Pasting, textural and thermal properties of resistant starch prepared from potato (*Solanum tuberosum*) starch using pullulanase enzyme

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Abstract Pullulanase enzyme (40 U/g, 10 h) was used for enzymatic hydrolysis of potato starch which was autoclaved (121 °C/30 min), stored under refrigeration (4 °C/24 h) and lyophilized. Comparison of morphological, pasting, textural and thermal properties among native hydrolysed starch (V2) and gelatinized hydrolysed starch (V3) prepared using pullulanase enzyme on potato starch (V1) were studied. The round, elliptical, irregular and oval shape with smooth surface of V1 was replaced with amorphous mass of cohesive structure leading to loss of granular appearance in V2 and V3. The percentage of amylose and resistant starch content of V2 (27.16 %) and (24.16 %); V3 (51.44 %) and (29.35 %) was higher when compared to V1 (22.17 %) and (3.62 %). The swelling power of V1 observed at 60 °C (0.85 %) and 95 °C (8.64 %) were significantly different from V2 at 60 °C (4.97 %) and 95 °C (7.66 %) and that of V3 at 60 °C (5.82 %) and 95 °C (7.5 %). Significance difference in water solubility (7.62 %) and absorption capacity (6.11 %) was noted in V3 when compared with V1 and V2 owing to amylose/amylopectin content. Increase in water solubility and absorption capacity along with decrease in swelling power of V2 and V3 was noted due to hydrolytic and thermal 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 ($P < 0.05$).

Keywords *Solanum tuberosum* · Enzymatic hydrolysis · Pullulanase and resistant starch

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
TDF	Total dietary fiber
IDF	Insoluble dietary fiber
SDF	Soluble dietary fibre
SP	Swelling power
T ₀	Onset temperature
T _p	Peak temperature
T _c	Conclusion temperature
ΔH _{gel}	Enthalpy of gelatinization
PHI	Peak height index
R	Gelatinization range

Introduction

In the world, India ranks second in the production of potato with the highest consumption among various age groups in various forms without channelizing the consumption of potato to its functional ingredients. Raw potato contains 15 % of starch and 3–4 % of Resistant Starch (RS) but RS content was increased up to 7 % during cooking and nearly 13 % upon cooling (Englyst et al. 1992). Potato starch is widely used in the food industry as a coating, blending, thickening agent etc., owing to its bland flavor and white nature (Madsen and Christensen 1996). Though potato starch do not owe to the functional ingredient in diet, the sensorial quality of the food recipe is always met with availability of such a starch with

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functionality that would be a boon in the food industry, such as an exceptionality is the RS derived from potato.

RS is one of the enriched functional ingredients, which exists in four different groups such as RS1, physically inaccessible to digestion entrapment in a non-digestive matrix (Haralampu 2000); RS2, native and ungelatinized starch granules as in raw banana and potato; RS3, retrograded starch (Muir and O'Dea 1992; Sajilata et al. 2006) and RS4, chemically modified starch (Englyst et al. 1992; Sajilata et al. 2006). RS2 is the form present in potato (Sajilata et al. 2006) with the lack of alpha-amylase enzyme in human system owing to the indigestibility in small intestine yet providing beneficial action in the large intestine with increase in short chain fatty acids with the help of microflora of the large bowel (Haralampu 2000; Muir et al. 1995; Thompson 2000).

Quoting the beneficial potential of RS2 available in potatoes, improving the yield for the availability of usage in the food industry being the need of the hour RS from potato has a bland taste, white in colour and predominantly insoluble (Sajilata et al. 2006) which would blend in the most of the commercially processed foods including French fries, similes, potato wedges, pastas and noodles being the major form of consumption of potatoes (Yue and Waring 1998).

Though recovery of RS using alcoholic alkaline methods dented the significance and availability of RS as a functional ingredient in potato the need to have a increased yield in RS with the beneficial functionality of pasting, texture and better thermal stability and also increased crispness would improve the usage of RS in food product development (Nugent 2005). With this as the basis, the present study was focused to yield the RS present in potato using Pullulanase enzyme (Polesi and Sarmento 2011). Pullulanase enzyme has been used in recovery of RS in various legumes, vegetables and cereals along with many other enzymes including α -amylase (Zhang and Jin 2011). Though much studies are not available on the usage of pullulanase enzymes in recovery of potato RS, the objective of the present was framed at quantitative of RS from potato using pullulanase enzyme and evaluating the pasting, texture and thermal capability of the derived RS compared to the potato starch used in the food industry.

Materials and methods

Materials

Fresh white potatoes (*Solanum tuberosum*) were procured from local market. The exclusion criteria included that potatoes which were scalded, block spotted were excluded as these criteria would directly owe to the starch content of potato. The selected potatoes were washed, wiped free from dirt and stored at 4 °C. Pullulanase enzymes from *Bacillus acidopulvificus* (Promozyme 400 L) were purchased from Sigma chemical company, USA. RS assay kit

was purchased from Megazyme International Ireland Limited, Ireland.

Isolation of starch

The potato starch was isolated from potatoes using the methodology described by Singh and Singh (2001). The procured potatoes were scrubbed; eyes and all bruises were pitted out and peeled. The peeled potatoes were cut in to pieces of 4 cm² and further dipped in potassium metabisulphite (0.35 g/L) solution to avoid browning. Homogenate slurry was extracted from potato pieces using a hand blender. The slurry was then filtered through muslin cloth. The residue left on the muslin cloth was washed with distilled water, until only small amounts of starch were passing the muslin cloth. Filtrate was collected in a glass beaker and residue was discarded. Further the filtrate was passed through sieves (60 and 100 US) and was left undisturbed for 4 h. By decanting the supernatant liquid a solid layer of starch settled down and was reslurried in distilled water. The process was repeated for 4–5 times until the supernatant was transparent. The starch cake formed was collected and dried at a temperature of 40 °C in a hot air oven.

Preparation of resistant starch

Enzymatic hydrolysis

Enzymatic hydrolysis of potato starch was carried out by the method of Polesi and Sarmento (2011). The potato starch suspension (10 % w/w db) was taken in sodium acetate buffer (0.1 M and pH 5.2). The mixture was added to pullulanase enzyme (40 U/g dry starches) and incubated in water bath at 60 °C for 10 h. The resultant 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.

Preparation of resistant starch

The starch samples, Potato 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.

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.

Scanning electron microscopy

The morphological studies 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 micrographs were obtained by assembly of the samples on aluminum stubs with double side adhesive tape to which the samples were fixed and covered with a thin gold layer.

Physico-chemical characteristics

Chemical analysis

The moisture content of potato starch (V1) was determined by gravimetric heating (130 ± 2 °C for 2 h) using a 2–3 g sample. Proximities including ash, protein, and fat were analyzed according to AACC methods 08–01, 46–13 and 30–25. The starch content in potato starch was carried out using the methodology described by Hodge and Hofreiter (1962).

Amylose content

The amylose content of samples V1, V2 and V3 were determined using the method described by McCready et al. (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 color 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.

Total dietary fibre (TDF)

TDF of the samples V1, V2 and V3 was 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 et al. (1983). Shortly, in this method the enzymatic hydrolysis of starch and protein were performed in three steps: gelatinization in the presence of a 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 4 volumes of 95 % ethanol and recovered by filtration.

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

The water solubility and absorption capacity of samples (V1, V2 and V3) were performed using the method described by Anderson et al. (1969). Briefly, sample 0.5 g was mixed with 6 ml of distilled water, and then it was centrifuged. After centrifugation the sample was kept in a water bath at 30 °C with continuously stirring for 30 min, then 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: $WSI = (\text{weight of dry solids in supernatant} / \text{weight of dry sample}) \times 100$; $WAC = \text{weight of wet sediment} / (\text{weight of the dry sample} - \text{weight of the dry solids})$.

Swelling power (SP)

The swelling power of samples (V1, V2 and V3) was performed using the method described by Leach et al. (1959). Briefly, 0.5 g of Sample was dispersed in distilled water followed by heating at 60 °C and 95 °C for 30 min in a boiling water bath. Then the suspensions were cooled and centrifuged at 5,000 rpm for 15 min, from that the supernatant water was decanted and the residue weighed. The SP was determined as: $SP = \text{weight of sediment} / \text{weight of dry sample solids}$.

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 distilled water. The following parameters are paste temperature, peak viscosity; breakdown viscosity, final viscosity and setback viscosity were examined from viscoamylograph.

Textural characteristics

The textural properties of RVA gels were determined using the method Lovedeep Kaur et al. (2007) with texture profile analyser (HDP/BS blade of texture analyzer (TA) TA – HD plus, Stable Micro Systems, Surrey). The starch (V1, V2 and V3) pastes 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 gels 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/sec during two penetration cycles. From the texture profile curve, Hardness, Cohesiveness, Gumminess, Adhesiveness, Springiness, Chewiness and stringiness were calculated.

X-ray diffraction

The samples (V1, V2 and V3) were submitted to an X-ray diffractometer (Shimadzu XRD 7000) with Cu K α radiation at speed of 2°/min at a diffraction angle 2 θ of 4 and 50° at 40 kV and 30 mA. The XRD profiles were classified according to patterns described by Zobel (1964).

Thermal analysis

The thermal properties of sample (V1, V2 and V3) were performed using the method described by Gao et al. (2011) with Differential Scanning Colorimetry (TA-Q20 DSC). Gelatinization temperatures were measured and recorded on Differential Scanning Colorimetry (DSC). Weighed 6 mg of the samples in the DSC pans and the samples were scanned from 40 °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.

Statistical analysis

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

Results and discussion

Morphological characteristics

SEM micrographs of V1 when compared to V2 and V3 differed significantly. Micrographs (Fig. 1) of the V1 studied showed round, elliptical, irregular 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 et al. 1996; Morris 1990; Ratnayake and Jackson 2007). This change in the

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

amorphous structure leads to the rejection in the activity of alpha amylase on the retrograded starch.

Chemical composition

Native potato starch (V1) contains starch (91 %), moisture (6.3 %), protein (0.5 %), fat (0.2 %) and ash (1.3 %) content, indicating that the isolated starch (V1) were quite pure and this chemical composition was comparable with other starch studies (Hoover and Manuel 1995; Abdel-Rahman et al. 2008).

Table 1 Resistant starch, amylose, total dietary fiber, swelling power, water absorption capacity and water solubility index of V1, V2 & V3

Type		V1	V2	V3
RS (%)		3.62 $\pm$ 0.2 ^c	24.16 $\pm$ 0.9 ^b	29.35 $\pm$ 0.9 ^a
Amylose (%)		25.17 $\pm$ 1.1 ^c	42.33 $\pm$ 0.7 ^b	51.44 $\pm$ 2.1 ^a
TDF (%)	IDF (%)	18.86 $\pm$ 0.5 ^c	22.01 $\pm$ 0.4 ^b	23.90 $\pm$ 2.0 ^a
	SDF (%)	12.13 $\pm$ 0.6 ^b	13.20 $\pm$ 0.6 ^b	15.23 $\pm$ 0.7 ^a
SP (%)	SP 60 °C	0.85 $\pm$ 0.1 ^c	4.97 $\pm$ 0.2 ^b	5.82 $\pm$ 0.3 ^a
	SP 95 °C	8.64 $\pm$ 0.2 ^a	7.66 $\pm$ 0.2 ^b	7.50 $\pm$ 0.1 ^b
WAC (%)		0.70 $\pm$ 0.1 ^b	6.09 $\pm$ 0.1 ^a	6.11 $\pm$ 0.2 ^a
WSI (%)		1.83 $\pm$ 0.1 ^c	5.33 $\pm$ 0.2 ^b	7.62 $\pm$ 0.2 ^a

Values with the same superscripts in a row did not differ significantly ($p < 0.05$) by DMRT

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, V1 potato starch, V2 retrograded enzyme hydrolyzed native starch, V3 retrograded enzyme hydrolyzed gelatinized starch

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)

Type	V1	V2	V3
Pasting temp (°C)	157.33±1.5 ^a	nd	nd
Peak time (min)	7.12±0.2 ^a	6.48±0.5 ^b	7.10±0.3 ^a
Peak viscosity (cP)	5722.00±37.5 ^a	218.33±2.5 ^b	217.33±2.5 ^b
Hold viscosity (cP)	4657.33±22.5 ^a	207.66±4.1 ^b	206.00±5.0 ^b
Final viscosity (cP)	5403.00±13.1 ^a	233.33±6.8 ^b	230.00±9.5 ^b
Break down (cP)	1042.00±48.7 ^a	15.00±1.0 ^b	6.00±1.0 ^b
Set back (cP)	865.33±8.0 ^a	26.66±2.0 ^b	26.66±2.5 ^b

Values with the same superscripts in a row did not differ significantly ($p < 0.05$) by DMRT

V1 potato starch, V2 retrograded enzyme hydrolyzed native starch, V3 retrograded enzyme hydrolyzed gelatinized starch, nd not detected

Physico-chemical properties

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

The amylose content of V1 (25.17 %), was found to be increased in V2 (42.33 %) and V3 (51.44 %) which were the resulting retrograded starch of V1 after the treatment with pullulanase enzyme before and after gelatinization respectively. The increase in amylose and RS content of V3 followed by those of V2 could be attributed to the effect of pullulanase enzyme on debranching of α -(1–6) linkage of amylopectin (BeMiller and Whistler 2009; Leong et al. 2007) which is converted into small chain linear polysaccharides like amylose molecules (Li et al. 2011; Polesi and Sarmento 2011).

The content of RS in V3 (29.35 %) was found to be significantly higher when compared to the V1 (3.62 %) and V2 (24.16 %). The RS type varies in V1, V2 and V3 as the RS expressed in V1 and V2 is RS2 type and the hydrothermal treated sample V3 contains RS3 type which is retrograded

Table 3 Textural properties of retrograded potato starches: hardness, cohesiveness, adhesiveness, gumminess, springiness, chewiness and stringiness[#]

Type	V1	V2	V3
Hardness (N)	0.41±0.006 ^a	0.10±0.012 ^b	0.08±0.006 ^c
Cohesiveness	0.38±0.002 ^b	0.54±0.027 ^a	0.53±0.011 ^a
Adhesiveness (Ns)	-14.27±0.54 ^c	-3.30±0.18 ^a	-4.10±0.11 ^b
Gumminess (N)	0.15±0.004 ^a	0.17±0.21 ^a	0.04±0.001 ^a
Springiness (s)	1.08±0.03 ^a	0.93±0.49 ^b	0.96±0.019 ^b
Chewiness (Ns)	0.17±0.007 ^a	0.03±0.006 ^b	0.04±0.002 ^b
Stringiness	6.66±0.20 ^a	6.67±0.28 ^a	5.76±0.09 ^b

Values with the same superscripts in a row did not differ significantly ($p < 0.05$) by DMRT

V1 potato starch, V2 retrograded enzyme hydrolyzed native starch, V3 retrograded enzyme hydrolyzed gelatinized starch

enzyme hydrolyzed gelatinized starch. The RS formed in V2 and V3 could be reported as RS3. Similar studies are widely reported for the formation of RS through the strong gel network formation on retrogradation (Jane and Chen 1992). The significantly increased V3 would aid in enhancing better potential of RS in human system as it is mostly in the RS3 form in the regular cooked food consumption.

The TDF content (both IDF & SDF) of V1 and V2 samples were 30.99 % and 35.21 % respectively, and shown to be lower than that of V3 (39.13 %). The increase in TDF of V3 with more thermal stability could be attributed to the amylose retrogradation with the effect of treatment to which V3 was subjected.

The SP of V1 observed at 60 °C (0.85 %) and 95 °C (8.64 %) was significantly varied from SP of V2 at 60 °C (4.97 %) and 95 °C (7.66 %) and that of V3 at 60 °C (5.82 %) and 95 °C (7.50 %). The observation of the decrease in the SP of V2 and V3 irrespective to increase in temperature could be attributed to the effect of gelatinization and autoclave process, which was done during the preparation of V2 and V3.

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

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

WAC is a phenomenon in wet heat treatment of starch sample. In this study, the gelatinization induced by heating and autoclaving has increased WAC in V3 (6.11 %) when compared to V1 (0.70 %). The WAC of V2 (6.09 %) was also similar to V3 (6.11 %) as both V2 and V3 have expressed the gelatinization process. The results of the study coincide with that of RS obtained from corn (Koksel et al. 2007) and chick pea starch (Polesi and Sarmento 2011) and the increased water activity in retrograded starch is noted. Increase in water solubility in the result of change in molecular structure or any mechanism leading to easy mobility of starch components resulting as a process of gelatinization and retrograded where leaching of starch is noticed (Colonna and Mercier 1983; Govindasamy et al. 1996). Similarly the WSI of V3 (7.62 %) was significantly differed from V2 (5.33 %) and V1 (1.83 %)

followed by V2 as V2 and V3 have undergone hydrolytic process when compared to V1. The enzymatic hydrolysis has contributed to the increased water solubility in V3.

Pasting properties

Pasting properties of V1, V2 and V3 are presented in Table 2 and Fig. 2. Sample V1 showed higher pasting viscosity than V2 and V3 ($P < 0.05$) indicating the typical pasting characteristics of native starch, which is comparable with other studies Singh and Kaur (2004), Miao et al. (2009a). Gelatinization and enzymatic hydrolysed starch (V2 & V3) have increased formation of short linear chain molecules and RS content which could lead to the decrease in the pasting viscosity of V2 & V3 along with the reduced ability of forming gel (Polesi and Sarmento (2011) and Gelencser et al. (2008)).

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. Similar pattern was observed in the preparation of RS from chickpea starch (Polesi and Sarmento 2011).

Textural properties

The RVA gels (V1, V2, and V3) were analysed using texture profile analysis after 24 h of retrograded storage at 4 °C and resulted data are shown in Table 3. More peak viscosity sample (V1) has greater hardness when compared with V2 and V3 ($P < 0.05$), and the higher peak viscosity which may be caused by presence of large granule and lower levels of amylose. The results suggest that there was significant variation present between all samples (V1, V2 & V3) with respect of all texture parameters like hardness, cohesiveness, adhesiveness, gumminess, springiness, chewiness and stringiness.

Table 4 Thermal properties of retrograded potato starches: transition temperatures (T_0 , T_P , T_C), enthalpy of gelatinization (ΔH_{gel}), peak height index (PHI) and gelatinization range (R)

Type	V1	V2	V3
T_0 (°C)	51.6±0.8 ^b	57.5±2.5 ^a	60.0±1.5 ^a
T_P (°C)	97.03±0.7 ^b	96.2±0.5 ^b	102.6±1.2 ^a
T_C (°C)	154.3±4.5 ^b	158.9±1.2 ^{ab}	160.9±1.3 ^a
ΔH_{gel} (J/g)	275.1±6.6 ^c	320.3±5.1 ^b	343.5±5.2 ^a
PHI (J/g.C)	6.6±0.2 ^b	7.5±0.3 ^a	7.6±0.1 ^a
R (°C)	101.1±1.7 ^a	101.3±1.6 ^a	103.4±2.2 ^a

Values with the same superscripts in a row did not differ significantly ($p < 0.05$) by DMRT

T_0 onset temperature, T_P peak temperature, T_C conclusion temperature, ΔH_{gel} enthalpy of gelatinization, PHI peak height index ($(\Delta H_{gel}/(T_P - T_0))$), R gelatinization range ($T_C - T_0$), V1 potato starch, V2 retrograded enzyme hydrolyzed native starch, V3 retrograded enzyme hydrolyzed gelatinized starch

Fig. 4 Differential scanning calorimetry (DSC) thermograms of V1 (potato 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 et al. 1999).

X-ray diffraction

The X-ray diffraction pattern of all sample (V1, V2 & V3) are shown in Fig. 3. Sample V1 showed C-type crystallinity pattern, with intermediate intensity peak at diffraction angles of $2\theta=16.9^\circ$ and strong peaks at $2\theta=22.1^\circ$ & 24.1° and this type of crystallinity was very similar to mixture of type A and B crystallinity patterns (Miao et al. 2009b). The crystallinity pattern for V2 and V3 showed type B with weak peaks $2\theta=16.9^\circ$ and 17.2° , intermediate peaks at $2\theta=22.0^\circ$ & 22.0° and strong intensity peaks at $2\theta=24.1^\circ$ & 24.2° which were similar with wheat and corn RS crystalline pattern (Eerlingen et al. 1993; Mun and Shin 2006). Type B crystallinity pattern was formed due to retrogradation at low temperature. It can be observed at $2\theta=20.9^\circ$ & 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 highest crystallinity value for V3 could be due to the highest RS content, which can be enhanced by gelatinization, enzymatic treatment and retrogradation.

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 enthalpy for V3 is higher, and it is directly

proportional to the RS content. 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 (275.1 J/g) when compared with V2 (320.3 J/g) & V3 (343.5 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 (Tester and Morrison 1990; Cooke and Gidley 1992). Lower enthalpy shows the less stability of the crystals (Chiotelli and Meste 2002). The thermal properties of starches were influenced by granule shape, amylopectin chain length and crystalline regions (Noda et al. 1996; Stevens and Elton 1971; Singh and Kaur 2004). Differences of R values between samples suggest that the presence of transforms in crystalline regions of the starch granules.

Conclusion

Potato starch when subjected to enzymatic hydrolysis by pullulanase increased the formation of RS and amylose content significantly. Along with this one there was increase in the thermal stability, water solubility, water absorption capacity and decrease in the swelling power and pasting property of RS which is a desirable attribute for retrograded starch. The increased RS content is inversely propositional to the viscosity of starch. Though isolation, identification, quantification and morphological studies of RS1 and RS2 are enormous the typical mode and exhibiting the functional property of RS into human system is RS3. This kind of enhanced RS content from potato using pullulanase would help in new food product development and also in food processing industries as an adjunct.

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