

## Effect of acetylation treatment on the physicochemical and morphological properties of three sweet potato starches (*Ipomoea batatas*)

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**Abstract.** Kusumaningsih T, Firdaus M, Handayani DS, Juneasri FTI, Ananta FM. 2023. Effect of acetylation treatment on the physicochemical and morphological properties of three sweet potato starches (*Ipomoea batatas*). *Biodiversitas* 24: 3038-3044. Irrespective of their promising applications, native starches still present some limitations for food industry purposes as a result of their unsatisfactory physicochemical characteristics. Nevertheless, these shortcomings can be overcome with the proper modification efforts. In this study, three different starches of sweet potatoes (white, yellow, and purple) were isolated from *Ipomoea batatas* (L.) Lam. have been modified by employing an acetylation reaction using acetic anhydride at concentrations of 3%, 4%, 5%, 6%, and 7%. The change in the Degree of Substitution (DS) value along with the presence of carbonyl group peaks and a decrease in the intensity of the hydroxyl group peaks after acetylation confirmed the success of the starch modification. DS showed a maximum value at 7% acetic anhydride concentration in acetate starch of 0.0492, 0.0509, and 0.0718 for white, yellow, and purple acetate sweet potatoes, respectively. The DS value of starch acetates demonstrates results that are consistent with the Food and Drug Administration (FDA) regulations (less than 2.45%), indicating that modified starches have the potential to be used in the food industry. Surface morphology analysis using the Scanning Electron Microscopy (SEM) instrument also confirmed the success of the acetylation process, where the acetylated starch surface became rougher than the original starch surface. Several physicochemical properties, including amylose, amylopectin, swelling power, water solubility, and Water Binding Capacity (WBC), were determined and showed that starch acetate has favorable characteristics for further applications in the food industry.

**Keywords:** Acetylation, *Ipomoea batatas*, physicochemical, starch ester, sweet potato

### INTRODUCTION

With an annual production rate of around 6.01%, sweet potatoes are becoming one of the essential tropical tuber crops in Indonesia. White, yellow, and purple sweet potatoes are three of the different varieties, and each has a different starch content of 28.79%, 24.47%, and 22.64%, respectively (Ginting et al. 2014). Sweet potato is a tuber that is a high source of carbohydrates, apart from wheat, rice, corn, potatoes, and cassava (Rozi et al. 2021). Furthermore, sweet potatoes are also enriched with numerous nutrients. Generally, sweet potato starch is utilized as the primary ingredient in foods like noodles, bakeries, snacks, and candies (Saputro et al. 2019). Apart from the applications, native sweet potato starch has several drawbacks, including poor water solubility and poor resistance to extreme processing conditions, which restrict its potential applications. As a result, in order to overcome the shortcomings and tailor it to the specific application in the industry, various modifications have been made.

Native starch, the most ubiquitous renewable polymer, has a granular structure and each granule is made up of a combination of amylose and amylopectin. Starch

modification includes the process of altering the shape/form of granules and the molecular composition of amylose and amylopectin by manipulating hydrogen bonds in a controlled manner (Cornejo-Ramírez et al. 2018). In general, there are three types of modifications, i.e., physical, chemical, and enzymatic, that have been considered in order to advance starch properties.

The three-dimensional structure and morphology of starch can change as a result of physical modifications. Physical modifications are possible through grinding, changing humidity, temperature, pressure, pH, and radiation treatment. Wang et al. 2020, have investigated the effects of ultrasound and microwave exposure on the physicochemical and functional properties of chestnut starch. Regardless of whether there were single or multiple modifications, the results showed a decrease in swelling strength, viscosity, and adhesion temperature. Then, with the aid of different enzymes, starch can also be enzymatically modified by changing the molecular weight, branch chain length distribution, and amylose/amylopectin ratio. Furthermore, the stability of the freeze-thaw gel is also impacted by this modification, which also prevents retrogradation during storage. Many enzymes can be used for starch modification, such as

cyclodextrin glycosyltransferase,  $\alpha$ -amylase,  $\beta$ -amylase, branching enzyme (glucan transferase), and de-branching enzyme (Bangar et al. 2022).

Of the three methods, chemical modification has received special attention because it offers several special benefits, such as the non-destructive nature of the method used and the potential to enhance the functional value of the modified starch. Generally, this modification will be highly associated with the presence of novel functional groups in the native starch through several reactions, such as esterification, cross-linking, and etherification. In this case, the hydroxyl (OH) groups in starch will play an essential role. Esterification is an effective starch modification method that has been applied in the food, petrochemical, textile, and pharmaceutical industries. For example, in US and EU countries, several types of starch esters, such as acetylated distarch adipate (ADiSP), starch sodium octenyl succinate, starch acetate, hydroxypropyl starch, and starch phosphate, have been used for food applications (Tian et al. 2018). Kapelko-Żeberska et al. (2022) heated starch with citric acid at 40°C and obtained low-substituted esters. It was observed that there were changes in physical and chemical properties, including increasing the degree of esterification, swelling power, solubility in water, and decreasing the viscosity of the original starch. The esterification reaction on resistant starch obtained from corn, potatoes, and sweet potatoes was also carried out by Na et al. (2021) using 2 M L-malic acid (pH 1.5) for 12 hours at 130°C. The results showed that the starch treated with malic acid showed an increase in the content of resistant starch compared to the control. Through heating, this esterification reaction results in partial hydrolysis and rearrangement of the helical structure of the crystal area.

At the industrial level, among the various types of esterification, acetylation is widely used in starch modification as it is a relatively simple process that significantly improves the physicochemical properties of starch. This modification can be carried out by using an esterification reaction between starch and acetic anhydride at low temperatures. The three free OH groups on C2, C3, and C6 of the starch molecule can be substituted with an acetyl group during the acetylation reaction. Acetyl groups can stimulate the reduction of interactions between the amylopectin and amylose outer chains, resulting in novel properties for the polymer. Starch acetate can provide desired thickening, reduced gelatinization temperature,

increased viscosity, and freeze-thaw stability, as well as a reduced retrogradation rate (Olagunju et al. 2020). Previously, the acetylation process on sweet potato starch had been carried out by Das et al. (2010) using vinyl acetate and double modification using propylene oxide and adipic acid anhydride. However, they did not mention which sweet potato variety was modified. Therefore, inspired by previous reports and given the fact that there is no study on the acetylation of three starches isolated from white, yellow, and purple sweet potatoes, these starches were isolated and modified by the acetylation technique using acetic anhydride. This research will focus on the comparison between the physicochemical properties and morphological properties of native starch and acylated starch.

## MATERIALS AND METHODS

### Chemicals

Sweet potatoes were obtained from IELS Organic Foods Sleman, Yogyakarta, Indonesia, whereas acetic anhydride (96.5%), n-hexane,  $\text{CuSO}_4$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{H}_2\text{SO}_4$  (95–97%), NaOH,  $\text{H}_3\text{BO}_3$ , indicator Methyl Orange, HCl (37%), and  $\text{I}_2$  were obtained from Merck.

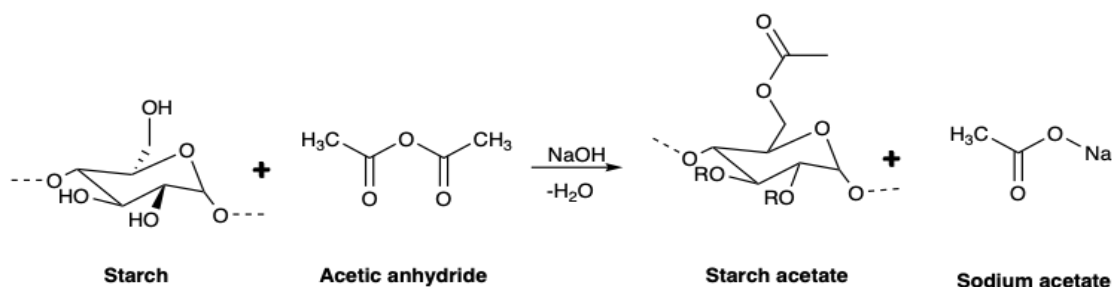
### Procedures

#### *Sweet potato starch isolation*

All starches were isolated according to the previously developed method (Kusumaningsih et al. 2022).

#### *Sweet potato starch esterification*

The general esterification procedure of sweet potato starch is illustrated in Figure 1A total of 375 mL of distilled water was used to dissolve 100 grams of sweet potato starch, which was then stirred for 60 minutes at 25°C. Afterward, 3.0% NaOH was added to the suspension in order to adjust the pH level to 8.0, and acetic anhydride with various concentrations (3, 4, 5, 6, and 7%) was added dropwise. After the acetic anhydride addition was completed, the reaction was allowed to continue for 10 minutes. The slurry was adjusted to pH 4.5 with 0.5 N HCl, washed with distilled water and ethanol, and dried in an oven at 40°C. Finally, the starch was ground, filtered after drying, and stored in an airtight container for further analysis.



**Figure 1.** Starch acetylation reaction with acetic anhydride (Masrukan 2020)

### Degree of Substitution (DS)

A total of 6.5 mL of distilled water and 1 gram of starch acetate were combined, and the mixture was then neutralized with a few drops of 0.1 M NaOH. Then, three drops of Phenolphthalein indicator (PP) were added to obtain a faint pink color. The aliquot was then added with 2.6 mL of 0.5 M NaOH while continuously stirring for 35 minutes. The solution mixture was titrated with 0.05 M HCl until the pink color disappeared. Unmodified natural starch was used as a blank. The calculation of DS can be determined using Equation 2 by first calculating the value of the Degree of Acetylation (DA) using Equation 1 (Olagunju et al. 2020).

$$DA (\%) = \frac{V(\text{blank}-\text{sample}) \times 0.043 \times N \times 100}{\alpha} \quad (1)$$

Where:  $V_{\text{blank}}$ ,  $V_{\text{sample}}$ , 0.043, N, and  $\alpha$  represent the volume of HCl required for blank titration (mL), the volume of HCl required for sample titration (mL), the molecular weight of an acetyl group, the normality of acid titer, and the weight of starch (g), respectively. According to Equation 1, the DS was calculated as follows (Equation 2):

$$DS = \frac{(162 \times DA)}{(4300 - DA)} \quad (2)$$

Where: 162 is the molecular weight of anhydrous glucose.

### Physicochemical properties

The physicochemical properties of starch acetate were determined by determining its amylose and amylopectin content (Siroha et al. 2019), swelling and solubility (Iheagwara 2013), Water Binding Capacity (WBC) (Olagunju et al. 2020), surface morphological analysis with the Scanning Electron Microscope (SEM-EDX JEOL JSM-6510LA) tool, and functional group analysis with the IR Prestige-21 spectrometer (Shimadzu) tool.

## RESULTS AND DISCUSSION

### Isolation and esterification of starch

To begin with, all sweet potato starches were isolated by soaking them in distilled water. This method was preferred because water is a low-cost, widely available solvent and does not affect the structure of starch (Kusumaningsih et al. 2022). The yield of starches was 79.04% for white, 83.88% for yellow, and 77.48% for purple. Afterward, the chemical modification was performed with the aid of NaOH as a catalyst and acetic anhydride as an acylating agent, as illustrated in Figure 1. In this reaction, OH groups are replaced by acetate groups via an addition-elimination mechanism. Owing to the different levels of reactivity, the primary OH at C6, which is located on the exterior surface, is more easily acetylated, while the secondary OH at C2 and C3 are more challenging to be acetylated due to steric hindrance (Hassan et al. 2020). Cuenca et al. (2020) have investigated this phenomenon using a Nuclear Magnetic Resonance (NMR) study on the acetylation of cassava starch, which revealed that C6 was more easily acetylated than other sites.

### Physicochemical properties of starch acetate

In this work, this modification was done by varying the concentration of acetic anhydride. The physicochemical properties of acetate starch, including DS (A), amylose content (B), amylopectin (C), swelling power (D), solubility (E), and WBC, are shown in Figure 2.

### Degree of Substitution (DS)

As can be seen in Figure 2A, the optimum condition was achieved at a concentration of 7% acetic anhydride. The higher the concentration of acetic anhydride added to the starch, the higher the DS value (0.07%). Das et al. (2010) acylated starch derived from sweet potatoes obtained from Sangrur, Punjab, India, using vinyl acetate ranging from 4% to 10%. This process was followed by dual modification using propylene oxide (7%) and adipic acid anhydride ranging from 0.05% to 0.12%. However, the DS value only ranged between 0.018-0.058% and 0.020-0.034% for acetylated and dual-modified starch samples, respectively. Thus, the DS value in this study was higher than in the previous study by Das et al. (2010). Based on Figure 2, increasing the DS value of starch will result in improved starch emulsifying capacity, more stable emulsion stability, and improved starch digestibility. According to the Food and Drug Administration (FDA) regulation, the DS value of starch acetate for the food industry is < 2.45% (Olagunju et al. 2020). The results indicate that the resulting starch acetate complies with FDA regulations.

### Amylose and amylopectin content

Table 1 shows the amylose and amylopectin levels of native starch, while Figures 2B-C exhibit the levels of starch acetate. It is observed that yellow sweet potato starch has the highest amylose (28.17%) (a mostly linear polymer) content and the lowest amylopectin (71.83%) (a highly branched polymer) content. Meanwhile, starch acetate shows an increase in amylose content with an increase in DS, which is related to the depolymerization of branched amylopectin molecules with an increase in amylose content due to the acetylation reaction. For example, the amylose content of yellow sweet potato starch has increased from 28.17% in native starch to 31.00% in acetylated starch. This is because the addition of the acetyl group is thought to interfere with the functionality of amylose and amylopectin. Since amylose digests more slowly than glucose, the higher amylose content found in the acetylated starch suggests that modified starch may be able to contribute to beneficial effects on human health (reduction of glycemic and insulin impact) (Das et al. 2010; Olagunju et al. 2020).

### Swelling and solubility

The swelling power and solubility of native and acylated starches are displayed in Table 1 and Figures 2D-E, respectively. When acetylation was performed on native starches, the swelling power was significantly increased, possibly due to the presence of hydrophilic substituting groups that retained water (Shaari et al. 2021). However, with increasing acetic anhydride concentrations, the swelling power decreased. This is due to the presence of hydrophobic acetyl groups in relatively large quantities, so

it is difficult for water to be absorbed into the starch granules. Afterward, the amount of amylose in the starch contributes to its solubility. Table 1 and Figure 2E depicts the solubility of native and acylated starches, respectively. Overall, the results showed that as DS increased, solubility decreased. The decrease in starch solubility is due to less amylose leaching, which can be attributed to stronger interactions between amylose and amylopectin molecules, which prevent amylose leaching from granules. The introduction of acetyl groups, primarily in C6, increased the molecular weight of starch, which may make it challenging to leach amylose from the starch granule. In the food industry, most of the industrial applications involve highly substituted starch acetate ( $DS > 2.0$ ), which is hydrophobic in nature. In general, swelling and solubility measurements have been used to describe the molecular arrangement of starch granules. Differences in swelling and solubility between the original sweet potato starch and starch acetate samples indicate that they have distinct functional properties and can be used in a variety of food-related applications (Lee and Lee 2017).

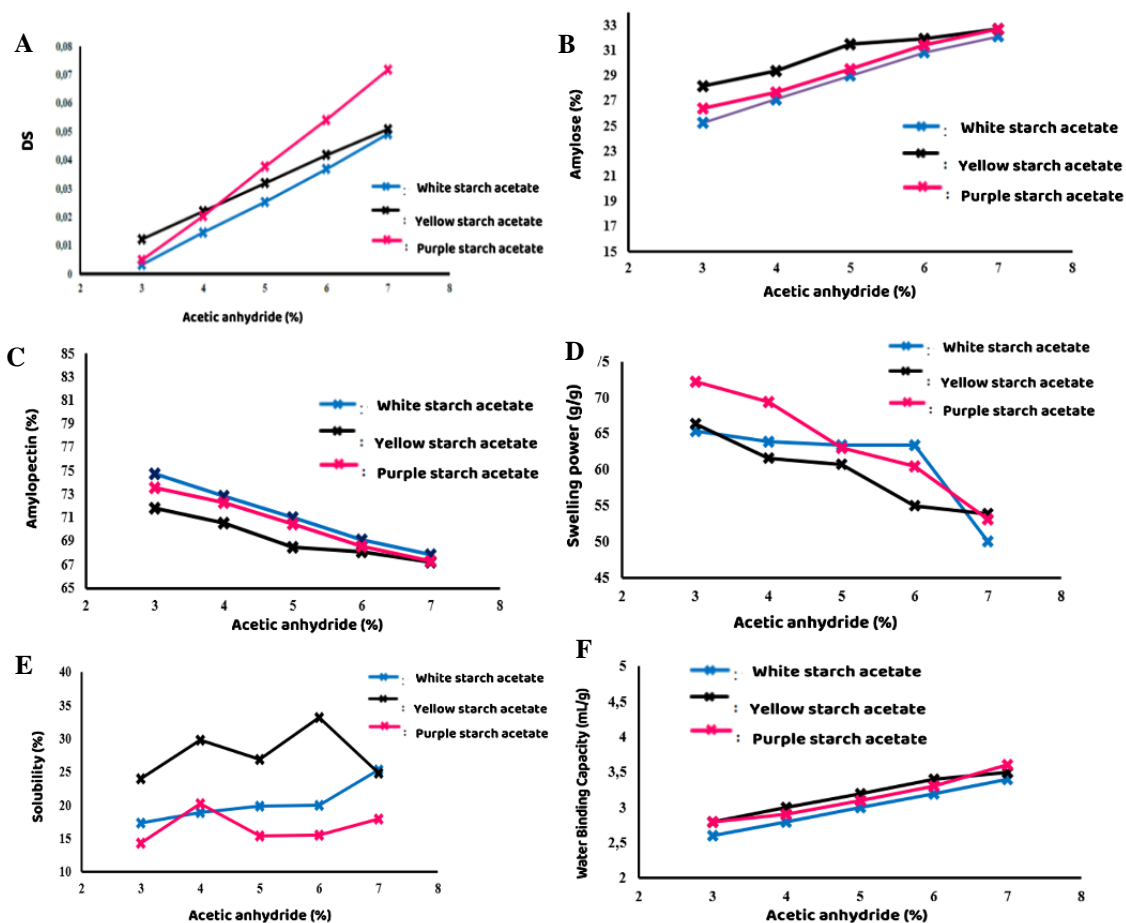
#### Water binding capacity

In food processing applications, WBC is a crucial parameter. Low WBC values are ineffective at retaining water, whereas high WBC values can cause food products

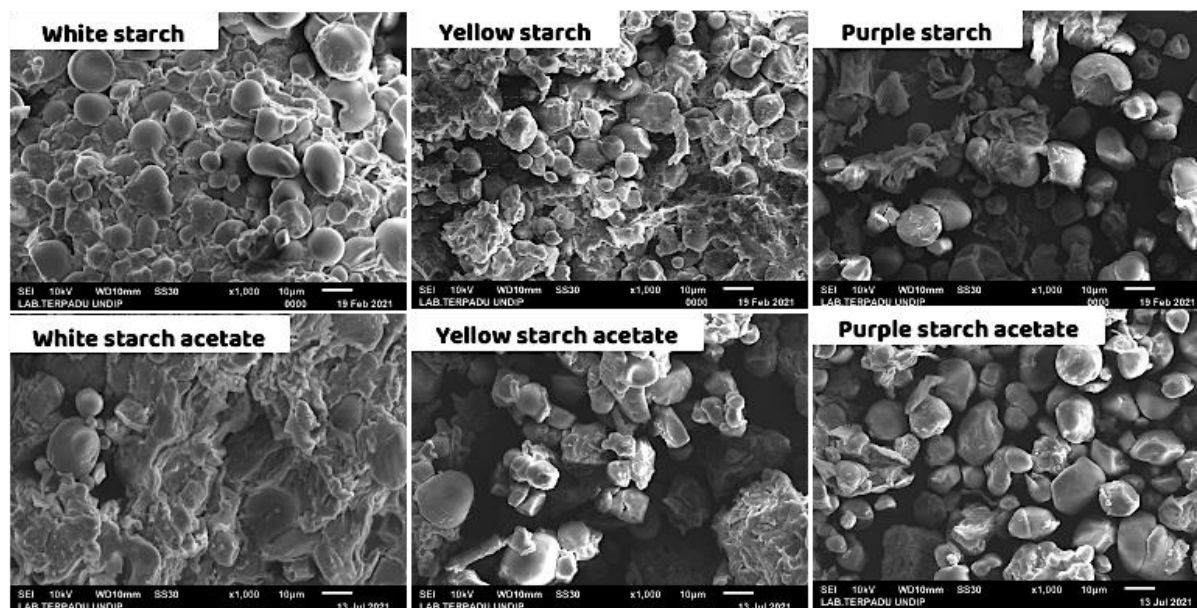
to become brittle and dry during storage (Ferawati et al. 2021). Amylopectin is generally responsible for the WBC of starch, so structural changes in amylopectin during the modification process will affect the final WBC value. Table 1 and Figure 2F depict the WBC of native and acylated starches, respectively. Modification of acetylation can increase the WBC value of native starch, where this value increases along with the increase in DS value. This is caused by the presence of hydrophilic groups, which can form hydrogen bonds and retain water molecules.

#### Surface morphology

According to Figure 3, there are no significant differences in granule morphology between natural starch and modified starch (at 7% acetic anhydride concentration). The SEM micrographs show that the surface of the native starches is still smooth, with no signs of cracks. This result was in accordance with previous works (Babu et al. 2015; Kim et al. 2020). On the other hand, modification results in the formation of concavities, which cause the starch surface to become slightly rough and deformed. Tupa et al. (2013) mentioned that the surface of acetylated starch granules was rougher than that of native starch. Structural changes in modified starch granules are clearly visible with high DS values. Some starch granules are more likely to rupture as DS increases and weakens intermolecular hydrogen bonds.



**Figure 2.** A. DS, B. Amylose content, C. Amylopectin, D. Swelling power, E. Solubility, and F. WBC of starch acetates



**Figure 3.** Surface morphology of native and modified starches (at 7% acetic anhydride concentration) by SEM with 1000x magnification

**Table 1.** Amylose, amylopectin, swelling power, and solubility of white, yellow, and purple sweet potato starches

Parameters	Sweet potato starch		
	White	Yellow	Purple
Amylose (%)	24.63	28.17	26.44
Amylopectin (%)	75.38	71.83	73.56
Swelling power (g/g)	7.06	8.60	7.46
Solubility (%)	1.50	2.00	2.70
WBC (mL/g)	1.60	2.00	1.80

### Functional group analysis

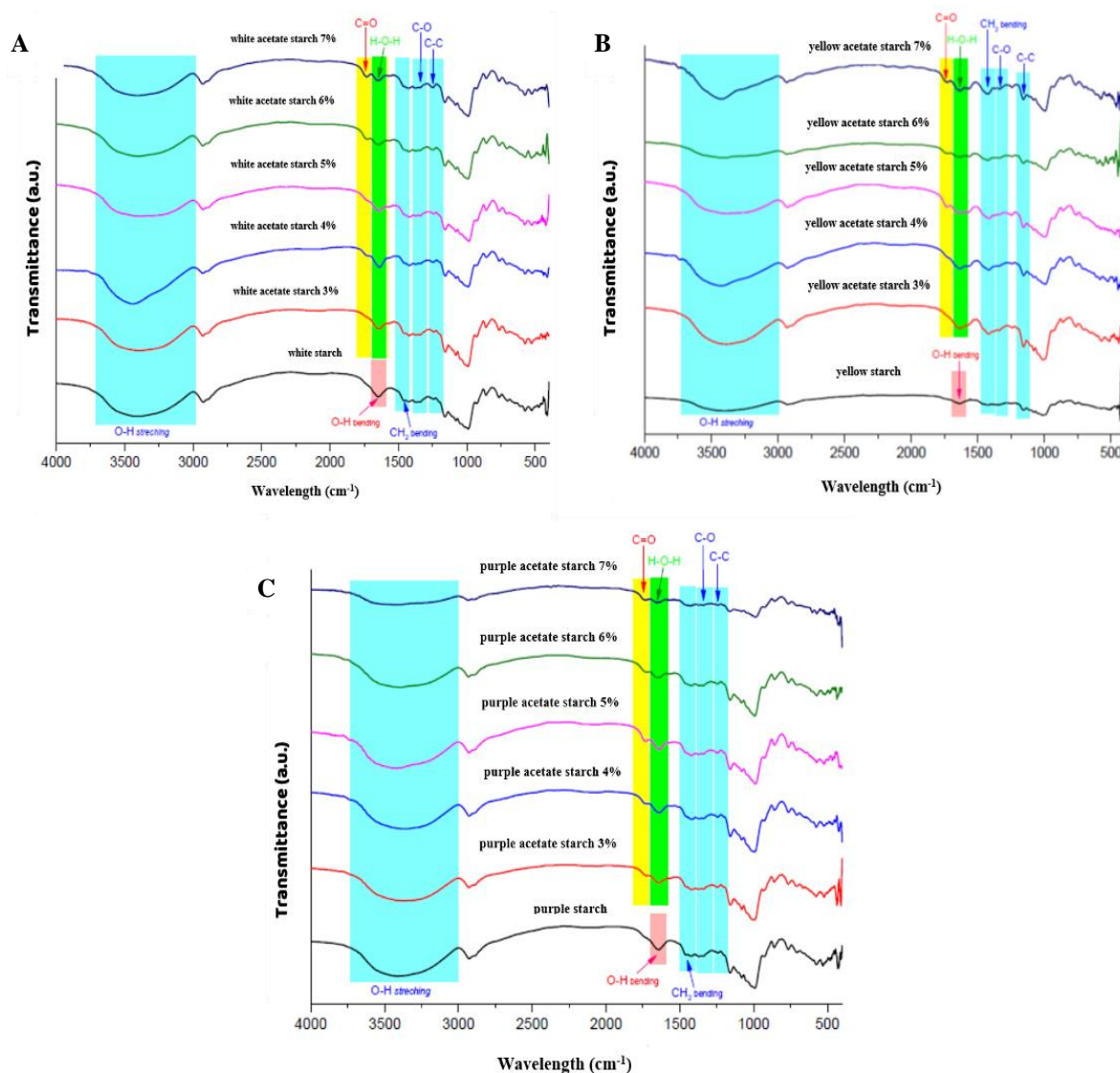
The FTIR spectra of all starches are presented in Figure 4. For all native starch spectra, an extremely broad band appears at 3100-3400  $\text{cm}^{-1}$  indicating the presence of -OH groups with hydrogen bonds. They also exhibit peaks of O-H bending at 1660  $\text{cm}^{-1}$  and C-O stretching at 1260-920  $\text{cm}^{-1}$ . Moreover, several characteristic absorption peaks that appear at 1160, 1085, and 1020  $\text{cm}^{-1}$  are associated with the stretching of the C-O band. Meanwhile, the FTIR spectra of starch acetate show a new absorption peak of C=O bond vibration at around 1750  $\text{cm}^{-1}$ , indicating that acylated starch has been successfully formed through the esterification reaction. Furthermore, the relative intensity of this band increased proportionally as the concentration of acetic anhydride increased. In contrast, a noticeable decrease in peak intensity at 3100-3400  $\text{cm}^{-1}$  after modification suggests that -OH groups participated in the reaction. When acetylation was carried out, the starch samples were washed several times before drying in order to remove residual reagents and by-products. The absence of peaks in the 1760-1850  $\text{cm}^{-1}$  area indicates that there is no remaining unreacted acetic anhydrous (Olagunju et al. 2020).

### Future prospect

Due to their continuous availability and affordability, sweet potatoes can be used as a potential starting material

for starch production. Although native starch shows some drawbacks for application in the food industry due to its poor physicochemical properties, chemical starch modification such as acetylation is possible to overcome these drawbacks. The outcome of the experiment demonstrates the composition and physicochemical properties of white, yellow, and purple starches isolated from *Ipomoea batatas* (L.) Lam. are considerably impacted by chemical modification. According to the FDA regulations, the DS value of the modified starches is allowed for further application for edible purposes ( $\text{DS} < 2.45\%$ ) (Olagunju et al. 2020). In addition, the acetylation treatment also improved the swelling power and WBC of the starch, indicating that the modified starch has different functional properties and can be used in a variety of food-related applications. Therefore, it is expected that this endeavor can increase the use value of the three different starches derived from *I. batatas* starch is a crucial raw material used as a food additive in the food industry, for instance, helping to control the consistency, stability, and texture of soups and sauces, to prevent gel breakdown during processing, and to lengthen the shelf life of goods. These modified starches can be used in food applications to develop a wide range of products depending on the desired level of quality, which can be assessed with regard to applications. In general, these results are in line with previous studies, which state that the physicochemical properties of starch will increase after a chemical modification process, especially acetylation (Das et al. 2010; Olagunju et al. 2020; Ferawati et al. 2021; Shaari et al. 2021). Furthermore, when compared with the results of previous studies by Das et al. (2010), the results of this study have a major advantage in terms of reaction time efficiency. While this method only takes 60 minutes at room temperature, the acetylation method with the double modification method performed by Das et al. (2010) requires 24 hours of reaction time.





**Figure 4.** FTIR spectra of: A. White, B. Yellow, and C. Purple sweet potato starches, along with their modified starch acetates

In conclusion, starch acetates that meet FDA standards derived from three different types of sweet potatoes (white, yellow, and purple) with different DS have been successfully prepared. The successful chemical modification was supported by FTIR spectroscopy analysis and DS calculations. The optimal condition of acetylation treatment was achieved at a concentration of 7% acetic anhydride. Compared with the original starch, the physicochemical properties of starch acetate have changed considerably. Morphological analysis by SEM revealed that there were no significant differences between native and acylated starch granules. However, the surface of acetylated starch is slightly rougher than native starch. Acylated potato starch exhibits better functional properties, including better-swelling power, solubility, and WBC, which can be attributed to structural changes at the molecular level. Thus, this modification has succeeded in changing the initial properties of native starch, thereby expanding its application potential for further use in the food industry, such as a stabilizer, thickener, filler, etc.

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