EVALUATION OF REACTION CONDITIONS FOR CARBOXYMETHYLATION OF MUNG BEAN STARCH USING MONOCHLOROACETIC ACID

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ABSTRACT

Carboxymethyl mung bean starch (CMS) was synthesized under different reaction conditions. The influence of sodium hydroxide concentration, monochloroacetic acid (MCA) concentration, type of organic solvent, reaction time, and temperature were evaluated for degree of substitution (DS). Optimal DS of 0.59 was obtained at 50 °C, 120 minutes in isopropanol-starch ratio of 7.5 mL/g. The ratio of sodium hydroxide and monochloroacetate acid moles to anhydroglucose unit (AGU) moles for the optimal DS were 2.4 and 1.5. 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 1710 cm⁻¹ corresponding to the vibrations of featured functional C=O group in CMS structure.

Keywords: Carboxymethylation, carboxymethyl starch, monochloroacetic acid, mung bean, modified starch.

1. INTRODUCTION

Natural starch is used in many fields because it's biodegradable, renewable and low cost. However, due to some other unsatisfactory features such as low mechanical properties, poor solubility leads to limited use of natural starch. Therefore, modified starch is a good way to improve its functional properties [1, 2]. In particular, carboxymethylation is a modification method that has been studied in recent years [1, 3-5].

Carboxymethyl starch (CMS) is formed by the reaction between starch and monochloroacetic acid (MCA) in an alkaline environment at the right temperature and time. A starch carboxymethylation occurs in two steps. The first step is the hydroxyl groups of the starch molecules are activated and changed into the more reactive alkoxide form:



The second step is the etherification process:

$$\xrightarrow{ONa}_{OH} \xrightarrow{O}_{OH} + Cl \xrightarrow{O}_{OH} + NaOH \longrightarrow \xrightarrow{O}_{OH} \xrightarrow{O}_{OH} + NaCl + H_2O$$
(2)

The properties of the produced carboxymethyl starch is mainly determined by the degree of substitution (DS), which is the average number of carboxymethyl functions groups in the polymer. DS can be controlled by adjusting the reaction parameters during carboxymethylation as the ratio of sodium hydroxide and monochloroacetate acid moles to anhydroglucose unit (AGU) moles, type of organic solvent, temperature and reaction time. Various studies on the carboxymethylation of different starches were performed to optimize the reaction conditions to increase product yield and reaction efficiency [6-9].

CMS can be used as a stabilizer for vegetable products, soft drinks, and as a preservative for meat, vegetables, and fresh fruits. Kittipongpatana *et al.* studied using CMS as a coating for pills and a gel base in the pharmaceutical industry [4]. El-Sheikh studied using CMS to create a new photosynthesis process to stabilize silver nanoparticles. In this author's study, CMS is used as an environmentally friendly water-soluble polymer [5].

The raw materials from conventional plant sources are widely used for modification of starch such as corn, wheat, rice, potato and cassava [10]. However, the increase in the demand for starch in basic food products has a great impact on the provision of these natural resources. Current starch research tends to focus on finding new starch sources from non-conventional sources such as jackfruit seeds [11], mung bean [4, 12], yam [7, 13], amaranth [14], etc. This not only contributes to reducing the pressure on conventional sources but also creates new sources of raw materials, satisfying the increasing human demand for starch.

Mung bean (*Vigna radiata* L.) is a legume of Asian origin, now widely cultivated throughout Asia, Australia, New Zealand, and Africa. Mung bean is a very starchy seed in human nutrition because it contains high amounts of carbohydrates (62-63%) and proteins (24%). Starch is the major carbohydrate component (22-45%), presenting high levels of amylose which gives interesting properties for applications and uses. Mung bean also contains other ingredients such as fat, ash, fibre, vitamins, etc. [15-17]. Mung bean is mainly used in food to make mung bean vermicelli, mung bean cakes. There are no previous publications on synthesis and characterization of carboxymethyl mung bean starch in the literature. Therefore, we present in this study, the synthesis as well as the influences of reaction parameters on synthesis and characterization of carboxymethyl mung bean starch. We are convinced that the information presented in this paper will contribute significantly to the literature and will be useful for further research in this area.

2. MATERIALS AND METHODS

2.1. Materials

This investigation using mung bean seed is collected in An Hao district, An Giang province. Other chemicals known as monochloroacetic acid (MCA), sodium hydroxide, sodium bisulfite, hydrochloric acid, and all organic solvent were pure analyzed chemicals which were purchased from Bach Khoa Chemical Company, Vietnam.

2.2. Experimental

2.2.1. Isolated and recovery of mung bean starch

Mung bean starches are raw material to produce carboxymethyl starches. Starch is extracted according to Chang *et al.* (2006) method with some modifications [12]. Mung beans were soaked overnight in steeping liquor (water containing NaOH 0.1% and Na₂SO₃ 0.2%) at room temperature. After steeping, the swollen and softened beans were washed before being ground with water to destroy seed-cell structures and to release the free starch. The slurry was filtered through a steel sieve to remove filtered sediments. The obtained filtrate was settled for about 16 hours, the upper layer containing protein was removed. The starch was washed with water five times. Afterwards, the starch was rinsed with 85% ethanol and dried at 50 °C for 10 hours.

2.2.2. Preparation of carboxymethyl mung bean starch

The preparation of CMS was carried out by the method suggested by Volkert *et al.* (2004) with some modifications [3]. 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) and NaOH was added to the mixture and it was stirred. The reaction was performed within the appropriate temperature and time period. At the end of the carboxymethylation, the reaction mixture was neutralized the pH to 7 using H_2SO_4 and NaOH solutions. Then, the slurry was filtered and washed 5 times with 85% ethanol until the solution rinses off the chloride ion (tested with AgNO₃ solution). Starch product was dried in the oven at 50 °C for 10 hours. The degree of substitution (DS) of carboxymethyl mung bean starch was determined by the method of Spychaj *et al.* (2013) [8].

2.2.3. Morphological and structural characteristics of starch

Starch granule morphology was observed by scanning electron microscope (SEM) using equipment of FM-6510LV (JEOL-Japan). The starch samples were dehydrated by drying in an oven at 50 $^{\circ}$ C for 5 days and then observed under a scanning electron microscope.

Fourier transform infrared (FTIR) spectra of starch were recorded with a Nicolet Impact 410 FTIR spectrometer in the frequency range 4000 - 400 cm⁻¹ using potassium bromide (KBr) disks prepared from powdered samples mixed with dry KBr in a ratio of 1:30.

3. RESULTS AND DISCUSSION

3.1. Effect of various reaction time

The influence of carboxymethylation reaction time to the DS is presented in Fig.1. The results showed that the DS value of carboxymethyl starch gradually increased with increasing reaction time. This was explained that increasing time of reaction enhanced the contact and the diffusion capacity between MCA agent and starch molecules, hence carboxymethylation was enhanced [18]. In the present investigation, the increase in the DS value was not remarkable after 120 minutes. The DS at 120 minutes were 0.46, whereas prolonging the reaction for another 60 minutes only increased the DS and to 0.47. On the other hand, using the ANOVA analysis method (P = 0.05) showed that there was no statistically significant difference between the DS values when the reaction time was longer than 120 minutes. Therefore, it can be concluded that 120 minutes is the optimal carboxymethylation time.





Fig.1. Effect of various reaction time on the DS (Starch: 10 g; temperature: 40 °C; n_{MCA}/n_{AGU}: 1.5; IPA/starch: 8 mL/g, n_{NaOH}/n_{AGU}: 2.0; solvent: IPA)



^{a,b,c,...}: The statistically difference between DS values (P = 0.05)

3.2. Effect of various reaction temperatures

The influence of different temperature levels on DS is presented in Fig. 2. The results showed that the DS values reached a maximum at 50 °C when the carboxymethylation temperature was enhanced from 30 to 60 °C. The increase in temperature within the temperature range of 30-50 °C facilitated increasing the number of molecules with high activation energy, consequently, the rate of reaction increased and was favourable for high DS [7]. However, when carboxymethylation temperatures exceeded 50 °C, the DS value decreased. Bi *et al.* (2008) explained that mung bean starch was gelatinized and was destroyed the granular structure when the temperature rises above 50 °C [19]. In addition, the results also showed that the CMS product samples was gelatinized and became yellow when the temperature was higher than 50 °C. This result is similar to the previous studies on cocoyam starch [20], potato starch [21], kudzu root starch [22]. Based on these considerations, the optimum carboxymethylation temperature was selected at 50 °C.

3.3. Effect of various MCA/AGU molar ratios

The effect of various MCA/AGU molar ratios on DS is presented in Fig. 3. The results showed that when MCA/AGU molar ratios increased, the DS values increased and reached a maximum at MCA/AGU of 1.5. The increase in DS could be attributed to increased contact between the etherifying agent and the starch molecules as the concentration of MCA increased [18]. At the MCA/AGU molar ratio higher than 1.5, favour glycolate formation and as a result, decreasing the DS of carboxymethylation as already indicated. This finding is supported by reports in previous studies [19, 23].

3.4. Effect of various IPA/starch ratios

The effect of IPA/starch ratio to the DS is presented in Fig. 4. The starch dissolves in an appropriate amount of solvent for better separation, diffusion, and adsorption of etherification agents [13]. The DS value was the highest at the IPA/starch ratio of 7.5. 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 value. On the other hand, the higher the ratio of IPA volume to starch mass (> 7.5 mL/g) was, the lower contact between the etherification agent and the starch molecules, making the carboxylmethylation reaction unfavorable and resulting in a decrease of the DS value [22].



3.5. Effect of various NaOH/AGU molar ratios

The effect of various molar ratios of NaOH to starch on the DS is presented in Fig. 5. The carboxymethylation reaction is carried out in an alkaline environment because this is a favourable environment for etherification. According to the survey results, when nNaOH/nAGU ratio increases from 1.2 to 2.4, the DS increases, this proves that the alkaline environment acts as a swelling agent to facilitate the diffusion and penetration of etherification agents to the grain structure of starch [14, 24]. However, the DS value decreases gradually when nNaOH/nAGU is greater than 2.4. This is explained by the strong alkaline environment causing the starch to gelatinize and the contact between MCA and starch is inhibited in the reaction mixture. On the other hand, high NaOH concentration will increase the likelihood of sodium glycolate byproducts reducing the effectiveness of the reaction. This result is consistent with studies on pigeon pea starch [14] and potato starch [24, 25].





3.6. Effect of different solvents on the DS

The DS value of carboxymethyl starch depends on the reaction medium. In the carboxymethylation reaction, solvent provides the accessibility of etherifying agents to the reaction centre of starch rather than glycolate formation [6, 26]. Many organic solvents had studied for use as the reaction media for the starch carboxymethylation process [6, 20, 22, 27].

The effects of solvents isopropanol, ethanol, methanol, acetone, dimethylformamide on DS in this investigation are shown in Fig. 6. The optimal DS of reaction were obtained in isopropanol solvent when other parameters were kept constant. The DS follows the order: isopropanol > ethanol > methanol > acetone > dimethylformamide. The other works investigated the influence of various organic solvents on starch carboxymethylation reaction also concluded that isopropanol gave a rise to the highest DS such as the study on corn starch of Kamel *et al.* (2007) [6], potato starch of Tijsen *et al.* (2001) [28] and cassava starch of Jie *et al.* [29]. This means that isopropanol was the best choice for the carboxymethylation of starch.

3.7. Structural characteristics of starch

3.7.1. Scanning electron microscope (SEM)

Scanning electron microscopy was used to investigate the granule morphology of both the mung bean starch as well as the carboxymethyl mung bean starch. The results of the investigation are presented in Fig. 7. Most of the starch particles have a free-flowing oval or round shape, separated particles, relatively smooth grain surface without indication of erosion or indents (Fig. 7a, c). This proves that the method of extraction and drying does not cause starch destruction. Micrographs of mung bean starch obtained have a similar oval or round shape with the results of other authors [12, 30, 31].



a. Mung bean starch (×2000)



c. Mung bean starch (×5000)



b. Carboxymethyl mung bean starch (×2000)



d. Carboxymethyl mung bean starch (×5000)



After carboxymethylation, the starch granule is not significantly different from the raw mung bean starch granule, the starch grain structure remains the same but the grain surface is no longer smooth like natural starch, the grain surface is cracked, many alveolar holes (Fig. 7b, d). 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 carboxymethyl process only takes place on the surface of starch granules without affecting the arrangement of starch structure. Similar research results were found for yam starch [7], cassava starch [23].

3.7.2. Infrared spectra

The FTIR spectroscopy method was used to confirm the effectiveness of the carboxymethylation process [32]. The FTIR spectra of mung bean starch and carboxymethyl mung bean starch was shown in Fig. 8. The absorption band at the wavenumber of 3400 cm⁻¹ is typical for the stretching vibrations of –OH groups. The band at about 2935 cm⁻¹ is assigned to the -CH₂ symmetrical stretching oscillations, and the band at the 1085 cm⁻¹ peak and the 1159 cm⁻¹ peak is characteristic for the symmetric valence oscillation of the C-O-C bond. The band at about 1427 cm⁻¹ and 1371 cm⁻¹ is attributed to -CH₂ scissoring and -OH bending vibration. Besides, the carboxymethyl mung bean starch sample, an additional carboxyl group (C=O) is present at 1710 cm⁻¹ peak, indicating that carboxymethylation has occurred on the mung bean starch molecules. Similar observations are reported for carboxymethyl starch which was created from yam starch [8], kudzu root starch [22], and rice starch [33].



Fig. 8. FRIR of mung bean starch (MS) and carboxymethyl mung bean starch (CMS)

4. CONCLUSION

The carboxymethyl starch product was obtained from the reaction of mung bean starch and monochloroacetic acid in the presence of sodium hydroxide. The influences of the reaction time, reaction temperature, the molar ratio of NaOH/AGU, the molar ratio of MCA/AGU, the ratio of IPA/starch on the degree of substitution (DS) were studied. The highest value of the DS (0.59) was obtained when the carboxymethylation was performed at 50 °C for 120 minutes with the optimal molar ratio of NaOH/AGU and MCA/AGU is 2.4 and 1.5, respectively. The best organic solvent using for carboxymethylation process is IPA. Compared with mung bean starch, the surface structure of the carboxymethyl mung bean starch particle is no longer smooth, there are small cracks, many alveolar holes. The infrared results appear oscillation of the C=O group at a wavenumber of 1710 cm⁻¹ which proves that carboxymethylation has occurred. The results of this study will expand the range of applications of modified starches from non-traditional sources in many different sectors of the industries.

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TÓM TẮT

KHẢO SÁT CÁC YẾU TỐ ẢNH HƯỞNG ĐẾN QUÁ TRÌNH CARBOXYMETHYL HÓA TINH BỘT ĐẬU XANH

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Nghiên cứu này đánh giá các yếu tố ảnh hưởng đến quá trình carboxymethyl hóa tinh bột đậu xanh (MS) trong dung môi hữu cơ bằng tác nhân axit monocloaxetic (MCA) với sự tham gia của natri hydroxit. Các thông số khảo sát tối ru sau thực nghiệm bao gồm: thời gian phản ứng 120 phút; nhiệt độ 50 °C; tỷ lệ mol MCA/AGU (đơn vị glucose) là 1,5; tỷ lệ mol NaOH/AGU là 2,4; tỷ lệ isopropanol (IPA)/tinh bột là 7,5 mL/g và dung môi sử dụng là IPA. Sản phẩm tinh bột đậu xanh carboxymethyl (CMS) tạo thành trong điều kiện tối ru có độ thế (DS) là 0,59. Ảnh SEM của các hạt CMS xác định bằng kính hiển vi điện tử quét (SEM) cho thấy cấu trúc hạt tinh bột vẫn giữ nguyên nhưng bề mặt xuất hiện nhiều lỗ nhỏ và không còn mịn như hạt tinh bột đậu xanh tự nhiên. Phổ hồng ngoại biến đổi Fourier (FTIR) của CMS xuất hiện peak hấp thụ ở bước sóng 1710 cm⁻¹ tương ứng với dao động nhóm C=O đã chứng tỏ quá trình carboxymethyl hóa diễn ra trên các phân tử tinh bột đậu xanh.

Từ khóa: Axit monocloaxetic, carboxymethyl hóa, đậu xanh, tinh bột biến tính, tinh bột carboxymethyl.