Physicochemical properties of phosphate butyrate arenga starches from dual modifications

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Abstract. Native arenga starch has infirmity physicochemical properties so that its use is limited to functional food. Dual modification is a starch processing to improve physicochemical properties of starch. The objective is to determine the optimum concentration of sodium trimetaphosphate (STMP) based on the physicochemical properties of the phosphate butyrate arenga starch (PBAS) from dual modification of butyrylation and phosphorylation. The method of making PBAS is using butyric anhydride 5% (w/v) and STMP concentration variation consisting of 2%, 4%, 6%, 8%, 10% and 12% (w/w) of the starch weight repeated three times and native arenga starch as a comparison. Physicochemical properties analyzed were percent butyryl, degree of substitution (DS), phosphate content, binding of butyrate phosphate with fourier transform infrared (FTIR), water, starch, amylopectin, amylose and ash contents. The results showed that the optimal STMP concentration range of 4-6% based on percent butyryl, DS and phosphate content that produce PBAS suitable for functional food ingredients. Binding of the functional group of PBAS formed a new peak in the FTIR spectra at wave number 1365.60 cm⁻¹ which indicates the presence of phosphate (P=O) groups of STMP on starch molecules. The water content of PBAS decreased with increasing STMP concentrations. The amylose, amylopectin and ash contents of PBAS were changed after the process of butyrylation and phosphorylation. PBAS is potential as a functional food ingredient due to its butyrate and phosphate contents.

1. Introduction
Arenga plant (Arenga pinnata Merr.) is one source of starch that has the potential to be developed, but the use of native arenga starch as food and non-food ingredients is still limited because it has poor physicochemical characteristics such as not heat-resistant, acidic and easily retrograde. The physicochemical characteristics of arenga starch can be improved with starch modification. Starch

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modification can be done by physical, chemical (etherification, esterification, oxidation and cross linking), enzymatic and dual modification which have different modified starch characteristics [1].

The results of the previous researches have proven that the butyrylation of arenga starch to produces butyrate arenga starch that has better physicochemical properties than native arenga starch [2]. Acetate arenga starch with a degree of substitution (DS) 0.016-0.105 can be used as a functional food ingredient [3]. The DS (0.033-0.249) of acetate arenga starch has better physicochemical properties than native arenga starch and has the potential to substitute wheat flour as a constituent of food products [4]. Acetate arenga starch can substitute up to 50% of wheat flour in bread making [5]. According to [6], the dual modification starch has slow paste retrogradation characteristics, is hydrophobic and hydrophilic, and its use is quite extensive both as food and nonfood. The research aims to determine the optimal concentration of sodium trimetaphosphate (STMP) and evaluate the physicochemical properties of the phosphate butyrate arenga starch (PBAS) as a result of dual modification by butyrylization and phosphorylation. Butyrylation process uses butyric anhydride and the phosphorylation uses STMP at different concentrations.

2. Methods

2.1. Materials

The research materials were arenga starch obtained from Palolo District, Sigi Regency, Central Sulawesi Province, 98% butyric anhydride from Sigma-Aldrich, STMP, sodium hydroxide (NaOH), hydrochloric acid (HCl) and potassium hydroxide (KOH) from Merck. Distilled water, 75% ethanol, acetic acid, and other analytical reagents were obtained at the Agroindustry Laboratory of the Faculty of Agriculture, Tadulako University.

2.2. Butyrylation and phosphorylation of arenga starch

Butyrylation was done according to the method of [2] and the phosphorylation was done according to the method of [7] with a few modifications. The suspension consisted of arenga starch (50 g) and distilled water (112.5 ml) stirred with a magnetic stirrer for one hour at room temperature. Butyric anhydride of 5% (v/w) was added dropwise until it ran out while maintaining pH 10 by adding 3% NaOH at the room temperature for 40 minutes. The suspension was increased to pH 10.5 by adding 3% NaOH while still stirring. The STMP of 2%, 4%, 6%, 8%, 10%, and 12% (w/w) was added while stirring for 30 minutes at the room temperature. The pH of the suspension was reduced to 4.5±5.5 by adding 0.5 N HCl to stop the reaction. The suspension was precipitated and washed with distilled water three times and with ethanol once. The modified starch was dried using an oven at 50°C for 12 hours until the water content was 10% to 12%, and then mashed and filtered with 100 mesh sieves to obtain PBAS for the analysis material.

2.3. Analysis method

2.3.1. Percent butyryl and degree of substitution. Percent butyryl and degree of substitution were done according to the method of [8] with a slight modification. The phosphate butyrate arenga starch (1.0 g) was put into 250 mL Erlenmeyer and added with 50 mL of 75% ethanol. The starch dispersion was heated and agitated at 50°C in a water bath for 30 minutes and cooled at room temperature. After cooling, 40 mL of 0.5 M KOH solution was added to the starch suspension and shaken using a shaker for 30 minutes at the room temperature. After being shaken, the excess alkali was titrated using 0.5 M HCl solution and phenolphthalein as an indicator until the pink color disappeared. As a blank, native arenga starch was used. The value of the DS can be obtained through the following equation:
DS = \frac{162 \, W}{100 \, M - (M-1) \, W} \quad (1)

\%
\text{Butyryl (W)} = \frac{(\text{Blank} - \text{Sample}) \, \text{mL} \times M \, \text{HCl} \times 0.071 \times 100}{\text{sample dry weight (g)}} \quad (2)

Where:
\( W \) = Weight of the substituent bound and replacing the hydrogen group in OH group (% weight)
\( M \) = Substituent molecular weight (butyryl group = 71)

2.3.2. Phosphate content. The observation of phosphate content was carried out using 1.5 g of starch samples weighed using an analytical scale. The weighed starch was then put into the crucible and heated using a furnace to ashes. After that, 10 ml of HCl 1: 3 solution was put into the crucible. While being filtered, it was put it into a 50-ml flask to mark it with the distilled water. The crucible was then piped as much as 5 ml, and then put into a test tube. 0.5 ml of concentrated P (phosphate) reagent was added to the test tube and then the solution was left alone for 15 minutes. The absorption of the solution was then measured using a spectrophotometer with a wavelength of 693 nm. The value of phosphate content can be obtained by the following equation:

\[
\text{Phosphate content (\%)} = \left( \frac{\text{ppm curve} \times \text{extract volume} \times \text{conversion factor}}{\text{sample weight}} \right) \times 100
\]

2.3.3. Analysis of binding of butyryl and phosphate groups. The FTIR spectra of native starch and PBAS was measured using the KBr method as suggested by [9]. The sample was mixed with KBr with a starch/KBr ratio of 1: 4. The mixture was compressed to obtain transparent pellets. Then, the sample was exposed to infrared light with a spectrometer (MIDAC, prospect 269, Costa Mesa, CA, USA). Each spectrum was analyzed in the range of resolution of 500-4000 cm\(^{-1}\).

2.3.4. Water content. The observation of the water content was done using the empty crucible which was cleaned, labeled and heated in the oven at a temperature of 105°C for 15 minutes, and then weighed using an analytical scale. The mashed sample was then weighed in a 2 g crucible. Furthermore, the crucible and its content were heated in an oven at 105°C for 2 hours. The crucible was then transferred to a desiccator, cooled and weighed using an analytical scale. After that, the crucible was reheated in the oven until a constant weight was obtained (successive difference in increments of less than 0.2 mg). The value of the water content of the materials can be obtained through the following equation:

\[
\text{Water content of material (\%)} = \left( \frac{\text{BS} + \text{BCK}}{\text{BS}} \right) \times (\text{BC}+1) \times 100
\]

Where:
BCK = Empty Crucible Weight (g)
(BC+I) * = Crucible Weight with Content after Heated (g)
BS = Sample Weight (g)

2.3.5. Amylose and amylpectin contents. The observation of amylose content was done with a starch sample that was weighed as much as 0.1 g, put in a beaker glass, and then added with 1 ml of 95% ethanol and 9 ml of 1N NaOH solution. After that, it was heated for 10 minutes on a hot plate, cooled, transferred
into 100-ml volumetric flask, added with distilled water to the tera mark, and then shaken. The 5 ml solution was taken and put into a 100-ml volumetric flask. A total of 1 ml of 1 N acetic acid and 2 ml of 0.2% iodine solution were added. After that, distilled water was added to the tera mark which was then shaken and left alone for 20 minutes. After that, the absorbency of the solution was measured with a wavelength of 625 nm. The amyllopectin content was determined with the following formula: 100% - amylose content.

2.3.6. Ash content. The observation of the ash content was done with the heating method in the furnace. The ash crucible was heated in the furnace and then cooled in a desiccator. The cold crucible was then weighed with an analytical scale. This process was repeated until a constant weight was obtained. After that, 2 g of starch sample was poured into the crucible, which were then put into the furnace. Ashing was carried out in two stages. The first was at the temperature of approximately 400°C. At this stage, the furnace door was left open because the burned material will emit smoke. Heating was continued at 500°C with the furnace door closed. The heating was done until gray ash was obtained. After that, the crucible containing ash was cooled in a desiccator. The cold crucible was then obtained. The value of the ash content of the materials can be obtained through the following equation:

\[
\text{Ash content (\%) = } \frac{(B \text{S}+B \text{CK})-(B \text{C}+1)^*}{B \text{S}} \times 100\%
\]

Where:
BCK = Empty Crucible Weight (g)
(BC+I) * = Crucible Weight with Content After Heated (g)
BS = Sample Weight (g)

2.3.7. Data analysis. The observation variables were analyzed with Analysis of Variance (ANOVA). If the ANOVA test provided information that the treatment given has a very significant effect, the advanced test using the 1% Honestly Significant Difference (BNJ) test would be done to determine differences in the average value of the treatment.

3. Results and discussion

3.1. Percent butyryl and degree of substitution
The percent of butyryl and DS are significantly influenced by STMP concentrations. The average percent butyryl and DS PBAS at different STMP concentrations are shown in Figure 1. The highest percent butyryl (2.47%) and DS (0.058) were found at 6% STMP concentrations and the lowest percent butyryl (1.58%) and DS (0.037) found at 2% STMP concentrations. Percent butyryl and DS at 6% STMP concentration were not significantly different from STMP concentrations of 4%, 8%, and 10%, but significantly different from other STMP concentrations.
Percent butyryl and DS increased with increasing STMP concentrations ranging from 2%-6% and then decreased until the end of the concentration. According to [10], the percent phosphate and DS of phosphate breadfruit starch increased with increasing STMP concentrations. The addition of STMP concentration causes an increase in mechanical energy and the reaction of the phosphate group to substitute for the OH group due to the weakening of the hydrogen bond. It is assumed that the increase in the concentration of reagents will accelerate the reaction, thereby increasing the value of DS. According to [11], DS of acetate breadfruit starch increases with increasing concentration of acetic anhydride. This is presumably due to the high concentration of acetic anhydride, which causes the faster movement of molecules and the contact between the reactants and the material to be more intense, giving a greater chance for the substituted acetyl groups on the hydroxyl groups in starch molecules.

3.2. Phosphate content
The PBAS phosphate content at various STMP concentrations can be seen in Figure 2. STMP concentrations significantly influence phosphate content in PBAS. The highest phosphate content of 0.0036% was found in PABF concentration of 4% STMP and the lowest of 0.0028% was found in the concentration of 2% STMP. The PBAS phosphate content tends to increase with increasing STMP concentrations of 2%-6%. The phosphate contained in starch molecules indicates phosphorus incorporation in butyrate arenga starch molecules to obtain a dual modification of arenga starch. According to [12], cross-linked tapioca starch phosphate content increased with increasing concentrations of STMP and sodium tripolyphosphate (STPP) mixture.
Figure 2. The phosphate content of phosphate butyrate arenga starch at different STMP concentrations

According to [13] reported that the phosphate content of hydroxypropyl phosphate arrowroot starch increased with increasing concentrations of given phosphate salts. According to [14], the modified canna and yam starch phosphate contents are higher than their native starch and the phosphate content also increases with increasing concentrations of POCl3 compounds.

3.3. Binding of butyrate and phosphate groups
FTIR spectra of native arenga starch (a) and PBAS with various STMP concentrations i.e. STMP 2% (b), STMP 4% (c), STMP 6% (d), STMP 8% (e), STMP 10% (f) and 12% STMP (g) are shown in Figure 3. Hydroxy (OH), C-H and H2O groups on native arenga starch have a wave number peaks of 3448, 2932 and 1651 cm⁻¹, respectively. The similar thing is also found in native corn starch [15] and native waxy potato starch [16].

The PBAS spectra (Figures b, c, d, e, f, g) have a new peak at the wave number 1365.60 cm⁻¹ which indicates the presence of vibrations of the phosphate group (P=O) of the STMP compound. This shows that butyrate arenga starch added with STMP results in phosphorus incorporation in starch molecules. According to [17], sago starch modified with STMP has a new peak at wave number 1261.38 cm⁻¹ which indicates the presence of vibrations of phosphate groups.
3.4. Water content
The water content of native arenga starch and PBAS at various STMP concentrations is shown in Figure 4. STMP concentrations significantly influence the water content of the PBAS. The highest water content of 12.72% was obtained in native arenga starch and the lowest of 8.22% found at 10% STMP concentration. The water content of PBAS tends to decrease with increasing STMP concentrations. This happens because phosphate compounds can form cross bonds in starch molecules thereby reducing the ability of water to diffuse into starch molecules. According to [18], acetate arenga starch has lower water content compared to native arenga starch does. This is because the OH group in the starch molecule is substituted by the non-polar acetyl group so the ability to absorb water is low.

Figure 3. FTIR spectra profile of native arenga starch (a) and phosphate butyrate arenga starch with different STMP concentrations: STMP 2% (b), STMP 4% (c), STMP 6% (d), STMP 8% (e), STMP 10% (f) and STMP 12% (g).

Figure 4. The water content of phosphate butyrate arenga starch at different STMP concentrations.
3.5. Chemical composition

STMP concentrations significantly influence amylose, amyllopectin and ash contents in native arenga starch and PBAS. The average values of amylose, amyllopectin and ash contents of native arenga starch and PBAS at various STMP concentrations are shown in table 1. The highest amylose content of 33.25% was found in PBAS concentration of 4% and the lowest 25.34% was obtained in PBAS concentration of STMP 12%. The amylose content of PBAS tends to increase with increasing STMP concentrations of 2%-6%, and then decreases to STMP concentration of 12%. The pattern of the amylose content increase is not linear, but it is quadratic. This happens because phosphate compounds can form stable cross bonds at 2%-6% STMP concentration and at higher concentrations hydrolysis occurs in the PBAS molecule.

| Treatments  | Amylose (%) | Amylopectin (%) | Ash (%) |
|------------|-------------|-----------------|---------|
| Native starch | 29.45 ± 0.418<sup>c</sup> | 70.55 ± 0.418<sup>c</sup> | 0.29 ± 0.016<sup>ab</sup> |
| STMP 2% | 26.81 ± 0.743<sup>b</sup> | 73.19 ± 0.743<sup>d</sup> | 0.35 ± 0.036<sup>b</sup> |
| STMP 4% | 33.25 ± 0.285<sup>a</sup> | 66.75 ± 0.285<sup>a</sup> | 0.35 ± 0.049<sup>b</sup> |
| STMP 6% | 32.09 ± 0.221<sup>d</sup> | 67.91 ± 0.221<sup>b</sup> | 0.24 ± 0.086<sup>ab</sup> |
| STMP 8% | 28.42 ± 0.413<sup>c</sup> | 71.58 ± 0.413<sup>c</sup> | 0.22 ± 0.049<sup>ab</sup> |
| STMP 10% | 27.32 ± 0.472<sup>b</sup> | 72.68 ± 0.472<sup>d</sup> | 0.21 ± 0.011<sup>a</sup> |
| STMP 12% | 25.34 ± 0.406<sup>d</sup> | 74.66 ± 0.406<sup>d</sup> | 0.32 ± 0.053<sup>ab</sup> |

Means with the different letters in the same column are significantly different (P<0.05).

According to [13] reported that amylose content is higher than native starch at a ratio of 2% STMP concentration with 5% STPP. According to [19], the amylose content of orange phosphate sweet potato starch increases with increasing STPP concentration and immersion time. This is presumed because during the immersion process, a new amylose (glucose polymerization) is formed as the result of the cutting of amyllopectin branch bonds by enzyme activity. The highest amyllopectin content was 74.66% found in the PBAS concentration of 12% and the lowest 66.73% was found in the PBAS with STMP concentration of 4%. The amyllopectin PBAS content decreases with increasing STMP concentrations of 2%-6%, and then increases to 12% STMP concentrations. [20] reported that the phosphorylation of amyllopectin in rice starch is easier than amylose, so that the amyllopectin molecules combine to produce large size molecules.

The highest ash content of 0.35% was found in PBAS concentrations of 2% and 4% and the lowest 0.21% was obtained in PBAS with STMP concentrations of 10%. The ash content of PBAS increases to a 4% of STMP concentration, and then decreases to 10% of STMP concentration. The decrease in ash content up to 4% of STMP concentration is due to perfect phosphorylation in starch molecules while an increase in STMP concentration up to 10% results in the hydrolysis of PBAS by STMP solution which is lost during washing.

4. Conclusion

The dual modification of arenga starch by butyrylation and phosphorylation produces phosphate butyrate arenga starch which has better physicochemical properties than native arenga starch. The STMP concentration of 4%-6% added to the butyrate arenga starch produces the best phosphate butyrate arenga starch, which can be applied as a functional food ingredient. Phosphate butyrate arenga starch has a DS range, phosphate, water, amylose, amyllopectin and ash contents, which are suitable for application as food ingredients. The physicochemical properties make phosphate butyrate arenga starch have wider uses for food.
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