Effect of acetyl esterification on physicochemical properties of chick pea (*Cicer arietinum L.*) starch

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Abstract Acetyl esterification of isolated Bengal gram starch was carried out using acetic anhydride as reactant. Modification of native starch at variant concentrations of acetic anhydride (6, 8 and 10 %, w/w) resulted in modified starch with 2.14, 3.35, 4.47% acetyl content and 0.082, 0.130 and 0.176° of substitution (DS) respectively. The acetyl esterification of native starch brought significant changes in physicochemical properties with respect to pasting behavior, granule morphology, thermal properties and retrogradation profile. Acetyl modifications of native starch increased swelling capacity, water absorption power and oil absorption capability by 17, 13 and 20 % respectively. Acetylation has decreased pasting temperature, pasting time, final viscosity and set back viscosity due to increase in amylose content, hydrogen bonding and porosity of starch granule. The acetyl modification was confirmed by IR spectra with the presence of an ester carbonyl group ($C = O$) at 1720.3 cm^{-1} and absorption band at 174.8 cm^{-1} . In DSC evaluation there was decrease in T_o , T_p , T_c and ΔH of acetylated starch than native starch which resulted in reduced retrogradation by 56 %.

Keywords Bengal Gram · Acetylation · Pasting Properties · Swelling · Gelatinization · Retrogradation

Introduction

Pulses originate from dicotyledons kernels of plants, principally of family Leguminosae (Hoover and Sosulski 1991). These pulses are the indispensable portions of common diets, since these are rich and inexpensive source of quality protein,

carbohydrate, vital nutrients like B-Complex vitamins, minerals etc. (Jood et al. 1988). Protein content among various pulses varies widely from 16 to 42 % and carbohydrate from 58 to 84 %. Some of the pulses provide good amount of fats also viz. Soybean. Pulses are being used to prepare variety of processed foods but amid various food legumes Garbanzo bean (*Cicer arietinum L.*), also known as Bengal gram/Chick pea/Ceci bean/Chana is most popular. Global Bengal gram production was approximately 8.1 million tons in 2006–2007 (FAO-Food and Agriculture Organization of the United Nations 2007) and India produces more than 70% of the global Bengal gram production. Generally, Bengal gram possesses a sweet & mellow taste. Flour of husked garbanzo bean is termed as ‘Besan/Bengal gram flour’ and is used for making a wide variety of sweet/savoury refreshments and munchies. Nearly each part of chick pea finds its use in some or other food/feed preparation. Bengal gram flour is also used in combination with wheat flour for making special kind of chapaties called as *Missie Roti* (Sharma et al. 2006). Some of the fermented snacks like *Dhokla*, a popular dish of Gujrat is also made from Besan after natural fermentation. Traditionally it is being used for making diverse food preparations and commercially it is the part of various fried snacks, fermented snacks, flaked snacks, extruded snacks, roasted snacks, sweet snacks, soups, curries, sweets and condiment mixtures.

Significant attempts have been made to apprehend the nutritional composition (Jórck I 1996) and commercial viability of Bengal gram flour. But usage of its starch moiety was not explored much for many reasons including high amylose content which in terms limit its application due to excessive retrogradation (Rege and Pai 1996), low solubility, swelling index, improper granule dispersability, high gelatinization temperature, high syneresis and resistance to enzymatic hydrolysis (Hoover et al. 2010). There is a huge demand for base

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materials such as starch for diversified industrial applications, hence, legume starches may be the unique stuffs for such commercial need (Adebawale et al. 2009). Chemical modification (substitution/cross linking) of native starches not only improves their resistance against retrogradation and syneresis but also increases paste viscosities, freeze thaw stability and paste clarities (Tuschoff 1987; Li et al. 2011). Esterification of native starches by way of inserting acetyl group produces a starch having film forming, adhesive, thickening, stabilizing, gelling, encapsulating, moisture retaining, texturizing and shelf life extending properties better than its native forms (Aggarawal et al. 2004; de Graaf et al. 1995). Presently many industries of food and non-food sectors are using low degree substituted starches to enhance various significant qualities such as rapid cooking at low temperatures because of high swelling, solubility and low gelatinization temperatures (Liu et al. 1999; Wang and Wang 2001). Considering commercial potentials of Bengal gram, present study was undertaken to isolate, modify (acetylated) and characterize its starch. Acetylation of starch is a chemical substitution reaction that results into granular acetylated product (modified starch) at low temperature for various thickening need (Wurzburg 1986). During investigation the physicochemical profile and process influencing factors like swelling power, solubility, pasting properties, oil and water binding capacities, pattern of retrogradation of the native and modified Bengal gram starch were studied. The information gathered out of the present study will help to provide sufficient knowledge to improve the utilization of milled products of Bengal gram in general and native & modified starches in particular for various food and non-food applications. This will also sustain many related industries to standardize various food process protocols and recipes for desired product with demanding nutritional requirement.

Materials and methods

Raw material and preparation of flour

Husked Bengal gram (Annigeri-1) (*Cicer arietinum L.*) splits had been purchased from grain market (APMC) of Mysore, Karnataka (India). Cotyledons were screened for defective grains and cleaned carefully for any objectionable materials such as fodder, straws and dust using high pressure air. Nearly identical dried splits grinded in a centrifugal mill (Retsch GmbH ZM200, Germany) using a sieve of 0.75 μ size to prepare fine flour (Besan). The temperature during grinding was restricted to maximum 40 °C using chilled water circulator coupled with pulverizer.

The flour was defatted and stored in paper/Al foil/polyethylene (PFP) pouches at ambient temperatures for starch isolation.

Isolation of starch in alkaline medium

Starch from defatted Bengal gram flour was isolated applying wet milling protocol (Paramahans et al. 1980) with slight modifications. 2 kg of flour was soaked in 6 L of 0.06 M NaOH solution for 4 h at ambient temperature, supernatant decanted and residue washed 4–5 times repeatedly for alkali amputation. Starch milk was passed through 150 mesh size and centrifuged at 2,500 rpm for 10 min (Model 7780 kuboto corporation, Japan). The slimy protein layer from the top was scrapped-off carefully. The starch cake was obtained after vacuum filtering the starch milk on a G2 grade sintered Buchner funnel. Subsequently starch cake was suspended in 95 % ethanol for 1 h, finally filtered on a Whatman no. 1 filter paper using Buchner funnel. The cake was dispersed and dried under vacuum at 40 °C for 72 h to a moisture level of about 3–5 %.

Preparation of acetylated starch

Acetylated starch was prepared using anhydrous acetic anhydride as reactant (Sodhi and Singh 2005) with slight modification. 1 kg isolated native starch was dispersed in 1.25 L of distilled water with constant shaking for 1 h at 25 °C. pH of the resultant slurry was adjusted in alkaline zone (8.0–8.3) using 0.75 M NaOH solution. Slurry was reacted with acetic anhydride (drop wise) w/v (6, 8 and 10 %) maintaining pH in the range of 8.0–8.4 using 0.75 M NaOH solutions. The reaction was continued for next 10–12 min after acetic anhydride addition. The slurry was then adjusted to a pH of 4.5 with 0.5 M HCl. After sedimentation, residue was repeatedly washed with distilled water to remove acid residue. Finally it was washed with ethanol followed by drying at 50 °C to a moisture level of about 3–5 %.

Quantification of Acetyl percentage (Ac %) and degree of substitution (DS)

Acetyl percentage and degree of substitution in the starch samples was estimated according to the method of Wurzburg (1986). Acetylated starch (1 g) was taken in a 250 ml iodine flask and 50 ml of 75 % Ethanol in distilled water was added. The loosely stoppered flask containing sample was heated to 50 °C for 30 min with continuous agitation. It was cooled and 40 ml of 0.5 M KOH was added. The excess alkali was back titrated with 0.5 M HCL using phenolphthalein as an indicator.

The solution was left for 2 h and again titrated for any additional alkali leech out. A blank using native starch was also carried out. DS is defined as the average number of sites per glucose unit that possess a substituted group (Whistler and Daniel 1985).

$$\text{Ac\%} = \frac{[(V_b - V_s) \times M \text{ of HCl} \times 0.043 \times 100]}{\text{Wt.}}$$

$$\text{DS} = \frac{(162 \times \text{Ac\%})}{[4300 - (42 \times \text{Ac\%})]}$$

V_b	Volume of blank
V_s	Volume of sample
Ac %	Acetyl percentage
M	Molarity
Wt	Weight of the sample
DS	Degree of Substitution

Physico-chemical profile

Moisture, protein, ether extractable fat, total ash and carbohydrate content of the starches were estimated as per AACC International (2003). The pH of 20 % starch slurry (50 ml) was measured using a microprocessor based pH meter (Model cyber scan pH 1,500, Eutech Equipments Pvt. Ltd, Chennai, India) (Larsonneur 1993).

Amylose estimation

Amylose in the native and modified samples was estimated as per the method described by Cready and Harrid.

(Cready and Harrid 1943). Sample (100 mg) weighed into a standard flask (100 ml) and alcohol (1 ml) was added to wet the starch. Subsequently water (10 ml) and 10 % NaOH (2 ml) was added. The solution was heated on a water bath until a clear solution formed and volume was makeup. From the above solution, 1 ml of the alkaline starch suspension was transferred to the standard flask (100 ml), 20 ml of water and 2–3 drops of 6 N acetic acid was added to acidify the sample. The content was mixed well, iodine solution (0.25 %) added and volume made with distilled water. The intensity of blue color was estimated using spectrophotometer (Shimadzu UV-2550, Japan) at 620 nm. The standard graph was plotted using standard amylose. The amylose (%) calculated using the slope of standard graph.

Light transmittance (paste clarity)

Paste clarity was studied using the method of Bhandari and Singhal (2002), with modifications. Fifty milligrams (on dry weight basis) of native and modified starches were suspended separately in 5 ml of distilled water, using 10 ml test tubes.

The test tubes were then heated in a boiling water bath (with occasional shaking) for 30 min. After cooling to ambient temperature, the percentage transmittance (%) was determined at 650 nm against a water blank using a spectrophotometer (Hewlett–Packard spectrophotometer 5989A).

Water absorption, oil absorption, swelling power and solubility

Water absorption and oil absorption capacities were determined using the procedures of Beuchat (1977), while swelling power and solubility patterns were determined as described by the method of Unnikrishnan and Bhattacharya (1981).

Image analysis

Image analyzer (BX51 model U-LH100HG, Japan) was used for morphological study of the native and modified starches.

Retrogradation study

Percentage syneresis in the starch gel was measured using the method described by Sodhi and Singh (2005). 5 % starch suspension (w/v) was heated at 90 °C for 30 min in a temperature controlled water bath subsequently chilled using ice-water bath to 25 °C for accelerated agglomeration. The starch samples were stored at 4 °C for a week. Syneresis was measured as the amount of water released after centrifugation at 4,500 rpm for 15 min.

Pasting properties

Viscosity profile was determined with a rapid rotating visco analyzer (RVA-4D, Newport Scientific instruments Pvt.

Ltd. Warie Wood, Australia equipped with varistaltic pump for cooling). Measurements were as per standards for RVA analysis (AACC International 2003). Samples were prepared by mixing starch (value corrected to 14 % moisture) with distilled water in Al canister. The mixer was stirred manually for 1 min to assist homogenous starch dispersion. Each sample was treated under same temperature-time conditions: heating from 50 to 95 °C at the rate of 6 °C/min (after an equilibration time of 1 min at 50 °C), a holding period of 5 min at 95 °C, cooling from 95 to 50 °C at the rate of 6 °C/min and a holding phase of 2 min at 50 °C. The constant rotating speed of paddle was 160 rpm except for first 10 s (960 rpm). Pasting parameter such as peak viscosity (PV), break down viscosity (BD = PV-HPV), set back viscosity (SB = CPV-HPV), pasting temperature, hot paste viscosity (HPV, Minimum viscosity at 95 °C) cool paste viscosity (CPV, Final viscosity at 50 °C) were automatically computed and recorded. All the measurements were carried out in triplicate.

Thermal analyses

Thermal profile parameters like onset temperature (T_o), maximum/peak/melting temperature (T_m), completion temperature (T_c) and enthalpy (ΔH) of starches were determined with a differential scanning calorimeter (DSC-TA 2100, TA Instruments Inc., New Castle, DE, USA), equipped with a mechanical refrigeration system. The instrument was used for all measurements with a sealed empty pan as reference using 40 ml/min nitrogen gas as flushing agent over the head. The DSC was calibrated for temperature and heat flow using indium [$mp = 156.6^\circ\text{C}$ and $\Delta H_m = 28.5\text{ J/g}$] as reported by Haque and Roos (2004). For the determination of the phase transition, DSC was operated in the normal mode from -20 to $+130^\circ\text{C}$, with a heating rate of 10°C/min and with one empty pan as reference. Samples (4 mg) with $10\text{ }\mu\text{l}$ water were closed in hermetic aluminium pans in a dehumidified room to avoid moisture uptake along with the empty pan. The pans were sealed hermetically using a sample encapsulation press (TA Instruments Inc., New Castle, DE, USA). The onset temperature of the transition was defined as onset temperature (T_o), the maximum peak temperature as the melting temperature (T_m), the end point of the transition as the completion temperature (T_c), and enthalpy (ΔH) was considered as the area of the endotherm peak. The data were analyzed using the Universal analysis V.3.0G software (TA Instruments Inc., New Castle, DE, USA), which is provided with the DSC instrument. All analyses were run in triplicate.

IR spectrum

IR spectra were recorded using a thermo Nicolet FTIR spectrometer (model 5700, Madison, WI) fitted with a single bounce attenuated total reflectance (ATR) accessory with ZnSe crystal.

Statistical analysis

Three replications were carried out for each measurement and the data obtained were analyzed statistically for analysis of variance (ANOVA) one way using completely randomized design and least significant difference at $p \leq 0.05$ using stat software release 8.0 package (Stat Soft, Tulsa, OK, USA).

Results and discussion

Starch isolation and proximate profile

Bengal gram flour (besan) was characterized to have 22.4 % protein, 6.1 % fat, 7.52 % moisture, 2.14 % ash, 26.4 % amylose and 61.84 % carbohydrate. Isolation of starch from Bengal gram flour was quite difficult and time consuming

because of its protein solubility and fibers content which formed a flocculum layer on the top. During centrifugation these fractions have hampered sedimentation and in some attempts resulted as brown precipitate with white starch cake. Such observations were also reported by Hoover and Sosulski (1985; Bressani and Elias 1979). In the present investigation wet milling method was applied to get a yield of $41.25 \pm 1.25\%$ of comparatively pure starch (Table 1). In other studies Moreira de Oliveira et al. (2009) reported 28 % yield, El Faki et al. (1983) reported 36 % yield from the chick pea with high purity and suggested that the yield and purity of isolated starch depends on its particle size distribution. Proximate profile of native and modified starch showed high purity ($>98\%$) as protein and ether extractable fractions were negligible (Table 2). After acetylation the protein and fat proportions were further reduced, which may be attributed to the repeated washing and other process related treatments. The granules of both the moieties were oval to cylindrical in the geometry but not spherical. Granules were simple with smooth surface and there was no evidence of cleaves or cracks (Huang et al. 2007). The effective radius of maximum granules observed were in average $38.72\text{ }\mu\text{m}$ (Fig. 1). Similar findings were reported by Mukhtarova and Lovacheva (1972) and Moreira de Oliveira et al. (2009). Amylose content of native starch was 24.12 % but it was significantly lower than acetylated starch (29.45 %). Result showed that Bengal gram starch falls under the category of normal starch (as amylose content was in the range of 15–30 %). It seems from the results that there was molecular de-polymerization of branched amylopectin because of acetylation and due to this phenomenon there was increase in the concentration of linear amylase chains. The yield of acetylated starch from the native starch was found to be $80.50 \pm 2.55\%$ (Table 1). This loss of yield during modification is due to repeated washings and filtrations.

Percentage acetyl (%Ac) content and degree of substitution (DS)

Acetylation is a substitution reaction for bringing many desired attributes in the indigenous starches (Betancur et al.

Table 1 Physicochemical profile of native and acetylated Bengal gram starch

Parameters	Starch yield	Amylose	pH (20 % slurry)
NBGS	41.25 ± 1.25^a	24.12 ± 1.65^a	7.10 ± 0.37^a
ABGS	80.50 ± 2.55^b	29.45 ± 1.84^b	7.01 ± 0.15^a
(From Native starch)			

NBGS, Native Bengal gram starch; ABGS, Acetylated Bengal gram starch. Values expressed are means ($n=3$) $\pm$ standard deviation. Means in the same column followed by different superscripts differed significantly ($p \leq 0.05$)

Tables 2 Proximate composition of native and acetylated Bengal gram starch

Parameters	Moisture, (%)	Crude protein, (%)	Total ether extractable, (%)	Carbohydrate, (%)	Total ash, (%)	Average granule size, (μm)
NBGS	3.14 ± 0.03^a	0.093 ± 0.01^a	0.46 ± 0.02^a	98.78 ± 1.54^a	0.661 ± 0.12^a	39.11 ± 10.66^a
ABGS	4.19 ± 0.06^b	0.021 ± 0.15^b	0.08 ± 0.01^b	99.41 ± 1.26^a	0.480 ± 0.08^b	38.33 ± 10.85^a

NBGS, Native Bengal gram starch; ABGS, Acetylated Bengal gram starch. Values expressed are means ($n=3$) $\pm$ standard deviation. Means in the same column followed by different superscripts differed significantly ($p \leq 0.05$)

1997). Three different concentrations (6, 8 and 10 % w/v) of acetic anhydride were used for modification using NaOH as catalyst of the reaction at ambient temperature conditions. The percent acetyl value increased from 2.14 to 4.47 % with the concentration of acetic anhydride (6 to 10 %). Subsequently, the degree of substitution (DS) from 0.082 to 0.176 in acetylated starches (Table 3). Vasanthan et al. (1995) has also reported similar finding with present investigation. The DS is the average count of sights per glucose unit with substituted groups. The resulted starch was characterized as low substituted starch. The products with low substitution (0.01 to 0.2 DS) is approved by the USFDA for food applications. The investigation suggested a high selectivity for starch acetate at increased temperature, starch and alkali concentration (de Graaf et al. 1995). However, Singh et al. (2004) mentioned that further increase in acetic anhydride (up to 12 %) did not increase the percent acetyl content of potato starch. Formation of highly substituted sago starch acetate using acetic anhydride in organic solvent was reported by Singh et al. (2011).

Physico-chemical properties

The physicochemical properties of native and modified starches are summarized in Table 4. Swelling power/index measures both inter and intra granular water absorption. In this study acetylation brought a significant changes in the swelling power (single stage swelling) of

Bengal gram starch by +17 %, water absorption by +13 % and oil absorption by +20 % at temperature of 90 °C. Though granule swelling was substantially increased but solubility was decreased. Similar observations were observed by Mbougung et al. (2012) in acetylated potato and cassava starches and by Agunbiade and Longe (1999) in legume starches. Below 60 °C temperature pulse starches are reluctant to swell and amylose leaching but this is significantly effective above 70 °C. Amylose fraction in Bengal gram starch is very compactly distributed within non crystalline zone of the granules and may facilitate a strong interaction between neighboring amylose successions. Moreira de Oliveira et al. 2009; Yadav et al. (2007) quoted opposite results for swelling power and solubility of chick pea starch at 90 °C. The propensity of high swelling may be attributed to the inclusion of acetyl group into the starch which facilitated water retention due to additional intra molecular hydrogen bonds (Tester et al. 2000). The enhanced capacity to absorb water/oil and swelling may be attributed to the insertion of acetyl group that facilitated access of water to amorphous domain by hydrogen bond distribution, stearic hindrance and inter-granular disorganization. Acetylated starch showed more capacity to retain water thus lesser degree of retrogradation than native starch. For this feature amylose-amylose chain association may be accountable. The percent transmittance for fresh and stored native as well as acetylated samples showed decreased values. The acetylated starch formed an opaque gel on

Fig. 1 Granule morphology of native and acetylated Bengal gram starch. (a). Native Bengal gram starch ($39.11 \pm 10.66 \mu\text{m}$). (b). Acetylated Bengal gram starch ($38.33 \pm 10.85 \mu\text{m}$)

a. Native Bengal gram starch ($39.11 \pm 10.66 \mu\text{m}$)

b. Acetylated Bengal gram starch ($38.33 \pm 10.85 \mu\text{m}$)

Table 3 Percent acetyl content (Ac %) and degree of substitution (DS) of acetylated Bengal gram starch at different concentration of acetic anhydride

Parameters	ABGS		
	With acetic anhydride treatment		
	6 %	8 %	10 %
Ac (%)	2.14 ^a	3.35 ^b	4.47 ^c
DS	0.082 ^a	0.130 ^b	0.176 ^c

ABGS, Acetylated Bengal gram starch; Ac (%), Acetyl percentage; DS, degree of substitution. Values expressed are means ($n=3$). Means in the same row followed by different superscripts differed significantly ($p \leq 0.05$)

cooling. These results are contrary to the finding of Byoungseung and Hye (2011). Above effect may be explained with the findings of Hoover and Sosulski (1986) about paste clarity that strength of associative bonding forces within the micellar network decreased and there was an increase in the amount of water bound with the starch molecule. These finding of the present investigation were supported by the research work of many predecessors (Adebowale et al. 2006).

Characterization studies with IR spectra

The IR spectra exhibited bands that begin largely from the rhythmic movement of amylose and amylopectin (Yadav et al. 2007). The O–H stretch ($3294.2\text{--}3301.1\text{ cm}^{-1}$), C–H stretch ($2928.2\text{--}2929.6\text{ cm}^{-1}$), and the skeletal mode vibration of the glycosidic linkage ($994.1\text{--}995.2\text{ cm}^{-1}$) were clearly seen for native and modified starch samples. The variation in the intensity of some FT-IR bands and their ratios were shown in Fig. 2 which may be corroborated to the modification. Native starch sample showed a band at 1633.4 cm^{-1} whose intensity increased in modified samples, indicating changes in crystallinity. The sharp band at $2,928\text{ cm}^{-1}$ is attribution of C–

H stretches associated with the ring hydrogen atoms (Yadav et al. 2007). Intensity changes in the C–H stretch range could be attributed to variations in the amount of amylose and amylopectin present in starches (Young 1984). The IR spectral data provided evidence for acetylation by the presence of an ester carbonyl group stretch (C = O) at 1720.3 cm^{-1} . The presence of absorption band in FT-IR at $1,748\text{ cm}^{-1}$ was considered as confirmation of the carbonyl group attachment by Singh et al. (2011), at 1733.25 cm^{-1} by Singh et al. (2004) in acetylated potato starch and at $1,226\text{ cm}^{-1}$ in acetylated barley starch by Bello Perez et al. (2010). In a similar study, with an increase in DS, increase of the carbonyl (C = O) group and a decrease of the hydroxyl (O–H) group, at about $1,750\text{ cm}^{-1}$ and $3,450\text{ cm}^{-1}$ (characteristic absorption peaks) respectively was observed in FT-IR analysis by Khalil Diop et al. (2011).

Rheological and pasting properties

The viscosity profile of the starch is one of the most hunted attribute to decide its commercial applicability. Rheological and melting properties were recorded using RVA for quick gelatinization profile (Table 5). Starch when heated in hydrated media experiences an order disorder phase transition called gelatinization in a temperature range distinctiveness of the individual starch source consequently pasting temperature is an indicator of the minimum temperature required to cook the sample. After acetylation the pasting temperature and pasting time reduced significantly (Xie et al. 2010; Mirmoghtadaie and Kadivar 2009). The above phase transition is associated with the diffusion of water into the granule, water uptake by the amorphous background region, hydration and radial swelling of the starch granule, loss of birefringence, uptake of heat, loss of crystalline order, uncoiling and dissociation of double helix and amylose leaching. The slurry of starch starts depicting viscosity trait during onset of heating cycle from $50\text{ }^{\circ}\text{C}$ onwards to $95\text{ }^{\circ}\text{C}$. Both starches showed increase in the viscosity on increase in the temperature which may be attributed to the removal of water from the exuded amylose by the granules as they swell (Ghiasi et al. 1982). During viscosity analysis both the starches showed sharp peaks on observation. Pasting properties of acetylated starch revealed that viscosity profile after modification improved significantly as pasting temperature and pasting time were reduced appreciably. Peak viscosity (PV) and break down (BD) viscosities were raised affirming stable gel as rupturing and fragmentation due to continuous stirring was sustained. During the final cycle of cooling ($95\text{ to }50\text{ }^{\circ}\text{C}$) the viscosity further increased owing to the alignment of the amylose chains. Final viscosity (FV), trough and set back (SB) viscosity of the acetylated starch was much lower compared to native starch indicating weaker gel formation on cooling cycle (Byoungseung and Hye 2011). These

Table 4 Oil absorption and water absorption capacities of native and acetylated starch

Sample	NBGS	ABGS
Water absorbed (g/g)	0.98 ± 0.01^a	1.11 ± 0.01^b
Oil absorbed (g/g)	0.55 ± 0.01^a	0.66 ± 0.01^b
% Transmittance (0 Days)	2.13 ± 0.12^a	1.70 ± 0.05^b
% Transmittance (7 Days)	2.55 ± 0.04^a	2.00 ± 0.00^b
Swelling power (%) at $90\text{ }^{\circ}\text{C}$	33.17 ± 0.12^a	38.84 ± 0.18^b
Solubility (%)	2.03 ± 0.02^a	1.55 ± 0.02^b

NBGS; Native Bengal gram starch, ABGS; Acetylated Bengal gram starch. Values expressed are means ($n=3$) $\pm$ standard deviation. Means in the same row followed by different superscripts differed significantly ($p \leq 0.05$)

Fig. 2 FTIR Spectra of Native and acetylated Bengal gram starch

results do not aligned with the findings of Moreira de Oliveira et al. (2009) who observed high viscosity and set back but low shear thinning of acetylated chick pea starch. Reduced SB viscosity depicts its resistivity

towards retrogradation. Lower SB viscosity also demonstrated that acetylation lowers syneresis (oozing of water/exudation of water during frozen storage) and this may be attributed to acetyl group incorporated, resulted

Table 5 Pasting characteristics of native and acetylated Bengal gram starch

Samples	Peak, (CP)	Trough, (CP)	Break down, (CP)	Final viscosity, (CP)	Setback, (CP)	Pasting time, (Min.)	Pasting Temp, (°C)
NBGS	4356.5±28.49 ^a	3171.5±17.38 ^a	1185±108.89 ^a	7191.5±116.67 ^a	4020.1±70.71 ^a	4.70±0.04 ^a	75.15±0.0 ^a
ABGS	4544.1±10.72 ^b	3035.2±11.53 ^b	1509±60.81 ^b	5757.5±386.79 ^b	2722.5±115.26 ^b	4.27±0.00 ^b	69.82±0.53 ^b

NBGS, Native Bengal gram starch; ABGS, Acetylated Bengal gram starch

Values expressed are means ($n=3$) ± standard deviation. Means in the same column followed by different superscripts differed significantly ($p\leq 0.05$)

in increased water retention within starch granules of cold stored gel. The decreased syneresis would make substituted pulse starches suitable for improving the textural quality of frozen foods.

Thermal properties and gelatinization profile

Melting and re-crystallization profile of acetylated starch was generated using DSC and summarized in Table 6.

Gelatinization transition temperatures and ΔH_{gel} have been shown to be influenced by the molecular architecture of crystalline region, which corresponds to the distribution of amylopectin short chains (Degree of Polymerization) not by the proportion of crystalline region which corresponds to the amylose to amylopectin ratio. During analysis it was observed that acetyl treatment reduced T_o (onset temperature), T_p (peak temperature), and T_c (concluding temperature). The results find similarity with the findings of Babic et al. (2009) and Nunez Santiago et al. (2010). It may be due to the presence of hydrophilic substituting acetyl groups and increased hydrogen bonding within starch molecules resulting in lower gelatinization parameters to their native counter parts. Percent retrogradation of acetylated starch was reduced by 56 % in comparison to its native starch. A low T_o , T_p , T_c and ΔH_{gel} reflex the presence of abundant amylose or short amylopectin chains. ΔH reflects the trouncing of double helical arrangement rather than the loss of crystallinity. It also reflects the totality of crystallinity of amylopectin in

terms of quality and quantity. Introduction of acetyl group in starch granules leads to the shattering consequently loss of relative crystallinity of starch granules. This molecular disorder resulted in lower submission of thermal energy for transition phase hence there was significant decrease in enthalpy of gelatinization (ΔH_{gel}) and enthalpy of retrogradation (ΔH_{ret}) as well. This decrease in ΔH_{ret} indicates about unarranged and less organized pattern of retrogradation. The behavior observed may be due the introduction of acetyl group in to polymer shackles which subvert the granular structure causing an increase in swelling volume and decrease in gelatinization temperature (Tester and Debon 2000). The degree of heterogeneity of the crystallites in the starch granules is responsible for the gelatinization temperature. It was also observed that gelatinization range after acetylation increased significantly compared to native starch. In certain cases it was reported that acetylation helps in providing thermal stability to the modified starches (Singh et al. 2011). In addition to the initial starch gelatinization properties the recrystallization and retrogradation after refrigerated storage is important for starch to be used in food industry. This is known property of gelatinized starch that they tend to recrystallized and ooze water (syneresis), if cooled, and if heated again causes melting of aligned amylopectin molecules. During this transitional phenomenon enthalpy of retrogradation was recorded that indicates order–disorder transition of crystallites (Adebawale and Lawal 2003).

Table 6 Gelatinization and retrogradation properties of native and acetylated starch

Gelatinization	T_o	T_p	T_c	ΔH_{gel}	PHI	R	
NBGS	63.08 ^a	67.83 ^a	71.27 ^b	1.5990 ^b	0.34 ^b	9.50 ^b	
ABGS	59.46 ^b	65.66 ^b	72.50 ^a	3.3250 ^a	0.54 ^a	12.40 ^a	
Retrogradation	T_o	T_p	T_c	ΔH_{ret}	PHI	R	% R
NBGS	32.66 ^a	35.00 ^a	38.44 ^a	0.1717 ^a	0.07 ^a	4.68 ^b	10.74 ^a
ABGS	30.32 ^b	33.88 ^b	36.55 ^b	0.1505 ^b	0.04 ^b	7.12 ^a	4.53 ^b

NBGS, Native Bengal gram starch; ABGS, Acetylated Bengal gram starch

Values expressed are means ($n=3$). Means in the same column followed by different superscripts differed significantly ($p\leq 0.05$)

Conclusion

Acetylation of isolated Bengal gram starch can be done by acetic anhydride which was confirmed in the present study with characteristic peak at 1720.3 cm^{-1} in FTIR spectrum. Increasing trend in percent acetyl content and degree of substitution in the modified Bengal gram starch was observed with increasing concentration of acetic anhydride up to 10 %. Esterification has resulted in to significant change in various physicochemical properties of pure Bengal gram starch such as better rheological profile with respect to lesser retrogradation, lower energy and duration of gelatinization, lesser syneresis hence more stability to gel. The modification has also attributed positively to enhance oil, water absorption power and swelling index. Hence such modification can be useful in improving the textural limitations of native starches during processing and storage of baked products, RTE snacks, frozen and instant foods.

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