Carboxymethyl Jackfruit Seed Starch: synthesis, characterization, and influence of reaction parameters

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Abstract. Carboxymethyl jackfruit seed starch (CMS) was synthesized under different reaction conditions. The influence of sodium hydroxide concentration, monochloroacetic acid (MCA) concentration, IPA-water ratio, solvent-starch ratio, reaction time, and temperature were evaluated for degree of substitution (DS). Results have shown that the optimal DS of 0.68 was obtained at 50 °C, 90 minutes in solvent-starch ratio and isopropanol-water were 8:1 and 10:0. The ratio of sodium hydroxide and monochloroacetate acid moles to anhydroglucose unit (AGU) moles for the optimal DS were 1 and 1. Scanning electron microscope (SEM) of CMS particles showed the starch grain structure remains the same but the surface appeared many alveolar holes and no longer smooth as MS. Fourier transform infrared spectra (FTIR) of CMS and MS confirmed that carboxymethylation takes place on native starch molecules when the absorption band appears at a wavenumber of 1643 cm⁻¹ corresponding to the vibrations of featured functional C=O group in CMS structure.

1. Introduction
Jackfruit (Artocarpus heterophyllus L.) consists of edible bulbs of white to yellow fleshy tissue and seeds, and is widely grown in tropical regions, including Vietnam [1]. The seeds account for 8–15% of the total weight of fruit and are rich in carbohydrate and protein content [2]. They also contain several minerals, saponins, lignans, phytonutrients and isoflavones [3]. As jackfruit seeds are composed of 63–80% carbohydrate, they can be used for the production of starch. Jackfruit seeds contain 70–85% total starch when dry [4]. Jackfruit seed starch (JFS) consists of 25 to 45% amylose content and 45 to 80% amylopectin content [5]. However, jackfruit seed is often discarded as agricultural wastes during processing of jackfruit products.
In our previous study, we attempted the valorization of this abundant agricultural waste by performing extraction of starch from the seeds collected at a local jackfruit growing area. However, the obtained starch was not modified, thus possessing limited physical properties and carrying little implications in industrial applications.

Scientists so far have found the way of processing the native starch to modify its structure and properties to improve its utilization and economic efficiency. Different methods of starch modification have been being studied such as acid hydrolysis, oxidation, acetylation, esterification, etc. Carboxymethylation of starch (CMS) consists of (1) activation of starch molecules and (2) etherification with monochloroacetic acid (MCA) in an alkaline environment (Fig. 1).

The properties of CMS is mainly determined by the degree of substitution (DS). CMS is mostly employed in pharmaceutical, textile, environmental, and food industries (2018). In the present study, we carried out carboxymethylation on starch extracted from jackfruit seeds under optimized conditions. The obtained carboxymethyl jackfruit starch was then analyzed for composition, morphology and structure by using Scanning Electronic Microscope and Fourier transform-infrared spectroscopy. These findings play an essential role in extending the applications of CMS at industrial scale.

![Figure 1. Carboxymethylation of starch (A) activation of starch molecules and (B) etherification with monochloroacetic acid (MCA) in an alkaline environment](image)

2. Methods and materials

2.1. Materials
A total amount of 5 kg of health Jackfruit seed was received from Tien Giang province (Vietnam), washed, dried and stored in refrigerator.

2.2. Starch extraction
Starch was extracted followed the procedure described by Luciano et al [6] with slight modifications. The white aril and brown skin of seeds were removed. Then, they were ground with water (1:5 of ratio) for 3 minutes. Next, the ground slurry was allowed to settle for about 16 h to remove protein in starch, and then removed the supernatant. The settled starch was collected and centrifuged (5000 rpm)
for 10 min. Afterwards, the starch was washed for two times with ethanol (1:4 ratio) and dried at 400 °C for 18 h and stored in a desiccators.

2.3. Carboxymethyl jackfruit starch preparation

The preparation of CMS was performed according to the method of Volkert et al. (2004) with some modifications [7]. MCA was dissolved in the appropriate volume of IPA and neutralised with aqueous sodium hydroxide. The mixture was stirred vigorously until became homogenous. Starch (10 g, dry weight) was mixed with NaOH and reaction occurred within the appropriate temperature and time period. After carboxymethylation has completed, the mixture was neutralized the pH to 7 using H₂SO₄ and NaOH solutions. Then, the slurry was filtered and washed 5 times with 85% ethanol for removing the chloride ion (tested with AgNO₃ solution) from the solution. Starch was collected and dried in the oven at 50 °C for 10 hours. The degree of substitution (DS) and the reaction efficiency (RE) of carboxymethyl jackfruit starch were calculated using the method of Spychaj et al. (2013) [8].

2.4. Analytical method

2.4.1. Compositional analysis. CMS (0.5 g) was measured for the total protein, fat (Soxhlet and Ash content) and moisture was determined by following the Kjeldahl system (P = N x 6.25), calcination at 600 °C and an Infrared Moisture Determination Blace (Ohaus MB45) at 104-105 °C for 2 minutes, respectively. Total carbohydrate content was measured by the formula below:

\[
\% \text{ Carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ protein} + \% \text{ fat}) \quad [9] \quad (\text{Eq. 1})
\]

2.4.2. Morphological and structural characteristics of starch. The samples were mounted on studs, sputter coated with gold (Balzers, JFC-1600) and was visualized by a JSM-6390LV Scanning Electronic Microscope (JEOL, Tokyo, Japan).

The FTIR absorption spectra of CMS was proceeded using the KBr disc technique with a Nicole 510 FT-IR in the range of 4000–400 cm⁻¹

2.4.3. Analysis of data. The result (mean ± standard deviation) were determined using the Microsoft Excel system (Microsoft Inc., Redmond, WA, USA) combining with one-way variance analysis (ANOVA) study in the Statgraphics system with 5% of significance standard.

![Diagram](Figure 2. Synthesis and analysis of carboxymethylated jackfruit seed starch)
3. Result and discussion

3.1. Approximate CMS composition

The table 1 demonstrated that approximate composition of starch and CMS samples calculated from Eq. 1. The jackfruit seed starch yield was 21.8±0.7% based on wet basic, which was relatively equal to by Tongdang (18.2%) [10]. There is no difference in approximate composition in starch and CMS.

| Parameters    | Jackfruit seed starch | CMS          |
|---------------|-----------------------|--------------|
| Moisture      | 8.99 ± 0.24           | 9.4 ± 0.17   |
| Ash           | 0.8 ± 0.11            | 0.08 ± 0.01  |
| Lipid         | 0.215 ± 0.02          | 0.19 ± 0.03  |
| Total protein | 3.79 ± 0.18           | 3.8 ± 0.18   |
| Carbohydrate  | 86.21 ± 0.26          | 86.23 ± 0.18 |

3.2. Effect of various IPA/water ratios

The effect of IPA/water ratio to the DS is presented in Figure 3. An attempt of polysaccharide modification applying starch content in a range 6:4 -10:0 (v/v) resulted in conclusion that 10:0 ratio was the most appropriate for DS (0.63), RE (63.23). An increase of water content resulted in more intensive by products formation and starch hydrolysis (CMS molar mass decrease). Water ratios (6:4, 7:3, 8:2, 9:1) caused granules agglomeration that hindered homogenous mixing during reaction and as a consequence DS of the final product decreased. The effect of water content was reported in a few works [11], [12], where similar trend was observed.

![Figure 3](image-url)  
**Figure 3.** Effect of different IPA/water ratios to CMS on the DS and RE

3.3. Effect of various IPA/starch

The effect of IPA/starch ratio to the DS is presented in Figure 4. The starch dissolves in an appropriate amount of solvent for better separation, diffusion, and adsorption of etherification agents [13]. The DS and the RE values were the highest at the IPA/starch ratio of 8:1 (v/w). After the critical ratio, the DS value was reduced when solvent content was higher increase. This was explained that when the solvent content was too small, the suspension was concentrated and interfered to the carboxymethylation process. Therefore, when the IPA/starch ratio was increased, the reaction was easier and increases DS and RE values. On the other hand, the higher the IPA volume to starch mass
The ratio (9:1, 10:1 v/w) was lower contact between the etherification agent and the starch molecules, making the carboxymethylation reaction unfavorable and resulting in a decrease of the DS and RE values.

![Figure 4](image)

**Figure 4.** Effect of various IPA/starch ratios to CMS on the DS and RE

### 3.4. Effect of various nMCA/nAGU ratios

The effect of nMCA/nAGU ratio to the DS is presented in Figure 5. When the starch is carboxymethylated, all carboxymethyl groups are provided by MCA. DS increased proportionally with MCA concentration, whereas RE increased only when the nMCA/nAGU ratio increased between 1 and 2. However, excessively high MCA concentration could cause sodium glycolate reaction which results in decreasing DS and RE. This finding is supported by previous literature [14], [15], yet contrast to the study on cocoyam starch carboxymethylation [16].

![Figure 5](image)

**Figure 5.** Effect of various nMCA/nAGU ratios to CMS on the DS and RE

### 3.5. Effect of various nNaOH/ nMCA ratios

The effect of nNaOH/ nMCA ratio to the DS is presented in Figure 6. Increasing NaOH/MCA ratio from 0.5 to 1 w/w increased DS and RE. NaOH forms alkaline environment for the reaction and acts as a swelling agent to facilitate diffusion of the etherifying agent into starch granules. Further increasing NaOH concentration de-activates sodium mono chloroacetate and hence its consumption in the side reaction. This result agreed with previous studies on carboxymethyl corn and amaranth starch [17].
3.6. Effect of reaction temperatures

The effect of temperature to the DS is presented in Figure 7. The effect of different temperatures (30, 40, 50, 60, 70°C) on DS and RE were investigated. Results have shown that increasing temperature promoted etherifying agents solubility and facilitated starch molecules swelling and reactants diffusion [18]. Increasing temperature enhanced the energy to a higher level than the activation energy, thus enhancing DS and RE. However, as gelatinization occurred at 60°C, it became difficult to remove the product. DS and RE were slightly declined at above 50°C.

3.7. Effect of reaction time

The effect of temperature to the DS is demonstrated in Figure 8. The DS and the RE increased proportionally with reaction time due to the enhanced contact between the etherifying reagent and starch molecules. Prolonged reaction time promotes starch swelling and thus improved reactants homogeneity. However, DS and RE significantly declined after 90 min of reaction.
3.8. Structural characteristics of starch

3.8.1. Scanning electron microscope (SEM). The results of the investigation are presented in Figure 9. Most of the starch particles have a free-flowing oval or round shape, separated particles. This proves that the extraction and drying methods did not result in starch destruction. Micrographs of mung bean starch obtained have a similar oval or round shape with the results of other authors [19], [20], [21]. The grain surface is cracked, many alveolar holes. It can be said that during carboxymethylation, starch granules are exposed to strong alkaline media resulting in deformed particle surfaces, causing granular disintegration. This proves that the carboxymethylation process only takes place on the granular surface without affecting the arrangement of starch structure. Similar research results were found for yam starch [13], cassava starch [22].

Figure 9: SEM of carboxymethyl jackfruit seed starch with various magnifications a) 1000, b) 5000

3.8.2. FTIR spectroscopy. The use of FTIR spectroscopy method was to confirm the effectiveness of the carboxymethylation process [23]. The FTIR spectra of CMS is presented in Figure 10. The CMS had the functional groups: hydroxyl group (−OH stretching) at 3400 cm⁻¹, methyl group (−CH₂ stretching vibrations) at 2934 cm⁻¹, carbonyl group (C=O stretching) at 1643 cm⁻¹, -CH₂ scissoring at 1400 cm⁻¹ and -OH bending vibration at 1392 cm⁻¹. Similar observations are reported for carboxymethyl starch which was created from yam starch [13], kudzu root starch [24], and rice starch [25].
4. Conclusion
The production and characterization of carboxymethyl derivatives of jackfruit seed starch are performed and optimized. Optimal DS of 0.68 was achieved with a reaction efficiency of 68.41% at 50°C after 90 min in isopropanol reaction medium when IPA/water was 10:0 (v/v) with the optimal molar ratio of 1:1 for both NaOH/AGU and MCA/AGU. FTIR results showed oscillation of the C=O group at 1710 cm\(^{-1}\) which proves that carboxymethylation has occurred. The choice of starch was due to the growing demand for new biomaterials in various industries. Furthermore, jackfruit seed is low-cost and widely distributed in the tropics.

5. References
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