Preliminary Study on the Synthesis of Phosphorylated Mung Bean Starch: The Effect of pH on the Physicochemical and Functional Properties

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ABSTRACT

*Mung bean (*Vigna radiate *L.) is a grain legume widely cultivated in tropical and sub-tropical regions. Mung bean seeds contain a significant amount of carbohydrate (63%-w/w) and are easily digested compared to seeds from other legumes. Mung bean starch has the potential to be used as thickener or gelling agents in food industries. Certain functional properties of mung bean starch, however, still need to be improved. In this research, a preliminary study was performed to upgrade mung bean starch properties using phosphorylation reaction. In particular, the effect of starch suspension pH (6–10) on the functional properties of the modified products was investigated. Phosphorylation was carried out at 130 °C, for 2 h using sodium tripolyphosphate (STPP) with an intake of 5%-w based on dry starch. The phosphorylated products were subsequently washed with water and dried. The experimental results show that the P-content of the phosphorylated mung bean starch is accessible in the range of 0.04–0.08%. The solubility (6.09–11.37%-w/w) and swelling power (9.88–11.17 g/g) of the modified starch products have been improved compared to native starch (solubility = 6.06 %-w/w, swelling power = 8.05 g/g). Phosphorylation also proved to increase peak viscosity, paste clarity, and water absorption/oil absorption capacity of the products.*

Keywords: *modified starch; mung bean; phosphorylation; starch*

ABSTRAK

*Kacang hijau (*Vigna radiate *L.) merupakan kacang-kacangan yang banyak dibudidayakan baik di daerah tropis maupun subtropis. Biji kacang hijau mengandung karbohidrat dalam jumlah yang cukup besar (63%-b/b) dan lebih mudah dicerna dibandingkan dengan biji kacang-kacangan yang lain. Pati kacang hijau memiliki potensi untuk digunakan sebagai agen pengental ataupun pembentuk gel dalam industri pangan. Namun, beberapa sifat fungsional dari pati kacang hijau harus lebih ditingkatkan. Pada penelitian ini, akan dilakukan studi awal untuk meningkatkan sifat fungsional pati kacang hijau menggunakan reaksi fosforilasi. Pengaruh pH suspensi pati (6–10) terhadap sifat fungsional produk termodifikasi akan diteliti lebih lanjut. Fosforilasi berlangsung pada suhu 130 °C selama 2 jam menggunakan sodium tripolifosfat (STPP) sebanyak 5%-b basis pati kering. Produk fosforilasi kemudian dicuci dengan air dan dikeringkan. Hasil penelitian menunjukkan bahwa kandungan P dalam pati kacang hijau termodifikasi berada pada rentang 0,04–0,08%. Kelarutan (6,09–11,37%-b/b) dan kekuatan mengembang (9,88–11,17 g/g) pada produk pati termodifikasi mengalami peningkatan dibandingkan dengan pati alaminya (kelarutan = 6,06%-b/b, kekuatan mengembang = 8,05 g/g). Fosforilasi juga terbukti dapat meningkatkan viskositas puncak, kejernihan pasta, dan daya serap air/minyak pada produk yang diperoleh.*

Kata Kunci: *modifikasi pati; kacang hijau; fosforilasi; pati*

INTRODUCTION

Starch is the most abundant reserve carbohydrate mainly found in seed, root, tuber, and fruit of plants [1]. Among the available sources, only maize, potato and cassava are currently utilized as major commercial starch sources. In fact, there are still a lot of potential starch resources that has not gained much attention such as mung bean (*Vigna radiate* L.). Mung bean, also known as green gram or golden gram, is mainly cultivated in South Vietnam [2]. Mung bean seeds contain 50–60% carbohydrate with starch as the major carbohydrate component and are easily digested compared to seeds from other legumes [3].

Starch contributes significantly to the textural properties of many foods and is widely used as gelling agent and thickener in food and industrial applications. However, starch granules are normally insoluble in cold water, and native starches also have a marked tendency to lose their viscosity and thickening power on cooling [4]. Phosphorylated starches are designed to overcome those shortcomings. Starch phosphates

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prepared by chemical methods give clear pastes of high consistency, good freeze-thaw stability, and high swelling power [5-8]. A lot of research papers have been published on the phosphorylation of various type of starch, such as corn starch, rice starch, wheat starch, and pinhao starch [9-12]. However, there is no study has been reported on the phosphorylation of mung bean starch.

The present preliminary study aims to investigate the potential use of phosphorylated mung bean starch for food thickener application. In particular, the effect of starch suspension pH on the Degree of Substitution (DS) of the products was evaluated. Relevant functional and physicochemical product properties, such as rheological properties, paste clarity, swelling power, solubility, and water/ oil absorption capacity were also determined and compared with the properties of native mung bean starch.

EXPERIMENTAL SECTION

Materials

Mung bean starch was purchased from PT. Hadian Global Gemilang, Bogor, Indonesia, and was used after a purification step performed according to the procedure described by Cho and Kim [13] with some modifications. Briefly, mung bean starch was mixed with 0.5 %b/v of NaOH solution at room temperature for 1 h (500 g starch/l solution). Then the suspension was filtrated and dried at 40 °C until constant weight. The phosphorylation was carried out using analytical grade of sodium tripolyphosphate/STPP (Sigma Aldrich) and sodium sulfate (Merck). Vanadate molybdate reagent, hydrochloric acid, and sodium hydroxide (analytical grade) were purchased from Merck.

Instrumentation

The Degree of Substitution (DS) of the products and paste clarity were measured using a UV-vis spectrophotometer (Thermo Scientific, GENESYS 20, United States). Pasting properties were recorded on a Rapid Visco Analyzer/RVA (Tecmaster Newport Scientific Pty. Ltd., Australia), with initial temperature of 50 °C, increased to 95 °C with a rate of 6 °C/min. The temperature of the sample was hold at 95 °C for 5 min before it was cooled back to 50 °C with a rate of 6 °C/min. Centrifuge (Hettich, EBA 21, United States) was used for starch phosphorylation, solubility, swelling power, water absorption and oil absorption capacity analysis.

Procedure

Synthesis of phosphorylated mung bean starch

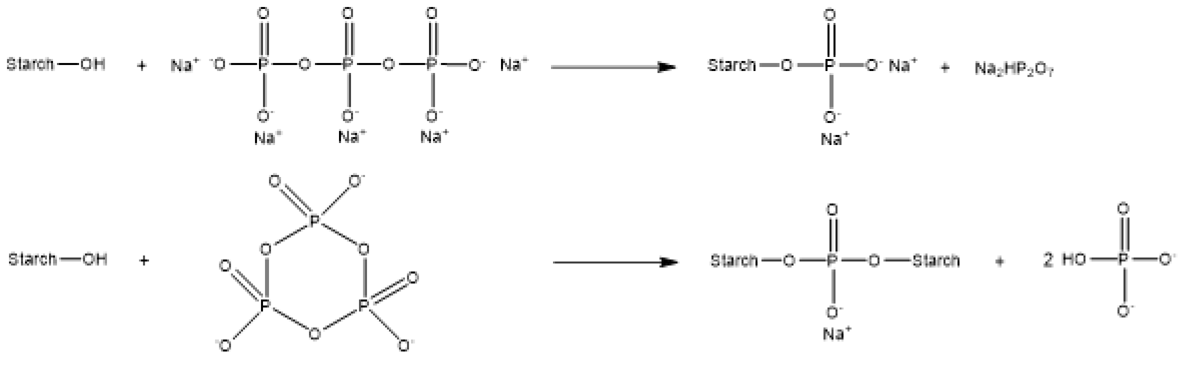
The mung bean starch was phosphorylated according to the method described by Lim and Seib [8] with a slight modification. STPP (0.5 g) was dissolved in 10 mL of water containing 0.5 g of sodium sulfate. The pH of the solution was adjusted between 6 and 10 by adding 5% aqueous solution of HCl or NaOH. Ten grams of mung bean starch (dry basis) was dispersed in the solution at a room temperature and the pH was readjusted with 5% aqueous solution of HCl or NaOH. The starch mixture was stirred for 1 h before it was dried in a tray dryer at 45 °C until the water content reached 10%-w/w. The dryer temperature was increased to 130 °C. and the solid was heated for 2 more hours. The products were subsequently cooled to room temperature, dispersed in 25 mL of RO water (pH of the mixture was adjusted to 6.5), and centrifuged (1500 rpm, 10 min). The washing steps were repeated two times, and the collected solid was finally dried at 45 °C until constant weight. Each experiment was replicated to ensure the reproducibility of the data.

Product characterization

DS of the products. The DS of the phosphorylated products was determined spectrophotometrically by the vanadomolybdophosphoric acid method [11]. A mixture of 0.12 g of Na_2CO_3 diluted in 2 mL of boiling water and 0.2 g of starch phosphate was dried at 100 °C and subsequently ashed in a muffle furnace (550 °C, 9 h). After the sample was cooled to 25 °C, it was hydrolyzed using 4.8 mL of 4%v/v aqueous solution of HCl. The solution was filtered and diluted with water up to 100 mL. The resulting filtrate (7.5 mL) was mixed with 2.5 mL of vanadate-molybdate reagent, and its transmittance was measured using a UV-vis spectrophotometer at 435 nm.

Paste clarity. The clarity of starch paste was determined according to the method described by Stahl et al. [9]. Starch suspension (1 g/100 mL RO water, pH = 6.5) was heated in a water bath at 95 °C for 30 min and subsequently cooled to 25 °C. Paste clarity is defined as the percentage of transmittance at 650 nm against a water blank in a UV/VIS spectrophotometer.

Solubility and swelling power. Swelling power and solubility were determined after heating a starch suspension (0.5 g/15 mL RO water) at 85 °C for 30 min as described by Li and Corke [12]. The suspension was subsequently centrifuged for 15 min and the separated starch paste was weighed and dried. Swelling power



Scheme 1

and solubility were calculated using equation 1 and 2, respectively [9].

$$\text{Swelling Power}=\frac{\text{weight of sediment paste (g)}}{\text{weight of initial sample (g, dry basis)}*\left(1-\frac{\text{Solubility}}{100}\right)} \quad (1)$$

$$\text{Solubility}=\frac{\text{weight of soluble starch (g)}}{\text{weight of initial sample (g, dry basis)}}\times 100\% \quad (2)$$

Water and oil absorption capacity. The water and oil absorption capacity were determined based on a published report [14] with a slight modification. Starch sample (1 g) was mixed with 10 mL of RO water or commercial palm cooking oil in a centrifuge tube and was kept at room temperature for 1 h. The sample was subsequently centrifuged (200 x gravitational acceleration, 30 min), and the water or oil volume in the sediment was measured. The absorption capacity was calculated as the mass of water or oil absorbed (gram) per gram of starch sample.

Rheology and pasting properties. Rheology and pasting properties of native and modified starches were examined in a *Rapid Visco Analyzer/* RVA. The starch sample (3.45 g) was suspended in a 25 mL of aquadest and heated to an initial temperature of 50 °C. The temperature was gradually increased (with a heating rate of 6 °C/min) to a temperature of 95 °C. The sample was hold at 95 °C for 5 min and gradually cooled to 50 °C (with a cooling rate of 6 °C/min).

RESULT AND DISCUSSION

Starch phosphorylation was conducted at 130 °C using an intake of 5%-w/w STPP based on dry starch. A schematic representation of the phosphorylation of starch with STPP is provided in Scheme 1. After subsequently isolated from the reaction mixture and dried, the modified starch product was obtained as white solids. Phosphorylation of starch will result in a product with more polar characteristic. The presence of phosphate groups significantly increases the hydrophilicity of starch molecules while at the same time reduces the inter- and intra-molecular hydrogen bond between hydroxyl groups of the anhydroglucose units.

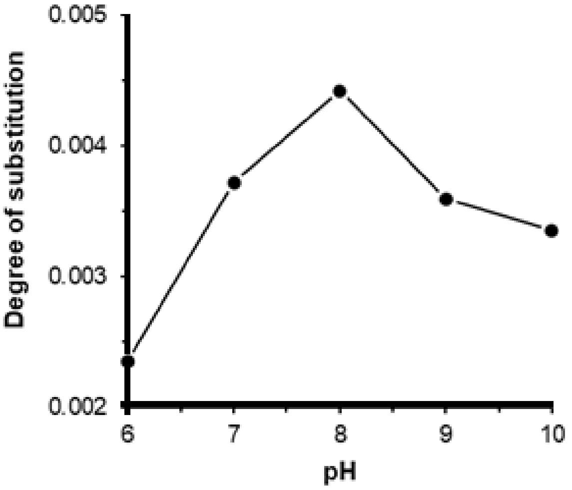


Fig 1. Degree of substitution (DS) of phosphorylated mung bean starch

Degree of Substitution (DS) of Phosphorylated Mung Bean Starch

The DS of phosphorylated products was determined spectrophotometrically, and the experimental results are shown in Fig. 1. Native mung bean starch contained 0.0129% P (dry starch basis). Phosphorylation of mung bean starch with 5% STPP at pH 6–10 (130 °C, 2 h) gave products with 0.045–0.084% P and the DS of the products was accessible in the range of 0.002–0.004. As the pH of the suspension was raised from 6 to 8, the P-content of the products was increased, but for pH > 8, the P-content decreased steadily. This trend was generally in agreement with the results obtained by Muhammad et al. [15] working with sago starch. In their research, the P-content of sago starch was increasing until its highest value at pH 9 (P-content = 0.224%) and decreasing at higher

Table 1. Physicochemical properties of phosphorylated starch products

Reaction pH	Paste Clarity (%T)	Swelling Power (g/g)	Solubility (%)	Water Absorption Capacity (g/g)	Oil Absorption Capacity (g/g)
Native	23.75	8.05	6.06	1.07	0.79
6	37.85	9.88	7.25	1.23	0.88
7	46.50	10.38	7.31	1.23	0.97
8	54.25	11.17	11.37	1.31	1.15
9	42.05	10.31	8.56	1.21	0.96
10	37.50	10.17	6.09	1.14	0.99

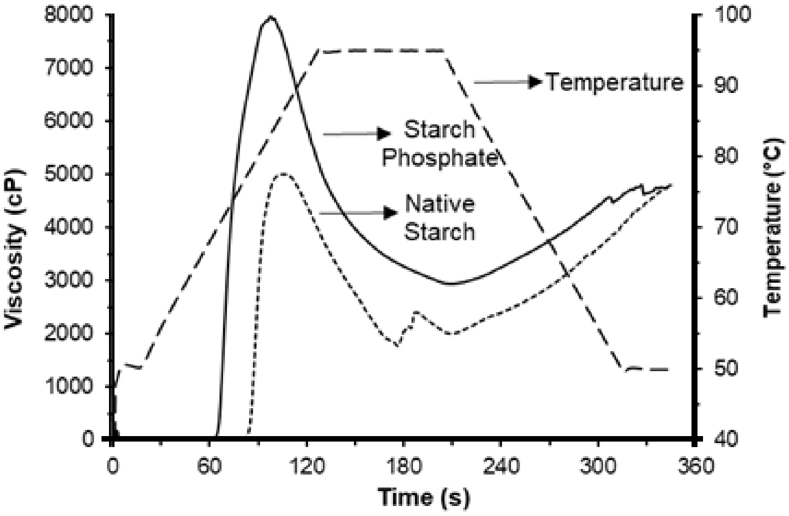


Fig 2. Viscosity profile of native and phosphorylated mung bean starch (P-content = 0.084%)

solution pH. All of the products obtained in this research contain P less than 0.084% (equivalent to the DS 0.004), which was still below the permitted level for food purposes according to Food Chemical Codex (0.4%) [16].

Product Characterization

The physicochemical and functional product properties for all phosphorylated products have been characterized. Initial phosphorylation suspension pH had a pronounced effect on the paste clarity of the resulting starch pastes (Table 1). Phosphorylation with STPP generally increased paste clarity of native mung bean starch. Lim and Seib [8] obtained a similar result and hypothesized that the higher paste clarity was caused by the increased level of hydrated molecules due to reduced interchain associations caused by repulsion between adjacent starch molecules in the presence of negatively charged phosphate groups. When suspension pH was increased from 6 to 8, the clarity of starch paste was increased gradually, before decreasing pH of 8–10. As cross-linking of starch has been reported to reduce paste clarity [5], it was hypothesized that the level of cross-linking with STPP increased significantly at pH above 8.

The swelling power and solubility of the native and phosphorylated mung bean starch products were determined by mixing and heating starch/ water suspension to 85 °C. The mixture was then centrifuged, and mass of the swollen solid as well as the soluble starch was determined. Native mung bean starch has lower swelling power and solubility (8.05 g/g; 6.06%-w/w) than native corn starch, pinhao starch, and job tears starch (10.3–26.02 g/g; 4.4-22.07%-w/w) [9-8], but much higher compared to arrowroot starch (4.87 g/g; 1.669%-w/w) [6]. Phosphorylated mung bean starch products have comparatively higher solubility and swelling power compared to native starch. The presence of phosphate groups reduces the bond strength of the micellar structure, resulted in increase in solubility and swelling power of the phosphorylated starch [9]. Native mung bean starch had lower water absorption capacity (0.99 g/g) compared to potato, corn, and tapioca starch (7.9–10.4 g/g) [14]. From the results in Table 1, it was observed that phosphorylated starch products absorb more water and oil compared to the native starch. This results shared a similar trend with the previous report on the phosphorylation of corn and wheat starch [8]. The introduction of more hydrophilic phosphate groups attracts more water/oil from its surrounding [8] and increases the amount of water/oil trapped inside starch molecules.

The pasting properties of the suspensions of the native starch and the phosphorylated starch with highest P-content (0.084%) obtained at reaction pH 8 were determined using a *Rapid Visco Analyzer*/RVA equipment. The viscosity profiles of native and phosphorylated mung bean starch product were shown in Fig. 2. The results showed that phosphorylation decreased the initial pasting temperature of mung bean starch from 76.9 °C to 69.2 °C. While peak viscosity of the phosphorylated product (7973 cP) was significantly higher compared to native mung bean starch (5006 cP), final viscosity of native mungbean is relatively unchanged after phosphorylation. It was hypothesized that at its gelatinized state (at higher temperature), the presence of hydrophilic phosphate groups successfully entrapped more water into the starch matrix resulting in suspension with higher peak viscosity. After the suspension was cooled (at lower temperatures), the effect of starch retrogradation leading to expulsion of water from the starch matrix was far more significant compared to increased hydrophilicity due to the presence of phosphate groups. The final viscosity was therefore relatively unchanged [16]. Pasting properties measurement showed that phosphorylated mung bean starch might serve as a better food thickener for foods served at hot or warmer condition.

CONCLUSION

A preliminary study on phosphorylation of mung bean starch has been reported. The phosphorylation was conducted using a mixture of STPP at 130 °C at solution pH of 6–10. Modified products with phosphate content safe for food applications (DS = 0.002–0.004, equivalent to P-content of 0.045–0.084%) have been obtained. Phosphorylation of mung bean starch increases paste clarity, swelling power, solubility, and water/oil absorption capacity compared to native starch. Moreover, the phosphorylated mung bean starch paste has lower initial pasting temperature and higher peak viscosity. These results suggests that phosphorylation is a promising method to improve the functional properties of mung bean starch.

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