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Comparative study of native and modified starches isolated from conventional and nonconventional sources

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Abstract

Starch is a carbohydrate composed of chain of glucose molecule. Potato, corn, rice are the major sources for starch but they are also used as a food. Our aim was to extract starch from some nonconventional source like taro plant (*Colocasia esculenta*) and did some physical and chemical modification to enhance the properties of starch. Physical modification method of starch like Osmotic Pressure Treatment can change the morphological characteristics which clearly indicated the change into a folded structure after the treatment. Starch treated by cross-linking and acetylation methods to increase the properties like viscosity, swelling volume so that these starches can be used as food thickeners. Acetylated starches had shown high swelling and solubility, low gelatinization temperature which can be used commercially in food industry. In this study we showed that taro was the good source for starch extraction and native, modified taro starch can be commercially used as a food product.

Introduction

Starch is a carbohydrate made up of glucose units linked together by glycosidic bond. Plants store glucose as the polysaccharide starch (Whistler and BeMiller, 1997). It is the most vital carbohydrate in the human diet and is major constituent of staple foods such as potatoes, rice, wheat, cassava, and corn. Depending on the plant, starch contains 20 to 25% amylose and 75 to 80% amylopectin (Whistler and BeMiller, 1997; Bule´on *et al*., 1998). Amylose is primarily a linear chain of D-glucose units linked by α-1→4 linkages. However, some amylose molecules have about 0.3-0.5% of α-1→6 linkages (branches) (Takeda *et al*., 1990). Amylopectin is a branched polymer of glucose unit, linked with α-D-(1→4) glycosidic bond with the branching of α-D-(1→6) bond occur every 24 to 30 glucose units (Wurzburg, 1986; Bule´on *et al*., 1998).

Modified starches are used in many processed food because their functional properties are improved over those of the native starches. Many physical modification processes like annealing (ANN) and heat-moisture treatment (HMT) causes a physical modification of starches without a gelatinization or damage of the starch granules, also it does not affect sizes, shapes and birefringence. Both treatments cause completely different alteration of the internal granule structure (Chung *et al*., 2009). Physical modification method like osmotic pressure treated starches showed changes in starch chain interactions, granule swelling, amylase leaching, viscosity, gelatinization temperatures, retrogradation, acid and enzyme hydrolysis (Pukkahuta *et al*., 2007).

The industrially used starch was isolated from native sources like potato, corn and sometimes rice, but these sources are very expensive. Taro contains 13-29% starch, 63-85% moisture and some other residues like riboflavin, vitamin C, ash etc. This project was aimed to isolate starch from nonconventional as well as underexploited plant like taro because it is cheaper than other native sources. Its physical and chemical properties were compared with potato and corn starches. Physical and chemical modifications of potato, corn and taro starch had done to improve some properties which will be helpful for its industrial application.

Materials and Methods

Materials

Sodium bisulfite ($NaHSO_3$), Barium chloride ($BaCl_2$), Sodium sulphate (Na_2SO_4), Acetic anhydride ($(CH_3CO)_2O$), Sodium hydroxide (NaOH) and Hydrochloric acid (HCl) were acquired from Qualigens®. Dimethyl sulfoxide (DMSO), Iodine (I), Methanol (CH_3OH), Phenol (C_6H_5OH), Sulfuric acid (H_2SO_4) and 1-Propanol (C_3H_7OH) were obtained from MERCK®. Phosphoryl chloride ($POCl_3$) was obtained from Sigma Chemical.

Method of starch isolation

The tubers and roots of taro (*Colocassia esculenta*) were peeled, washed, diced, placed in

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ice-cold water containing 100 ppm of $NaHSO_3$ and homogenized at low speed in a blender. The slurry was filtered through a filtering cloth, the residue washed with a small amount of water, and then the filtrate was centrifuged at 3,000×g for 10 min. The supernatant and the brown layer of material on the surface of the sediment were removed, and further purification was achieved by repeated suspension in water and centrifugation. The purified starch was dried at 35^{o}C (Hoover and Hadziyev, 1981). The same method was used for isolation of Kufri Chandramukhi potato and sweet corn starches which brought from local market in Kolkata, India.

Physical modification of starches

100 g (dry basis) of starch was suspended in 200 mL saturated Na_2SO_4 solution (100 g Na_2SO_4: 200 mL distilled water), in a 500 mL conical flask. The starch solution heated upto 120°C, which corresponds to the calculated osmotic pressure of 341atm (345 bar) (assuming Na_2SO_4 dissociated completely) for 15, 30 and 60 min. After the heating process was over the flasks were cooled down to room temperature. Then the starch was taken out of the conical flask and washed with distilled water (8×500 mL) to remove Na_2SO_4 by centrifugation at 4552×g for 10 min and this procedure repeated twice. After that, testing for any residues of Na_2SO_4 in the starch was performed by precipitation with $BaCl_2$ solution. Then the starch was dried at 60°C in hot air oven overnight (Pukkahuta *et al*., 2007). This process was followed for every starch sample.

Chemical modification of starches

The chemical modification of starches by cross-linking method with $POCl_3$ had done as described by Woo and Seib (1997). Another modification process, acetylation of starch with acetic anhydride was carried out by using Phillips *et al.* (1999) method. The percentage acetylation (Acetyl %) and degree of substitution (DS) were determined by using the method described in Ogawa *et al.* (1999). (See supplementary information)

Physicochemical properties of conventional, nonconventional and modified starches

Physicochemical characteristics like moisture content (Lyne, 1976), amylose content (Bates *et al*., 1943), swelling volume (Mat Hashim *et al*., 1992), leaching of amylose (Chrastil, 1987; Hoover and Vasanthan, 1994), leaching of carbohydrates (Dubois *et al*., 1956), reducing sugar (by Dinitrosalicylic Acid method), were estimated of conventional, nonconventional and modified starches. Starch granule shapes were observed and photographed by using an optical microscope (CX 31, Olympus®) and SEM (JSM 5200, JEOL® Scanning Microscope). Gelatinization and dissociation parameters were measured by using differential scanning calorimetry (Pyris Diamond TG/DTA, Made by Perkin Elmer®, a modulated thermal analyzer DSC). Viscosities (cps) of suspensions (7% taro, 5% poatao, 3% corn w/w) of native and physically modified starches were measured in different RPM by DV-I Prime Brookfield viscometer (RV-1 Spindle) at 30°C. (See supplementary information)

Results and Discussion

Physical and chemical properties of native and modified starches

Physical and chemical properties of isolated starches like potato, corn and taro starch and modified starches are shown in Table 1.

Moisture content

The moisture content of physically modified potato, taro and corn starch (modified for 60 min showed highest) was higher than their native form. The difference in moisture content will also depend on the extent of drying. The moisture content of cross-linking potato, corn and taro starch were lower than their native form. The moisture content of taro starch was lower than potato starch and higher than corn starch which showed that taro starch can be stored for longer time.

Amylose content

Modified starches contained higher amount of amylose than their native form. Native potato has 11% amylose content, then it increases to 14% by OPT-120^{o}C-15 min, then goes down to 10% under OPT-120^{o}C-30 min, and increases again to 11% after the treatment of OPT-120^{o}C-60 min. This profile followed by other starches also. OPT-120^{o}C-15 min and crosslinking taro starch contained higher amount of amylose than their native form. Amylose is present in the amorphous region and during modification, this region are mostly accessible than amylopectin side chains. Therefore, amylose content is changing due to modification and structural difference between amylose and amylopectin is considered to be the most important factor for starch property. With high amylose content, starch will show high volume

Table 1. Physical and chemical properties of native (taro, corn and potato starch) and modified starches

Starch	Treatment		Moisture Content (%)	Amylose content (%)	Swelling volume (mL)	Amylose Leaching (%)	Leaching of carbohydrate (µg)	Reducing Sugar (mg/ml)
Potato starch	Native		7±1	11.19±2	25±1	13.45±1.5	28.786±2	0.861±0.12
	Osmotic Pressure Treatment	15 min	17±1.2	14.077±0.5	27.5±1	12.202±0.5	117.41±1.6	0.157±0.08
		30 min	19.5±1.5	10±0.9	15±1.2	15.238±1.2	119.68±2	0.074±0.01
		60 min	24.5±1	11.25±2.5	22.5±3	6.607±0.8	98.6±2	0.0416±0.01
	Cross-Linking		2±0.9	18.273±1.9	20±1	1.125±0.7	88.96±3	0.241±0.02
	Acetylated		10.5±0.8	22.14±1.8	30±1	1.428±1	89.19±2	0.074±0.02
Corn starch	Native		3.5±1	7.91±1.3	20±2	9.226±1.2	12.24±1	0.462±0.01
	Osmotic Pressure Treatment	15 min	15.5±1.2	12.142±2	25±1.5	19.583±2	117.86±2	0.138±0.002
		30 min	15±2	11.5±0.5	23±1.6	29.166±2.2	117.75±2	0.115±0.005
		60 min	17.5±1.6	13.69±0.9	22.5±1.3	20.654±2	117.64±3	0.097±0.004
	Cross-Linking		1.5±0.5	16.13±0.6	10±1	1.428±0.6	88.06±2.1	0.01±0.001
	Acetylated		2.5±0.2	13.63±1	25±1.3	1.785±0.02	87.83±2.3	0.037±0.004
Taro starch	Native		4.5±0.5	2.14±0.05	10±1	13.27±1.6	20.286±1.3	0.046±0.002
	Osmotic Pressure Treatment	15 min	4±0.2	5.89±0.2	35±2.3	3.988±0.5	118.32±2	0.0833±0.008
		30 min	4±0.09	3.392±0.09	27.5±1.2	8.3928±0.26	120.36±3	0.0972±0.005
		60 min	6±0.1	4.28±0.08	22.5±1.5	4.583±0.07	118.43±3	0.055±0.004
	Cross-Linking		1±0.02	12.142±1	5±0.5	0.595±0.02	88.5±2	0.055±0.005
	Acetylated		2±0.02	7.619±0.6	15±2	1.071±0.05	89.64±3	0.241±0.009

expansion and a high degree of flakiness on the other hand if food prepared by less amylose content starch, the food become moist and sticky and hard to chew.

Swelling behavior of starches

Starch swells in hot water which shows the magnitude of starch chain interactions within the gelatinized granules (Hoover, 2001). When starch was dispersed into water and heated up then the water penetrates into the starch granule until the granule is fully hydrated. In hydrated condition, the hydrogen bonding between the amylose and the amylopectin maintains the integrity of the granule and it begins to swell from the centre. Native potato has a swelling volume of 25 ml, then it increases to 27.5 ml by OPT-120^{o}C-15 min, then goes down to 15 ml under OPT-120^{o}C-30 min, and increases again to 22.5 after the treatment of OPT-120^{o}C-60 min. For taro starch, its native form has a swelling volume of 25 ml, then it increases to 35 ml by OPT-120^{o}C-15 min, then goes down to 27.5 ml under OPT-120^{o}C-30 min, and decreases further to 22.5 after the treatment of OPT-120^{o}C-60 min. Potato starch showed the highest swelling behavior, followed by corn starch then taro starch. Potato starch have high level of phosphate groups which shows the highest swelling ability because of the repulsion between the negatively charged phosphate groups in neighbouring amylopectin chains, which weakens the hydrogen bonding between chains and leads to rapid hydration and swelling (Yuan *et al.*, 2007). Acetylation increased the swelling factor of all the starches compared with their corresponding native starches, whereas cross-linking decreased the swelling factor. Cross-linking reinforces the structure of starch granules and limits water absorption which restrict the mobility of starch chains in the amorphous region.

Amylose leaching

Leaching of amylose at 60^{o}C was increased in 1.13% in 30 min modified potato starch and decreased in 2.03% in 60 min modified potato starch than native potato starch. Leaching of amylose was increased in all modified corn starch and decreased in modified taro starch. The amylose assay used in the leaching experiments assumes that acetyl groups do not interfere with iodine binding and blue color formation. If the temperature and heating period were increased then amount of amylose leaching was also increased. In case of every starch, decreased amylose leaching was observed after acetylation and cross-linking because these methods had done at 25^{o}C which was quite low temperature (Noranizan *et al.*, 2010).

Carbohydrate leaching and reducing sugar

Leaching of carbohydrate increased 4.07, 9.62, 5.83 times respectively in modified potato, corn and taro at 95^{o}C. Leaching of carbohydrate of native potato was 28.75 µg, then it increased upto 117.41 µg by OPT-120^{o}C-15 min, then further increased upto 119.68µg under OPT-120^{o}C-30 min, and then decreased to 98.6µg after the treatment of OPT-120^{o}C-60 min and for crosslinking and acetylated starch it increased to 88.96 µg, 89.19 µg respectively. Leaching of carbohydrate of native corn, taro starch were 12.24 µg, 20.286 µg respectively which increased upto 117 µg, 118 µg after the treatment of OPT-120^{o}C and ~88 µg after the treatment of crosslinking and acetylation. Leaching of carbohydrate increased because after modification, the starches were degraded, so that the molecular weight of the cleaved fractions decreased (Mohd Adzahan *et al.*, 2009). The carbohydrate leaching of the cross-linking and acetylated corn, taro and potato starches were higher than their native form but comparatively lower than physically modified

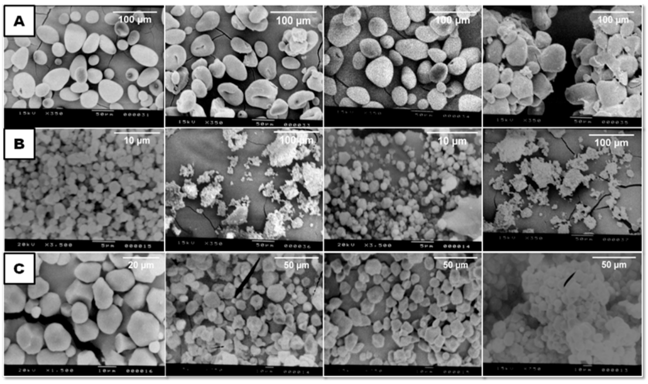

Figure 1. SEM of (A) potato starch, (B) taro starch, and (C) corn starch in its native, OPT-120˚C-30 min, acetylated and cross-linked form (left to right).

starches because amylose-lipid complex existing in cross-linking and acetylated starches inhibits swelling, cracking and dispersion of the granules. All the samples were heated at 95°C for 30 minutes but the starches were not showing similar behavior because starch contained lipid complexes which require different and more severe heat treatment to allow leaching of carbohydrate polymer as indicated by the solubilisation values (Noranizan *et al*., 2010). The reducing sugar content in modified starches was lower than the native starches.

Gelatinization

Native potato, taro starch and modified potato, taro starch gelatinized nearly 65˚C, 68˚C and 55˚C, 60˚C respectively. This DSC results showed that starches were modified because gelatinization temperature (Tp) of native starches and modified starches were different. DSC result also showed that isolated taro starch had gelatinization property which means the intermolecular bonds of starch could break down in presence of water and heat. The Tp depends on the degree of cross-linking of the amylopectin (Jenkins and Donald, 1998).

Structural changes conformation by SEM

Native potato starch granules were oval in shape without any hollow area inside and heterogeneous distribution (Figure 1A). Native corn starch granules were round in shape and had a homogeneous distribution (Figure 1C). Native taro starch granules were hexagonal in shape and very small in size (Figure 1B). After osmotic pressure treatment starch granules were contorted to a folded structure, showed birefringence and hollow area inside the starch was observed, which confirms the presence of non-gelatinized granules. Folding was highly observed in 30 min modification. From the SEM pictures it was observed that the structures of starches were modified.

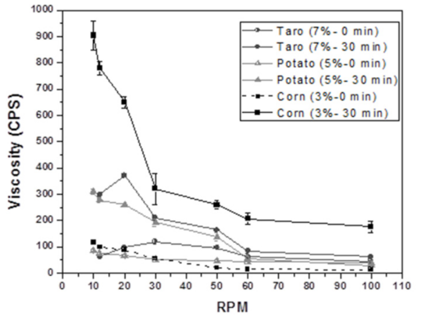

Figure 2. Viscosity (cps) of suspensions of native and modified OPT-120˚C 30 min 5% w/w potato starch, 3% w/w corn starch and 7% w/w taro starch.

Viscosity

30 min OPT potato starch (5% w/w), corn starch (3% w/w) and taro starch (7% w/w) had shown the higher viscosity than their native form (Figure 2). If the granule size of starch is large then it shows higher viscosity because the physical size of the granule makes it more sensitive to shear. Potato starch has both small and large granules and same composition of amylose and amylopectin but the gelatinization properties of the large and small granules do not show significant performance differences.

Percentage acetylation and the degree of substitution

The corn starch showed the highest % of acetylation and potato starch showed the lowest % of acetylation. % of acetylation for acetylated potato, corn and taro starches were 0.688, 1.204, 1.439 respectively and degree of substitution (DS) were 0.026, 0.046 and 0.055 respectively. DS was calculated for acetylated starch with 8% acetic anhydride. Wu and Saib (1990) obtained DS of 0.1 in an acetylated di-starch phosphate from waxy maize a barely starches, using 8% acetic anhydride on a starch basis. Betancur *et al.* (1997) have found acetyl % between 1.5-2.34 and DS Values of 0.057-0.09 using 8% modifying reagent, in 40 Canavalia starch suspensions. The differences in acetyl content reported from various studies may be due to difference in reaction conditions and starch source used.

Physical and chemical modified starch

Studying the physicochemical properties of the OPT-potato starch 30 min 120°C had lower swelling volume, higher amylose leaching and carbohydrate leaching properties and amylose content was similar to the native potato starch. OPT-corn starch 30 min 120°C had lower moisture content, higher amylose content than native starches and higher amylose leaching and carbohydrate leaching properties. OPT-taro starch 30 min 120°C had also lower moisture content and lower swelling volume than other OPT modifications. Its amylose content, amylose leaching, carbohydrate leaching properties were higher than the native taro starch.

Chemically modified acetylated starch and cross-linked starch improves the cooking properties (cooking loss decreases) and eating quality (softness, stretch-ability and slipperiness increase) of different foods like noodle, spaghetti. Cross-linking process changes some functional properties of starches which permits a wide range of applications. Acetylated starches with low degree of substitution (DS) are broadly used in food industries for several years because of important characteristics such as high swelling and solubility, low gelatinization temperature, and good cooking and storage stability (Liu *et al*., 1999; Wang and Wang, 2002). The acetylated starches are also less susceptible to retrogradation because the amylose fraction which is mainly responsible for starch retrogradation, is modified. The physicochemical properties of acetylated starches depend on their chemical structures, DS and acetyl group distributions. The DS increased with decreasing starch granule size dimension which confirms from the SEM images. Taro starch had the highest DS value and the dimension of the starch granule was lowest and vice versa applicable for potato starch and corn starch. The DS of the isolated amylose populations of differently sized granule fractions were constant. Amylose is mainly located in amorphous region and the branched chains of Amylopectin are located in crystalline region. Acetylation occurs in all amorphous regions and only in the outer lamella of crystalline regions so that the specific surface area of starch granule increases which are in the agreement with the assumption of Biliaderis (1982) for the acetylation in starch granules.

Conclusions

The results of the study suggested that the physical–chemical properties of taro starch were more or less similar with other starches. Hexagonal shaped Taro starch had higher amylose leaching and carbohydrate leaching properties than potato and corn starches. The properties of smaller stable granules (higher digestibility) and medium amylose contents of taro starch could be used for the manufacture of food products. Its lower swelling power displayed the more stable granule structure. The higher viscosity of 7% taro starch exhibited greater retrogradation tendency, which was an important factor for starch used to prepare diet shake, but it may come across as stringy and slimy when consumed. Although this property limits the applications of food storage for long duration, this starch may be suitable for food products (e.g. glass noodle) where greater retrogradation tendency is required. High amylose starch shows gelling properties while high-amylopectin starch like taro would be the choice if viscosity is needed. The morphological characteristics of OPT starch granules change into a folded structure after the treatment. The narrow DSC curve of OPT starch can be an indicator of a better starch and shows that the large scale production of modified starch is possible. Taro, potato and corn starch are generally not used as starch thickeners because of low swelling, low viscosity development, and low shear resistance which can improved by cross-linking method. The DS increased with decreasing starch granule size dimension which confirms from the SEM images. Taro is a very cheap source for starch extraction and its physical and chemical properties are more or less similar like native potato and corn starches. By physical and chemical modification of taro starch we improve some properties which will be helpful for industrial application.

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