Effect of different types of phosphorylating reagent on the synthesis of modified tapioca starch

A K Sugih*, I. Christabella, H Kristianto and S Prasetyo
Chemical Engineering Department, Parahyangan Catholic University, Jl. Ciumbuleuit 94, Bandung 40141, Indonesia

*Email: asaf.k.sugih@unpar.ac.id

Abstract. Tapioca starch is isolated from the root of cassava (*Manihot esculenta*) plant and currently ranked second among commercial starch produced worldwide. Tapioca starch has very low residual impurities, bland flavor, and superior gel properties. Native tapioca starch has been widely used for daily food preparation. Chemical modifications of native starch structure, however, is often needed before starch can be utilized for industrial applications. In this research, a preliminary study on the use of different types of phosphorylating reagents (Sodium Tripolyphosphate/STPP, Sodium Trimetaphosphate/STMP, and mixture of both reagents) on the synthesis of modified tapioca starch was performed. Phosphorylation of tapioca starch was performed at a temperature of 130 °C, initial suspension pH of 9, and an intake of STPP of 5 %-w/w and/or STMP of 2 %-w/w based on dry starch. The experimental result shows that modified starch products with Degree of Substitution (DS) of 0.0084-0.0132 (correlated to P-content of 0.169-0.258 %-w/w) were obtained. The use of both STPP and STMP phosphorylating reagents gives starch products with increased solubility. Phosphorylation with all types of reagent also increases water and oil absorption capacity as well as paste clarity of tapioca starch. The result suggests that STPP and STMP are potential phosphorylating agents for modified tapioca starch preparation.

1. Introduction
Starch is a polysaccharide playing important role in human diet. Starch is manufactured by extraction from seed, fruit, stem, and root of higher plants. Tapioca starch is a major commercial starch in Indonesia isolated from the root of cassava (*Manihot esculenta*) plant. Cassava root is the fourth staple food worldwide [1], and tapioca starch ranked second among commercial starches produced globally [1,2]. Southeast Asian countries (mainly Thailand and Indonesia), and Brazil are currently the largest tapioca starch producers [3]. Tapioca starch has low level of lipid and protein compared to cereal starches [4]. It has rather low amylose content, and is characterized by high molecular weights of amylose and amylopectin. Tapioca starch also has bland flavour and superior gel forming ability [4]. These excellent properties make tapioca starch an interesting material for direct use as well as for a starting material for modification into specialty products.

Native starch is a versatile material for daily cooking and food preparation. Its use in food and non-food industries, however, in many cases is limited by inferiorities of its functional properties [5]. To improve starch characteristics, several physical and chemical modification methods have been proposed [5,6]. Esterification of starch with phosphate (also known as phosphorylation) is a chemical method commonly used to improve hydrophilicity of starch [6]. Phosphorylated starch gives clear paste with high consistency and good freeze-thaw stability and emulsifying properties [7]. Depending
on the type of reagents and applied reaction conditions, phosphorylation may result in monostarch phosphate or distarch phosphate products. Distarch phosphate is a type of cross-linked starch, and its presence even at a very low level can drastically change pasting and gel properties of the products. Research performed on corn, wheat, sago, pinhao, and rice starch phosphorylation has been reported [7-10] and modified starch products with low phosphate content has been allowed to be used in food industry [11]. While study on phosphorylation of such an important global commodity as tapioca starch will impart significant scientific as well as industrial impact, as far as we know no research on this topic has been reported to date.

The objective of this research is to preliminarily study the potentiality of applying chemical phosphorylation to improve tapioca starch characteristics. In particular, an examination on the effect of different esterification reagents (STPP, STMP, and mixture of STPP+STMP) on the Degree of Substitution (DS) of the products was reported. Selected functional product properties important for industrial food applications were also measured and compared to native tapioca starch.

2. Materials and Methods

2.1. Materials

Commercial grade tapioca starch manufactured by Tirta Kencana (Bogor, Indonesia) was used without further purification. Two phosphorylating reagents used were technical grade sodium tripolyphosphate (STPP) and analytical grade sodium trimetaphosphate (STMP) from Sigma Aldrich, Singapore. Sodium sulfate, hydrochloric acid, sodium hydroxide, vanadate molybdate, potassium hydrogenphosphate, and sodium carbonate (all chemicals were of analytical grade) were obtained from Merck (Jakarta, Indonesia).

2.2. Tapioca Starch Phosphorylation

Phosphorylated tapioca starch was synthesized according to a procedure applied previously for corn, wheat, and sago starch [7,8] with slight modification. A salt solution containing the phosphorylating reagents (5 %w/w STPP, or 2%w/w STMP, or 5 %w/w STPP + 2%-w/w STMP, based on dry starch), 12 ml of RO water, and 5% of sodium sulfate (dry starch basis) was prepared. Acid or base solution (HCl or NaOH, 10%) was added to adjust pH of the solution to 9. Tapioca starch (10 g, dry basis) was mixed with the phosphorylating reagent solution and pH was adjusted back to 9. Starch dispersion was stirred for one hour at room temperature before it was dried inside a tray dryer at a temperature of 45 °C. After starch mixture reached moisture content of 10 – 15% w/w, temperature of the tray dryer was subsequently increased to 130 °C and the solid was heated for 2 more hours. The obtained phosphorylated starch products were then cooled to room temperature and mixed with 25 ml of RO water (pH of the mixture was adjusted to 9). The mixture was subsequently centrifuged for 10 minutes at a rotational speed of 1500 rpm, and the collected starch solid was re-dispersed in 25 ml of RO water. This washing sequence was repeated three times. Starch phosphate products were finally dried inside a tray drier at 45 °C until constant weight. Each experiment was replicated to ensure reproducibility of the result.

2.3. Product Characterisation

Several important chemical properties (DS and P-content) as well as functional properties (swelling power, solubility, water/ oil absorption, and paste clarity) were selected (after consulting previous reports on starch modification [7-10, 12-17]) to characterize phosphorylated and native tapioca starch products.

2.3.1. Degree of Substitution (DS) and Phosphorus Content (P-content) Characterization. Determination of Degree of Substitution (DS) and P-content of starch products were performed by spectrophotometry at λ = 385 nm based on a previously reported procedure [9].
2.3.2. Swelling Power and Solubility Measurement. Swelling Power (SP) and Solubility (Sol) of the native and modified tapioca starch were determined according to a published method [12] with slight modification. Starch product (0.5 g) was suspended in 15 ml of RO water at a temperature of 85 °C (100 rpm, 30 minutes) before subsequently centrifuged at a rotational speed of 3000g for 15 minutes. Starch paste was separated from the supernatant, weighed, and subsequently dried at 100 °C in a tray dryer until constant weight. SP (g/g) and Sol (%-w/w) of the starch products were calculated as follows:

\[ SP = \frac{w_{sd}}{w_i \times (1 - \frac{S}{100})} \]  
\[ Sol = \frac{w_{ss}}{w_i} \times 100\% \]

in which:
- \( w_{sd} \) = weight of sediment paste (g)
- \( w_{ss} \) = weight of soluble starch (g)
- \( w_i \) = weight of initial sample (g)

2.3.3. Water and Oil Absorption Capacity Measurement. Water and Oil Absorption Capacity (WAC, g/g and OAC, g/g) of starch products were determined according to a published method [13] with slight adaptation. Starch samples were mixed with RO water or palm cooking oil (1 g starch in 10 ml of water or oil), kept for 1 hour at room temperature, and subsequently centrifuged at a rotational speed of 3000g for 30 min. The weight of water or oil in the sediment was measured, and the absorption capacity was calculated as the mass of water or oil absorbed.

2.3.4. Paste Clarity Measurement. Paste clarity of gelatinized starch suspensions was determined based on a previously reported method [10]. Starch samples were suspended in RO water (1 %-w/v, pH = 6.5), heated at 95 °C for 30 min, and subsequently cooled to 25 °C for 1 hour. Paste clarity was determined as percentage of transmittance of the starch suspensions measured by spectrophotometry at \( \lambda = 650 \) nm.

3. Result and Discussion
Tapioca starch was phosphorylated with STPP (5 %-w/w) or STMP (2 %-w/w), or mixture of both reagents (5 %-w/w % STPP + 2 %-w/w STMP). Starch and phosphorylating reagents were mixed in the presence of Na\(_2\)SO\(_4\) at an initial pH level of 9 before the reaction was further conducted at 130 °C. The products were washed three times using RO water before finally dried at 45 °C in a tray dryer.

Degree of Substitution (DS) and P-content of the modified products were determined spectrophotometrically, and the result is shown in Figure 1 and 2. Degree of Substitution of modified tapioca starch products is accessible in the range of 0.0084 - 0.0132. The use of STMP is more effective than STPP reagent, as the use of 2 %-w/w STPP reagent (only 40% of the intake of STPP reagent) resulted in product with DS = 0.050, or about 60% of the value obtained when using STPP as phosphorylating reagent (DS =0.084). A similar trend was observed in previous works on corn, sago, and wheat starch phosphorylation [7,8]. It is hypothesized that STMP gives higher P-substitution than STPP because it has more polyphosphate groups on the starch chains. Initial attack of starch on the cyclic STMP resulted in starch tripolyphosphate, while initial reaction with STPP only gives starch mono and diphosphates [7,8]. Deetae, et al [14] working on phosphorylation of sago starch hypothesized that at pH = 9.5 the use of STPP will result in simple starch esterification, while the use of STMP resulted in crosslinked product. The use of combined STPP+STMP reagents resulted in P
incorporation nearly equal to the sum of DS obtained on single use of STPP and STMP (DS = 0.0132), as was also reported previously for wheat, corn, and sago starch [7,8,14].

Phosphorus content of modified tapioca starch is in the range of 0.169 - 0.258 % w/w. These values are in the range obtained in our previous works on phosphorylation of sago, arrowroot, and sweet potato starch (0.065-0.250 % w/w) [15-17], as well as in previous research on phosphorylation of corn, sago, and wheat starch (0.052 – 0.320 % w/w) [7,8]. Phosphorylated tapioca starch products synthesized using STPP and mixture of STPP and STMP have P content below the maximum limit allowed for food application purposes according to Food Chemical Codex (0.4 %) [11]. The use of STMP alone, however, result in product that cannot be used in food application as the P content is above the maximum limit of 0.04 % in Food Chemical Codex [11].

![Figure 1. Degree of substitution (DS) of native and modified tapioca starch products.](image1)

![Figure 2. Phosphorus content of native and modified tapioca starch products.](image2)

Several relevant functional properties of native and phosphorylated tapioca starch products were characterised and the result is shown in Table 1. Native tapioca starch has swelling power of 47.67 g/g and solubility of 19.01 % w/w, comparatively higher than our previous results when working with other root/ tuber (sago, arrowroot, and sweet potato) starch (3.78-7.36 g/g and 1.67-2.33 % w/w, respectively) [15-17]. Swelling power of native tapioca starch is also higher than native seed (corn,
pinhao, and job’s tears) starch reported by previous researchers (10.3-26.02 g/g) [10, 12] while its solubility is higher than corn and job’s tears starch (4.4-8.8 %.-w/w) [10, 12], but slightly lower compared to pinhao starch (22.07 %.-w/w) [10]. The use of STPP and mixture of STPP+STMP reagents increases swelling power and solubility significantly, while the use of STMP only results in moderate increase of swelling power but very high (about 2.5 times) increase in solubility of the modified products.

Water and oil absorption capacity (WAC and OAC) of native tapioca starch (3.04 g/g and 3.02 g/g, respectively) are higher compared to arrowroot and sweet potato starch (0.847-1.33 g/g and 0.572-1.4 g/g, respectively) [16,17]. Introduction of phosphate groups into starch molecule increases WAC and OAC of tapioca starch. As in the case of swelling power, the use of STMP only slightly improves WAC and OAC, while STPP and mixture of STPP/STMP resulted in products with significantly higher WAC and OAC values. Improvement in swelling power as well as absorption capacity of phosphorylated starch products is hypothesized to occur due to increased hydrophilicity and reduced intermolecular bond of starch molecules in the presence of phosphate groups [10].

### Table 1. Physicochemical and functional properties of native and phosphorylated tapioca Starch.

| Product               | Swelling Power (g/g) | Solubility (%.-w/w) | Water Absorption Capacity (g/g) | Oil Absorption Capacity (g/g) | Paste Clarity (%.-T) |
|-----------------------|----------------------|---------------------|---------------------------------|-------------------------------|---------------------|
| Native Starch         | 47.67                | 19.01               | 3.04                            | 3.02                          | 11.23               |
| Phosphorylated Tapioca Starch | STPP            | 56.43               | 28.67                           | 3.54                          | 3.45                | 27.2               |
|                       | STMP                 | 52.28               | 47.65                           | 3.11                          | 3.05                | 15.25              |
|                       | STPP+STMP            | 67.12               | 55.99                           | 3.76                          | 3.51                | 41.48              |

Paste clarity of native and modified tapioca starch products was determined spectrophotometrically. Native tapioca starch has lower paste clarity (T = 11.23%) compared to sweet potato, wheat, and corn starch (T = 15.30%, 24%, and 34%, respectively) [7,17]. The use of STPP and mixture of STPP+STMP phosphorylating reagents resulted in products with considerably higher clarity. It is proposed that the presence of negatively charged phosphate groups increases repulsion between more hydrophilic starch molecules. As the result, hydrated swollen starch particles with high light transmittance are formed [7]. On the contrary, reaction of starch and STMP at pH above 8 presumably gives products with some degree of crosslinking [7]. The presence of crosslinking in starch molecules strengthens the granules and prevents swelling of hydrated starch particles [8] resulting in products with only small increase in paste clarity.

### 4. Conclusions

A preliminary investigation on the synthesis of tapioca starch phosphate using three types of phosphorylating reagents (STPP, STMP, and mixture of STPP+STMP) has been reported. Phosphorylation is performed at an initial pH of 9 and reaction temperature of 130 °C. The use of STPP and mixture of STPP+STMP reagents resulted in modified tapioca starch products safe for food applications (DS = 0.0084-0.0132, or P-content of 0.169-0.258 %.-w/w), while P-content of phosphorylated product obtained using STMP as reagent (0.105%-w/w) exceeds the maximum limit allowable for food purposes according to Food Chemical Codex. All phosphorylated tapioca starch products have improved swelling power, solubility, water and oil absorption capacity, as well as higher paste clarity compared to native starch. Starch phosphate products has potential applications in food, pharmaceutical, paper, and textile industries. We are currently working on further investigation on the effect of using various process conditions on the DS of the products, as well as comprehensive characterization (pasting properties, freeze thaw stability, thermal and granular properties) of modified tapioca starch, and the result will be reported in forthcoming papers.
5. References

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