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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

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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.

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

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

2.5.5. Swelling power (SP)

The swelling powers of samples (V1, V2 and V3) were performed using the method described by Nattawat, Narumol, and Ornamphai (2009). Sample (0.2 g) was dispersed in water (20 ml). 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 sediment was used to calculate the swelling power. The supernatant were dried and measured the weight of dissolved starch in supernatant.

$$\text{swelling power} = \frac{\text{weight of sediment}}{\text{weight of dry starch} - \text{weight of dissolved starch}}$$

2.5.6. Pasting properties

The viscoamylographic property of samples (V1, V2 and V3) were performed using the method described by Polesi and Sarmento (2011) with 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 including paste temperature, peak viscosity; breakdown viscosity, final viscosity and setback viscosity were examined from viscoamylograph.

2.5.7. Textural characteristics

The textural properties of RVA gels were determined using the method Kaur, Singh, McCarthy, and Singh (2007) with texture profile analyzer (HDP/BS blade of texture analyzer (TA) TA-HD plus, Stable Micro Systems, Surrey). The starch paste (V1, V2 and V3) formed in the canister by RVA testing were transferred in cylindrical plastic tubes (25 mm diameter, 40 mm depth). After cooling at room temperature for 1 h, the paste were covered with aluminum foil and stored at 4 °C for 24 h. Then tubes were brought to room temperature before performing the test. The texture profile analysis was performed on the samples in tubes at room temperature. Then each gel sample inside the tube was penetrated (to a depth of 16 mm) with a cylindrical probe (5 mm in diameter). Force–time curves were obtained at a crosshead speed of 1.5 mm/s during two penetration cycles. From the texture profile curve, Hardness, Cohesiveness, Gumminess, Adhesiveness, Springiness, Chewiness and Stringiness were calculated.

2.5.8. XRD and relative crystallinity

The samples (V1, V2 and V3) were submitted to an X-ray diffractometer (Shimadzu XRD 7000) with Cu Kα radiation at speed of 2°/min at a diffraction angle 2θ of 4° and 50° at 40 kV and 30 mA. The XRD profiles were classified according to patterns described by Zobel (1964). RC of the starches was quantitatively estimated following the method of Nara and Komiya (1983) using software (Origin version 7.5, Microcal Inc., Northampton, MA, USA). The graphs were plotted between angles 2θ of 4° and 30° and smoothed with the “Adjacent Averaging” tool.

2.5.9. Thermal analysis

The thermal properties of samples, (V1, V2 and V3) were performed using the method described by Qunyu, Suling & Huali (2011) with Differential Scanning Colorimetry (TA-Q20 DSC), Gelatinization temperature of the samples (V1, V2 and V3) were measured and recorded on Differential Scanning Colorimetry (DSC). Water (14 μl) was added with a micro syringe to starch samples (6 mg dry basis) in the DSC pans, which were then sealed, reweighed and kept at room temperature for 24 h to ensure equilibration of the starch samples and water. The samples were scanned from 35 °C to 200 °C at heating rate of 10 °C/min and an empty pan was used a reference. The values of onset (T_o), peak (T_p) and final (T_f) gelatinization temperatures and enthalpy (ΔH) were obtained from the thermographs of the samples using Universal Analysis 2000 3.9A software.

2.6. Statistical analysis

All analysis was done in triplicate. The data were subjected to one way ANOVA to analyse the significance of difference in all data and Duncan’s Multiple Range Test (DMRT) ($P \leq 0.05$) to analyse the significance of difference between mean values of samples using SPSS 18 software (SPSS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Morphological characteristics

SEM micrographs (Fig. 1) of the V1 studied showed round, elliptical, irregular, kidney and oval shape with smooth surfaces. V2 and V3 resemble an amorphous mass of cohesive structure, leading to the loss of granular appearance. The change in appearance from granular to amorphous is due to the consequence of gelatinization temperature where the coupled starch granules forms sponge like structure leading to double helix in the inner region of the retrograded starch (Escarpa, Gonzalez, Manas, Garcıa-Diz, & Saura-Calixto, 1996; Morris, 1990; Ratnayake & Jackson, 2007). This change in the amorphous structure leads to the rejection in the activity of alpha amylase on the retrograded starch.

3.2. Chemical composition

Red kidney bean starch (V1) consisted of starch (90%), moisture (8.5%), protein (1.3%), fat (0.5%) and ash (0.7%), indicating that the isolated starch (V1) were quite pure and these constituents were comparable with other legume starch studied (Abdel-Rahman, El-Fishawy, El-Geddawy, Kurz, & El-Rify, 2008; Hoover & Manuel, 1995; Wani et al., 2010).

3.3. Physico-chemical properties

The amylose, RS content, total dietary fibre, swelling power, water solubility, water absorption index and relative crystallinity, of samples V1, V2 and V3 are shown in Table 1.

The amylose content of V1 (25.33%) was found to be significantly lower than V2 (48.66%) and V3 (67.66%). Further, V3 (67.66%) was found to be significantly greater than V2 (48.66%). The RS content of V3 (42.34%) was significantly higher when compared with V2 (31.47%) and V1 (21.27%) owing to the increased amylose content in Samples V2 & V3 are the resultant retrograded starch of V1 upon the treatment of pullulanase enzyme before and after gelatinization respectively. The considerable increase in RS content in V2 followed by V3 would be attributed by the effect of pullulanase enzyme on debranching of α-(1–6) linkage of amylopectin (BeMiller, Whistler, & Starch, 2009; Leong et al., 2007) which is converted into small chain linear polysaccharides like amylose molecules (Polesi & Sarmento, 2011; Suling et al., 2011). Similar studies are also widely reported for the formation of RS through the strong gel network formation on retrogradation (Jane & Chen, 1992). Legumes contain RS2 (granular starch) and during thermal process RS2 converts to RS3 by retrogradation process (Polesi & Sarmento, 2011). The RS formed in V2 and V3 is subjected to retrogradation which ensures the conversion of RS2 to RS3.The TDF content (both IDF & SDF) of V1 and V2 samples were 32.63% and 32.09% respectively, which were significantly lower than that of V3 (35.75%). The increase in TDF of V3 which is gelatinized, enzymatically hydrolysed retrograded starch could be attributed by the amylose retrogradation.

The swelling power of V1 observed at 85 °C (7.13%) and was significantly different from the SP of V2 at 85 °C (5.93%) and that of V3 at 85 °C (5.44%). Swelling power ascertains the

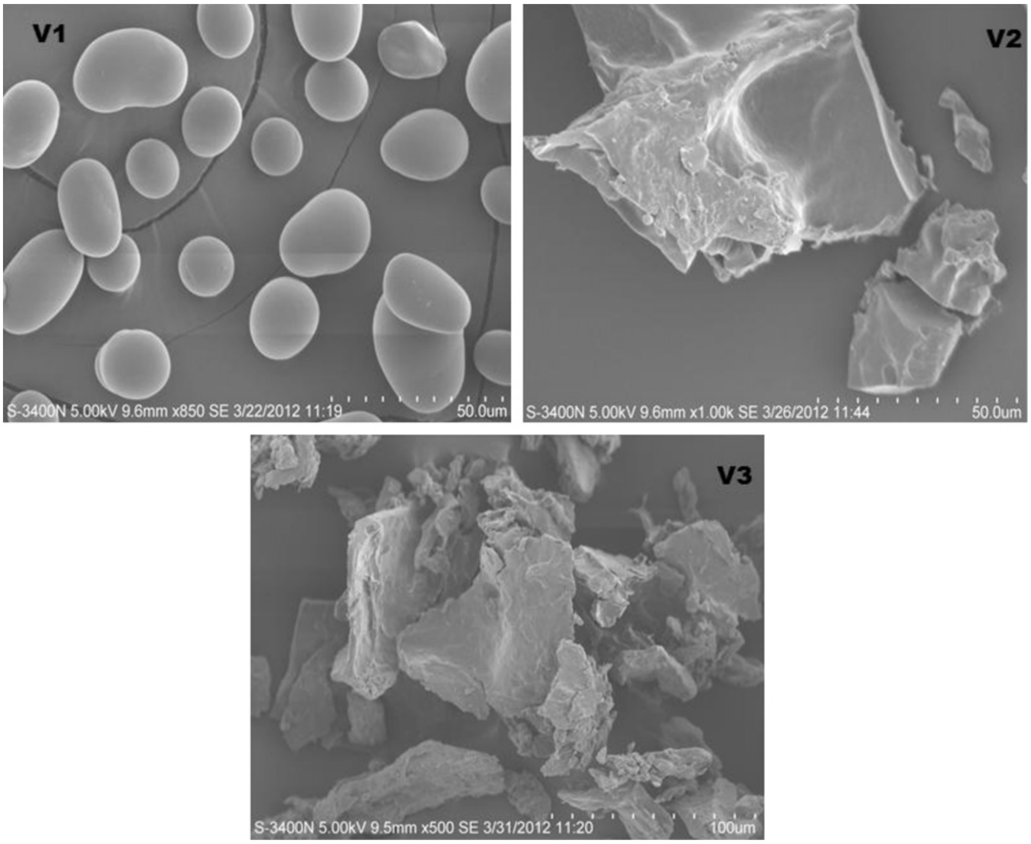

Fig. 1. Scanning electron micrographs (SEM) of V1 (red kidney bean starch), V2 (retrograded enzyme hydrolyzed native starch) & V3 (retrograded enzyme hydrolyzed gelatinized starch).

magnitude of interaction among the starch chain within the amorphous and crystalline region. Distribution of molecular weight, degree of debranching, length of branches and the conformation of the molecules (Singh, Singh, Kaur, Sodhi, & Gill, 2003) along with ratio of amylose and amylopectin places a very vital influence on the extent of water and starch interaction (Hoover, 2001). The decreased swelling power noted in V3 at both the temperature is owed by the subjection of the starch to gelatinization (95 °C) prior to enzyme hydrolysis followed by retrogradation.

There was a significant difference in WSI and WAC of V1, V2 and V3 ($P < 0.05$). The significant increase in WSI (15.05%) and WAC (6.70%) of V3 and WSI (13.0%) and WAC (6.13%) of V2 when compared to WSI (2.50%) and WAC (1.84%) of V1 could be interpreted by the variations in amylose/amylopectin content, extraction procedures and thermal treatment observed among V1, V2 and V3 in the present study (Table 1).

3.4. *Pasting properties*

Table 2 and Fig. 2 summarize the pasting properties of V1, V2 and V3. V1 was observed with higher pasting viscosity than V2 and V3 ($P < 0.05$) indicating the typical pasting characteristics of native starch, which is dependable with the investigation by Singh, Sandhu, & Kaur (2004) and Miao, Jiang, & Zhang (2009). Gelatinization and enzymatic hydrolysed starch (V2 & V3) have increased formation of short linear chain molecules and RS content which could be substantiated to the decrease in the pasting viscosity of V2 and V3 along with the reduced ability of forming gel (Gelencser, Juhasz, Hodsagi, Gergely, & Salgo, 2008; Polesi & Sarmento, 2011).

The pasting temperature was non detectable for V2 and V3 which could be owed to the destruction of starch granules when subjected to autoclave at 121 °C during resistant starch preparation.

Table 1
Amylose, resistant starch, total dietary fiber, swelling power, water absorption capacity, water solubility index and relative crystallinity V1, V2 & V3.[#]

Parameter		Type		
		V1	V2	V3
Amylose (%)		25.33 ± 2.3^c	48.66 ± 1.1^b	67.66 ± 3.2^a
RS (%)		21.27 ± 0.9^c	31.47 ± 0.9^b	42.34 ± 1.1^a
TDF (%)	IDF (%)	20.50 ± 1.1^b	22.63 ± 0.8^a	18.53 ± 0.9^c
	SDF (%)	12.13 ± 0.6^b	9.46 ± 0.6^c	17.23 ± 0.7^a
SP (%)		7.13 ± 0.1^a	5.93 ± 0.2^b	5.44 ± 0.1^c
WAC (%)		1.84 ± 0.1^c	6.13 ± 0.1^b	6.70 ± 0.1^a
WSI (%)		2.50 ± 0.1^c	13.0 ± 0.05^b	15.05 ± 0.1^a
RC (%)		35.72 ± 1.0^c	41.22 ± 1.3^b	45.88 ± 1.4^a

RS: resistant starch; TDF: total dietary fiber; IDF: insoluble dietary fiber; SDF: soluble dietary fibre; SP: swelling power; WAC: water absorption capacity; WSI: water solubility index; RC: relative crystallinity; V1: red kidney bean starch; V2: retrograded enzyme hydrolyzed native starch; V3: retrograded enzyme hydrolyzed gelatinized starch.

[#] All data were means of triplicates. Values with the same superscripts in a row did not differ significantly ($P < 0.05$) by DMRT.

Table 2
Pasting properties of V1, V2 & V3: pasting temperature (°C), peak time (min), peak viscosity (cP), hold viscosity (cP), final viscosity (cP), break down (cP) and set back (cP).#

Parameter	Type		
	V1	V2	V3
Pasting temp (°C)	77.9 ± 0.5^a	Nd	Nd
Peak time (min)	7.12 ± 0.2^a	6.48 ± 0.5^c	7.10 ± 0.3^b
Peak viscosity (cP)	591.66 ± 5.8^a	222.33 ± 5.1^b	194.33 ± 4.7^c
Hold viscosity (cP)	550.66 ± 5.3^a	227.66 ± 4.4^b	189.33 ± 5.8^b
Final viscosity (cP)	742.66 ± 2.0^a	252.66 ± 6.9^b	233.33 ± 7.6^b
Break down (cP)	7.63 ± 1.1^a	1.53 ± 0.5^b	1.8 ± 0.2^b
Set back (cP)	164.33 ± 8.7^a	39.66 ± 2.0^b	25.66 ± 2.0^c

V1: red kidney bean starch; V2: retrograded enzyme hydrolyzed native starch; V3: retrograded enzyme hydrolyzed gelatinized starch; nd: not detected.

All data were means of triplicates. Values with the same superscripts in a row did not differ significantly ($P < 0.05$) by DMRT.

Table 3
Textural properties of retrograded red kidney bean starches: hardness, cohesiveness, adhesiveness, gumminess, springiness, chewiness and stringiness.#

Parameter	Type		
	V1	V2	V3
Hardness (N)	0.37 ± 0.005^b	0.11 ± 0.007^c	0.09 ± 0.01^a
Cohesiveness	0.39 ± 0.005^c	0.47 ± 0.01^b	0.77 ± 0.009^a
Adhesiveness (Ns)	−10.62 ± 0.24^b	1.90 ± 0.04^a	−12.47 ± 0.46^c
Gumminess (N)	0.14 ± 0.003^a	0.22 ± 0.29^a	0.28 ± 0.009^a
Springiness (s)	1.02 ± 0.02^c	1.72 ± 0.41^a	1.18 ± 0.077^b
Chewiness (Ns)	0.14 ± 0.003^b	0.10 ± 0.004^c	0.35 ± 0.005^a
Stringiness	6.82 ± 0.07^c	7.67 ± 0.14^b	8.51 ± 0.10^a

V1: red kidney bean starch; V2: retrograded enzyme hydrolyzed native starch; V3: retrograded enzyme hydrolyzed gelatinized starch.

All data were means of triplicates. Values with the same superscripts in a row did not differ significantly ($P < 0.05$) by DMRT.

3.5. Textural properties

The RVA gels of samples (V1, V2, and V3) were analysed using Texture profile analysis after 24 h of retrograded storage at 4 °C and resulted data were showed in Table 3. Peak viscosity of sample (V1) was significantly greater than sample V2 and V3. This increase in peak viscosity of V1 related to the greater hardness when compared with V2 and V3 ($P < 0.05$). The increase in peak viscosity and hardness of V1 is attributed to the size of the starch granule and lower content of amylose when compared to V2 and V3 which is subjected to enzymatic hydrolysis. Further other texture parameters like hardness, cohesiveness, adhesiveness, gumminess, springiness, chewiness and stringiness were also found to be significantly different.

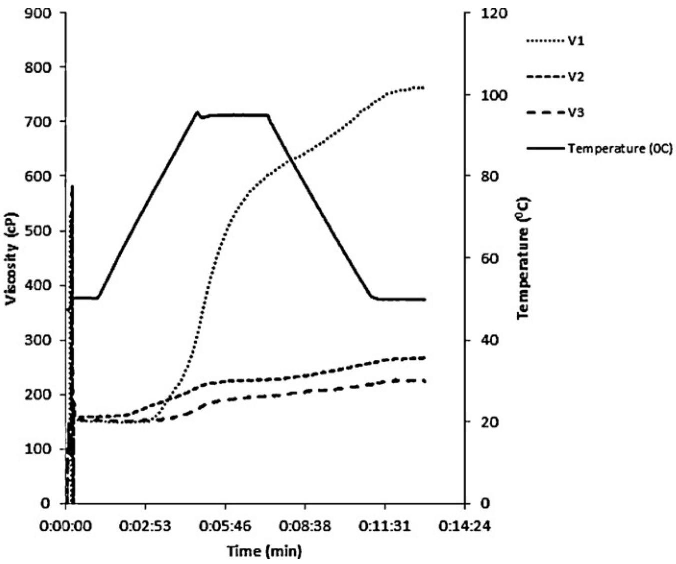

Fig. 2. Typical RVA starch pasting curves for of V1 (red kidney bean starch), V2 (retrograded enzyme hydrolyzed native starch) & V3 (retrograded enzyme hydrolyzed gelatinized starch).

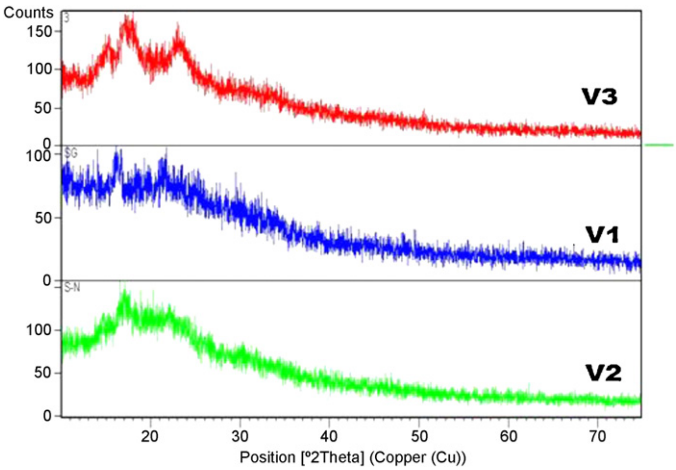

Fig. 3. X-ray diffraction pattern (XRD) of V1 (red kidney bean starch), V2 (retrograded enzyme hydrolyzed native starch) & V3 (retrograded enzyme hydrolyzed gelatinized starch).

The Difference in textural properties of all sample gels were influenced by rigidity in gelatinized starch, amylose content as well as interaction between the dispensed and continuous phase of the gel, which in turn is dependent on the amylose and amylopectin structure (Yamin, Lee, Pollak, & White, 1999).

3.6. XRD and Relative Crystallinity

The crystallinity of all sample (V1, V2 & V3) are showed in Table 1 and Fig. 3. Sample V1 showed C-type crystallinity pattern, with intermediate intensity peak at diffraction angles of $2\theta = 15.1^\circ$ and 15.9° and strong peak at $2\theta = 16.8^\circ$ and this type of crystallinity was very similar to mixture of type A and B crystallinity patterns (Miao, Zhang, & Jiang, 2009) and some authors also find c-type crystallinity pattern in legumes (Hughes, Hoover, Liu, & Donner, 2009). The crystallinity pattern for V2 and V3 showed type B with weak peaks $2\theta = 15.2^\circ$, intermediate peaks at $2\theta = 16.4^\circ$ and 18.0° and strong intensity peaks at $2\theta = 20.9^\circ$ and 21.2°which were similar with wheat and corn RS crystalline pattern (Eerlingen, Crombez, & Delcour, 1993; Mun & Shin, 2006).

Type B crystallinity pattern was formed due to retrogradation at low temperature. It can be observed at $2\theta = 20.9^\circ$ and 21.2° where similar pattern was also observed in sago starch (Leong et al., 2007) treated with pullulanase, autoclaved and retrograded. Comparing with V2, V3 showed strong intensity peaks because of the recrystallization with retrogradation. The relative crystallinity of V1 (35.72%) and V2 (41.22%) are lower when compared with V3 (45.88%). The highest crystallinity value for V3 could be due to the highest RS content, which can be enhanced by gelatinization, enzymatic treatment and retrogradation.

3.7. Thermal properties

Thermal analysis of V1, V2 and V3 were performed by DSC and the resulted data are represented in Table 4 and Fig. 4. The peak temperature for V1 was lower, when compared with V3 due to the gelatinization and retrogradation process. The Endothermic

Table 4
Thermal properties of retrograded red kidney bean starches: transition temperatures (T_0; T_P; T_C), enthalpy of gelatinization (ΔH gel), peak height index (PHI) and gelatinization range (R).[#]

Parameter	Type		
	V1	V2	V3
T_0 (°C)	57.5 ± 2.5^{ab}	58.1 ± 1.4^{b}	60.5 ± 0.7^{a}
T_P (°C)	98.3 ± 2.3^{b}	99.03 ± 0.7^{b}	103.0 ± 1.6^{a}
T_C (°C)	162.4 ± 5.0^{a}	161.8 ± 4.5^{a}	163.54 ± 4.7^{a}
ΔH gel (J/g)	293.3 ± 6.1^{b}	312.7 ± 2.7^{a}	346.8 ± 6.0^{a}
PHI (J/g.C)	7.9 ± 1.0^{a}	8.0 ± 0.04^{b}	8.1 ± 0.2^{a}
R (°C)	104.8 ± 6.2^{a}	103.7 ± 4.5^{a}	102.7 ± 5.1^{a}

T_0: onset temperature; T_P: peak temperature; T_C: conclusion temperature; ΔH gel: enthalpy of gelatinization; PHI, peak height index ((ΔH gel/($T_P - T_0$)); R, gelatinization range ($T_C - T_0$); V1: red kidney bean starch; V2: retrograded enzyme hydrolyzed native starch; V3: retrograded enzyme hydrolyzed gelatinized starch.

[#] All data were means of triplicates. Values with the same superscripts in a row did not differ significantly ($P < 0.05$) by DMRT.

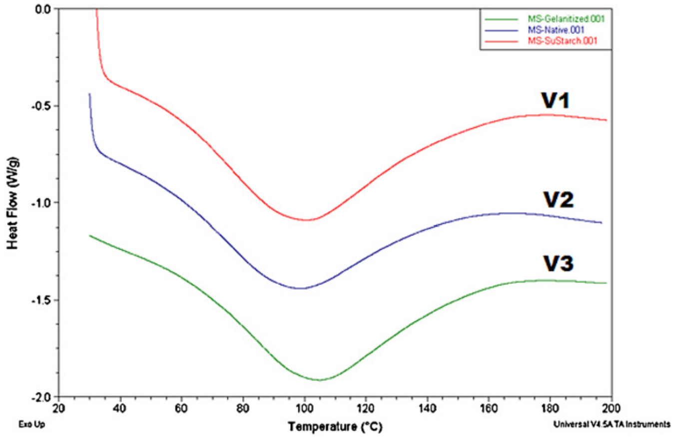

Fig. 4. Differential scanning calorimetry (DSC) thermograms of V1 (red kidney bean starch), V2 (retrograded enzyme hydrolyzed native starch) & V3 (retrograded enzyme hydrolyzed gelatinized starch).

enthalpy for V3 is higher, and it is directly proportional to the RS content. The peak temperature of V3 was similar with other legume studies (Suling, Gao, & Ward, 2011) and the RS rich samples (V2 & V3) shows broad peaks when compared with native starches because of its re-association of amylose upon retrogradation. Haralampu (2001) reported that DSC of retrograded amylose shows thermal activity from 100 to 165 °C due to the amylose retrogradation. The enthalpy of gelatinization was observed to be lowest for V1 (293.3 J/g) when compared with V2 (312.7 J/g) and V3 (346.8 J/g). Enthalpy of gelatinization gives an overall measure of crystallinity (quality and quantity) and is an indicator of the loss of molecular order with the granule that occurs on gelatinization (Cooke & Gidley, 1992; Tester & Morrison, 1990). Lower enthalpy shows the less stability of the crystals (Chiotelli & Meste, 2002). The thermal properties of starches were influenced by granule shape, amylopectin chain length and crystalline regions (Noda, Takahata, Sato, Ikoma, & Mochinda, 1996; Singh & Kaur, 2004; Stevens & Elton, 1971). Differences of *R* values between samples suggest that the presence of transforms in crystalline regions of the starch granules.

4. Conclusion

Red kidney beans (RKB) starch when subjected to enzymatic hydrolysis by pullulanase increased the yield of RS significantly. In food industries the requisite for processed starch include greater water absorption, solubility, and crystallinity along with higher thermal stability. Similarly the retrograded enzyme hydrolysed gelatinized starch of RKB has shown increased water absorption and solubility with decreased starch viscosity. The increased RS content is inversely propositional to the viscosity of starch. The retrograded enzyme hydrolysed gelatinized starch of RKB can be used as a source of RS from legumes which meet the favourable prerequisite of a modified starch in food processing industries and also a functional ingredient in food product development.

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