Effect of heating temperature on citric acid-locust bean gum synthesis

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RESEARCH ARTICLE

Effect of heating temperature on citric acid-locust bean gum synthesis

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Abstract

Background: Citric acid-locust bean gum (CA-LBG) is a derivative of locust bean gum. Its synthesis has been carried out previously using UV light as an energy source. Heat is an alternative energy source that is simple and affordable. **Objective:** To determine the effect of temperature on the synthesis of CA-LBG. **Method:** The synthesis of CA-LBG was achieved through the esterification process of citric acid (CA), locust bean gum (LBG), hydrochloric acid (HCl), and water bath. The temperatures used were 20°C, 40°C, 60°C, and 80°C for 30 minutes. CA-LBG was synthesised chemically and physically. **Result:** At 20°C, the viscosity of the mucilago was high, so mucilago was difficult to precipitate. At 80, the viscosity of the mucilago was very low, which affected the precipitation and denaturation process. The temperatures reported for further synthesis were 40°C and 60°C. FTIR spectra showed carbonyl ester as a specific group at 40°C (1738.6 cm^{-1}) and 60°C (1735.1 cm^{-1}). Evaluation of the effect of 40°C and 60°C heating on the swelling index was 3.09% and 3.15%; pH was 4.82 and 4.88; and yield was 39.76% and 37.18%, respectively. **Conclusion:** The higher temperature increased the UV wavelength, Mp, pH, and swelling index and decreased the yield.

Introduction

Citric acid-locust bean gum (CA-LBG) is a derivative of locust bean gum (LBG) (Hadinugroho *et al.*, 2019). CA-LBG is a product of the esterification of citric acid (CA) with LBG. CA-LBG products have been applied to tablet formulations as disintegrating agents and negative matrices (Hadinugroho *et al.*, 2022; 2023). LBG is a natural polymer derived from galactomannans endosperm, a member of the Leguminosae family.

LBG contains mannose and galactose (4:1) as monomers (Dey *et al.*, 2013; Kaity *et al.*, 2013; Alves *et al.*, 2016). The advantages of LBG are that it is inert, safe, non-toxic, biocompatible, biodegradable, and available in nature. The C6 atom of the LBG monomers has the opportunity to bind to acidic compounds (Samavati *et al.*, 2007). CA is one of the weak acid compounds that have the potential to react with LBG. The O atom in the carbonyl group of CA has the potential to be protonated and form a positive C atom (Karadag *et al.*, 2001; Palit, 2009; Dey *et al.*, 2013). CA's carboxylic group replace the OH of the C6 atom of mannose and galactose.

Acid compounds such as hydrochloric acid (HCl) act as catalysts for polymer synthesis. HCl induces protonation of the O atom of the carbonyl group to form a positive C atom. HCl can accelerate the release of the OH group on the C6 atom of monomers (Colas, 2005; Bhattacharya *et al.*, 2008).

Esterification requires energy for the positive C atom to bond with the O atom in C6 monomers (Hadinugroho *et al.*, 2017; 2019). Previous studies on CA-LBG synthesis reported esterification using UV radiation as an energy source (Hadinugroho *et al.*, 2019). The choice of energy source influences the quality of the CA bond with LBG. The success of the synthesis gives new characteristics of CA-LBG, including glass transition temperature, endothermic temperature, degree of crystallinity, solubility, and viscosity (Hadinugroho *et al.*, 2019; Hadinugroho *et al.*, 2022). The problem identified in previous studies was that UV radiation requires a long time to synthesise CA-LBG

(Hadinugroho *et al*., 2019; Hadinugroho *et al*., 2022; Hadinugroho *et al*., 2023). The synthesis temperature affects the characteristics of the CA-LBG mucilago mass. Mucilago, with high viscosity, inhibits the extraction of CA-LBG during separation with distilled water (Hadinugroho *et al*., 2019; Hadinugroho *et al*., 2022; Hadinugroho *et al*., 2023). This research is expected to provide information regarding the effect of temperature on the synthesis of the CA-LBG mucilago mass. The novelty of this research is that temperature is used as an alternative energy for CA-LBG esterification so that the synthesis method becomes simple and affordable. The success of this method is expected to provide opportunities for industrial-scale production of CA-LBG and utilisation of CA-LBG in various fields.

Methods

CA-LBG synthesis

LBG (4.52×10^{-6} mol), was soaked in hot distilled water (100 ml) and left to swell (after which CA (4.76×10^{-3} mol) and HCl (1.20×10^{-2} mol) were added and stirred until homogeneity was achieved. The CA-LBG mucilago was heated in a water bath at the design temperatures (20°C, 40°C, 60°C, and 80°C) for 30 minutes. The CA-LBG mucilago was precipitated with acetone and washed repeatedly with acetone water (1: 1). The CA-LBG precipitate was dried at ambient temperature. Dry CA-LBG was pollinated and synthesised. This synthesis method adopts previous research (Hadinugroho *et al.*, 2019; Hadinugroho *et al*., 2022; Hadinugroho *et al.*, 2023).

Viscosity

CA-LBG mucilago was poured into a 200 ml beaker glass. The spindle was mounted and the speed was regulated on a Brookfield viscometer (Brookfield, Model LVDV-I Prime, AP6510416, USA). The spindle was inserted into the CA-LBG mucilago up to the limit mark and pushed the power button. The spindle rotated until a stable viscosity and torque <10% were obtained. The CA-LBG mucilago viscosity was measured on a Brookfield viscometer monitor.

Fourier transform infrared

A certain amount of CA-LBG powder was placed on the diamond plate (UATR 10.4.3., Perkin Elmer Spectrum, USA). The stick was turned on, then the infrared spectra appeared clear. Spectra were recorded at a wavelength of 400-4000 cm^{-1}. The spectra were analysed for the functional groups present.

UV spectrophotometer

The CA-LBG powder (50 mg) was dissolved with distilled water (10 mL) in a volumetric flask. The mixture was stirred for 60 minutes (Corning LSE Vortex Mixer, USA) and filtered. The filtrate was placed in a cuvette and the spectrophotometer holder (Hitachi U-1100, Japan). The spectrophotometer monitor read the UV spectra and the wavelengths (λ).

Melting temperature

The CA-LBG powder was filled into the capillary tube (±2 mm) and placed in the melting point holder. The temperature was monitored within the range of 140°C to 170°C (1°C per minute). The CA-LBG melting point (Mp) is displayed on a melting point equipment monitor (Optimelt MPA 100, USA).

pH

The remaining filtrate from UV spectrophotometer observations was used to test the pH. The pH meter had been calibrated at pH 4.0, 7.0, and 10.0. The electrodes of the pH meter were cleaned, dried, and immersed in the CA-LBG filtrate. The results of the examination can be seen on the pH meter's monitor.

Swelling index

CA-LBG powder (25 mg) was placed on filter paper, weighed (Mettler Toledo AL204, Switzerland) and placed in the funnel. Hot distilled water (50ml) was poured and stirred until it swelled. The filter paper with the swollen CA-LBG was weighed, and the swelling index, the difference between CA-LBG swelling with the initial powder multiplied by 100%, was determined (Gulrez *et al.*, 2011).

Yield

The synthesised CA-LBG powder was weighed carefully in an analytical balance (Mettler Toledo AL204, Switzerland). The ratio of the weight of the CA-LBG powder to the sum of the weights of CA and LBG multiplied by 100% is the yield.

Results

The esterification mechanism began with the protonation of the citric acid carboxylic group to create a C positive. This atom binds to the O atom on the C6 atom of the monomers of LBG. An O atom from the protonated OH ($^{+}OH_2$) produces loose OH and loses H_2O to produce ester (CA-LBG). The C=O ester group appears as a typical group of CA-LBG, which is not visible on LBG (Hadinugroho *et al*., 2017, Hadinugroho *et al*., 2019). Heating at a controlled temperature was needed as the bond energy of the C atom was positive with the C6 atom of the LBG monomers, so the esterification time was shorter. Details of the experimental conditions and mechanisms are shown in Table I and Figure 1.

Table I: Details of mucilago and powder character of CA-LBG

Condition code	Temperature [°C]	Heating time [minute]	Mucilago character: Viscosity [cP]	Mucilago character: pH	Powder character: C=O [cm-1]	Powder character: λ [nm]	Powder character: Mp [°C]	Powder character: pH	Powder character: Swelling index [%]	Powder character: Yield [%]
A	20	30	545.30 ± 5.48	0.90	-	-	-	-	-	-
B	40	30	228.86 ± 5.30	0.86	1738.6	203.50	125-135	4.82	20.37 ± 0.40	39.76 ± 0.09
C	60	30	17.48 ± 0.22	0.81	1735.1	204.00	135-155	4.88	20.88 ± 0.31	37.18 ± 0.16
D	80	30	2.52 ± 0.22	0.83	-	-	-	-	-	-

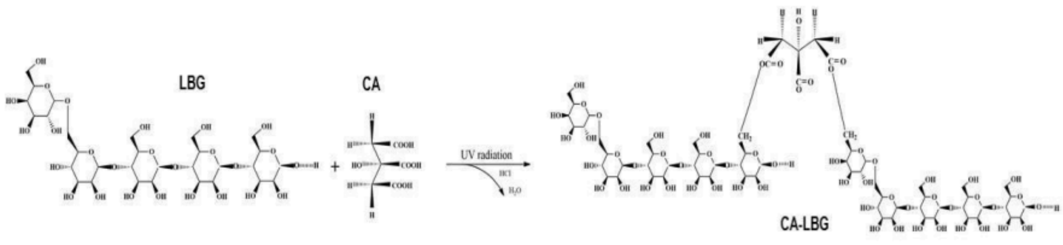

Figure 1: CA-LBG esterification mechanism

The viscosity of the CA-LBG mucilago before heating was 545.62 cP ±4.03. The CA-LBG viscosity with the influence of temperature 20-80°C showed a decreasing profile starting at 545.30 cP; 228.86 cP; 17.48 cP; to 2.52 cP. The pH of the mucilago at a synthesis temperature of 20°C-40°C showed a pH < 1.0, which confirmed the acidic state of the mucilago before heating. The pH of the CA-LBG synthesised at 40°C was 4.82; at 60°C, the pH value was 4.88. The CA-LBG pH values from both synthesis heating temperatures were weakly acidic. The profile of the relationship between the temperature, viscosity and pH is presented in Figure 2.

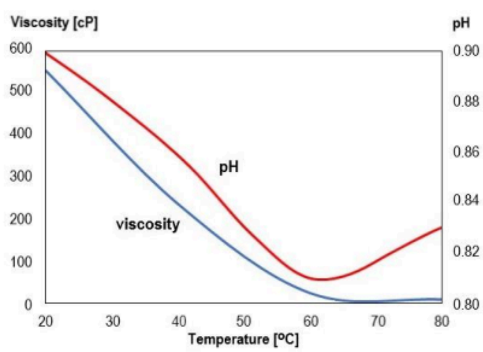

Figure 2: Profile of the effect of temperature on the viscosity and pH of the CA-LBG mucilago

The FTIR spectra at 3011.86 cm^{-1} and 3323.20 cm^{-1} show the O-H of carboxylic acids. Wavenumber at 2929.08 cm^{-1}; 2924.08 cm^{-1}; 2853.80 cm^{-1}; and 2857.20 cm^{-1} indicates C-H from CA-LBG. The CA-LBG-specific group, namely C=O ester, appeared at 1740.10 cm^{-1} and 1739.22 cm^{-1}. The CA-LBG infrared spectra are presented in Figure 3. The wavelength of CA-LBG from heating to a temperature of 40°C appears at 203.50 nm and a temperature of 60°C appears at 204.50 nm in the ultraviolet wavelength range.

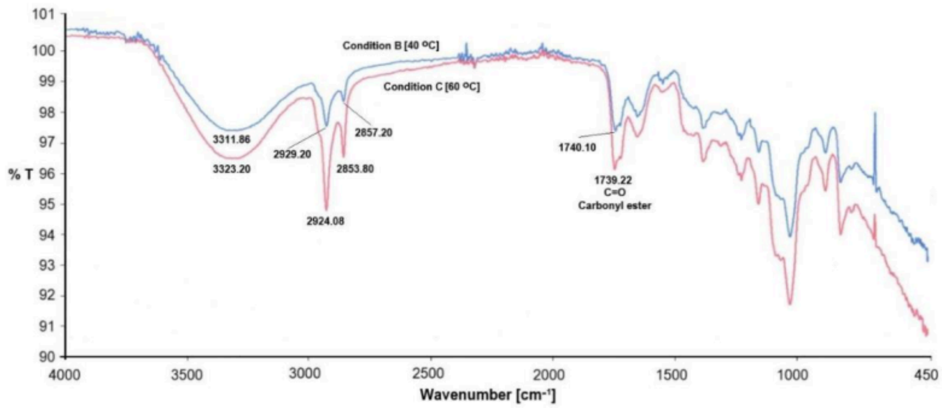

Figure 3: CA-LBG infrared spectra at 40°C and 60°C

The melting point of CA-LBG produced at 40°C was 125°C-135°C and 60°C was 135°C-155°C. The swelling index value of the CA-LBG synthesised at 40°C was 20.37, and at 60°C was 20.88. This value indicates the ability of CA-LBG to trap the swelling solvent. The yield value of CA-LBG synthesised at 40°C was 39.76 %, and at 60°C was 37.18. This value indicates the amount of CA that can bond strongly to LBG and is resistant to sedimentation and washing processes.

Discussion

This experiment showed that temperature has excellent potential as an energy source for CA esterification from LBG. The temperature reduced the initial mucilago viscosity from 545.62 cP (unheated) to 2.52 cP. The decrease in viscosity due to temperature is presented in Table I and Figure 2. Temperature decreases the bond strength between atoms in mannose and galactose so that the O atom on the C6 atom of the monomers of LBG has the potential to bind to the C positive in the carboxylic group of CA.

In this study, the energy produced at the low temperature (20°C) was not strong enough to reduce the bond strength between atoms in mannose and galactose, hence the decrease in mucilago viscosity was insignificant. The energy produced at 40°C and 60°C reduced the bond strength between the atoms in mannose and galactose to provide a greater chance of bonding between the O atoms and the C positive of CA. The presence of CA in LBG decreased the ability of mannose and galactose to trap the swelling solution. This ability showed the specific character of CA-LBG. (Hadinugroho, *et al.*, 2022; 2023). The energy at a high temperature (80°C) was powerful enough to reduce the bond strength between the atoms in mannose and galactose. Hence, the O atom had a very large opportunity to bind to the C positive of CA. This enormous energy may also break bonds between atoms in mannose, galactose, or CA (Hadinugroho *et al.*, 2019; Hadinugroho *et al.*, 2022; Hadinugroho *et al.*, 2023).

High temperatures for a long time may damage the bonds between the CA-LBG atoms that have been formed. The esterified mucilago's final viscosity determines the CA-LBG deposition's success (Hadinugroho, *et al.*, 2019; 2022; 2023). Mucilago, which was too viscous, was difficult to precipitate and tended to physically trap the CA from not reacting. This condition also makes it difficult for washing to be free of unreacted CA and HCl. Non-viscous mucilago is difficult to precipitate because the bonds between atoms are broken when it interacts with acetone and water. This condition is because the CA-LBG formed may not trap the swelling solution. After all, the bonds between the atoms have been broken.

The temperature of 60°C produces the lowest pH of the mucilago. Heating to a temperature of 60°C causes the solubility of CA to be higher, and there is stretching of the bond between the mannose and galactose atoms, making the dissolved CA more homogeneous. This condition will give a more acidic pH value. At 80°C, the pH of mucilago tends to increase because the bonds between atoms of CA are damaged due to the large amount of energy supplied.

Subsequent synthesis was only carried out on the mucilago, which produced CA-LBG. The homogeneity of CA-LBG was evaluated based on the pH of CA-LBG and the pH value of the washing solution during manufacturing. The CA used in the synthesis had a pH of 2.02, while LBG had a pH of 5.85. CA-LBG had a pH of 4.82 and 4.88, which fell between the pH values of the CA and LBG. In addition, repeated washing until pH 6.8-7.0 (pH acetone-distilled water) ensures that the CA-LBG precipitate is free of CA and that the LBG does not react. The infrared spectra showed the presence of the C=O ester group (1740.10 cm^{-1} and 1739.22 cm^{-1}) as a specific group that LBG does not have. The C=O ester group indicates successful esterification of CA-LBG.

In previous studies, the C=O group appeared at 1735 - 1743 cm^{-1}) (Hadinugroho *et al.*, 2019; Hadinugroho *et al.*, 2022; Hadinugroho *et al.*, 2023). On heating at 40°C and 60°C, the spectra were clear, smooth, and without damage. This shows that the two temperatures did not damage the bonds between atoms of CA-LBG. The UV wavelength of the spectrophotometer analysis of CA-LBG (203-204 nm) was lower than that of galactomannan (205 nm) (Matsuda *et al.*, 2016) and CA (210 nm) (Krukowski *et al.*, 2017). This wavelength shift indicates CA's presence in LBG, which gives it a new character. Increasing esterification temperature causes the wavelength to increase due to the increasing number of C mannose and galactose atoms that bind to the protonated C=O groups of CA.

The CA-LBG Mp (125°C-155°C) fell between LBG and CA because CA-LBG underwent decomposition. The higher the heating temperature, the faster the Mp due to the increasing number of O atoms of OH in mannose and galactose that bind to the C positive of the CA. This condition also applies to the CA-LBG pH parameter. The higher the heating temperature, the higher the pH.

The swelling index parameter showed that the strength of the atomic bonds in CA-LBG could withstand swelling solutions. The increase in esterification temperature causes the melting index to increase because the temperature at 60°C may form stronger bonds between atoms than at 40°C. At 60°C, itgave a lower yield than at 40°C. This is because less CA was tightly bound to LBG but with strong bonds between the O atoms of OH and the C positive of CA.

Conclusion

The temperature of 40°C-60°C is suitable for the esterification of CA with LBG. The higher the heating temperature, the lower the CA-LBG mucilago's final viscosity. The temperature of 60oC was the highest, decreasing the pH of the CA-LBG mucilago. The higher the esterification temperature, the higher the UV wavelength, Mp, pH, and swelling index and the decrease in the yield of CA-LBG synthesis.

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Conflict of Interest

The authors declare no conflict of interest.

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