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Effect of Cross-Linking and Pre-Gelatinization on Morphology and Crystallinity Characteristics of Unripe Banana Starch (*Musa balbisiana*)

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ABSTRACT

Starch is a complex carbohydrate with various applications in food and other industries. The properties of starch depend on its morphology and crystallinity, which different methods can modify. This study examined the effects of two types of modifications, crosslinking, and pre-gelatinization, on the morphology and crystallinity of unripe banana starch (Musa balbisiana var. Abu). Scanning electron microscopy (SEM) and X-ray diffraction (XRD) were used to analyze starch granules' shape, size, structure, and degree of order. This study revealed that cross-linking and pre-gelatinization altered the morphology and crystallinity of unripe banana starch in different ways. In morphological analysis, cross-linking slightly reduced the granule's size, while pre-gelatinization increased the surface roughness of starch granules. Pre-gelatinization also disrupted the granular structure and transformed the crystallinity pattern from A-type to C-type. Neither modification affected the crystallinity index of unripe banana starch. These results suggest that cross-linking and pre-gelatinization can be used to tailor the properties of unripe banana starch for specific applications.

INTRODUCTION

Starch, a complex carbohydrate consisting of two types of glucose polymers (amylose and amylopectin), is produced by most green plants for energy storage [1]. It is widely distributed in various foods, such as maize (75%), cassava (14%), wheat (7%), and potato (4%) [2], and has many applications in food, paper, pharmaceutical, and other industries [3,4]. One of the sources of starch is bananas, especially unripe ones, which are believed to contain a large amount of starch [5]. Banana starch has a high content of resistant starch. This indigestible carbohydrate acts as fiber and may have health benefits such as improving colon health, increasing satiety, and lowering blood sugar levels [6].

Starch modification can be performed by physical, chemical, or enzymatic methods to improve the functionality, stability, or digestibility of native starch. Some examples of modified starches are hydroxypropylated starch, oxidized starch, and acetylated starch [7]. Two common types of starch modification are gelatinization and cross-linking. Suri and Singh [8] stated that gelatinization is a type of physical modification that does not involve any chemical and can be obtained by physical treatment that involves heating and drying of starch to make it soluble in cold water, which can improve the viscosity,

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texture, and stability of the starch-based product [7,8]. Cross-linking, on the other hand, is a chemical modification that involves forming bonds between the glucose units of starch molecules, which can enhance the resistance of starch to heat, shear, and ad [9,10]. The properties of starch are also affected by its morphology and crystallinity.

Cordenunsi-Lysenko *et al.* [11] mentioned that starch morphology refers to the shape, size, and structure of starch granules or grains, which vary depending on the plant source and the environmental conditions. Starch morphology can be classified into four types: compound grains, bimodal simple grains, uniform, simple grains, and mixed configuration. Starch crystallinity is a measure of the degree of order or regularity of the glucose chains in starch granules [12], which can be determined by different techniques, such as X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). Starch crystallinity determination is essential as it influences the gelatinization, retrogradation, solubility, and enzymatic hydrolysis of starch. This study investigated the effects of pre-gelatinization and cross-linking, two types of starch modification, on the morphology and crystallinity of native starch. It was aimed to understand how these modifications alter the shape, size, structure, and degree of order of starch granules.

MATERIALS AND METHODS

Materials

The unripe banana sample (maturity index 2), *Musa balbisiana* var. Abu was purchased from local farmers in Kota Kemuning, Shah Alam, Selangor, Malaysia. The chemicals used in the analysis including sodium sulfate (Sigma-Aldrich, USA), sodium tripolyphosphate (Sigma-Aldrich, USA), sodium trimetaphosphate (Sigma-Aldrich, USA), sodium hydroxide (Systerm, Malaysia) and hydrochloric acid (Systerm, Malaysia) were purchased from Biotek Abadi Sdn. Bhd.

Production of banana flour

The method described by Falodun *et al.* [13] referred to banana flour production with minor modifications. After washing and peeling, a slicer was used to cut the banana into slices of 2-3mm thickness. Slices were frozen overnight at -40 ° C in a freezer (Sanyo, MDF-U5412, Japan). Then, they were freeze-dried at -50°C and 0.1m Pa using a freeze dryer (Martin Christ, Alpha 1-4 LD plus). The dried banana slices were then ground with a dry blender to produce flour and stored in sealed containers for further analysis.

Isolation of Banana Starch

Following a method from Agama-Acevedo *et al.* [14] with minor changes, unripe banana starch was isolated. Banana flour obtained from the previous method was mixed with 1% sodium sulfate solution (pH 4.5) at a 1:10 ratio and incubated at room temperature for 18 hours in an incubator shaker (Innova 40, New Jersey, USA). The mixture was then filtered through a muslin cloth and a 200-mesh nylon filter to separate the starch from the residue. The residue was rinsed several times during the filtration to increase the starch yield. The filtrate was centrifuged at 3000 rpm for 15 minutes, and the starch was collected. The starch was dried in an oven (Memmert, Germany) for 24 hours at 45 ° C. Lastly, it was ground with a pestle and mortar to obtain finely powdered starch that can pass through a 100-mesh screen and stored in glass jars. The starch yield was calculated using the following formula:

$$Starch\ yield\ (\%) = \frac{W1}{W2} \times 100$$

Where W1 is the weight of starch isolated from a known weight, and W2 is the weight of banana flour used.

Cross-linking of starch

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Following Park *et al.* [15], approximately 5 g of starch was dispersed in 75 ml of distilled water with sodium sulfate (10%, w/w starch basis) and STMP/STPP (12%, 99: 1 ratio). The dispersion was set to pH 9.52 with 0.1 N NaOH and heated at 50.6 ℃ for 2 hours at 200 rpm in a shaking water bath (Technohouse, Germany). After cooling, 0.1 N HCl was added to reach pH 6.0. Starch was centrifuged (Kubota 5420, Japan) at 3,600 rpm for 10 minutes and washed with distilled water (100 ml, three times). The sample was dried at 35 ° C for 24 hours, powdered with pestle and mortar, sieved through a 100 mesh screen, and stored in a glass jar.

Pregelatinization of Starch

This study followed the pre-gelatinization method of Waliszewski *et al.* [16]. The starch sample (6 g) was mixed with distilled water (20 ml) and heated on a hotplate at 50.1 ° C for 14.40 minutes with slow stirring. The starch was then dried in an oven (Memmert, Germany) at 40 ° C for 48 hours and collected. The dried starch was ground using a pestle and mortar, sieved through a 100-mesh screen, and stored in glass jars at room temperature.

Determination of starch morphology using scanning electron microscopy

A scanning electron microscopy instrument with model JSM-5410LV (JEOL, USA) with a large field detector was used to analyze the starch sample's granule surface, shape, and size. The sample was mounted on a copper stub with adhesive tape and gold-coated under vacuum. The acceleration voltage was 15 kV, and the vacuum mode was 0.7-0.8 torr. The images were captured at 2000 magnifications.

Determination of the degree of crystallinity using X-ray diffraction (XRD)

The starch sample was stored in a sealed container before analysis. XRD patterns were recorded on a Siemens D-500 diffractometer in reflection mode from 5 ° to 40° 2θ. Each sample was measured thrice. The degree of crystallinity was calculated as follows:

$$\text{Degree of crystallinity, \%} = \frac{integrated\ area\ of\ crystalline\ peak}{integrated\ area\ of\ graph} \text{ x } 100$$

Where the integrated areas were obtained after smoothing and fitting the original peak using the OriginLab software.

RESULTS AND DISCUSSION

Morphological Properties of native and modified starches

SEM examined the granular structure of native and modified starch to elucidate the structural alterations induced by modification treatments. Yang et al. [17] reported that the genotype, cultivation, and amylose-amylopectin ratio affected the starch morphology. In contrast, the shape, size, and damage variation were related to the hydrolysis time, starch processing, and modification of chemicals. Figure 1 shows the SEM images of native, cross-linked, and pre-gelatinized starches at 2000x magnification.

Native starch showed a smooth, non-porous granule surface with an oval and elongated shape. This was consistent with Yang *et al.* [17], who found that native banana starch had smooth, irregularly shaped granules with various shapes and sizes, such as rod, oval, and spheroid. The smooth surface of the starch granules indicated that the processes involved in starch preparation did not damage the starch structure. The length of native starch granules ranged from 13.5 to 33.2 μm. Oktaviana and Saepudin [18] reported that tapioca starch had round and smooth granules with sizes between 4 to 35 μm.

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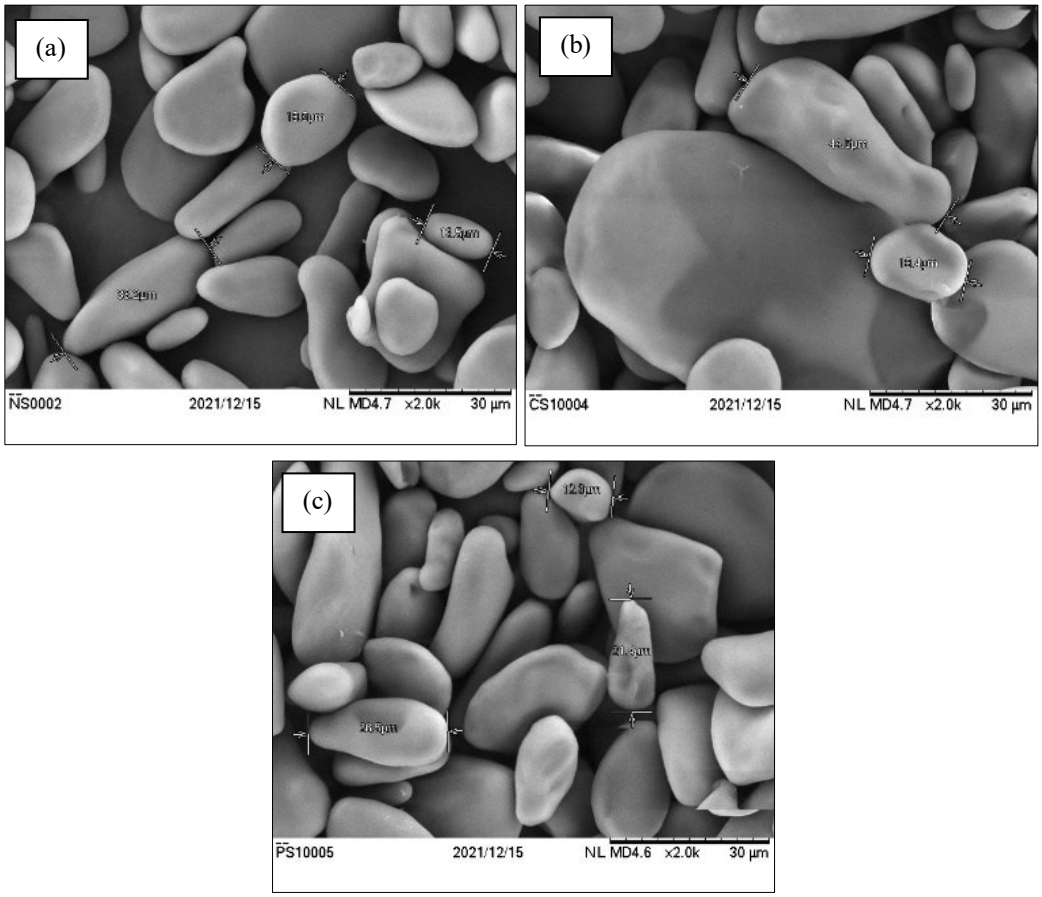

Fig.1. SEM images of native (a), cross-linked (b), and pre-gelatinized (c) starches.

Cross-linked starch exhibited slight changes in granular structure and minor granule damage, especially in the large granules. Black zones and large grooves on the granule surface were also observed, suggesting a rough surface. This result agreed with Korkut and Kahraman [19] and Rao and Parimalavalli [20], who observed that cross-linked tapioca and corn starch had rougher and more irregular granule surfaces than their native counterparts. Omojola *et al.* [21] also noted similar findings with minor granule aggregation and distortion of cross-linked cola starch. The black zones or grooves observed implied slight fragmentation [22], and the minor damages were due to a lower level of cross-linking introduced [23]. Moreover, the SEM images showed that cross-linked starch had more compacted and larger granules, ranging from 18.4 to 44.5 μm, compared to native starch.

Pre-gelatinized starch displayed more noticeable changes as deep grooves on the granule surface and more aggregated granules were detected, along with shrinking in granule size. This might be attributed to the disintegration and subsequent release of soluble constituents during the thermal process of pre-gelatinization [24]. The reduction of average length size range from 13.5 to 33.2 μm of native starch to 12.3 to 26.5 μm, with closely packed granules, was due to mild heat treatment, gelatinization, and rupture occurrence during the modification process, which was hypothesized to affect the granule size [25]. Furthermore, the treatment temperature disrupted the hydrogen bond between starch molecules and destroyed the amylopectin crystallinity, resulting in bond rearrangement within the granules, leading to

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granule integrity loss [22,24]. Wijanarka *et al.* [26] and Adewumi *et al.* [27] described similar effects of pre-gelatinization on gay flour and cassava starch, respectively.

Crystallinity properties of native and modified starches

The crystalline structure and characteristics of the starch granules were investigated by X-ray diffractometer. Chang *et al.* [28] reported that the crystalline structures of starch were classified into three types of XRD patterns: A, B, and C. The XRD patterns of native and modified starches are shown in Figure 2, while the crystallization percentages are given in Table 1.

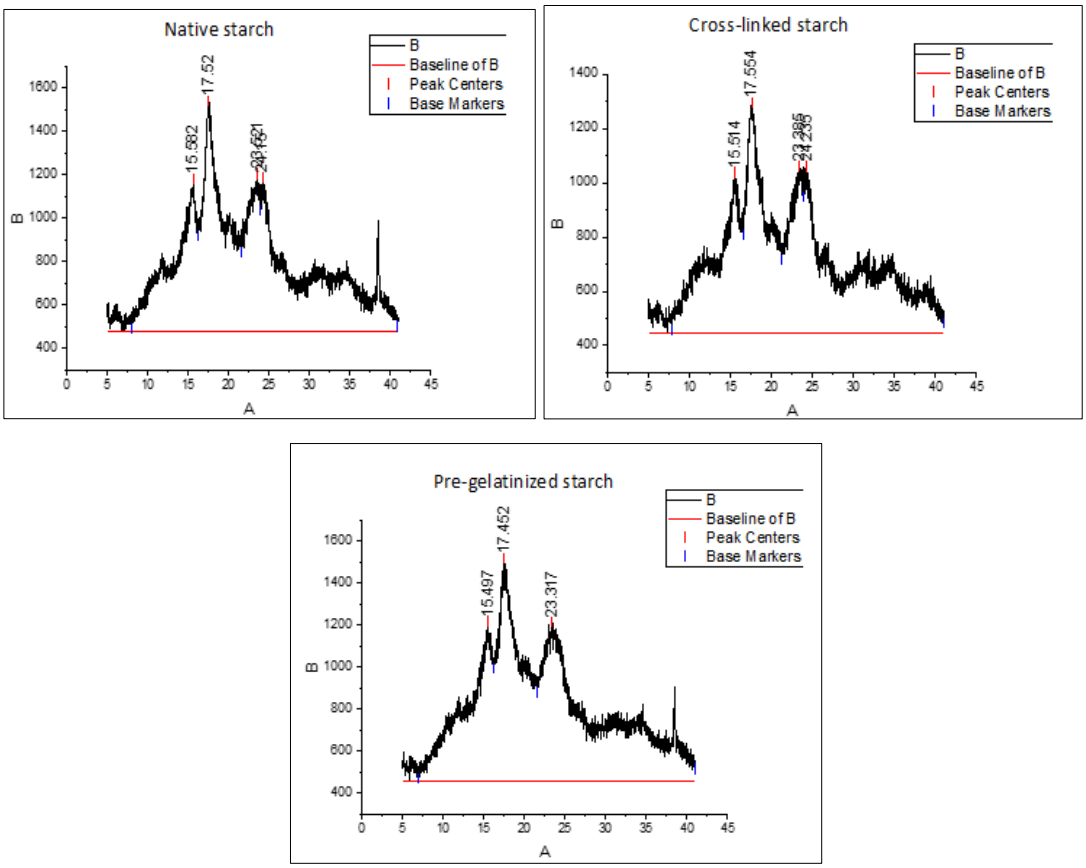

Fig. 2. XRD Spectra curves of native and modified starches

The XRD patterns of both native and cross-linked starch showed similar features, with solid diffraction peaks of 2θ at 15° and 17° and a small unresolved doublet at 23° and 24°. These peaks indicated the characteristic A-type crystallinity of the starch (corresponding to the double helices). This implied that the cross-linking process did not alter the native banana starch crystallinity, which was consistent with the findings of Korkut and Kahraman [19] for tapioca starch. Koo *et al.* [29] also found that the cross-linked corn starches maintained their XRD patterns when the ratio of STMP to STPP was less than 12%. This suggested that the cross-linking treatment only substituted the functional groups in the starch amylose and amylopectin chains, thereby enhancing the starch granule stability without creating new crystalline regions [30]. Therefore, it indicated that the cross-linking mainly occurred in the amorphous regions, as confirmed

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by Sriprablom *et al*. [6]. However, Marta *et al.* [31] reported that native and cross-linked banana flour exhibited B-type crystallinity.

On the other hand, pre-gelatinized starch displayed firm peaks of 2θ at similar angles of 15°, 17° and 23°, but without any double peaks. This revealed a C-type crystallinity of the starch, which agreed with the result of Remya *et al*. [32]. The lack of peak at 24° indicated that the gelatinization during the pre-gelatinization modification weakened the starch granules and slightly affected the starch crystal properties. Li *et al*. [33] observed a shift from B-type to C-type crystallinity of buckwheat starch after pre-gelatinization using a twin drum drier.

The crystal structure of banana starch was an essential factor that influenced its functional properties, and it depended on various factors such as the source variety, composition, growing condition or isolation technique [34,35], and the branching patterns of amylopectin in the starch granules [32]. Starches with short amylopectin branches (dp; degree of polymerization 23-29) showed A-type XRD patterns, while starches with long amylopectin branches (dp 30-44) showed B-type patterns, and starches with intermediate amylopectin branches (dp 26-20) showed C-type patterns [35]. Chen *et al*. [36] also stated that shorter branches (dp 6-12) might cause defects in the crystal structure as they could not form perfect double helices.

Table 1: Degree of crystallinity (%) of native and modified starches

Starch sample	Native	Cross-linked	Pre-gelatinized
Degree of crystallinity, (%)	$65.40^{a} \pm 5.93$	$65.29^{a} \pm 5.25$	$64.18^{a} \pm 2.01$

As the XRD patterns were almost identical, there was no significant difference in the degree of crystallinity between both modified starches and the native starch (Table 1). This was similar to the results reported by Shi *et al*. [37] for pea starch. The lower degree of crystallinity of the pre-gelatinized starch suggested its weaker crystal structure due to the thermal treatment during the modification process. The relative degree of crystallinity of starch also depended on factors such as amylose-amylopectin ratio, average chain length of amylopectin units, crystal size, and orientation of double helices to X-ray beam [36,38]. The area under the fitted curve in Figure 2 represented the amorphous content of the starch, and the total area under the peaks represented its crystalline content. The linear amylose chains contributed to the amorphous character, while the double helices of amylopectin formed crystalline structures [39]. Therefore, a higher proportion of amylopectin in the starch granules resulted in higher crystallinity.

CONCLUSION

To summarize, the modification process resulted in changes in the morphology and crystallinity of native starch. The morphological damage was more evident in pre-gelatinized starch than in cross-linked starch, as shown by the disrupted granular structure and the increased surface roughness. Pre-gelatinization also affected the crystallinity pattern of native starch, transforming it from A-type to C-type, which indicated a rearrangement of the amylose and amylopectin chains. However, neither modification significantly impacted the crystallinity index of native starch, as measured by X-ray diffraction.

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AUTHOR'S CONTRIBUTION

Nurul Nor Izzuani carried out the research project and wrote the draft of the article. Siti Roha designed the experimental framework and supervised each research progress. Anida reviewed and approved the revisions for article submission.

CONFLICT OF INTEREST STATEMENT

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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