The red kidney bean starch suspension (10%, w/w db) was taken in sodium acetate buffer (0.1 M and pH 5.3). The mixture was added to pullulanase enzyme (40 U/g dry starches) and incubated in water bath 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, Red kidney bean starch (V1), Native hydrolyzed by enzyme(V2) and gelatinized hydrolyzed by enzyme(V3) in suspensions (10%, w/w dry basis) were autoclaved at 121 °C for 30 min, cooled to 4 °C and stored at this temperature for 24 h. The samples (V1, V2 & V3) were then lyophilized.

2.3.3. Determination of resistant starch content

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. Scanning electron microscopy

The appearance of the V1, V2 and V3 were evaluated using the technique described by Polesi and Sarmento (2011) with scanning electron microscope (HITACHI Model S-3000H). The assembly of the samples was performed on aluminum stubs with double side adhesive tape to which the samples were fixed and covered with a thin gold layer.

2.5. Physico-chemical characteristics

2.5.1. Chemical analysis

The moisture content of red kidney bean starch (V1) was determined by gravimetric heating (130 ± 2 °C for 2 h) using a 2–3 g sample. The ash, protein, and fat were analyzed according to AACC methods 08–01, 46–13 and 30–25.

2.5.2. Amylose content

The amylose content of samples V1, V2 and V3 were determined using the method described by McCready, Guggolz, Silviera, and Owens (1950). Briefly, 100 mg of the sample was added to 1 ml of distilled ethanol and 10 ml of 1 N NaOH followed by the incubation for overnight at room temperature. The incubated mixture was made up to 100 ml with distilled water and aliquot of 2.5 ml was taken for titration against 0.1 N HCL by adding 20 ml distilled water and three drops of phenolphthalein. The end point of disappearance of pink colour was observed and 1 ml of iodine reagent was added and made up to 50 ml and read the colour at 590 nm using UV-Visible Spectrophotometer (UV-1800, Shimadzu, North America). The amount of amylose present in the sample was calculated from the standard curve for standard amylose solution at 20–100 μg/ml. The blank was prepared with 1 ml of iodine reagent diluted to 50 ml of distilled water.

2.5.3. Total dietary fibre (TDF)

The total dietary fibre (TDF) of the samples V1, V2 and V3 were measured as the sum of water-soluble and water-insoluble fractions, based on digestion of the sample (1 g) with digestive enzymes, using the method described by Asp, Johnson, Hollmer, and Siljestrom (1983). Briefly, this method illustrates the enzymatic hydrolysis of starch performed in three steps: gelatinization in the presence of Termamyl (heat stable α-amylase) (100 mg, 90 °C, 15 min, pH 6.0), treatment with pepsin (100 mg, 40 °C, 60 min, pH 1.5) and incubation with pancreatin (100 mg, 40 °C, 60 min, pH 6.8). The Insoluble Dietary Fibre (IDF) was recovered by filtration with celite as a filter aid. Then soluble dietary fibre (SDF) was precipitated from the filtrate with four volumes of 95% ethanol and recovered by filtration.

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

The water solubility index and absorption capacity of samples (V1, V2 and V3) were performed using the method described by Anderson, Conway, Pfeifer, and Griffin (1969). 0.5 g of sample was mixed with 6 ml of distilled water into a centrifuge tube. After continuously stirring for 30 min in water bath at 30 °C, the suspension was centrifuged at 3000 × *g* for 10 min. The supernatant was placed in a petridish and dried at 105 °C for 4 h to obtain the dry solids weight, and the wet residue of the centrifugation was also weighed.

$$\text{WSI} = \frac{\text{weight of dry solids in supernatant}}{\text{weight of dry sample}} \times 100$$

$$\text{WAC} = \frac{\text{weight of wet sediment}}{\text{Weight of the dry sample} - \text{weight of the dry solids}}$$