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Extraction and Modification of Starch from Purple Taro Tuber, Colocasia esculenta B. Tini, Through Acetylation Method: Optimization of the Acetylation Process

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Abstract

In this study, starch was extracted from non-conventional purple taro tuber, *Colocasia esculenta B. Tini* and modified via acetylation method. The extracted starch was acetylated under varying acetic anhydride concentrations, reaction temperature, and time. The acetylation experiments were designed by Box–Behnken Design (BBD) whereas the effects of the variables on the acetylation process were studied at a significance of $p < 0.05$ with response surface methodology (RSM). The experimental results were subjected analysis of variance to come up with a quadratic model equation. The quadratic model showed that an acetic anhydride concentration of 9.70%, reaction temperature of 30.94 ºC, and reaction time of 15.17 min, were found to be optimal to obtain a maximum yield of 40.31% acetyl content and 2.34 degree of substitution (DS). The amylose and amylopectin content, as well as the proximate composition of the native starch of purple taro tuber were determined according to the Association of Official Analytical Chemists (AOAC). The proximate composition of native and acetylated starches of purple taro tuber showed the moisture, crude protein, crude fat, ash, amylose, and amylopectin contents of 64.60 ± 3.68 & 2.95 ± 0.24%, 3.90 ± 0.33 & 0.97 ± 0.09 %, 2.96 ± 0.17 & 0.60 ± 0.22 %, 4.30 ± 0.47 & 0.50 ± 0.29 %, and 30.18 ± 0.67% & 69.82 ± 0.33% contents, respectively. On a dry weight basis, the purple taro tuber yielded 16.7 ± 0.16 % starch. The native starch (NS) and acetylated purple taro tuber starch (APTS) were characterized by determining their pH, hydration capacity, swelling power and solubility, SEM, and XRD. The pH and hydration capacity of APTS were found to be 7.30 and 2.90 ± 1.17, respectively. The SEM image showed the native and acetylated purple taro tuber starches had a polygonal, semi–oval, and irregular shapes. The native and acetylated purple taro tuber starch showed a B–type XRD pattern.

1. Introduction

Starch is a natural, cheap, available, renewable, and biodegradable polymer produced by many plants as a source of stored energy [1]. It is a carbohydrate consisting of a large number of glucose units joined together by glyosidic bonds [2]. Starch is composed of two polymers of D-glucose: 20-30% amylose with linear structure and 70-80% branched amylopectin [3]. It has great nutritional, pharmaceutical and industrial significances due to its unique physical, chemical and functional properties [4]. It contributes 50-70% of the energy in the human diet, providing a direct source of glucose, which is an essential substrate in brain and red blood cells for generating metabolic energy [5]. Indeed, the availability of a reliable source of starch from agriculture is considered to have been an important factor in human development [6]. It contributes greatly to the textural properties of many food stuffs and is widely used in food and industrial applications as a thickener, colloidal stabilizer, gelling agent, bulking agent and water retention agent [7]. Starch, the principal source of dietary calories to the world's human population, is the major polysaccharide reserve material present in photosynthetic tissues and in many types of plant storage organs such as seeds, tubers, roots, and fruits [8]. The conventional sources of starch such as cereal starches (wheat, rice, maize) and tubers (potato, cassava and sweet potato) have been the major focus for research and industrial application [9]. Starch isolation has been limited to a few traditional crops, such as corn, wheat, rice, potato, and cassava. However, the new botanical sources are emerging as unconventional sources of starch, including roots, pulses, and tubers from local origins, increasing their cost effectiveness [10]. Therefore, starch can currently be extracted from non–conventional botanical sources with different physicochemical, structural, and functional features that can extend the range of industrial applications. Among the raw materials that can be used as new sources of extraction of starch are tubers such as the taro tuber. Taro tuber is used similar to potato tuber in diverse regional dishes with an important carbohydrates supply, because it was reported that taro tuber contains 70–80% starch in dry basis [11]. Native taro tuber contains high starch yield (around 83.5% on a dry weight basis; amylose content of around 20.7%, granule size less than 6.63 µm, and good compaction property [5].

The native starches have very limited industrial applications due to their low water solubility, limited swelling power, higher syneresis, poor granule dispersibility, high gelatinization temperature, and resistance to enzymatic hydrolysis [12]. Thus, modification of the native starch is necessary in order to overcome these limitations. Furthermore, modification of native starch can be useful in the development of tailor made properties of starch suitable for specific applications [13]. Different methods have been used to modify the native starch characteristics including physical, chemical, and enzymatic modification methods [14].Physical native starch modification methods include hydrothermal treatments, pre-gelatinization, and non-thermal processes [14].Chemical modification method is commonly achieved through derivatization such as etherification, esterification, cross-linking, and grafting [14]. Enzymatic degradation by food-grade enzymes, such as amyl glucosidase, pullulanase, a–amylase, b-amylase, and isomerase, is used in the production of maltodextrin, modified starches, or glucose and fructose syrups [14]. Acetylation is one of the most commonly used chemical modification methods to improve the compaction and flow ability of native starch, pre-requisites for a direct compression (DC) excipient [5]. The modified (acetylated) native starch has been tested for its good binding functionality, powder flow ability, compressibility, and high-dilution potential so that it achieves the desired properties for making tablets [5]. Acetylation can be obtained through esterification of native starch with acetic anhydride, vinyl acetate or acetic acid [15]. Acetylation involves the substitution mechanism where the free hydroxyl (OH^-) groups in the starch chains are replaced by acetyl ($CH3COO^-$) groups [16]. In this study, native starch was extracted from purple taro tuber and modified through acetylation method. The acetylation process parameters were optimized through the response surface methodology (RSM). The effect of the acetylation process parameters such as acetic anhydride concentration, reaction temperature, and reaction time on the acetyl content of the native starch and the degree of substitution (DS) has been studied and optimized.

2. Materials and methods

2.1. Materials

Acetic anhydride (99%), sodium hydroxide, potassium hydroxide, HCl (35%), H_2SO_4 (98%), n-hexane (95%), potassium sulfate, Anhydrous copper sulfate, phenolphthalein indicator (1%), boric acid, ethanol (96%), starch amylose, and iodine solution. All the chemicals used were of reagent grade and were used without further purification. UV spectrophotometer, x-ray diffraction (XRD), Scanning electron microscope (SEM), and pH meter were used to analysis and characterize the native starch and APTS. The response surface methodology (RSM) was performed to optimize acetic anhydride concentration, reaction temperature, and reaction time to obtain maximum acetyl content (A) and degree of substitution (DS) using Box-Behnken design. The design expert (version13.0.14) software was used to analysis both experimental design and statistical analysis of acetylation process parameters.

The primary raw material for this study was purple taro tuber (Figure 1). The raw material, purple taro tuber was collected from Arba Minch city in southern Ethiopia and transported to Bule Hora University, department of Chemical Engineering Laboratory. The purple taro tuber sample was properly cleaned and peeled using stainless steel knives, and the peeled purple taro tuber sample was washed, rinsed with deionized water, and then sliced. The slices were dried for 4 h in the oven at 105 ± 1°C [17]. The dried taro chips were powdered with a laboratory scale grinder and sifted through a 300µm sieve. Until the investigation of proximate analysis was carried out, the samples were sealed and placed in airtight containers.

2.2. Proximate composition analysis

The triplicate proximate composition analysis, like moisture content, ash content, protein content, and crude fat, was performed for the purple taro tuber sample and native starch according to the method of AOAC [18]. The amount of total carbohydrates were calculated by subtracting the percentages of moisture, crude protein, and crude fat from 100% [19]. On the other hand, the amylose content was determined through a simple colorimetric iodine method [20]. The amylopectin content was calculated as the difference between total starch and amylose contents [21].

Figure 1. Purple taro plant (a) and raw purple taro tuber (b).

2.3. Starch isolation from the purple taro tuber

Starch from purple taro tuber was extracted according to the method outlined by Taylor et al. [22] with slight modifications. The tubers were washed under tap water, peeled, and cut into slices of approximately 1 cm. The slices (10g) were soaked in distilled water (50 mL) for 2 h, and then the suspension was blended at a high-speed blender for 2 min. The slurry was filtered through a No. 35 wire mesh sieve. The filtrate was then allowed to settle at 25°C for 1h. The top layer was decanted off and precipitates were washed with 50 ml distilled water, 4 times to remove impurities. The filtered product was centrifuged at 3000 rpm for 10 min (4 times), and the resulting supernatant was decanted. The starch granules were washed 4 times with distilled water. The starch was dried at 45 ºC for 12 h, ground so that it passes through a 60 mesh sieve, and then stored in sealed plastic bags at 4ºC before use [23]. The percentage yield of the starch was determined according to the method described by the AOAC [18]. The percentage of starch yield was determined using Eq. (1):

$$\text{Native starch yield (\%)} = \frac{\text{weight of dry native starch}}{\text{weight of the sample}} \times 100 \qquad (1)$$

2.4. Preparation of acetylated purple taro starch

Acetylated starch was prepared by reacting native starch with acetic anhydride according to the procedures outlined in [24] and [26] with slight modifications. About 5.50 g native purple taro starches was dispersed in 45 mL distilled water and stirred for 15 min at 25 °C to obtain a uniform suspension. For the acetylation reaction to take place, the pH of the suspension must be maintained between 8.0-0.4 with 4% (w/v) aqueous NaOH solution [16]. Then, acetic anhydride (8 and 10%) was added dropwise to the stirred slurry using a magnetic stirrer, while maintaining the pH during the reaction within the range of 7.8–8.2 using 4% NaOH solution. The reaction mixtures were heated to (30 and 65 ^{O}C), and held at this temperature for 15 min and 30 min while being stirred. Finally, pH was adjusted to 4.5 using an HCl solution (0.10N). The product was washed free of acid three times with distilled water, and then dried in an oven drier at 50 °C. The dried acetylated starch was ground and passed through a 100-mesh standard sieve and placed in a sealed container, and stored at room temperature until used.

2.5. Determination of Percentage of Acetyl and Degree of Substitution

The degree of substitution (DS) of acetylated purple taro tuber starch (APTS) was determined according to the method reported in previous work [27]. Determination of the DS of APTS by titration involved complete basic hydrolysis of the ester linkages and titration of the excess alkali [27]. 2.0 g of APTS was weighed accurately with a digital balance and placed in a 250 mL Erlenmeyer flask, and 40 mL of distilled water was added upon mixing at room temperature. A few drops of 1% (v/v) phenolphthalein indicator were added, and the suspension was titrated with 0.05 M NaOH to a permanent pink endpoint. After the addition of 10 ml 0.50 M NaOH, the flask was stopped loosely with plastic wrap and shaken vigorously for 1h to complete the saponification reaction at room temperature. After shaking, the plastic wrap was carefully removed and washed down, along with the flask walls. The saponified mixture, containing excess alkali, was then titrated with a standard 0.50 M HCl to the endpoint as indicated by phenolphthalein. A blank, using the native purple starch, was also treated similarly. Acetyl content (A) was calculated as in Eq. (2).

$$A = \frac{(V_1 - V_2) \times 10^{-3} \times N \times 43 \times 100}{W} \tag{2}$$

Then, the acetyl content (A) was used to calculate the degree of substitution (DS) as in Eq. (3).

$$DS = \frac{162A}{43 \times 100 - (43 - 1) \times A} \tag{3}$$

where, V_1 is the volume (mL) of 0.50 M HCl used for titration of native starch, V_2 is the volume (mL) of 0.50 M HCl used for titration of APTS, M is the molarity of the HCl solution, W is the weight of the APTS (g/mol), 43 is the molecular weight of the acetyl group (g/mol), 162 is the molecular weight of an anhydrous glucose unit (g/mol).

2.6. Physicochemical properties of the native and acetylated purple taro tuber starches

2.6.1. Determination of starch hydration capacity

The hydration capacity of the starch was determined by using the method outlined in [28] with slight modifications. 1 g of starch was placed in a plastic centrifuge tube, 10 ml of distilled water was added, and then closed. The contents were shaken for 2 min and allowed to stand for 10 min, and immediately centrifuged at 1000 rpm for 10 min using a bench centrifuge. The supernatant was decanted, and the weight of the wet starch was recorded. The hydration capacity was determined using Eq. (4):

$$\text{Starch hydration apacity} = \frac{W_s}{W_d} \tag{4}$$

where, W_S is the weight (g) of the sample, W_d is the weight (g) of the precipitate.

2.6.2. Swelling Power and Solubility

The swelling power and solubility of the starches were determined by the method reported somewhere else [5] with slight modifications. Samples (0.50 g) were dispersed in 10 mL of distilled water in centrifuge tubes (reweighed). Then, the tubes were transferred into a thermostatically controlled water bath for 30 min at 20, 37, 50, 65, 75, and 85 °C, with shaking every 5 min, and then left to cool down. Then, the suspensions were placed in a centrifuge machine operated at 3000 rpm for 15 min, and the supernatants were decanted onto dried and pre-weighed petri dishes and dried in an oven for 2 h at 130°C. The solubility, S (%) and Swelling power (SP) were calculated using Eqs. 5 & 6, respectively.

$$S\,(\%) = \frac{W_T}{W_S} \times 100 \tag{5}$$

$$SP = \frac{W_P}{W_S \times (100 - S)} \times 100 \tag{6}$$

where, S is the starch solubility (%); SP is the starch swelling power; W_T is the weight (*g*) of soluble material in the supernatant; W_P is the weight (*g*) of the precipitate; W_S is the weight (*g*) of the starch sample.

2.6.3. Freeze-thaw stability

Freeze–thaw stability of the starches was determined by the method described in [20] with slight modifications. Starch (6% w/v, dry basis) was subjected to cold storage at 4°C for 16 h and then frozen at -16 °C. To measure freeze-thaw stability, the gels were frozen at –16°C for 24 h and were thawed at 25°C for 6 h and then refrozen at -16 °C. Five cycles of freeze-thaw were performed. The excluded water was determined by centrifuging at 1000 rpm for 20 min after thawing. The percentage syneresis was determined as the weight of exudates to the weight of the paste.

2.6.4. Determination of pH

The pH of the native and APTS were determined using the method described in [29]. 1% (w/v) starch was dispersed in water and shaken for 5 min, and then its pH was determined using a digital pH meter (Wagtech 3510, Hanna Instruments).

2.6.5. Starch crystalline structure

X–ray diffraction pattern (XRD) of the native and APTS were done using the methods described in [30] with slight modifications. An X–ray diffractometer operating at 40 kV with a target current of 30 (mA) was used for the analysis. The native and APTS samples were equilibrated at a temperature and relative humidity of 25 °C and 100%, respectively, in a low-temperature incubator (MTIE10, Labcon, South Africa) for 12 h. The equilibrated samples were tightly packed in a rectangular glass cell and scanned over a region of 5.000-65.000 (2θ) at a scanning speed of 2.000 (degree/min). The degree of relative crystallinity was calculated from the ratio between the peak area and the total diffraction area [31]. The degree of relative crystallinity was determined using Eq. (7).

$$X_C = \frac{A_P}{(A_P + A_b)} \times 100 \tag{7}$$

where, X_c is the relative crystallinity, A_p is the peak area, A_b is the basis area.

2.6.6. Morphological granular characteristics

The native and APTS granule morphological characteristics were determined by the method described in [32] with slight modifications. The native and APTS were mounted on carbon sample holders using double–sided sticky tape and were observed using a JCM-6000 Plus scanning electron microscope with the mode at 15.00 kV accelerating voltage. Micrographs at 600x and 1500x magnification are presented. Samples were not metalized since the microscopy equipment operates under high-vacuum conditions.

2.7. Experimental Design

The experimental design was determined by the method of [33] with slight modifications. Design-Expert software (version 13.0.14) was used for designing the statistical test, analyzing the results, as well as drawing the response surface plots. Response surface design was used to estimate the second–degree polynomial relationships between independent and dependent variables. On the basis of a single-factor test, the response surface test was designed by selecting the reaction temperature, reaction time, and concentration of acetic anhydride as independent variables, and acetyl content (A) and degree of substitution (DS) as response variables. The actual values of the factors were varied at three levels, namely −1, 0, and +1 corresponding to minimum, middle, and maximum. The factors and coded levels of the Box-Behnken test are shown in Table 1.

Table 1. Factors and coded levels of the Box-Behnken test.

Factors and levels	Concentration of acetic anhydride (A) (%)	Reaction temperature (B) (°C)	Reaction time (C) (min)
−1	8	30	15
0	9	47.5	22.5
1	10	65	30

3. Results and discussion

3.1. Proximate analysis of purple taro tuber

The comparison of the proximate composition of the purple taro tuber with the previous studies is shown in Table 2. Temesgen [34] reported the moisture content of taro tubers ranges from 60 to 83% depending on growth and harvest time. The moisture content in this investigation was 64.60 ± 3.68%, which was similar to the value for taro tuber observed by [11]. The taro tuber may contain a considerably high amount of total ash due to its high mineral content, ranging from 3.54 to 7.78% [34]. The purple taro tuber ash content in this investigation was found to be 4.3%, which is similar to the value reported by [11]. However, the ash contents of the taro reported by [35] were lower than in this study. The observed difference in the ash contents may be attributed to climatic factors, the soil type, and the variety differences. From the high ash contents of the taro samples studied, one can easily understand that taro could contain an appreciable quantity of minerals [36]. The purple taro tuber employed in this study had a lower protein content than the of protein content reported by [19]. Similarly, the protein content of the purple taro tuber used in this study was lower than that reported by [37]. However, compared to the protein content of taro corm reported by [38] the protein content in this study was higher. The observed difference in the protein contents might be related to climatic factors, the soil type, and the variety difference [19]. The crude fat content of the purple taro tuber used in this investigation was higher than the range of fat content reported by [34]. Similarly, the purple taro tuber has a larger crude fat content than the taro corm reported by [38]. Kaushal et al. [38] observed that the crude fiber content varied from 0.60 to 1.18%. When compared to the study done by [38], purple taro showed a higher crude fiber value (6.21 ± 0.163 %). However, [11] reported that the crude fiber content of the taro tuber of 6.8%. Thus, the crude fiber content of purple taro tuber was lower than the range of crude fiber contents that was reported by [11]. The carbohydrate content of taro corm ranged from 13.0 to 29.0% [38]. The carbohydrate content of the purple taro tuber was found to be 28.54 ± 5.823%, which is in agreement with the result reported by other authors for other taro tubers [38].

Table 2. The comparison of the proximate composition of purple taro tuber starch with the previous studies.

Parameter	Current study	Previous study	References
Moisture	64.60 ± 3.68	60.0-83.0	[34]
		65.20	[11]
Ash	4.30 ± 0.472	4.20	[11]
		0.90–1.37	[35]
Protein	3.90 ± 0.327	4.03–6.22	[19]
		4.50	[32]
		1.40–3.0	[38]
Fat	2.96 ± 0.17	0.30–0.60	[34]
		0.16–0.36	[38]
Fiber	6.20 ± 0.163	0.60–1.18	[38]
		6.80	[11]
Carbohydrate	28.54 ± 5.823	13.0–29.0	[38]

3.2. Purple taro starch yield and chemical composition

Table 3 shows the starch yield and chemical composition of purple taro tuber. The yield of the starch extract from the purple taro tuber was 16.7 ± 0.163%, with 87.85 g of dry starch (25°C) being produced from 525.94 g of the raw purple tar tuber. This value is lower than the 86.5% for the taro tuber that was previously reported by [39]. However, [38] observed that the starch yield from taro tuber was 13.0–29.0%. This result agrees with the current study. In comparison with the result reported by [40] for cassava starch (24.4%), the starch yield is lower. However, the result in this study is higher (14.49%) than for a potato tuber previously reported by [41].

The purple taro starch had 2.95 ± 0.24% moisture, 0.50 ± 0.29% ash, 0.97 ± 0.09% crude protein, and 0.60 ± 0.22% crude fat. The moisture content of purple taro starch, was found to be 2.95 ± 0.24% %, which is lower than the 8.96-10.12% reported for taro starch by [42], in which both were within the safe range for storing starches without a quality reduction. According to the study by [42], the crude protein, crude fat, and ash contents range from 0.33-0.62, 0.21-0.43, and 0.43-0.57, respectively for the various cultivars. The crude protein, crude fat, and ash contents were approximately similar to the current study. The low values of ash, lipid, and protein contents were found to be lower than those reported as typical of the native starch indicating that the material investigated was high purity [43]. The applications of starch depend on the amylose and amylopectin content, which have a significant impact on the chemical characteristics of the starch. The amount of amylose was measured using a standard curve created using absorbances at wavelength of 610 nm. The amylose and amylopectin content in this study were found to be 30.18 ± 0.67 and 69.82 ± 0.33%, respectively. In this investigation, the amylose content of purple taro starch was higher than the range of amylose content reported by Martins et al. [44]. The amylopectin content of purple taro starch was 69.82 ± 0.33%, which is lower than that of bun long taro cultivated in China (91.50 ± 4.10%) [45].

Table 3. The comparison of the chemical composition of the extracted purple taro starch with the previous studies.

Parameter	Current study	Previous study	References
Starch content	16.70 ± 0.16	86.50	[39]
		13.0-29.0	[38]
		24.40	[40]
		14.49	[41]
Moisture content	2.95 ± 0.24	8.96-10.12	[42]
Ash content	0.50 ± 0.29	0.43-0.57	[42]
Crude protein	0.97 ± 0.09	0.33-0.62	[42]
Crude fat content	0.60 ± 0.22	0.21-0.43	[42]
Amylose content	30.18 ± 0.67	26.45	[44]
Amylopectin content	69.82 ± 0.33	91.50 ± 4.10	[45]

3.3. Degree of substitution and acetyl content

Degree of substitution (DS) represents the average number of hydroxyl groups substituted per anhydroglucose unit. As each anhydroglucose unit has three hydroxyl groups available for substitution, the maximum possible DS is 3 [27]. The effect of acetic anhydride concentration on the acetyl content (%) and degree of substitution (DS) of purple taro starch at various concentrations (8% and 10%) at a constant reaction temperature (30°C) and reaction time (15 min) is shown in Table 4. The acetic anhydride concentration rises from 8 to 10%, the acetyl content rises from 18.86 ± 0.05-37.71 ± 0.47%, and the degree of substitution rises from 0.86 ± 0.05-2.06 ± 0.05. The increase in the DS could be reasonably attributed to increased contact between the starch molecules and the acetic anhydride [27]. The acetyl (%) and degree of substitution (DS) in acetylated purple taro starch were higher than those previously studied for potato and corn starches [46].

Table 4. Effect of acetic anhydride concentration on acetyl (%) and degree of substitution (DS) of purple taro starch at a constant reaction temperature (30 °C) and reaction time (15 min).

Acetic anhydride concentration (%)	Acetyl (%)	DS
8	18.86 ± 0.05	0.86 ± 0.05
10	37.71 ± 0.47	2.06 ± 0.05

The effect of reaction temperature on acetyl content (%) and degree of substitution (DS) at a constant acetic anhydride concentration (8%) and reaction time (15 min) is shown in Table 5. The reaction temperature increases from 30 to 65 °C, resulting in a decrease in the degree of substitution (DS) and acetyl content (%). The esterification of starch is an exothermic reaction; the higher temperature is unfavourable to the absorption of the esterifying agent by the starch and the formation of active reaction centers. Therefore, the acetyl content and degree of substitution (DS) decreased slowly with further increase in temperature [27].

The effect of the reaction time on acetyl content (%) and degree of substitution (DS) at a constant acetic anhydride concentration (8%) and reaction temperature (30°C) is shown in Table 6. When the reaction time is increased from 15-30 min, the acetyl content (%) and degree of substitution (DS) both decrease. The acetyl content (%) and degree of substitution (DS) decrease as the reaction time increases continuously. It is due to the complete consumption of the esterifying agent [27]. Similarly, [47] observed that the degree of substitution (DS) decreases as the reaction time increases continuously, which can be due to the complete consumption of the esterifying agent and intensified degradation of acetylated corn starch during the reaction process.

Table 5. Effect of reaction temperature on acetyl (%) and degree of substitution (DS) of purple taro starch at a constant acetic anhydride concentration (8%) and reaction time (15 min).

Temperature (°C)	Acetyl (%)	DS
30	35.27 ± 0.84	2.03 ± 0.104
65	26.66 ± 0.47	1.35 ± 0.29

Table 6. Effect of reaction time on acetyl (%) and degree of substitution (DS) of purple taro starch at a constant acetic anhydride concentration (8%) and reaction temperature (30°C).

Time (min)	Acetyl (%)	DS
15	30.39 ± 0.56	1.63 ± 0.05
30	12.09 ± 1.80	0.52 ± 0.06

3.4. Physicochemical properties of native and acetylated purple taro starches

3.4.1. Determination of starch hydration capacity

The hydration capacity of native starch was found to be 1.30 ± 0.75 (Table 7). When the starch was modified by the acetylation method, the starch hydration capacity increased (2.90 ± 1.17). When compared to the native starch, the APTS was found to have a greater water hydration capacity. Similarly, [48] observed that the acetylation of sword bean has a higher hydration capacity than the native starch. The acetylation of starch enhanced the water-binding capacity, because hydrophilic groups are incorporated into the native starch [48].

Table 7. Starch hydration capacity of native and acetylated purple taro starches.

	Native purple taro tuber starch	Acetylated purple taro tuber starch
Hydration capacity	1.30 ± 0.75	2.90 ± 1.17

3.4.2. Swelling power and solubility

The solubility and swelling power of native and APTS as a function of temperature were tested, and the results are shown in Figures 2 and 3. All of the starches' swelling and solubility properties increased as a result of the rising temperature. These findings are consistent with those made previously by [42, 49, 50]. In this study, the APTS had higher swelling power and solubility than the native starch. Ahmed et al. [12] observed that at all temperatures, the modified starches had higher swelling power than the native starch. According to the study by other authors [51], the higher the percentage of acetyl groups on the starch, the higher the percentage of swelling power and solubility observed. This result supports the fact that the introduction of hydrophilic acetyl groups allows the retention of water molecules because of their ability to form hydrogen bonds.

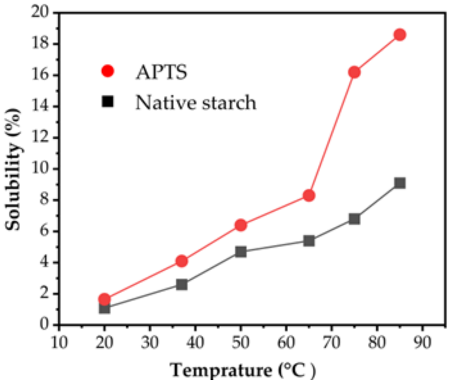


Figure 2. Solubility of native and acetylated purple taro starches.

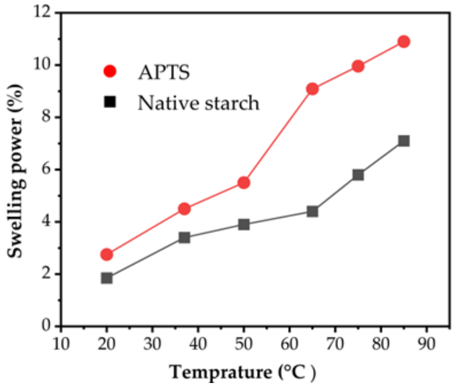


Figure 3. Swelling power of native and acetylated purple taro starches.

3.4.3. Freeze-thaw stability

The percentage of water released (syneresis) and the number of freeze-thaw cycles are used to measure the freeze–thaw stability of starch pastes. The freeze-thaw stability of native starch and APTS are shown in Figure 4**.** After modification using the acetylation method, syneresis was shown to decrease. This indicated that acetylation reduces syneresis. Han et al. [47] reported that the acetyl group interaction with other macromolecules causes a decrease in syneresis in acetylated corn starch. Lower values of syneresis of acetylated starches might be due to the acetyl groups, which cause steric hindrance in the reassociation of starch chains, thereby resulting in enhanced water retention capacity of the acetylated starch during storage [52].

3.5. Characterization of the Native and Acetylated Purple Taro Starches

3.5.1. Determination of pH

In this study, the pH values of the native starch and APTS were 7.50 and 7.30, respectively. Adebowale et al. [53] observed that the pH values of the native and acetylated bambara starch were 6.50 ± 0.501 and 6.10 ± 0.18, respectively. This reduction could be explained by the pH decrease to 4.5 after the acetylation and washing steps; the pH during acetylation was maintained between 8.0-8.40 [54].

3.5.2. Morphological characteristics of purple taro starch

Morphological characteristics of starches, such as size and shape, vary depending on the plant source. Besides the biological origin, the morphology of the starch granule depends on the biochemistry and metabolic routes occurring in the chloroplast or amyloplast, as well as the physiology of the plant and the agronomic and climatic conditions under which they were grown [57]. Microscopy, light microscopy, and scanning electron microscopy have played an important role in increasing the understanding of the granular structure of modified starches [14]. In this study, the native and APTS granules, which can be seen below in Figure 6 (a-b) were polygonal, semi-oval, and irregular in shape. SEM revealed no significant differences between the external morphology of native and acetylated starches. Ayucitra [58] reported identical results for acetylated corn starch, finding that acetylation did not significantly alter the granules' size, shape, or exterior appearance and that the results were independent of the degree of acetylation. The acetylated starches maintained their morphology because the reaction took place preferentially in the amorphous amylose-containing region of the granule and because the lack of pores or channels on the granule surface restricted acetic anhydride penetration, preventing the granule surface from going through drastic changes [59].

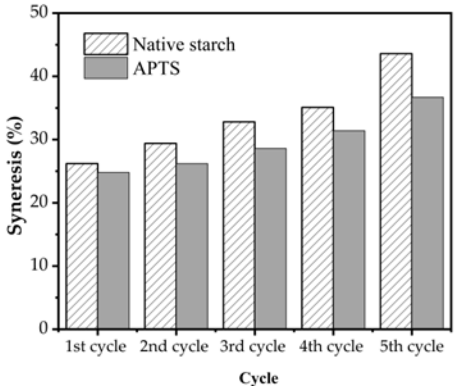
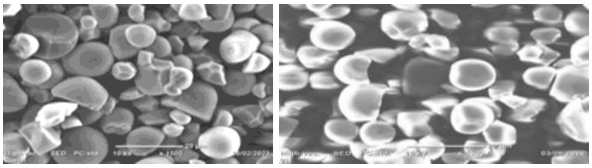


Figure 4. Syneresis of the native and acetylated purple taro starches.



(a) (b)

Figure 5. Scanning electron micrograph (SEM) of the native (a) and acetylated purple taro starch (b).

3.5.3. X-ray diffraction pattern

According to Sukhija et al. [60], most of the tuber starches exhibit 'B' type X–patterns, in which main peaks are centred at 2Θ around 5.6°, 15°, 17°, 19.7°, 22.2°, and 24°. Whereas, cereal starches exhibit 'A' type X–patterns, in which the main peaks are centred at 2θ around 15.3°, 17.0°, 18.0°, 20.0°, and 23.4°. X–ray diffraction measurement is performed to check if chemical modification alters the crystallinity of the starch [27]. The XRD patterns of native starch and APTS for the current study are shown in Fig. 7. Diffraction peaks for the B–type crystalline structure could be seen in the native purple taro starch at angles (2θ) of 5.6°, 15°, 17.4°, 19.7°, and 23.5°.

The XRD patterns of APTS did not change after acetylation reaction. This result suggests that acetylation reaction did not change the crystalline patterns of the native purple taro starch, indicating that the esterification reaction mainly occurred in the amorphous regions of the native purple taro starch. This observation agrees with the finding by [59], who confirmed that the esterification occurred primarily in the amorphous regions and did not change the crystalline pattern of starches.

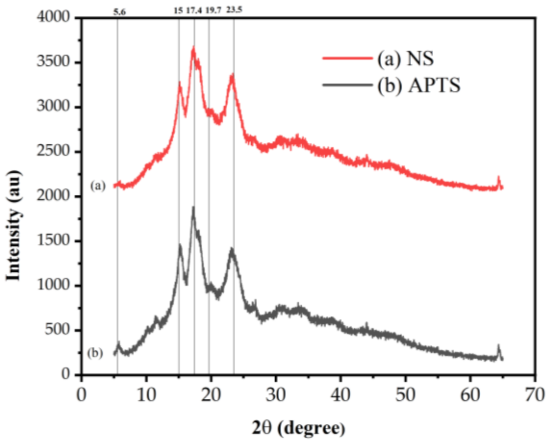


Figure 6. X-ray diffraction (XRD) patterns of the native and acetylated purple taro starches.

3.6. Analysis of the acetylation of purple taro starch

The native purple taro starch was modified by the acetylation method at different acetic anhydride concentrations (8-10%) in the presence of sodium hydroxide. The acetylation process was carried out at various reaction temperatures (30-65 °C) and reaction times (15-30 min) (Table 8).

Table 8: Acetyl content and degree of substitution of acetylated purple taro starch using Design Expert with Box-Behnken method.

Run	Con. (%)	Temp. (°C)	Time (min)	Acetyl (%)	Predicted	DS	Predicted
1	9	30	15	30.39	29.8363	1.63	1.6163
2	8	65	22.5	25.62	25.0174	1.29	1.2562
3	10	47.5	15	35.71	35.6611	2.06	2.0398
4	9	47.5	22.5	20.14	25.0214	0.94	1.2647
5	9	47.5	22.5	20.14	20.4100	0.94	0.9600
6	10	47.5	30	25.69	25.0444	1.31	1.2833
7	9	65	15	13.76	13.8190	0.60	0.6071
8	8	47.5	30	22.97	23.0189	1.12	1.1361
9	10	30	22.5	35.27	35.8726	2.03	2.0617
10	9	30	30	12.09	12.0320	0.52	0.5088
11	9	47.5	22.5	20.95	20.4100	0.99	0.9920
12	8	47.5	15	18.86	19.4036	0.80	0.8906
13	10	65	22.5	26.66	26.6499	1.35	1.3630
14	9	65	30	24.17	24.7237	1.19	1.2036
15	8	30	22.5	19.11	19.1201	0.89	0.8721

3.6.1. Analysis of Variance (ANOVA) for Taro Tuber Starch Acetylation

Analysis of variance (ANOVA) was used to examine the created quadratic model's statistical significance and goodness of fit, as well as the impact of each variable and its interactions. The significance of the relationship between various factors and response value was judged by the F and P-values [61]. A large F-value indicates that most of the variation can be explained by a regression equation, whereas a low P-value (< 0.05) indicates that the model is considered to be statistically significant [62]. The APTS have the F-value and P-values, which were obtained by the model are shown in Table 9. In the case of acetyl content (A) and degree of substitution (DS), F-value (151.16), P-value (< 0.0001) and F-value (232.03), P-value (< 0.0001) were obtained. These values imply that the model is significant. As a result, the model can sufficiently predict the acetyl content and degree of substitution based on the investigated factors affecting the process. The P-value less than (< 0.05) indicates model terms are significant. In this case, A, B, C, AB, AC, BC, and A^2 are significant model terms that indicate that the model is suitable for use in this experiment and suggest that all parameters influence the acetyl content and degree of substitution. The lack of fit test is a measure of failure of the model to represent the data in the experimental domain, at which points were not included in the regression or variations in the models cannot be accounted for by random error [63]. The lack–of–fit test value was not significant ($p > 0.05$), which also shows a good fit between experimental data and the model [64]. The "Lack of Fit F-value" of the acetyl content was 2.96 implies the lack of fit is not significant relative to the pure error. There is a 26.26% chance that a lack of fit F-value this large could occur due to noise. In the case of degree of substitution, the "Lack of Fit F-value" was 2.31 implies the lack of fit is not significant relative to the pure error. There is a 31.66% chance that a lack of fit F-value this large could occur due to noise. Not-significant lack of fit is desirable.

Table 9. ANOVA analysis of acetylated purple taro starch.

	% Acetyl		Degree of substitution		
Model terms	F-value	p-value	F-value	p-value	
Model	151.16	< 0.0001	232.03	< 0.0001	Significant
A-Acetic anhydride concentration	355.05	< 0.0001	613.23	< 0.0001	
B-Temperature	11.62	0.0191	36.08	0.0018	
C-Time	50.00	0.0009	95.26	0.0002	
AB	120.07	0.0001	213.94	< 0.0001	
AC	104.86	0.0002	183.14	< 0.0001	
BC	432.88	< 0.0001	529.64	< 0.0001	
A^2	277.38	< 0.0001	411.06	< 0.0001	
B^2	0.59	0.4781	3.76	0.1101	
C^2	2.63	0.1657	0.48	0.5185	
Lack of Fit	2.96	0.2626	2.31	0.3166	Not-significant
R^2	0.996		0.998		
Adj. R^2	0.997		0.993		
Pred. R^2	0.951		0.969		
CV	2.940		3.13		
Ad. pred.	42.321		51.373		
Mean	23.440		1.180		
SD	0.699		0.037		

The fitness and adequacy of the models were judged by the coefficient of R^2 and the significance of lack-of-fit. The R^2 is defined as the ratio of the explained variation to the total variation, a measure of the degree of fit [64]. The coefficient of determination (R^2) varied between 0 and 1.0. The closer to 1.0 the R^2 value, the better the model predictive [61]. The R^2 value of the acetyl content was 0.996, indicating that only 0.37% of the total variations could not be explained by the model. It implied that it was feasible to obtain the regression equation using the response surface method. In the case of degree of substitution, the value of R^2 was 0.9976, showing that only 0.24% of the total variations could not be explained by the model. It implies that it was feasible to obtain the regression equation using the response surface method. The adjusted R^2 is a corrected value for R^2 after the elimination of the unnecessary model terms. If there are many non-significant terms included in the model, the adjusted R^2 would be remarkably smaller than the R^2 [64]. The R^2 and adjusted R^2 values of the acetyl content were 0.9963 and 0.9897, respectively, demonstrating that the independent variables had high correlations with one another. In the case of degree of substitution, the values of R^2 and adjusted R^2 were 0.9976 and 0.9933, respectively, demonstrating that the independent variables had high correlations with one another. The difference between the values of adjusted R^2 and predicted R^2 were less than 0.2 [65]. The Predicted R^2 and Adjusted R^2 values of the acetyl content were 0.9507 and 0.9897, respectively; *i.e*, the difference is less than 0.20. In the case of degree of substitution, the value of Predicted R^2 and Adjusted R^2 were 0.9691 and 0.9933, respectively; i.e. the difference is less than 0.20. The adequate prediction compares the range of the predicted value at the design points to the average prediction error. Adequate prediction measures signal-to-noise ratio. A ratio greater than 4 is desirable [64]. The value of the adequate prediction for the acetyl content and the degree of substitution was 42.321 and 51.373, respectively. The coefficient of variation (CV) illustrates the ratio of the standard deviation of the estimate to the mean values of the observed dependent variables. The CV also represents the degree of reproducibility and repeatability of the models [64]. The CV value of the model could be considered reasonably reproducible (CV < 10%) [64]. The values of the CV for the acetyl content and the degree of substitution were 2.94 and 3.13, respectively, indicating that the experiments were more precise and reliable.

3.6.2. Response surface model equation

A model equation describes the mathematical link between the factors and the response. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as (+1) and the low levels are coded as (-1). The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. The final equations in terms of coded factors for acetyl content and degree of substitution are shown in Eqs. (8) and (9).

$$\begin{aligned}\text{Acetyl conten} = 20.41 + 4.60A - 0.83B - 1.77C - 3.78AB \\ - 3.53AC + 7.18BC + 5.98A^2 \\ + 0.2751B^2 - 0.5824C^2\end{aligned} \tag{8}$$

$$\begin{aligned}\text{DS} = 0.960 + 0.324A - 0.079B - 0.128C - 0.27AB \\ - 0.2505AC + 0.43BC + 0.39A^2 \\ + 0.04B^2 - 0.013C^2\end{aligned} \tag{9}$$

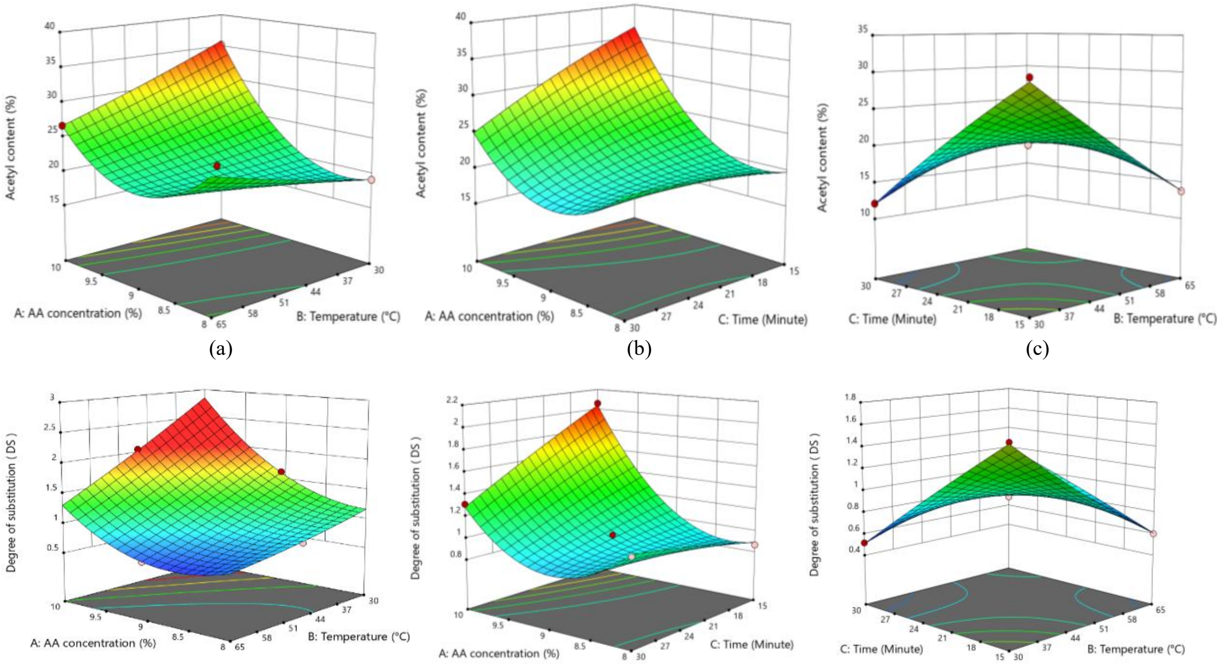
where, A is the acetic anhydride concentration (%), B is the reaction temperature (°C), and C is the reaction time (min) of acetylated purple taro tuber starch.

3.6.3. Interaction effects

The interaction between the process variables is graphically visualized by the 3D surface plots (Figure 7). The interaction effects of the variables can be effectively analysed by the shape of the surface or contour lines [67]. The interaction effect of acetic anhydride concentration (A) and reaction temperature (B) on the acetyl content is shown in Figure 7(a), and it was observed that acetyl content increased with a decrease in reaction temperature after it had decreased from a temperature of 65°C. However, when the acetic anhydride concentration increased from 8 to 10%, the acetyl content typically increased. Figure 7(b) shows the interaction effect of acetic anhydride concentration (A) and reaction time (C) on the acetyl content, which reveals that acetyl content increases with a decrease in reaction time. However, when the concentration of acetic anhydride increased from 8 to 10%, the acetyl content typically increased. Figure 7(c) represents the interaction effect between reaction temperature (B) and reaction time (C) on the acetyl content, which revealed that acetyl content increases (30.39%) with decreasing levels of both reaction time (15 min) and reaction temperature (30°C). Figure 7(d) depicts the interaction effect of acetic anhydride concentration (A) and reaction temperature (B) on the degree of substitution (DS), which revealed that the degree of substitution (DS) value increased with a decrease in reaction temperature after it had decreased from a temperature of 65°C. However, when the acetic anhydride concentration increases from 8 to 10%, the degree of substitution (DS) value typically increases. The interaction effect of acetic anhydride concentration (A) and reaction time (C) on the degree of substitution (DS) value is shown in Figure 7(e), and it was observed that the degree of substitution (DS) value increased with a decrease in reaction time. However, when the concentration of acetic anhydride increased from 8 to 10%, the degree of substitution (DS) value typically increased. Figure 7 (e) depicts the interaction effect of reaction temperature (B) and reaction time (C) on the degree of substitution (DS) value, which revealed that the degree of substitution (DS) value increased (1.63) with decreasing levels of both reaction time (15 min) and temperature (30°C).

3.6.4. Numerical optimization of purple taro starch acetylation process

Optimization of process parameters as a function of a desired degree of substitution (DS) and acetyl content (A) was performed with numerical optimization. The goal was to maximize the degree of substitution (DS) and acetyl content (A) of the APTS. From the software, to optimize the factors towards the response targets, the numerical optimization (desirability-based). Therefore, the predicted optimum responses (DS of 2.345 and acetyl content of 40.305%) and the corresponding levels of independent variables (acetic anhydride concentration 9.70%, reaction temperature of 30.95°C, reaction time 15.17 min) with desirability of 1.000 were obtained according to the set goals. Confirmatory experiments were successfully carried out in triplicate (n = 3) under optimal conditions to validate the best modification process conditions. The degree of substitution (DS) and acetyl content (A) in this confirmation experiment were found to be 2.94 ± 0.01 and 44.31 ± 0.08%, respectively. The experimental results are reasonably in agreement with the predicted results (percentage error < 5%) [65].



(a) (b) (c)

(d) (e) (f)

Figure 7. Response surface 3D plots effects for the acetylation of purple taro starch: (a) interaction effect of acetic anhydride concentration and reaction temperature on the acetyl content, (b) interaction effect of acetic anhydride concentration and reaction time on acetyl content, (c) interaction effect of reaction temperature and time on acetyl content, (d) interaction effect of acetic anhydride concentration and reaction temperature on DS, (e) interaction effect of acetic anhydride concentration and reaction time on DS, and (f) interaction effect of reaction temperature and time on DS.

4. Conclusions

In this study, the extraction and modification of starch from purple taro tuber have been conducted. Purple taro starch modification by the acetylation method was investigated and optimized using the Box-Behnken method. The study has shown that the experimental method can be used to identify the interaction effect of the individual parameters (acetic anhydride concentration, reaction temperature and reaction time) on the modification of taro tuber starch. The acetic anhydride concentration increased with an increase in both acetyl content (A) and degree of substitution (DS). However, both reaction temperature and reaction time were increased with a decrease in both acetyl content (A) and degree of substitution (DS). The acetic anhydride concentration of 9.70%, reaction temperature of 30.95°C, and the reaction time of 15.17 min were found as the optimum responses (DS = 2.35 and acetyl content = 40.31%). The physicochemical properties of native starch and acetylated purple taro starch were studied using different techniques. The SEM images show that native and acetylated purple taro starches were polygonal, semi–oval, and irregular in shape. x–ray diffraction patterns indicate that the native and acetylated purple taro starches are of B–type. The acetylated purple taro starch possesses a higher water hydration capacity when compared to native starch. The swelling power and solubility of the acetylated purple taro starch increased upon increasing the reaction temperature. The percentage of syneresis decreased with the number of cycles for the acetylated purple taro starch when compared to native starch. The acetylation reaction slightly improved the physicochemical properties of native purple taro starch. It can be concluded that acetylated purple taro starch can be used for industrial applications. However, further studies should be conducted on the utilization of acetylated purple taro starch for industrial applications.

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