

Evaluating The Cross-Linking Agents In Modifying Yam Starch For Resistant Starch Type-4 (RS4) Prebiotic Products in A Succinct Treatise

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Abstract: Yam (*Dioscorea* spp.) is an important staple crop whose starch is widely consumed but is generally susceptible to enzymatic digestion and may contribute to rapid glucose release. Chemical crosslinking offers a potential approach to convert yam starch into Type 4 resistant starch (RS4) by introducing covalent linkages that restrict starch-chain mobility and reduce accessibility to digestive enzymes. This review examines the main chemical reagents and modification systems used to develop RS4, with particular emphasis on their reaction mechanisms, structural effects, functional properties, safety considerations, and potential nutritional implications for yam starch. The review discusses phosphate-based systems, including sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), STMP/STPP combinations, and phosphorus oxychloride (POCl_3), alongside organic and other modifying systems involving citric acid, adipic acid, tartaric acid, epichlorohydrin, monosodium phosphate, and acetic anhydride. The reviewed evidence indicates that chemical modification can alter starch swelling, gelatinization, pasting behaviour, thermal and shear stability, and susceptibility to enzymatic hydrolysis, although the magnitude and direction of these effects depend strongly on starch source and reaction conditions. Resistant starch may also reach the colon and undergo microbial fermentation, potentially contributing to short-chain fatty acid production and changes in gut microbial composition. However, evidence specifically addressing chemically crosslinked yam starch

remains limited, and findings from other botanical starches cannot be directly generalized to yam. Future research should therefore focus on species-specific optimization of crosslinking conditions, food-safety assessment, standardized digestibility and fermentation studies, human glycemic-response evaluation, sensory quality, and scale-up. Overall, chemical crosslinking represents a promising but incompletely validated strategy for developing functional yam-based RS4 ingredients and foods.

Keywords: Yam starch; *Dioscorea* spp.; resistant starch type 4; chemical crosslinking; STMP; STPP; citric acid; starch modification; glycemic response; gut health.

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1.0 Introduction

Yam (*Dioscorea* spp.) is an important tropical and subtropical tuber crop in the family Dioscoreaceae and a major source of dietary carbohydrate, particularly in West Africa. *Dioscorea rotundata*, commonly known as white yam or 10-month yam, is one of the most widely cultivated yam species and has acquired diverse regional and cultural names across its cultivation areas. In Nigeria, where yam has considerable agricultural, nutritional, cultural, and socioeconomic importance, it is variously referred to as *ji* or *ji abana* among Igbo communities, *doya* among Hausa communities, and *isu* among Yoruba communities, while related yam species are known by different names in other parts of the world (Anuagasi & Okigbo, 2024). Yam tubers have long served as important starch repositories and are used in numerous traditional and processed food products. Beyond its role as a staple food, yam contributes to traditional medicinal practices and cultural heritage in several producing communities (Obidiegwu *et al.*, 2020).

Yam is also an important component of global food production. It ranks among the major edible tuber and rhizome crops and has been identified as the fourth most important crop in this broad category after cassava, potato, and sweet potato, emphasizing its contribution to food security and agricultural livelihoods in developing countries (Epping & Laibach, 2020; Cao *et al.*, 2021). Nigeria is a major yam producer, contributing a substantial share of global production. Earlier production estimates have reported worldwide yam production at approximately 88 million metric tonnes, with Nigeria accounting for a major share of the total (Awolu & Olofinlae, 2016). The high

starch content of yam tubers makes yam starch particularly important for traditional food preparation and offers an opportunity to develop value-added starch ingredients with improved nutritional and functional characteristics.

Despite its importance, native yam starch presents several nutritional and technological limitations. Its relatively rapid enzymatic digestibility can promote rapid conversion of starch to glucose following consumption and may consequently contribute to elevated postprandial glycemic responses (Amaraweera *et al.*, 2021). This characteristic is particularly relevant in metabolic disorders such as type 2 diabetes, where dietary strategies to moderate rapidly digestible carbohydrates are of considerable nutritional interest. Native yam starch may also exhibit inadequate thermo-mechanical stability, excessive swelling, paste breakdown, and retrogradation, thereby limiting its performance under some food-processing and storage conditions (Amaraweera *et al.*, 2021). These limitations have stimulated interest in starch modification approaches that can simultaneously improve technological functionality and nutritional quality.

Starch modification can be achieved through genetic, enzymatic, physical, and chemical approaches. Chemical modification is particularly versatile because the hydroxyl groups present in starch molecules provide reactive sites through which the molecular structure can be altered. Chemically modified starches have been investigated for a wide range of applications, including food products, packaging materials, adhesives, pharmaceutical formulations, agricultural products, superabsorbent materials, and wastewater-treatment applications (Amaraweera *et al.*, 2021). For food applications, however, attention has increasingly focused on modifications that not



only improve processing characteristics but also increase starch's physiological resistance to digestion.

One important nutritional strategy is developing resistant starch (RS), the fraction of starch that escapes digestion in the small intestine and becomes available for fermentation in the large intestine. Resistant starch is commonly classified into five forms, RS1–RS5, based on the structural or physicochemical mechanisms that resist digestion. Type 4 resistant starch (RS4) comprises chemically modified starches in which deliberate structural changes reduce susceptibility to digestive enzymes. Developing RS4 from staple crops such as yam is therefore of interest because it offers a potential way to modify the digestibility of a widely consumed carbohydrate while maintaining its role in conventional food systems.

Among chemical modification techniques, crosslinking is particularly important for developing RS4. Starch contains numerous hydroxyl groups that can participate in chemical reactions, and crosslinking involves forming covalent bridges between adjacent starch chains or molecular segments. These intermolecular linkages restrict polymer-chain mobility and can reduce excessive granule swelling, improve resistance to thermal and mechanical stresses, and modify starch gelatinization and pasting behaviour. Crosslinking may also reduce hydrolytic enzyme access to susceptible regions of the starch structure, thereby increasing resistance to enzymatic degradation. Crosslinking-induced structural reinforcement has been associated with improved starch stability under heat, acidity, and mechanical stress (Punia Bangar *et al.*, 2024).

Chemical crosslinking can involve different classes of reagents, and the choice of crosslinking agent strongly influences the final

properties of the modified starch. Phosphate-based reagents, including sodium trimetaphosphate (STMP) and sodium tripolyphosphate (STPP), are widely investigated because they can produce phosphate-mediated linkages between starch chains. Researchers have also explored other phosphate systems and combinations of phosphate reagents to modify starch functionality. Organic polycarboxylic acids, including citric acid, adipic acid, and tartaric acid, offer another group of potential crosslinking agents because their multiple carboxyl groups can react with starch hydroxyl groups under appropriate conditions. The different chemical structures and reaction mechanisms of these crosslinkers can result in variations in the degree of crosslinking, molecular organization, crystallinity, swelling behaviour, gelatinization, pasting characteristics, and enzymatic resistance of the resulting starch.

Previous research on chemically modified starches has demonstrated that crosslinking can substantially influence starch structure and functional properties. Crosslinked starches generally exhibit restricted swelling and improved resistance to processing conditions, while changes in molecular organization may increase their resistance to enzymatic hydrolysis. The formation of covalent bonds through chemical modification is particularly relevant to RS4 development because the modified structure can remain comparatively resistant to gastrointestinal digestion. Liu *et al.* (2025) emphasized the importance of chemical modification in the development of resistant starch with altered structural and digestive characteristics. However, the magnitude and direction of crosslinking effects depend on the starch's botanical source, the crosslinking reagent, reagent concentration, reaction conditions, and extent of modification.



The literature on yam starch modification therefore requires further consideration. Although several studies have investigated chemical modification of starches from cereals, roots, and tubers, systematic evaluation of different crosslinking agents specifically for converting yam starch into RS4 remains comparatively limited. In particular, researchers have paid insufficient attention to the comparative effects of phosphate-based and organic crosslinkers on yam starch structure, physicochemical properties, enzymatic digestibility, and resistant starch formation. This is important because differences in amylose content, granule morphology, crystalline organization, molecular architecture, and other compositional characteristics among botanical starch sources can influence their response to chemical modification.

A further knowledge gap concerns the relationship between the extent of crosslinking and the nutritional functionality of the resulting RS4. Increasing crosslink density may improve resistance to enzymatic hydrolysis, but excessive modification may adversely affect hydration, gelatinization, pasting characteristics, sensory quality, or other properties required for food applications. Similarly, although resistant starch can reach the colon and serve as a substrate for microbial fermentation, you cannot assume the prebiotic potential of a particular RS4 product solely from its chemical classification. Its physiological potential depends on how much it escapes small-intestinal digestion, becomes available to colonic microorganisms, and supports the production of beneficial fermentation metabolites. These considerations highlight the need to evaluate crosslinking agents not only by their ability to modify starch chemically but also by their effects on nutritional and technological performance.

Addressing these gaps is significant for the development of value-added yam-based functional foods. Identifying appropriate crosslinking systems could provide a means of improving the thermal and mechanical stability of yam starch while increasing its resistance to enzymatic digestion. Such modifications may contribute to the development of yam-based products with potentially lower rates of starch digestion and enhanced delivery of fermentable carbohydrate to the colon. The resulting knowledge may also assist food scientists and technologists in selecting suitable food-compatible crosslinking agents, optimizing modification conditions, evaluating safety and regulatory considerations, and developing functional starch ingredients with potential applications in glycemic management and colonic health.

Accordingly, this mini-review critically evaluates the principal chemical crosslinking agents used to modify yam starch for Type 4 resistant starch (RS4) production. This review emphasises phosphate-based and organic crosslinking systems, their reaction mechanisms, effects on the structural and physicochemical properties of yam starch, influence on enzymatic digestibility and resistant starch formation, potential prebiotic and physiological benefits, safety considerations, and suitability for developing functional yam-based food products.

2.0 Chemical Modification Agents

Among starch modification strategies, chemical treatment remains the most widely used because it covalently attaches functional groups to the carbohydrate backbone, driving profound changes in physicochemical and functional properties. Starch crosslinking, a specific category of chemical modification, yields Type 4 Resistant Starch (RS4). The crosslinking process establishes covalent networks (including ether, phosphodiester, and organic ester linkages) across adjacent glucan



polymers. These intermolecular bridges supplement the native hydrogen bonding within the crystalline domains, anchoring hydroxyl groups across neighboring chains and reinforcing overall granule architecture.

Reagents utilized for the chemical crosslinking of starches and flours are broadly classified into two categories: monofunctional and bifunctional (or polyfunctional) agents. Monofunctional reagents react with a single hydroxyl position on the anhydroglucose unit, introducing nonionic, cationic, or anionic pendant groups that disrupt retrogradation and enhance pasting clarity without bridging chains. Conversely, bifunctional and polyfunctional reagents possess two or more reactive sites, enabling simultaneous esterification or etherification across distinct hydroxyl groups on separate glucan chains. Common crosslinking agents include Sodium Trimetaphosphate (STMP), Monosodium Phosphate (MSP/SOP), Sodium Tripolyphosphate (STPP), Epichlorohydrin (EPI), Phosphoryl Chloride (POCl_3), Adipic Acid, Acetic Anhydride, and their synergistic combinations.

2.1 Chemical Crosslinking Agents for Type 4 Resistant Starch (RS4) Production

2.1.1 Sodium Trimetaphosphate (STMP)

Chemical modification is an important approach for tailoring the physicochemical, structural, and functional properties of starch. Unlike physical modification, chemical treatment can introduce new functional groups or covalent linkages into the starch matrix, thereby altering molecular organization, swelling behaviour, gelatinization, pasting properties, retrogradation, and susceptibility to enzymatic hydrolysis. Among chemical modification strategies, crosslinking is particularly relevant to developing Type 4 resistant starch (RS4) because it introduces covalent linkages between starch chains and

can restrict molecular mobility and enzymatic accessibility. Depending on the reagent employed, crosslinking may generate phosphate, ester, or ether linkages between adjacent glucan chains. These intermolecular connections reinforce the starch network and can alter hydroxyl-group accessibility, water penetration, granule swelling, and the susceptibility of glucan chains to digestive enzymes.

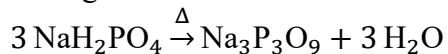
Chemical reagents used in starch modification can be broadly distinguished by their functionality. Monofunctional reagents generally introduce a single substituent onto the starch molecule without establishing a covalent bridge between two starch chains, whereas bifunctional and polyfunctional reagents possess two or more reactive sites and can form intermolecular or intramolecular bridges. This distinction is important because not every reagent used in chemically modified starch production is a true crosslinking agent. Important reagents and systems considered in starch modification include sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), monosodium phosphate (MSP), phosphorus oxychloride (POCl_3), epichlorohydrin (EPI), citric acid, adipic acid-based systems, tartaric acid, and acetic anhydride. Their individual reaction mechanisms and functional effects determine their suitability for producing starches with enhanced resistance to enzymatic digestion and, consequently, their potential contribution to RS4 development.

2.1.2 Sodium Trimetaphosphate (STMP)

Sodium trimetaphosphate (STMP) is one of the most extensively investigated phosphate-based reagents for chemical starch modification. It is a cyclic triphosphate that reacts with hydroxyl groups on starch under alkaline conditions to form phosphate ester linkages. STMP can be synthesized through the thermal condensation



of monobasic sodium phosphate according to the following reaction:



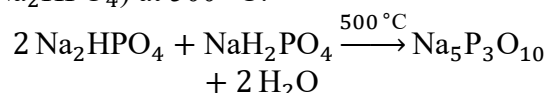
Under alkaline reaction conditions (pH 10.5–11.5), STMP undergoes nucleophilic ring-opening substitution, esterifying hydroxyl groups on adjacent glucan chains to form distarch phosphate diester linkages. These covalent bridges predominantly link the C-6 position of one anhydroglucose unit with the C-2 or C-3 position of a neighbouring unit (Chandak *et al.*, 2022). This phosphorylation mechanism generates intra- and intermolecular bridges that constrict the granule's internal network, restrict water uptake, and bolster thermo-mechanical stability, resulting in elevated gelatinization temperatures and reduced peak paste viscosity relative to native starch (Chandak *et al.*, 2022; Shaltout *et al.*, 2025).

A primary advantage of STMP over reagents such as epichlorohydrin is its superior safety profile: it is regulatory-approved for food applications and yields non-toxic reaction byproducts, making it a premier phosphorylating agent for functional food development (Shaltout *et al.*, 2025). Recent studies highlight its functional versatility: Korkut & Kahraman (2021) observed that STMP-crosslinked tapioca starch displayed controlled changes in digestibility, morphology, and crystalline integrity governed by reaction pH and temperature. Similarly, Sida-Arreola *et al.* (2024) combined STMP with sodium tripolyphosphate to dually modify oat and apple starches, reporting heightened freeze-thaw stability and altered rheological performance. Butrim *et al.* (2025) showed that single-stage cationization and STMP crosslinking of potato and corn starches preserved granular morphology across diverse temperature profiles, though phosphorylation efficiency declined at higher STMP

concentrations. Collectively, these findings confirm STMP's efficacy in generating thermally robust, enzyme-resistant RS4 for food, packaging, and bio-adsorbent platforms.

2.1.2 Sodium Tripolyphosphate (STPP)

Sodium tripolyphosphate (STPP) is a linear polyphosphate synthesized via thermal condensation of sodium dihydrogen phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) at 500 °C:



STPP behaves differently from cyclic STMP in starch modification because its molecular structure and reactive phosphate groups influence the nature and extent of phosphorylation. The reaction is strongly dependent on pH. Under some conditions, STPP may predominantly introduce monostarch phosphate groups, whereas under more strongly alkaline conditions it may participate in reactions leading to phosphate linkages between starch chains. Punia Bangar *et al.* (2024) discussed the influence of reaction conditions on phosphate-based starch modification and emphasized the importance of pH in determining the extent and nature of phosphorylation.

Evidence from broader biopolymer systems also demonstrates the ability of STPP to participate in crosslinking reactions. Bhatia *et al.* (2024) used STPP to crosslink sodium alginate–chitosan composite films and reported changes in film thickness, elongation at break, and moisture retention. Although these findings demonstrate STPP's crosslinking potential in non-starch polysaccharide matrices, they should not be interpreted as direct evidence of equivalent behaviour in yam starch. For starch-based RS4 production, STPP is particularly relevant when considered as part of combined phosphate modification systems, especially in association with STMP.



2.1.3 STMP/STPP Blends

STMP and STPP are frequently investigated together as a dual-phosphate modification system. The combination allows the different chemical characteristics of the two phosphate reagents to be exploited in a single modification process. In such systems, STMP can contribute substantially to phosphate-mediated crosslinking, whereas STPP can provide additional phosphorylation and influence the distribution of phosphate-containing structures. The proportions of the two reagents, reaction pH, temperature, and treatment time must be optimized to obtain the desired degree of modification and functional characteristics (Punia Bangar *et al.*, 2024).

Several studies have demonstrated the potential of STMP/STPP systems to increase digestive resistance and modify the functional characteristics of starches. Li *et al.* (2022) modified lotus seed starch using STMP/STPP and employed ^{31}P NMR to investigate phosphate-containing structures. Their results indicated that distarch phosphate formation increased with crosslinking dosage and was associated with changes in thermal stability and enzymatic digestibility. Fang *et al.* (2023) investigated STPP/STMP modification of purple potato starch and reported changes in pasting behaviour and structural characteristics associated with variations in degree of substitution. Malik *et al.* (2023) reported increased resistance to gastrointestinal digestion following STPP/STMP phosphorylation of mandua (finger millet) starch. Gu *et al.* (2024) further demonstrated that STMP/STPP modification produced source-dependent changes in the pasting and freeze-thaw properties of waxy wheat, maize, and tapioca starches.

These findings indicate that STMP/STPP systems can help manipulate starch structure and digestive behaviour. However, the

response remains dependent on the botanical source and modification conditions. Applying these methods to yam starch therefore requires assessing crosslinking efficiency, resistant starch formation, physicochemical properties, and enzymatic digestibility, rather than extrapolating directly from other starch sources.

2.1.4 Phosphorus Oxychloride (POCl_3)

Phosphorus oxychloride (POCl_3) is a highly reactive phosphorylating reagent that can form phosphate linkages between starch chains. Its high reactivity allows substantial structural modification at relatively low reagent concentrations. Under strongly alkaline conditions, POCl_3 can react with hydroxyl groups on neighbouring glucan chains to produce distarch phosphate linkages, while side reactions may result in monophosphate substitution. The reaction conditions, including pH and the presence of neutral salts, influence reagent distribution and the extent of starch modification (Punia Bangar *et al.*, 2024).

Because POCl_3 is highly reactive, careful control of dosage and processing conditions is necessary. Neutral salts such as sodium sulfate may be incorporated into the reaction system to influence granule integrity and reagent distribution. Punia Bangar *et al.* (2024) described the role of such reaction conditions in phosphate-mediated starch modification.

Studies involving different botanical starches demonstrate the effectiveness of POCl_3 in modifying starch structure. Ibikunle *et al.* (2022) investigated banana and plantain starches subjected to STPP phosphorylation followed by POCl_3 crosslinking and reported pronounced changes in physicochemical and pasting characteristics. Xu *et al.* (2024) investigated POCl_3 -crosslinked normal and waxy maize starches and observed changes in swelling power, solubility, pasting behaviour, pseudoplasticity, and viscoelastic properties.



Removing surface lipids further influenced crosslinking efficiency.

Although POCl_3 can produce substantial structural modification, its high reactivity and associated handling and regulatory considerations are important to consider in food applications. Therefore, its potential for RS4 development must be evaluated not only in terms of crosslinking efficiency but also in terms of processing control, residual reagent considerations, and food-safety requirements.

2.1.5 Monosodium Phosphate (MSP/SOP)

Monosodium phosphate (NaH_2PO_4), also referred to as MSP or SOP, is a monofunctional phosphate reagent that can react with starch hydroxyl groups to introduce phosphate ester substituents. Unlike polyfunctional phosphate reagents such as STMP, MSP does not principally establish intermolecular covalent bridges between separate glucan chains. It is therefore more appropriately classified as a phosphorylating or esterifying reagent than as a primary starch crosslinker (Gałkowska *et al.*, 2023).

Nevertheless, MSP can contribute to starch modification by altering charge characteristics, hydration behaviour, pasting properties, and molecular interactions. Rożnowski *et al.* (2021) investigated orthophosphate-based modification of potato starch and reported that reaction temperature and duration influenced retrogradation resistance and rheological properties. Sanyaolu *et al.* (2021) reported changes in the pasting and functional characteristics of cassava and red cocoyam starch following MSP treatment. Rohima *et al.* (2025) investigated MSP esterification combined with heat-moisture treatment in potato starches from different cultivation altitudes and reported improved thermal stability relative to native starch.

MSP can also be incorporated into more complex phosphate modification systems.

Saberian & Forouhar (2025) reported that optimizing mono- and disodium phosphate ratios before STMP crosslinking could produce phosphated distarch phosphate (E1413) with improved emulsion stability and reduced syneresis in stirred yogurt. Thus, although MSP should not be regarded as a principal crosslinker, its role as a phosphorylating reagent or component of multi-stage phosphate modification systems may be relevant to developing functional RS4 starches.

2.1.6 Epichlorohydrin (EPI)

Epichlorohydrin (EPI) is a bifunctional organochlorine reagent that can form ether linkages between starch chains. Under alkaline conditions, the epoxide functionality of EPI can react with starch hydroxyl groups, forming glycerol ether linkages that bridge adjacent glucan chains (Li *et al.*, 2023). These covalent bridges can reinforce the starch matrix and restrict molecular mobility, thereby affecting swelling, pasting behaviour, thermal stability, and resistance to enzymatic degradation.

EPI crosslinking has been associated with changes in paste clarity, acid and shear resistance, retrogradation, and freeze-thaw stability (Zhang *et al.*, 2022). Chen *et al.* (2022) reported that EPI modification could reduce susceptibility to α -amylase hydrolysis and increase resistant starch fractions. These characteristics demonstrate EPI's potential to produce structurally reinforced starches.

However, toxicological considerations constrain the potential use of EPI in food-oriented RS4 production. Epichlorohydrin can present health risks through ingestion, inhalation, and dermal exposure, particularly when unreacted residual material remains in the modified starch (Li *et al.*, 2022). Consequently, food applications require strict control of reaction conditions and residual levels. Food-compatible alternatives, including inorganic phosphate systems and organic



polycarboxylic acids, may therefore offer more attractive options for developing food-grade RS4 products (Li *et al.*, 2022).

2.1.7 Citric Acid

Citric acid is a tricarboxylic organic acid that has attracted considerable interest as a food-compatible reagent for starch modification. Under appropriate thermal conditions, citric acid can dehydrate to form reactive anhydride intermediates, which then react with starch hydroxyl groups to form ester linkages (Golachowski *et al.*, 2020). Depending on the extent of reaction, monoesterification or multiple esterification reactions can occur, with the latter potentially producing crosslinked starch structures.

Reaction temperature, acid concentration, reaction time, and starch substrate characteristics strongly influence the degree of modification. Cavallo *et al.* (2025) reported resistant starch contents approaching 100% under optimized experimental conditions for citric-acid-modified corn starch and identified reaction temperature as an important factor controlling crosslinking density. Abedi *et al.* (2025) similarly reported that freeze-thaw pretreatment could increase the degree of modification and that citric acid produced greater crosslinking density and slower digestion than the mono- and dicarboxylic organic acids examined.

Excessive acid concentration, however, may promote hydrolytic degradation rather than beneficial crosslinking. Chen *et al.* (2021) reported maximum crosslinking efficiency of 56% at 0.6 wt% citric acid in tapioca starch films, whereas higher concentrations resulted in localized acid hydrolysis and reduced structural integrity. Catalytic systems may also accelerate the otherwise relatively slow esterification reaction. Liang *et al.* (2025) demonstrated that p-toluenesulfonic acid could accelerate citric acid crosslinking and improve the water resistance of starch-based adhesives.

Researchers have also investigated citric acid modification in starch-based polymer blends. Jozinović *et al.* (2024) examined citric-acid-modified thermoplastic starch/poly lactide systems and highlighted the importance of balancing thermo-mechanical performance with biodegradability. For yam starch, citric acid is therefore of particular interest because its food compatibility, multiple carboxyl groups, and capacity to produce ester linkages make it a potential alternative to less food-compatible synthetic crosslinkers.

2.1.8 Adipic Acid and Acetylated Distarch Adipate Systems

Adipic acid is a dicarboxylic acid that can participate in starch esterification and crosslinking. In starch modification, adipic acid is often considered in mixed acetic acid/adipic anhydride systems rather than as a standalone crosslinker. Such systems can introduce adipate bridges together with acetyl groups, producing acetylated distarch adipate (E1422). The adipate bridges provide structural reinforcement, whereas acetyl substitution can modify interchain interactions, swelling, paste clarity, and retrogradation behaviour (Punia Bangar *et al.*, 2024).

The functional effects of adipate-based modification have been demonstrated in several non-conventional starch sources. Mohammadi *et al.* (2022) optimized modification of amaranth starch using an adipic acid/acetic anhydride mixture and reported increases in swelling power, solubility, viscosity, and clarity, together with a reduction in gelatinization enthalpy. Zakeri *et al.* (2023) investigated a similar mixed-anhydride system in quinoa starch and reported increased water absorption and structural stability. Mahmud (2022) combined adipic acid esterification with alkaline and mustard-oil treatments to develop modified biomaterials.



More recently, Abedi-Firoozjah *et al.* (2026) highlighted adipic acid as a potentially greener crosslinking reagent for biopolymer films and discussed its advantages relative to some synthetic crosslinkers. Nevertheless, for food-grade RS4 development, researchers must carefully evaluate the specific reaction system, degree of modification, residual reagents, and resulting digestibility. Evidence from amaranth, quinoa, and other starches provides useful mechanistic information, but targeted research is still required to establish the effectiveness of adipic acid-based systems for yam starch.

2.1.9 Tartaric Acid

Tartaric acid is a naturally occurring organic acid with multiple functional groups that can participate in starch esterification under suitable reaction conditions. It has attracted attention as a potentially biocompatible alternative to some synthetic crosslinking agents. A meta-analysis of 18 studies by Rahmadani *et al.* (2024) indicated that organic acid treatments generally increased resistant starch fractions while reducing rapidly digestible starch. The same analysis suggested that hydroxy-acid treatments, including citric and malic acids, may be particularly effective and that combining organic acid treatment with hydrothermal pretreatments such as heat-moisture treatment or annealing may further increase resistant starch formation.

The effectiveness of tartaric acid modification depends strongly on thermal activation and reaction conditions. Mansur *et al.* (2025) reported that, under the conditions examined, heating above 100 °C promoted conversion of tartaric acid to reactive forms that could esterify starch hydroxyl groups, whereas effective esterification did not occur at 25 °C. In their study, tapioca starch treated with 3 M tartaric acid at 150 °C for 6 h exhibited a reported resistant starch content of 97.5% and a degree of substitution of 0.744, together with

reduced pasting viscosity. These findings demonstrate the potential of tartaric acid modification, although the reported values are specific to the experimental conditions and starch substrate used.

Tartaric acid's potential has also been demonstrated in combined modification systems. Yu *et al.* (2024) investigated tartaric, citric, and malic acid treatments combined with heat-moisture treatment in potato starch and reported substantial increases in cooked resistant starch relative to native starch while retaining prebiotic activity during colonic fermentation. These findings are particularly relevant to the present review because they suggest a link between chemical modification, resistant starch formation, and subsequent microbial fermentation. Nevertheless, direct evaluation of tartaric-acid-modified yam starch remains necessary before assuming similar nutritional and functional outcomes.

2.1.10 Acetic Anhydride as a Monofunctional Modifying Agent

Acetic anhydride differs fundamentally from the bifunctional crosslinking agents discussed above. It is primarily a monofunctional esterifying reagent that introduces acetyl groups onto starch hydroxyl groups rather than forming covalent bridges between adjacent glucan chains. Acetylation is commonly carried out in aqueous starch slurries under mildly alkaline conditions, typically using NaOH or KOH as the catalyst (Subroto *et al.*, 2023).

Although acetic anhydride does not constitute a true structural crosslinker, acetylation can substantially modify starch functionality. The introduction of bulky acetyl groups can reduce interchain association and influence swelling capacity, water and oil retention, paste clarity, and retrogradation behaviour (Subroto *et al.*, 2023). Xu *et al.* (2025) compared acetic anhydride and vinyl acetate modification in six



underutilized starches, including proso millet, amaranth, foxtail millet, quinoa, buckwheat, and oat, and reported changes in pasting behaviour and substitution patterns. Kusumaningsih *et al.* (2023) similarly reported changes in functional and morphological characteristics following acetylation of pigmented sweet potato starches.

Acetic anhydride is relevant to crosslinked RS4 systems mainly because it is used in combination with bifunctional reagents. For example, acetylation can be combined with adipic acid-based modification to produce acetylated distarch adipate, thereby integrating the functional effects of acetyl substitution with the structural reinforcement provided by crosslinking. Consequently, acetic anhydride is better considered a complementary modifying reagent rather than a primary crosslinking agent.

2.1.11 Comparative Evaluation and Relevance to Yam Starch RS4

The chemical reagents discussed above differ substantially in their molecular structures, reaction mechanisms, food-safety considerations, and effects on starch functionality. STMP and STMP/STPP systems are particularly relevant to phosphate-mediated crosslinking and have been extensively investigated for modifying starch structure and digestive resistance. STPP can contribute to phosphate modification, although its behaviour depends strongly on reaction conditions, and it may be more effective in starch when combined with STMP. POCl_3 provides highly reactive phosphate crosslinking but requires careful consideration of processing and regulatory requirements. EPI can produce stable ether crosslinks and enhance enzymatic resistance, but its toxicological profile limits its attractiveness for food applications. Organic acids such as citric and tartaric acids provide potentially food-compatible alternatives, while

adipic acid-based systems offer combined crosslinking and acetylation effects. MSP and acetic anhydride, in contrast, are principally monofunctional modifying reagents and should not be classified as primary crosslinkers, although they may contribute to multi-stage or combined modification systems.

An important consideration is that much of the available evidence comes from starches from botanical sources other than yam. Studies involving tapioca, potato, maize, oat, apple, lotus seed, purple potato, finger millet, wheat, banana, plantain, cassava, cocoyam, amaranth, quinoa, and sweet potato demonstrate the broad potential of chemical modification, but the responses of these starches cannot necessarily be extrapolated directly to *Dioscorea* starch. Differences in amylose content, amylopectin organization, granule morphology, crystalline structure, lipid and protein content, and other minor constituents can influence the accessibility of reactive hydroxyl groups and the effectiveness of crosslinking reactions.

For yam starch, therefore, selecting a crosslinking agent should be based on several interconnected criteria: efficiency of covalent modification, ability to increase resistance to gastrointestinal enzymes, preservation or improvement of desirable processing properties, compatibility with food applications, safety and regulatory acceptability, and potential fermentability of the resulting RS4. The ultimate objective is not simply to maximize the degree of crosslinking, because excessive modification may adversely affect hydration, gelatinization, pasting, sensory characteristics, or other properties required for food formulation. Instead, an appropriate balance between structural modification, digestive resistance, technological functionality, and nutritional performance is required.

Overall, the available literature indicates that chemical crosslinking provides a promising



route for increasing the resistant fraction of starch and modifying its functional properties. However, comparative evidence specifically addressing STMP, STPP-based systems, phosphate reagents, EPI, citric acid, adipic acid-based systems, and tartaric acid in yam starch remains limited. This reinforces the need for targeted investigations of yam starch to establish how crosslinker chemistry, reaction conditions, structural modification, resistant starch formation, enzymatic digestibility, physicochemical properties, and potential colonic fermentation are interrelated. This comparative evaluation would provide a stronger basis for selecting appropriate crosslinking systems for developing food-compatible yam-based RS4 ingredients.

3.0 Summary of Crosslinking Reagents for RS4 Production

Based on the chemical modification systems reviewed in Section 2.0, Table 1 summarizes the principal reagents according to their functional class, predominant linkage type, reaction characteristics, effects on starch properties, and relevant food-safety considerations. The table distinguishes true crosslinking agents from monofunctional modifying reagents because not all chemicals used in starch modification directly establish intermolecular bridges. This distinction is particularly important in the context of Type 4

resistant starch (RS4), in which chemical modification must produce sufficient structural alteration to reduce susceptibility to gastrointestinal enzymatic hydrolysis.

The reported effects are dependent on starch source, reagent concentration, reaction pH, temperature, reaction time, and processing conditions. Therefore, do not interpret the properties listed in the table as universal outcomes for yam starch. In particular, MSP and acetic anhydride are primarily modifying/esterifying agents rather than true crosslinkers, whereas the extent of crosslinking by STPP is strongly dependent on reaction conditions.

4.0 Nutritional and Health Implications

4.1 Glycemic Management and Diabetes Control

The digestibility and glycemic response of yam-based foods vary according to species, cultivar, starch composition, processing method, and food preparation conditions. Riley *et al.* (2008) reported differences in starch digestibility and glycemic responses among yam varieties and prepared yam products, indicating that the physiological behaviour of yam starch cannot be attributed solely to the crop's botanical identity. These variations provide a basis for investigating chemical modification to further regulate starch digestion.

Table 1. Summary of chemical reagents used for starch modification and RS4 development

Agent	Functional reagent class	Principal linkage/type of modification	Reaction profile and mechanism	Principal effect on starch	Regulatory/safety considerations	References
STMP	Polyfunctional phosphate	Phosphate ester; may form distarch phosphate	Ring-opening phosphorylation under alkaline conditions; reaction conditions influence crosslink formation	Improves thermal and shear stability, restricts swelling,	Widely used for food starch modification under controlled	Shaltout <i>et al.</i> (2025)



				and can increase resistance to enzymatic digestion	and regulated conditions	
STPP	Polyfunctional phosphate	Predominantly phosphate monoester; crosslinking may occur depending on conditions	pH-dependent phosphorylation; extent of intermolecular crosslinking depends on reaction conditions	Alters water binding, pasting behaviour, and starch functionality; crosslinking efficiency may be limited when used alone	Used in food-related starch modification; application and dosage require regulatory control	Punia Bangar <i>et al.</i> (2024)
STMP/S TPP	Combined phosphate system	Mixture of monoester and diester phosphate structures	Combined phosphorylation/crosslinking; reagent ratio and reaction conditions require optimization	Can increase digestive resistance and modify thermal, pasting, and freeze-thaw properties	Relevant to food-grade starch modification; optimal formulation is system-dependent rather than universal	Punia Bangar <i>et al.</i> (2024)
POCl₃	Highly reactive phosphorylating/crosslinking reagent	Distarch phosphate and related phosphate structures	Rapid phosphorylation/crosslinking under alkaline conditions; salt concentration and pH influence reaction	Produces strong structural modification and can improve resistance to swelling and enzymatic hydrolysis	Highly reactive; use in food applications requires strict process and residual-level control	Punia Bangar <i>et al.</i> (2024)



MSP	Monofunctional phosphate	Monostarch phosphate	Phosphorylation of starch hydroxyl groups; does not primarily form intermolecular bridges	Can alter solubility, pasting behaviour, hydration, and thermal properties; may serve as a component of phosphate modification systems	Food-related phosphate modification requires appropriate regulatory control	Rožnowski <i>et al.</i> (2021)
EPI	Bifunctional organochlorine reagent	Ether linkage; distarch glycerol-type bridge	Nucleophilic ring-opening reactions involving starch hydroxyl groups	Produces relatively stable ether crosslinks and can improve acid, shear, and enzymatic resistance	Toxicological concerns associated with residual EPI restrict its attractiveness for food applications	Li <i>et al.</i> (2022)
Citric acid	Polycarboxylic acid	Ester and potential diester/crosslinked structures	Thermal conversion to reactive anhydride species followed by esterification of starch hydroxyl groups	Can increase structural stability and resistant starch under suitable reaction conditions	Food-compatible organic acid; reaction severity and residual acidity must be controlled	Golachowski <i>et al.</i> (2020)
Adipic acid-based systems	Dicarboxylic acid/mixed anhydride system	Adipate ester bridges with possible acetyl substitution	Esterification/crosslinking, commonly using mixed acetic-anhydride systems	Can improve structural stability, swelling behaviour, clarity, and other functional	Food applications require control of reagent levels and compliance with applicable	Mohammadi <i>et al.</i> (2022)



				l propertie s	additive regulations	
Tartaric acid	Hydroxycarboxylic acid	Ester/diester structures	Thermally activated esterification; reaction efficiency depends strongly on temperature and treatment conditions	Can increase resistant starch and alter thermal and pasting propertie s	Naturally occurring food acid; suitability for RS4 depends on controlled processing conditions	Mansur <i>et al.</i> (2025)
Acetic anhydride	Monofunctional anhydride	Acetyl ester	Alkaline acetylation of starch hydroxyl groups	Improves clarity and can modify swelling, pasting, and storage propertie s; does not crosslink starch by itself	Used as an acetylating reagent in regulated starch modification systems	Subroto <i>et al.</i> (2023)

Chemical crosslinking can introduce intra- and intermolecular covalent linkages within the starch matrix, thereby restricting polymer-chain mobility and reducing the accessibility of digestive enzymes to susceptible glucan regions (Fig.1). Evidence from non-yam starches indicates that phosphate crosslinking can reduce in-vitro starch digestibility (Zhang *et al.*, 2025). Furthermore, Bangar *et al.* (2024) reported lower postprandial glucose and insulin responses after consuming a crosslinked resistant starch preparation than control foods in a human study. These findings provide evidence that chemically modified resistant starch can influence carbohydrate digestion and postprandial metabolic responses, although the findings cannot automatically be extrapolated to crosslinked yam starch.

The nutritional rationale for developing yam-based RS4 is therefore based on the possibility of shifting a proportion of rapidly digestible starch (RDS) toward a chemically resistant fraction that escapes digestion in the small intestine. Such a shift could potentially reduce the rate of glucose release following consumption. Studies involving resistant starch have reported reductions in postprandial glycemic responses under specific experimental conditions (Mah *et al.*, 2018; Rosida & Susiloningsih, 2020). Runingsari *et al.* (2026) further discussed the potential application of RS4-enriched staple-food matrices as a strategy for modifying carbohydrate quality. However, it is important to distinguish potential nutritional functionality from demonstrated clinical efficacy. The ability of crosslinked yam starch to attenuate



postprandial glycemia, moderate insulin responses, or contribute to long-term glycemic control remains to be established through direct human studies. Consequently, crosslinked yam

starch should currently be regarded as a promising candidate for glycemic-management-oriented food development rather than as an established therapeutic intervention.

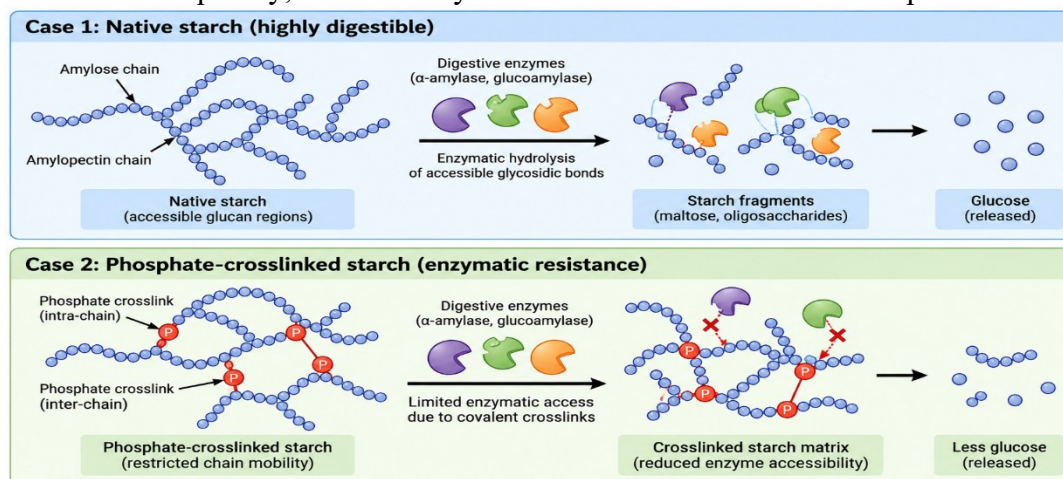


Fig. 1. Proposed mechanism of enzymatic resistance in phosphate-crosslinked starch

In Case 1, digestive enzymes hydrolyse accessible glycosidic regions of native starch and release glucose. In Case 2, covalent phosphate-mediated crosslinks restrict enzyme accessibility and reduce hydrolysis of the modified starch matrix, thereby increasing the fraction of starch that may resist small-intestinal digestion.

4.2 Gut Health, Short-Chain Fatty Acids, and Potential Colonic Effects

Resistant starch is defined as the fraction of dietary starch that escapes digestion and absorption in the small intestine and reaches the large intestine, where it may become available for microbial fermentation. Depending on its chemical structure and degree of modification, RS4 may therefore provide a substrate for selected colonic microorganisms (Hamanaka *et al.*, 2020).

Microbial fermentation of resistant starch can produce short-chain fatty acids (SCFAs), principally acetate, propionate, and butyrate. These metabolites are important products of carbohydrate fermentation and may influence the colonic environment and host-microbiota

interactions. Upadhyaya *et al.* (2016) reported that some crosslinked RS4 preparations increased cecal acetate and propionate concentrations in rats, although butyrate responses differed among preparations. In human studies, RS4 consumption has also been associated with changes in gut microbial composition and fecal SCFA concentrations. However, the magnitude and direction of these responses depend on the chemical structure and degree of crosslinking of the resistant starch.

The extent of fermentation may also be influenced by the degree of chemical modification. Le Thanh-Blicharz *et al.* (2014) and Upadhyaya *et al.* (2016) reported relatively low breath-hydrogen responses following consumption of highly crosslinked phosphate starches in specific experimental settings, suggesting that some highly crosslinked materials may be relatively resistant to microbial fermentation. Hamanaka *et al.* (2020) similarly demonstrated that the structural characteristics of chemically modified starch influence its digestive and fermentative behaviour. Thus, high RS4



content does not necessarily mean that all resistant material will be extensively fermented in the colon.

SCFAs and changes in colonic pH may influence the intestinal microbial environment. Scott *et al.* (2008), for example, demonstrated in an infant-gut context that fermentation products and lower faecal pH were associated with changes in potential-pathogen abundance, although those findings specifically involved *Bifidobacterium infantis* and human-milk oligosaccharides and should not be directly extrapolated to yam-derived RS4. Evidence from yam and resistant starch studies nevertheless supports further investigation. Martinez *et al.* (2010) reported that uncooked yam intake in mice reduced fecal pH, increased bifidobacteria, decreased *Clostridium perfringens*, and increased fecal SCFA production. Separate human RS4 research has also reported increases in *Bifidobacterium adolescentis*, although considerable inter-individual variability was observed.

These findings suggest that yam-derived resistant starch may have potential effects on the gut microbiota and colonic fermentation. Nevertheless, direct evidence for crosslinked yam starch remains limited. The effects of a particular RS4 preparation depend on its degree of crosslinking, chemical structure, accessibility to microbial enzymes, and interactions with the resident microbiota. Similarly, claims concerning epithelial integrity and colonic protection should be interpreted cautiously because evidence from yam starch and resistant starch has been generated using different animal models and preparations (Wang *et al.*, 2026). Therefore, targeted fermentation studies and well-controlled human trials are required to determine whether crosslinked yam starch can produce reproducible changes in microbiota composition, SCFA production, colonic environment, or epithelial function.

5.0 Recommendations for Future Research

Although chemical crosslinking provides a promising route for modifying yam starch toward Type 4 resistant starch (RS4), targeted empirical research is required to translate the demonstrated chemical and physicochemical effects into food products with validated nutritional functionality. Existing evidence indicates that the effectiveness of starch crosslinking is influenced by intrinsic structural characteristics, including amylose content, amylopectin organization, granule morphology, and crystalline structure. These characteristics vary among *Dioscorea* species and cultivars and may consequently affect reagent accessibility, crosslinking efficiency, digestibility, and functional properties. In addition, an appropriate balance must be achieved among RS4 yield, processing functionality, safety, sensory quality, and consumer acceptability. Future research should therefore prioritize the following areas.

5.1 Optimization of Crosslinker Combinations and Dual Modification Systems

Future studies should systematically compare single and combined modification systems, including STMP/STPP phosphate systems and mixed acetylation-crosslinking approaches such as acetic-adipic anhydride systems. Rather than assuming a universal optimum such as a 99:1 STMP/STPP ratio, experimental designs should establish the optimum reagent ratio specifically for each yam starch source. The effects of these combinations should be evaluated in relation to RS4 yield, degree of crosslinking, paste clarity, swelling power, retrogradation, thermal stability, rheological behaviour, and enzymatic digestibility.

5.2 Standardization of Species- and Cultivar-Specific Reaction Parameters

Systematic comparisons should be undertaken among major yam species, including *Dioscorea rotundata*, *Dioscorea alata*, and



Dioscorea esculenta. Differences in amylose-to-amylopectin ratio, granule size, crystallinity, and other compositional characteristics may influence the efficiency of chemical modification. Optimization should therefore consider reaction pH, temperature, starch-slurry concentration, reagent dosage, reaction time, catalyst concentration, and post-reaction washing conditions. Particular attention should be given to degree of crosslinking and phosphorus incorporation for phosphate-modified systems.

5.3 Development of Food-Compatible and Environmentally Preferable Modification Systems

Further research should evaluate food-compatible organic crosslinkers such as citric and tartaric acids, particularly when combined with physical or hydrothermal pretreatments such as heat-moisture treatment (HMT) and annealing. Such combinations may improve resistant starch formation while potentially reducing the amount of chemical reagent required. However, the environmental advantages of a reagent should be assessed using appropriate measures of reagent consumption, energy requirements, wastewater generation, and overall process efficiency rather than assumed solely from its natural origin.

5.4 Validation of In-Vitro and In-Vivo Nutritional Performance

Future studies should combine standardized in-vitro gastrointestinal digestion procedures, including the Englyst approach where appropriate, with controlled assessment of rapidly digestible starch, slowly digestible starch, and resistant starch fractions. Promising formulations should subsequently be evaluated using appropriate human studies measuring postprandial glucose and insulin responses. Where claims concerning prebiotic functionality are proposed, researchers should

additionally employ controlled colonic fermentation models and characterize microbiota composition and SCFA production. These complementary approaches are necessary to establish whether chemical resistance observed in vitro translates into meaningful physiological effects.

5.5 Evaluation of Food-System Integration and Sensory Quality

Modified yam starches should ultimately be incorporated into realistic food matrices, including reconstituted yam flour, baked products, extruded snacks, noodles, and other yam-based formulations. Evaluation should include water absorption, swelling, gelatinization, pasting behaviour, rheological characteristics, texture, colour, flavour, sensory acceptability, and storage stability. Because extensive crosslinking may improve digestive resistance while adversely affecting desirable processing or sensory characteristics, optimization should focus on the overall performance of the food product rather than RS4 yield alone.

5.6 Safety, Regulatory Compliance, and Residual-Reagent Assessment

Future studies should explicitly quantify residual reagents and reaction by-products, particularly for highly reactive systems such as POCl_3 and epichlorohydrin. Food-grade status should not be inferred solely from a reagent's chemical identity; compliance depends on intended use, concentration, processing conditions, residual levels, and applicable regulatory requirements. Comparative safety assessment should therefore accompany the evaluation of crosslinking efficiency when selecting reagents for food-grade yam RS4.

5.7 Linking RS4 Formation with Fermentation and Prebiotic Functionality

RS4 formation should be distinguished from proof of prebiotic activity. Future



investigations should determine the proportion of chemically modified yam starch that survives standardized small-intestinal digestion and subsequently assess its fermentability using representative human colonic microbiota. Measurements should include SCFA production, changes in microbial composition, gas production, and other relevant fermentation parameters. This would help determine whether a highly crosslinked yam starch that exhibits high enzymatic resistance also provides an effective substrate for beneficial colonic fermentation.

5.8 Translation from Laboratory-Scale Modification to Industrial Processing

Finally, validate laboratory findings at pilot and larger scales. Scale-up studies should examine mixing efficiency, reagent distribution, reaction uniformity, water and energy requirements, washing and purification requirements, drying costs, process yield, and reproducibility. Economic and environmental assessments should be integrated with nutritional and functional evaluations to identify crosslinking systems that are not only chemically effective but also technically feasible and commercially viable for yam-based RS4 production.

6.0 Conclusion

Taken together, the chemical crosslinking agents evaluated in this mini review converge on a unified structural transformation when applied to yam (*Dioscorea* spp.) and related tuber starches: the establishment of covalent phosphodiester, phosphomonoester, ether, or ester linkages that reinforce the internal hydrogen-bonded network of the starch granule. Regardless of the specific chemical reagent employed, this covalent reinforcement qualifies the modified polysaccharide as Type 4 Resistant Starch (RS4), as the resulting spatial reorganization sterically hinders

salivary α -amylase, pancreatic α -amylase, and brush-border glucoamylase from accessing α -(1,4) and α -(1,6) glycosidic bonds, thereby substantially curbing the rate and extent of enzymatic hydrolysis. For yam starch specifically, this molecular reinforcement directly resolves the nutritional limitations inherent in native yam starch, which exhibits rapid digestibility and triggers sharp postprandial blood glucose surges that complicate metabolic management for individuals with prediabetes or type 2 diabetes mellitus. By converting a major fraction of rapidly digestible starch (RDS) into resistant starch (RS4), crosslinking reduces the effective glycemic load of yam-based food matrices, flattening postprandial glucose and insulin excursions while supporting long-term insulin sensitivity. Furthermore, crosslinked yam starch that evades upper gastrointestinal digestion reaches the large intestine intact, serving as a fermentable substrate for anaerobic microbiota to produce short-chain fatty acids (SCFAs), particularly butyrate, which maintain mucosal integrity, lower luminal pH, and foster colonic health. Ultimately, chemical crosslinking using food-grade phosphate agents (STMP, STPP, POCl_3 , MSP), polycarboxylic acids (citric, adipic, tartaric acids), or mixed anhydride systems represents a mechanistically sound strategy for upgrading yam starch into a functional, diabetic-friendly ingredient that simultaneously supports glycemic regulation and colonic well-being.

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Compliance with Ethical Standards Declaration

Ethical Approval

Not Applicable

Competing interests

The author declares no known competing financial interests.

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Author's Contribution

Buhari Lenfa Nuhu conceptualized the review and coordinated manuscript preparation. Elizabeth Tomilayo Akinterinwa contributed to literature review, microbiological and prebiotic aspects, and interpretation. Ayo Akinterinwa contributed to literature acquisition, chemical cross-linking analysis, and manuscript development. Peter Michael Dass contributed to starch chemistry, critical analysis, and revision. Ebun Peace Oladele contributed to literature synthesis, editing, critical review, and final approval of the manuscript.

