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(Research Article)

Increase in physicochemical, amylase-amylopectin, and β-carotene in phosphorylated starch of Banggai Yam (*Dioscorea alata* L.)?

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Abstract

Limited knowledge exists regarding the effects of chemical modification on the relationship between structure and bioactivity in non-conventional starches, restricting their application as bioactive carriers. This study examines the impact of sodium tripolyphosphate (STPP) phosphorylation on the physicochemical properties and β-carotene retention of Banggai yam starch. Starch samples were treated with 2–10% STPP under alkaline conditions and subsequently analyzed for basic composition, amylose–amylopectin content, and β-carotene levels. Phosphorylation resulted in increased carbohydrate content and reduced protein levels, while *apparent amylose* content rose markedly (from approximately 45% to 57%), indicating structural alterations. β-carotene retention also improved (from about 0.031% to 0.037%), with no further increase observed at higher STPP concentrations, suggesting that initial modification suffices to stabilize the bioactive compound. These results demonstrate a direct association between phosphorylation-induced structural changes and enhanced retention, highlighting the potential of modified starch as a carrier for bioactive compounds. The study provides new insights into the interplay among structure, function, and bioactivity in phosphorylated starch, supporting its application in the development of functional foods.

Keywords: Amylose; Amylopectin; Banggai Yam; Phosphorylation; Starch; Β-Carotene

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1. Introduction

Starch is widely used in the food industry due to its biodegradable properties, abundant availability, and high application flexibility in complex food systems. (1,2). Nevertheless, the demand for modern foodstuffs is not limited only to technological functions (4), but also includes health aspects such as improved insulin sensitivity, cardiovascular health (5), glycaemic control, and bioactivity potential (6). In addition, the global trend towards clean labels and natural ingredients is further reinforcing the need for safe and sustainable starch modifications. (7–10). Therefore, innovations in starch modification are crucial for producing ingredients that are not only functional but also offer added nutritional and health value.

Phosphorylation of starch using sodium tripolyphosphate (STPP) is a chemical modification approach that involves a substitution and/or crosslinking mechanism, which can limit the mobility of polymer chains and modulate the interaction between the amylose and amylopectin fractions, thereby impacting the physicochemical and functional properties of starch. (11,12). The phosphorylation process is known to improve thermal stability. (13), reduce retrogradation (14,15) and improve the viscoelastic properties of starches through the formation of phosphate bonds between polymer chains (16). The crosslinking mechanism that occurs also contributes to increased resistance to process conditions such as high temperatures and pH extremes. (17). In addition, these modifications can affect

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molecular interactions in starch granules that impact changes in hydration and gelatinization properties. (18). Thus, phosphorylation is one of the effective strategies to engineer the structure and function of starch in a controlled manner.

Although phosphorylation modifications have been widely reported in conventional starches, the mechanistic relationship between the rate of modification, measured changes in amylose–amylopectin ratios, and their implications for the retention or stabilization of bioactive compounds is still not comprehensively understood, particularly in non-conventional starch sources. Most previous studies have focused more on physicochemical characteristics without linking them to the dynamics of molecular structure in depth. (19–21). On the other hand, the potential of starch as a carrier matrix for bioactive compounds such as β-carotene is still rarely explored in the context of chemical modification. (22). This gap suggests the need for a more integrative approach to understanding the relationship between the structure, composition, and bioactivity of modified starches.

Exploration of starches from underutilized local sources, such as Banggai yam (*Dioscorea alata* L.), offers an opportunity to uncover intrinsic characteristics that differ compared to commercial starch sources, as these starches can be physically or chemically modified to improve their functional properties, such as water absorption, solubility, and retrogradation stability, thus expanding their industrial applications (23). D. alata starch can be used as a thickener, stabilizer, and texturizer in products such as soups, sauces, and baked goods. The natural purple color of these yams, caused by the anthocyanin content, also adds aesthetic value and health benefits (24,25). In addition, the utilization of local resources supports the diversification of raw materials and biodiversity-based food security (26), potentially for use in bioplastics, cosmetics, and pharmaceuticals due to their biocompatibility properties (27). The application of phosphorylation using STPP was chosen because of its ability to produce a more controlled starch structure through the formation of phosphate bonds that can affect the molecular organization of starch (28).

Current research tends to focus on improving the functional properties of modified starches without deeply elucidating the relationship between structural changes due to phosphorylation and composition dynamics and bioactive potential, so this study aims to elucidate the relationship between the level of phosphorylation using STPP and changes in physicochemical characteristics, structural composition, and β-carotene content in Banggai yam starch. This approach is expected to provide a more comprehensive understanding of how chemical modifications affect molecular interactions in starches. (16,29,30). In addition, the integration of composition and bioactivity parameters provides a new perspective in starch studies that were previously more focused on technological aspects. (31). Thus, this research is not only descriptive but also analytical in explaining the phenomenon that occurs. This makes this research relevant in the context of the development of modern starch science.

2. Methods

2.1. Isolation of Banggai yam starch

The synthesis of Banggai yam Starch is carried out according to the method described by (28,29,32). Fresh Banggai yam tubers are peeled, washed, sliced, and blended with distilled water at a 1:3 (w/v) ratio. The pulp is filtered through a muslin cloth, and the filtrate is left for 12 hours to allow the starch to precipitate. The supernatant is discarded, and the precipitated starch is washed repeatedly with distilled water until the pH is neutral. The starch is then dried in the oven at 45 °C for 24 hours, ground, and sifted through an 80-mesh sieve. The starch obtained is stored in an airtight container for further modification.

2.2. Phosphorylation of Starch Using Sodium Tripolyphosphate (STPP)

Starch is chemically altered through phosphorylation using STPP at three concentrations: 2%, 6%, and 10% (b/b, based on dry weight of starch). A total of 100 g of starch is mixed with STPP solution in distilled water (225 mL) using a magnetic stirrer at room temperature for 1 hour, until a homogeneous mixture is formed. The pH of the mixture was maintained at 10.5 with 5% NaOH, and STPP was then added as per the treatment. The suspension is then incubated at 40 °C for 45 minutes with continuous stirring to allow a phosphorylation reaction to occur. After the reaction is complete, the mixture is diluted with 0.5 N HCl to adjust the pH to 4.5. Next, the mixture is precipitated, washed once with 95% ethanol, and then three times with distilled water to remove the remaining reagent and adjust the pH to neutral. The modified starch is dried at 50°C for 13 hours, ground, filtered through a 100-mesh sieve, and stored in a sealed container. The resulting product is called Phosphorylated Banggai Yam Starch (PBYS). Carbohydrate content is calculated according to (33). Lipid content was analyzed using the Soxhlet extraction method (33), and protein content (33). The crude fiber content was determined using the crude fiber method (33), Amylose content was determined using the spectrophotometry method (34), and β-carotene content was determined using the method (35).

2.3. Statistical analysis

All experimental data were analyzed using SPSS Statistics software (version 30, IBM Corp., Armonk, NY, USA). The result is expressed as the mean ± the standard deviation (SD) of the three independent replications. Data analysis was conducted using a one-way analysis of variance (ANOVA) to evaluate the effect of treatment on physicochemical parameters, amylose-amylopectin, and β-carotene PBYS, followed by the Duncan Multiple Distance Test (DMRT) at a significance level of $p < 0.05$ to identify significant differences between the average treatments.

3. Results

Table 1 shows the physicochemical composition, carbohydrate fractions, and β-carotene content of both native and phosphorylated Banggai yam starch at different STPP concentrations. The results are given as mean values with standard deviations (X̄ ± SD) to show how chemical modification affects starch properties**.**

Table 1 Physicochemical composition, amylose–amylopectin content, and β-carotene levels of native and STPP-phosphorylated Banggai yam starch

Content	Value (X̄ ±SD)			
	Native Starch	STPP		
		2%	6%	10%
Carbohydrates	77.06 ± 1.935a	80.3 5± 0.174c	78.35 ± 0.271a	79.66 ± 0.101bc
Lipid	2.65 ± 0.216b	4.72 ± 0.144c	2.53 ± 0.180b	0.58 ± 0.055a
Protein	4.72 ± 0.167b	3.93 ± 0,061a	3.97 ± 0.072a	3.93 ± 0.010a
Crude Fiber	0.77 ± 0.097a	0.90 ± 0,087a	0.81 ± 0.006a	0.86 ± 0.015a
Amylose	44.98 ± 0.648a	56.35 ± 0.898b	55.87 ± 0.248b	57.58 ± 0.288c
Amylopectin	32.08 ± 2.536a	43.65 ± 0.898b	44.13 ± 0.248b	42.42 ± 0.288b
β–carotene	0.0307 ± 0,00137a	0.0374 ± 0.00059b	0.0373 ± 0.00056b	0.0370 ± 0.00024b

Note: Values are presented as Mean ± Standard Deviation (X̄ ± SD). Different letters (a, b, c) within the same row indicate statistically significant differences.

This study evaluates the effects of varying concentrations of sodium tripolyphosphate (STPP) on the chemical properties of starch. The results indicate distinct changes in composition and starch fractions compared to native starch. STPP addition increases carbohydrate, amylose, amylopectin, and β-carotene contents, with the highest amylose observed at 10% STPP. Protein content decreases at all STPP concentrations, while lipid content increases at 2% but declines sharply at 10%. Overall, STPP treatment significantly ($p<0.05$) alters the nutritional and structural profile of the starch, demonstrating the effectiveness of this method for modifying starch properties for food industry applications.

4. Discussion

4.1. Effect of Phosphorylation on Proximate Composition of PBYS

Phosphorylation treatment using STPP has a significant effect on the proximate composition of Banggai yam, as shown in **Table 1**, with a pattern of changes indicating a reorganization of the starch matrix. Carbohydrate content increased from 77.06% in natural starch to 80.35% in 2% STPP treatment, then showed a moderate decrease at higher concentrations, although it remained above natural starch values. This nonlinear pattern indicates that the increase in carbohydrate content is likely not the result of the synthesis of new components, but rather a consequence of the reduction of the noncarbohydrate fraction during the reaction and leaching process, thereby increasing the relative value of the carbohydrate calculated by the by-difference method. The increase in carbohydrates in PBYS can be seen in Figure 1.

This phenomenon is not an absolute carbohydrate accretion, but rather an artifact of the by difference method, in which the reduction of non-carbohydrate fractions (proteins and lipids) leads to an increase in the relative proportion of carbohydrates, reflecting a redistribution of the starch matrix that is in line with a decrease in protein levels. (36). These nonlinear dynamics are influenced by molecular interactions and chemical reactions during modification, with low concentrations maximizing non-starch elimination, while high doses trigger partial degradation or elution of granules due to reorganization of amorphous-crystalline structures through phosphate bonds. (37). Its functional implications include improved starch purity that has the potential to optimize technological properties such as gelatinization and viscosity, although it requires evaluation of interactions with food matrices for functional applications; overall, STPP phosphorylation doubles as a modulator of structure and composition, enriching the applicative potential of modified starches. (38) (39).

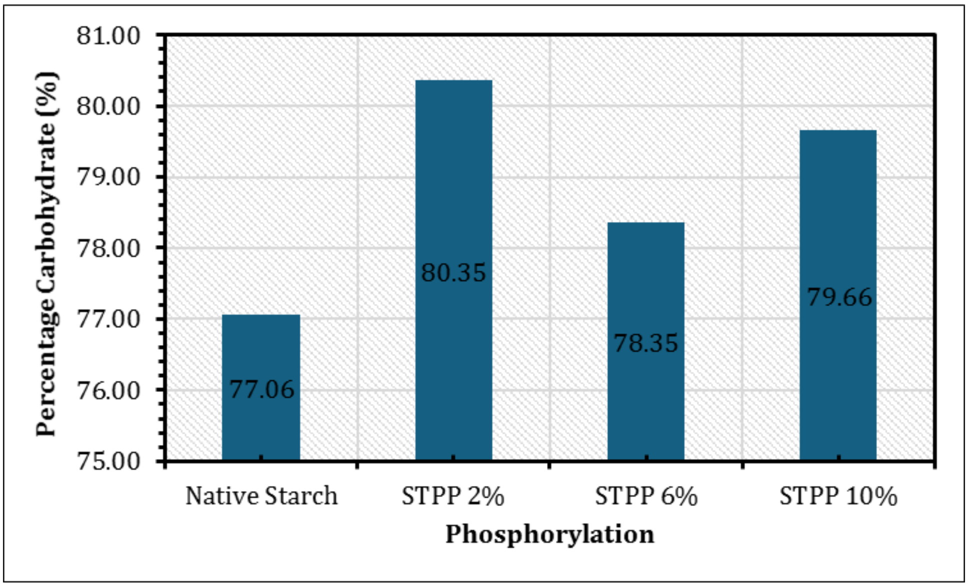

Figure 1 PBYS Carbohydrates

Phosphorylation treatment using STPP exerted a different effect on the minor components of Banggai yam starch, as shown by changes in lipid, protein, and crude fiber levels. Lipid levels increased from 2.65% in natural starch to 4.72% in 2% STPP treatment, then decreased to 6% concentration (2.53%) and significantly reached the lowest value at 10% (0.58%) (Figure 2). This nonlinear pattern indicates the existence of a competing mechanism during the modification process, where at low concentrations there is a release of bound lipids due to disturbance of the granule structure, while at higher concentrations alkaline conditions and intensive washing promote lipid lubrication or degradation (40). This shows that the stability of lipids in the starch matrix is greatly influenced by the intensity of the phosphorylation treatment and the reaction conditions used.

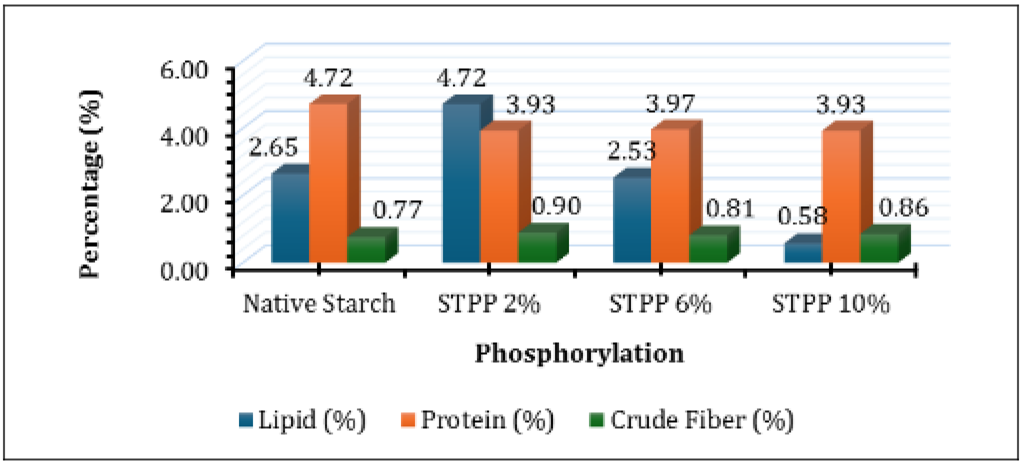

Figure 2 PBYS Lipids, Proteins, and Crude Fiber

In contrast to lipids, protein levels showed a relatively consistent decrease from 4.72% in natural starch to about 3.93–3.97% in the entire phosphorylation treatment. This decrease shows that the proteins contained in Banggai yam starch are mostly weakly bound or on the surface of the granules, so they are easily released in alkaline conditions during the modification and washing process. This reduction in protein levels has implications for increasing the purity of starch, but can also affect molecular interactions in the system, given that proteins can play a role in stabilizing granular structure and interacting with other components (41). Therefore, these changes are not only compositional but also potentially affect the functional properties of starches.

Meanwhile, crude fiber levels showed no significant change, with values ranging from 0.77% to 0.90% across treatments. This stability indicates that the coarse fiber component, which is generally composed of non-starchy polysaccharides with more complex structures, is relatively inert to chemical conditions during phosphorylation. Overall, these results confirm that phosphorylation more selectively affects reactive minor components such as lipids and proteins compared to crude fibers. The dynamics of these compositional changes have important implications for the functional properties of starches, including molecular interactions, stability during processing, and potential applications in food systems that require starch matrices with more controlled characteristics. (19).

4.2. Modulation of Apparent Amylose–Amylopectin Composition and Structural Implications

Phosphorylation of Banggai yam starch significantly modulated the structural composition, indicated by changes in amylose and amylopectin levels (Table 1), with a marked increase in amylose from 44.98% to 56.35–57.58% as STPP concentrations increased. This increase cannot be interpreted as an absolute increase in the amylose content but rather reflects a change in the ability to form complexes with iodine due to modifications in the molecular structure of starch. Phosphorylation through the formation of phosphate bonds can disrupt crystalline structure and increase amorphous fractions, thereby increasing the accessibility of linear chains to iodine reagents. (42–44). An increase in amylose and amylopectin is shown in Figure 3.

Interestingly, amylopectin levels also show significant improvements compared to natural starches, which theoretically contradicts the concept of the composition of the two main fractions of starch. This phenomenon indicates the limitations of differential-based quantification approaches, as well as the possibility of reorganization of the internal structure of starch granules that affects the distribution of analytically detected fractions. (45,46). Thus, these results confirm that the measured changes in composition reflect more changes in supramolecular structure than changes in absolute chemical composition.

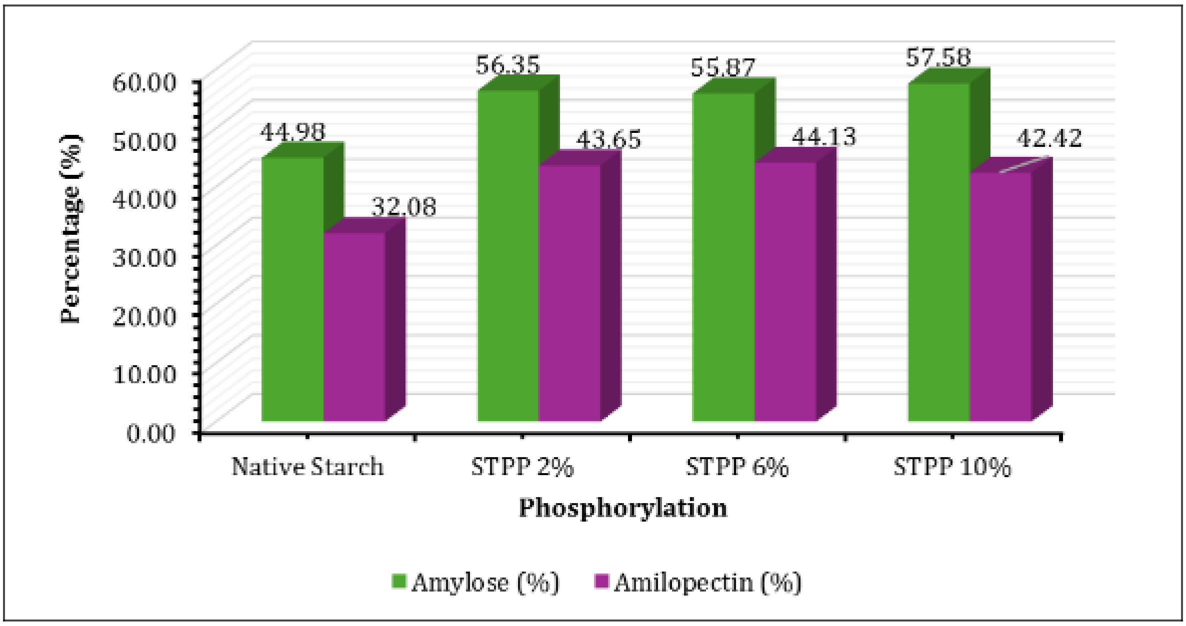

Figure 3 Amylose and Amylopectin PBYS

An important implication of these findings is that an increase in apparent amylose may contribute to the formation of more stable helical structures. (47), which potentially improves the ability to interact with hydrophobic molecules (48). This is an important basis for understanding the relationship between structural modification and retention of bioactive compounds in modified starch systems.

4.3. Enhancement of β-Carotene Retention and Its Structural Correlation

β-carotene levels showed a significant improvement across phosphorylation treatments compared to natural starches, with relatively stable values in the range of 0.0370–0.0374% with no significant differences between STPP concentrations (Table 1). This pattern suggests that the main effect of phosphorylation on β-carotene is not a linear dose-response (49), but rather is related to changes in starch matrix structure that have been achieved even at low concentrations (39). In other words, early modifications are sufficient to create conditions that favor β-carotene retention.

This increase in retention can be explained by its association with the increase in apparent amylose observed in the previous subchapter. The more exposed and organized structure of the amylose helix after phosphorylation has the potential to form an inclusion complex with β-carotene molecules that are hydrophobic in nature (50), thus protecting it from oxidative degradation (32). In addition, the formation of crosslinking can create a tighter and more stable matrix, which limits the diffusion of oxygen and improves the stability of bioactive compounds.

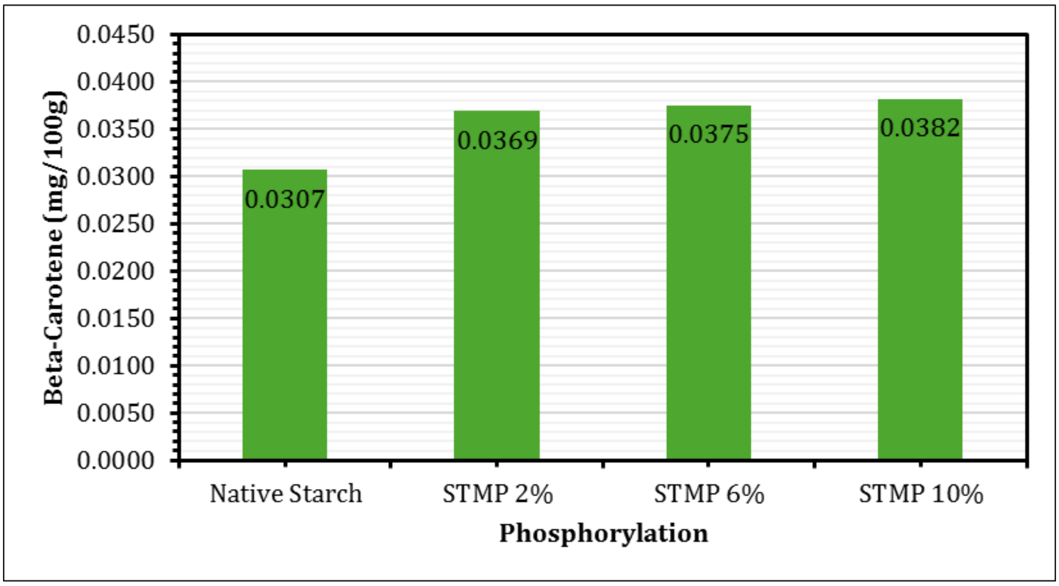

Figure 4 Gambar 4 β–Carotene PBYS

Overall, the association between increased real amylose and β-carotene retention suggests an important structure–function–bioactivity relationship in modified starch systems, which has the potential to be utilized in the development of starch-based functional foods.

5. Conclusion

Phosphorylation of Banggai yam starch with STPP altered its structure and composition, increased apparent amylose content, and enhanced β-carotene retention. These findings demonstrate a relationship among structure, function, and bioactivity, supporting the potential use of this starch as a functional matrix. Although the precise mechanisms remain to be fully elucidated, this study provides a foundation for the development of modified starch-based systems for bioactive compound delivery.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Author Contributions (CRediT)

- **RC:** Conceptualization, methodology, investigation, data curation, formal analysis, writing original draft, writing review and editing, and supervision; corresponding author.
- **DL:** Methodology, validation, investigation, writing, review and editing, and visualization.

All authors have read and approved the final version of the manuscript.

AI-Use Disclosure

Artificial intelligence (AI) tools were used in this study to assist with language refinement, grammar correction, and improvement of manuscript clarity. The authors retained full responsibility for the accuracy, integrity, and originality of the content. No AI tools were used for data generation, data analysis, or interpretation of results. All scientific conclusions and interpretations presented in this manuscript are solely those of the authors.

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