PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF VITATO-MODIFIED STARCH

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ABSTRACT

Starch is the major component of sweet potatoes, which can be used in food industries. Starch can be modified to produce resistant starch with various functions depending on their structural, functional and physicochemical properties. The physicochemical and functional properties of the modified starch could be used to suggest their application in industrial uses. Starch modifications are commonly treated by enzymatic, physical or chemical methods. In this study, native sweet potato (VitAto) starch (Ipomoea batatas L.) was treated with 3% citric acid at 45 °C for 6 hours and 2% α-amylase at 60 °C for 8 hours. Both modified starches showed a high yield recovery of 97%. The characterization of the modified starches including the amylose content, dextrose equivalent (DE), moisture content, water activity, water holding capacity, water absorption index and emulsion stability were analysed. The modified starches showed no significant differences ($p > 0.05$) for amylose and amylopectin content. While, the water absorption index, water holding capacity, moisture content, water activity and emulsion stability were significantly different ($p < 0.05$). These characteristics were useful for future study of the modified starch potential in industrial uses.

Keywords: Ipomoea batatas L., sweet potato starch, VitAto, modified starch, physicochemical properties

INTRODUCTION

Sweet potato or *Ipomoea batatas* L. is widely cultivated especially in Asia. Malaysia has cultivated sweet potato as an industrial crop for its sweet and tasty tuberous roots. There were 3,062 hectares planted area in 2018 and increased to 3,254 hectares in 2021 with a production of 52,225 mt to 53,614 mt (DOA, 2022). In 2023, sweet potato production was 47,922 mt, followed by cassava (39,387 mt), yam (7,153 mt) and potato (17 mt) (DOA, 2023). There are a few varieties of sweet potato that are cultivated in Malaysia including MARDI's varieties. Malaysian Agricultural Research and Development Institute (MARDI) introduced a few varieties of sweet potatoes such as Gendut (white), VitAto (orange), and purple sweet potatoes namely Anggun 1, Anggun 2, Anggun 3 and Lembayung which are claimed to contain more anthocyanin content, high resistance to pest and microbial attack (Nurul et al., 2019). VitAto is the most commercialized variety in Malaysia which is mainly planted in Perak, Kelantan and Selangor. Besides for fresh consumption, sweet potato can be processed into sweet potato flour or starch. Different sweet potato varieties may vary in different physicochemical properties which may also lead to different applications including the production of starch. Sweet potato has the advantage as a high starch-producing crop with 30–50% greater starch yield than rice, corn, and wheat starch sources (Rahman et al., 2003).

Starch is the major component of sweet potatoes, which can be used in food industries. Understanding the relationships between the functional properties and the structure of sweet potato starch in optimizing food and suggesting their application in industrial uses is very useful. Sweet potato starch can be used in many food and non-food products including paper, cosmetics, textile and adhesive industries. In food applications, sweet potato starches can be utilized as an ingredient in bakery products, confectionary products, and as food additives such as thickener, and stabilizer and it can be modified to produce resistant starch with various functions depending on their structural and physicochemical properties. The branched glucose polymers, amylose (AM) and amylopectin (AP) are the main components of starch. Normally, sweet potato starch contains 20–30% linear and slightly branched AM and 70–80% highly branched AP (Lai et al., 2016). Starch with variable AM content provides different structures and physicochemical properties that can be used in various applications.

Sweet potato starches can be modified to enhance starch performance by physical, chemical or enzymatic treatment. In this study, acid hydrolysis and enzymatic hydrolysis using α-amylase were carried out to modify sweet potato (VitAto) starch. The objective of this study was to determine the physicochemical and functional properties of the acid-modified starch and enzymatic-modified starch from the VitAto. Studying the relationships between the structure, physicochemical and functional properties of the modified sweet potato starches is very important to suggest their application in industrial uses.

MATERIALS AND METHODS

Materials

VitAto sweet potatoes were harvested from MARDI Bachok, Kelantan. The α-amylase enzyme from *Aspergillus oryzae* was purchased from Sigma-Aldrich (St. Louis, USA). All reagents, and chemicals used were analytical grade.

The VitAto sweet potatoes were washed with clean water to remove any foreign matter and were sanitized with a chlorine solution (200 ppm). Starch was extracted according to the method of Madzlan et al. (2012). The starch was then weighed and the starch yield and starch recovery were calculated according to these formulas:

$$Starch\ yield = \frac{extracted\ starch}{amount\ of\ sweet\ potato\ roots}\ x\ 100$$

$$Starch\ recovery = \frac{yield\ of\ starch}{starch\ content}\ x\ 100$$

Modification of starch

Acid-modified starch

Acid-modification of starch was carried out according to Babu et al. (2016). Starch slurry was prepared by dispersing 40.0 g (dry basis) of VitAto starch in 3% citric acid solution (0.15 M) and hydrolyzed in a water bath for 6 hours at 45 °C with constant stirring. The pH was then adjusted to 5.5 ± 0.2 by adding sodium hydroxide solution (5 %). The starch was washed three to four times with a two-fold volume of deionized water prior to filtration and the starch cake was dried in a blowing oven at 45°C until the moisture reached <10%. The dried starch was ground into powder, sieved to 150 µm size and packed in an airtight container until further analysis.

Enzymatic-modified starch

The solution of 30% (w/w) VitAto starch was preheated until it reached the enzyme optimum temperature (below the gelatinization temperature of 60 °C). Then, 2% (mg/g) α-amylase enzyme from *Aspergillus oryzae* was added and hydrolyzed for 8 hours. After hydrolysis, the pH was adjusted to 4.0 by adding H_2SO_4 solution slowly to inactivate the enzyme. The pH of starch suspensions will be adjusted back to pH 5-6 by washing the starch with deionised water and the starch residue will be isolated by filtration and dried in a blowing oven for 24 h at 45 °C. The dried starch was ground into powder, sieved to 150 µm size and packed in an airtight container until further analysis. The recovery yield of the modified starches was calculated according to the formula:

$$Recovery\ yield = \frac{dry\ weight\ of\ starch\ after\ hydrolysis}{dry\ weight\ of\ starch\ before\ hydrolysis}\ x\ 100$$

Characterization of modified starch

Characterization of starch will be carried out according to Babu et al. (2016) and Grace & Henry (2020) for the physicochemical properties (analysis of reducing sugar value, amylose/amylopectin content, moisture content, water activity), functional properties (water holding capacity, emulsifying activity, emulsion stability) and thermal analysis using Differential Scanning Calorimetry(DSC).

Moisture content

Moisture content was carried out according to AOAC (2005) method. The starch samples were dried in a hot air oven at 100 ± 5 °C to a constant weight and the moisture and dry matter were calculated according to AOAC (2005) and expressed in percentage. The water activity (a_w) was measured using an Aqua-Lab digital instrument. The determinations were carried out in triplicates.

Amylose/amylopectin content

Amylose content was measured according to the method of Hoover and Ratnayake (2001) by using a UV-spectrophotometer at 600 nm. The total amylose content of the modified starch was calculated based on the equation of the standard calibration curve with the standard deviation less than 0.01 The amylopectin content was calculated by difference.

Dextrose equivalent

Dextrose equivalent (DE) was determined according to Babu et al. (2016) by measuring the reducing sugar value using the dinitro salicylic acid (DNA) method. The diluted modified starch samples were filtered and the filtrate was mixed with DNA reagent and the reducing sugar value was measured with dextrose as standard. The DE value was calculated as follows:

$$DE = \frac{g\ reducing\ sugar}{g\ dry\ weight\ of\ starch}\ x\ 100\%$$

Functional properties

The water absorption index (WAI), water holding capacity (WHC) and swelling power (SP) were carried out according to the method of Mohd Hanim et al. (2014). 4.5 g of modified starches was suspended in 30 mL of deionised water in a 50 mL centrifuge tube. The slurry was mixed and vortexed for 1 minute and centrifuged at 3000 × g for 10 minutes. The supernatant was transferred carefully into a petri dish and evaporated overnight at 110°C. The calculation was carried out according to these formulas:

$$Water\ Absorption\ Index\ (WAI) = \frac{wet\ sediment\ weight}{dry\ sample\ weight}$$

$$Water\ Holding\ Capacity\ (WHC, \%) = \frac{dry\ supernatant\ weight}{dry\ sample\ weight}\ x\ 100$$

$$Swelling\ Power\ (SP) = \frac{wet\ sediment\ weight}{dry\ sample\ weight\ x\ (1 - \frac{WSI}{100})}$$

The emulsifying activity and stability of the modified starch were determined according to Babu et al. (2016). 10 mg/mL of starch dispersion was prepared and homogenized with 5 mL of refined sunflower oil for 1 minute. The emulsion was then centrifuged at 1100 x g for 5 minutes and the emulsifying activity and emulsion stability were calculated according to these formulas:

$$Emulsifying\ activity\ (\%) = \frac{height\ of\ the\ emulsified\ layer}{height\ of\ the\ total\ content}\ x\ 100$$

$$Emulsion\ stability\ (\%) = \frac{height\ of\ the\ emulsified\ layer\ after\ heating}{height\ of\ emulsified\ layer\ before\ heating}\ x\ 100$$

Thermal analysis

The thermal properties of the modified starches were determined according to Babu et al., 2014) with a slight modification. A 6.0 mg sample (dry basis) was weighed in an aluminium pan and 24 mg of deionised water was added and mixed well. The pan was sealed and allowed to stand for 1 h. An empty aluminium pan was used as a reference. The analysis of the modified starch was carried out using a Differential Scanning Calorimeter (DSC 214 Polyma Netzsch, Germany). The sample was subjected to a heating program over a range of temperatures from 10 to 125 °C and a heating rate of 5 °C/min. The onset, peak, and final temperatures (To, Tp, and Tc, respectively) and transition enthalpy (ΔH) were measured.

Statistical analysis

The data for physical and chemical characteristics were tested by conducting a one-way analysis of variance (ANOVA) using the SAS System, ver. 9.0 statistical software. When statistically significant differences were indicated, the Duncan New Multiple Range Test (DMRT) was employed to compare the modified starches. All values are expressed as mean ± standard deviation (SD) and a difference was considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Extraction of starch

Table 1 shows the moisture content, dry matter and starch yield of the starch extracted from fresh VitAto. The total starch content was also determined using Megazyme K-TSTA-50A (total starch assay procedure of AOAC 996.1 dan AACC 76-13.01) and the results showed that VitAto contained 11.49 % of starch. From Table 1, the extraction of starch showed that fresh VitAto can produce about 10.98% of starch and the starch recovery is about 94.20 %. These results were supported by another study that reported the starch extraction yield (yield from fresh sweet potato roots) is usually not more than 12% for traditional scale and not more than 15% for large scale (Rahman et al., 2003).

Table 1: The starch yield of VitAto starch

	Extracted VitAto starch (%)
Moisture content	3.96 ± 0.04
Dry matter	96.04 ± 0.04
Total starch content	11.49 ± 0.21
Starch yield	10.98 ± 0.32
Starch recovery	94.20 ± 2.73

Starch modification

Table 2 shows the recovery yield of the Vitato starch modification and physicochemical properties of the acid-modified and enzymatic-modified VitAto starches. The recovery yield of acid-modified (AMS) and enzymatic-modified (EMS) VitAto starch was more than 97% and did not show any significant difference. The starch yield was reduced as starch might be hydrolysed by citric acid and α-amylase during the hydrolysis reaction.

Table 2: Recovery yield and physicochemical properties of enzymatic and acid-modified starches

	Acid-modified starch (AMS)	Enzymatic-modified starch (EMS)
Recovery yield (%)	97.30 ± 0.51[a]	97.08 ± 0.42[a]
Moisture content (%)	9.88 ± 0.15[a]	8.78 ± 0.04[b]
Water Activity (aw)	0.43 ± 0.00[a]	0.33 ± 0.02[b]
Amylose content (%)	37.07 ± 2.40[a]	35.55 ± 1.93[a]
Amylopectin content (%)	62.93 ± 2.40[a]	64.45 ± 1.93[a]

Dextrose Equivalent (DE) (%)	0.40 ± 0.01[a]	0.39 ± 0.01[a]

Note: Experimental data are means ± standard deviation (SD) for triplicates. Mean values with the same letters within the row are not significantly different (p>0.05).

Physicochemical properties of modified starches

Brown (2011) reported that a water activity value below 0.6 can prevent microbial growth and the rate of quality change is slowed. These results showed that the low value of moisture content (< 10%) and the water activity (<0.5) of both EMS and AMS showed that the modified starch could be stored at room temperature.

The branched glucose polymers, amylose and amylopectin are the main components of sweet potato starch. Sweet potato starch normally contains 20–30% linear and slightly branched amylose and 70–80% highly branched amylopectin (Lai et al., 2016). The starch modification displayed a higher fraction of amylose. The amylose content of EMS and AMS was 35.55% and 37.07%, while the amylopectin content of EMS and AMS was 64.45% and 62.93%, respectively (Table 1). These results were supported by O'Brien and Wang (2008); amylopectin was preferentially hydrolyzed during enzymatic hydrolysis which increased the amylose ratio in starches. The degradation of amylopectin contributes to shorter polymer chains and increases the amylose content value. Another study also reported acid hydrolysis increases the amylose content of starch due to the de-polymerization of amylopectin fractions at a high acid concentration (Betancur and Chel 1997).

Dextrose Equivalent (DE) acts as an indicator for the degree of hydrolysis. DE value of native starch is 0. Starch modification either acid-hydrolysis or enzymatic-hydrolysis could increase the DE value of the starch. The DE values of EMS and AMS were 0.39% and 0.40%, respectively (Table 2). The result showed a low DE value as citric acid used for acid-hydrolysis was a weak organic acid, with a low concentration of 3% citric acid, hence the degree of hydrolysis of the starch seems to be lower and resulted in a lower DE value. The results were supported by Shariffa et al. (2017) who reported that the DE values of the modified starches (tapioca and sweet potato) were significantly increased due to the higher susceptibility of the modified starches to α-amylase during the hydrolysis process as compared to native starch. The disruption of the hydrogen bonds in modified starches had weakened the granule structure, hence allowing the enzyme to penetrate and degrade the α-1,4 and α-1,6 linkages of the starch molecule.

Functional properties of modified starches

The result of water holding capacity (WHC), water absorption index (WAI), swelling power (SP), emulsifying activity and emulsion stability is shown in Table 3. A significant difference was observed in the water holding capacity, water absorption index and swelling power between EMS and AMS. A previous study showed that VitAto starch has 0.19% WHC and SP and 1.8 WAI (Hasnisa et al., 2023). The water holding capacity values of native and modified starches were less than 1%. A slightly increasing water holding capacity of EMS is probably due to the interaction between amylose and/ or amylopectin. Hydrolysis might increase the low molecular weight starch fraction with hydroxyl groups that hold water molecules forming hydrogen bonds. While lower water holding capacity of AMS might be due to low citric acid concentration and weak organic acid used for acid-hydrolysis. EMS showed a higher water absorption index and swelling power (2.13) compared to AMS (1.76). This might be due to the interaction between amylose-amylose and/or amylopectin-amylopectin, decreasing intra-granular binding forces and reinforcement of the granule. A higher disruption of the crystalline structure of the starch after hydrolysis may interact with the hydrogen bonding between starch and water, thereby increasing the water absorption index and swelling power.

Table 3: Functional properties of enzymatic and acid-modified starches

	Acid-modified starch (AMS)	Enzymatic-modified starch (EMS)
Water Absorption Index (WAI)	1.76 ± 0.03[b]	2.13 ± 0.16[a]
Water Holding Capacity (WHC, %)	0.15 ± 0.01[b]	0.31 ± 0.03[a]
Swelling power (SP)	1.76 ± 0.03[b]	2.13 ± 0.16[a]
Emulsifying activity (%)	64.29 ± 0.00[a]	66.05 ± 2.18[a]
Emulsion stability (%)	90.85 ± 1.27[b]	94.08 ± 1.37[a]

Note: Experimental data are means ± standard deviation (SD) for triplicates. Mean values with the same letters within the row are not significantly different (p>0.05).

From Table 3, EMS exhibited a higher emulsifying activity and emulsion stability compared to AMS. Starch with high linear amylose exhibits emulsion properties (Babu et al., 2016). The high amylose starch may act as the interface between water and oil and linear amylose chains of starch granules are more favoured in stabilizing the emulsion system than branched amylose and/or amylopectin chains. The linear amylose fractions could improve the emulsion capacity and stability of the starch.

Thermal analysis

Table 4 shows the thermal properties of the acid-modified (AMS) and enzymatic-modified (EMS) starch. The results showed the onset (T_o), peak (T_p), conclusion (T_c) temperatures and enthalphy (ΔH) between AMS and EMS. AMS showed a lower onset temperature than EMS. This result was supported by Babu et al. (2016) who reported that acid-modified maize starch also displayed a low T_o. EMS showed a higher T_p than AMS with no significant difference while the T_c of AMS was significantly higher than EMS. The enzymatic treatment might hydrolysed the starch at amorphous region and resulted an increase in relative crystallinity of the starch and increased the gelatinization temperature than acid treatment. EMS (2.879 J/g) showed higher enthalphy than AMS (2.787 J/g) with no significant difference. ΔH measures the starch crystallinity and acts as an indicator for the loss of the structured molecules in starch granule during gelatinization. A higher degree of acid hydrolysis might resulted a greater loss of

structured molecules of starch than enzymatic-modified starch. A decreased ΔH of acid-modified starch might be due to their short amylopectin chain length which requires lesser energy to melt the crystalline structure and lesser number of double helices.

Table 4: Thermal properties of the acid-modified (AMS) and enzymatic-modified (EMS) starch

Sample	Gelatinization temperature (°C)			ΔH (J/g)
	T_o	T_p	T_c	
AMS	68.6[b]	79.7[a]	92.2[a]	2.787[a]
EMS	70.1[a]	80.5[a]	90.1[b]	2.879 [a]

Note: T_o, T_p, and T_c stand for onset, peak, and conclusion temperatures, respectively. ΔH (J/g) indicates enthalpy. Mean values followed by the different letters within the column are significantly different ($p < 0.05$).

CONCLUSION

Acid-modified or enzymatic-modified starch is produced to achieve desired functional and sensory properties. Acid or enzymatic hydrolysis scission the glucosidic linkages, thereby altering the structure and properties of the native starch. Hydrolysis reduces the molar mass and consequently increases the free aldehyde group content. It also increases the solubility of the granules, decreases viscosity, minimizes syneresis, and causes gel thermo-reversibility. This study showed no significant differences ($p>0.05$) for amylose and amylopectin content between both acid and enzymatic-modified starch. While, the water absorption index, water holding capacity, moisture content, water activity and emulsion stability were significantly different ($p<0.05$). The differences in the physicochemical properties and functional properties of both modified starches can be used to suggest their potential industrial uses. A few analyses such as pasting profile, structural properties and morphology surfaces were suggested to be carried out to get more information in understanding the modified starches characteristics to suggest their application in food or non-food industries.

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