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Functional Physical and Chemical Properties of Crosslinking Modified Starch

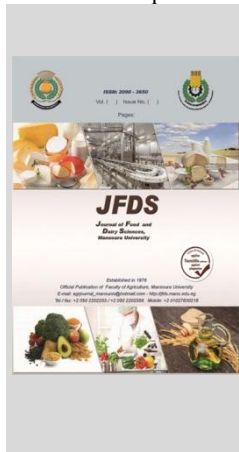
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ABSTRACT

Starch is a crucial component in food and other industries. Modified starch possesses superior qualities compared to native starch. Phosphorylation is one of the common modifications used to enhance the functional properties of starch and alter its characteristic behaviors, such as gelatinization, pasting, and retrogradation. Native corn starch was modified through cross-linking with tetra potassium pyrophosphate (TKPP) at three different concentrations 1%, 2%, and 3% to produce diphosphate starch. The resulting modified starches were characterized based on their chemical and physical properties. Modification with TKPP significantly ($P \leq 0.05$) reduced the moisture, acidity, and fat content of the starch samples, while increasing their protein and ash content compared to native starch. TKPP modification also led to a significant ($P \leq 0.05$) increase in viscosity, water absorption, and oil absorption values, with higher TKPP concentrations resulting in more pronounced effects. However, a significant decrease in gelatinization values ($P \leq 0.05$) was observed. Scanning electron microscopy showed that the starch granules in the native corn starch sample were smaller compared to those in the chemically modified starch, with notable alterations in granule morphology. The application of corn starch modified with 3% TKPP resulted in yogurt with similar properties firmness, viscosity, and syneresis—to that made using commercial starch.

Keywords: Corn Starch – Modified – Cross-linking – Phosphorylation.

INTRODUCTION

Starch, the second most abundant naturally occurring polysaccharide after cellulose, is found in the roots, seeds, and leaves of numerous plants, as well as in some algae. Typically, starch granules consist of two primary components: amylopectin—a highly branched polymer with numerous short chains linked to the linear portions of the macromolecule via α -(1 \rightarrow 6) linkages and amylose, a linear glucose polymer with α -(1 \rightarrow 4) linkages (Fan & Picchioni, 2020). The properties of starch, which determine its various applications, depend on the amylose content and the structural characteristics of amylopectin (Kong *et al.*, 2008).

Native starch granules have limitations in industrial applications due to poor solubility, retrogradation, syneresis, thermal decomposition, and high viscosity. These limitations have led to the development of various starch modification techniques (Liu *et al.*, 2011; Compart *et al.*, 2023).

The term “starch modification” refers to treatments that alter the structure and functionality of starch, including processes such as debranching, cross-linking, chain length modification, pre-gelatinization, and disproportionation. With more people wanting processed and easy-to-eat foods, the demand for altered starch has grown, leading to its use in many different industries. This expansion is fueled by new technologies and creative applications in both food and non-food areas. Because it has significant economic and social effects, modifying starch is predicted to become even more important in the future. Usually, these modifications are done outside of the plant using physical, chemical, and/or enzymatic techniques (Compart *et al.*, 2023).

Chemical alterations usually don't break down the main structure of starch quickly and effectively these changes happened depend on the kind and amount of the reacting substance and the environment in which the reaction takes place. The shape of the starch granules, the proportion of amylose to amylopectin, and where the starch comes from. Generally, chemical modifications of starch are categorized as cross-linking, esterification, etherification, oxidation, grafting, and acid hydrolysis (Wang *et al.*, 2020).

Crosslinking is one of the most commonly used methods for modifying native starch. It restricts the ability of starch granules to swell during heating and can also inhibit gelatinization. Even very low concentrations of crosslinking reagents can effectively modify starch (Cui, 2005). The efficiency of starch crosslinking depends on multiple variables, such as the starch source, the concentration and composition of the crosslinking reagent, degree of substitution, reaction time, temperature, and pH (Ashogbon & Akintayo, 2014; Blennow, 2015). Numerous crosslinking reagents have been identified. The Food and Drug Administration (FDA) has approved the use of phosphoryl chloride, sodium tri metaphosphate (STMP), adipic-acetic mixed anhydride, and combinations of STMP with sodium tripolyphosphate (STPP) as safe crosslinking agents for starch intended for food use. enhances dispersion clarity and results in higher viscosities (Wurzburg, 2006). Increasing phosphate content also reduces the pasting temperature while slightly raising the peak viscosity (Fortuna *et al.*, 1990). Therefore, this study investigates how cross-linking corn starch with tetra potassium pyrophosphate (TKPP), a food-grade phosphorylating agent, modifies its physicochemical

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properties including viscosity profiles, thermal stability, swelling power, pasting characteristics, water solubility index, and sediment volume formation. The research specifically examines TKPP's effects as an efficient cross-linker due to its ability to introduce phosphate groups into starch molecules, thereby altering their structural and functional properties relevant for food applications.

MATERIALS AND METHODS

Materials:

1-Corn Starch:

Food-grade native corn starch was sourced from the Egyptian Starch and Glucose Manufacturing Company, Mousturd Factory, Egypt.

2- Chemicals:

Tetra potassium pyrophosphate (E 450, food grade) was procured from Prayon S.A., Rue Joseph Wauters 144, 4480 Engis, Belgium. All other analytical-grade reagents were supplied by Sigma-Aldrich Co. (St. Louis, MO, USA).

Methods:

1. Preparation of Cross-Linked Corn Starch:

Di-starch phosphate (E 1412) was prepared by treating an aqueous slurry of native starch (21–22 Bu, approximately 460 g/L) with tetra potassium pyrophosphate (TKPP) under alkaline conditions (pH 10–10.5) for one hour at 25–30 °C. After cross-linking, the reaction mixture was neutralized to pH 7–8 using acid. The product was then thoroughly washed with water, dewatered, and dried. TKPP was added at concentrations of 1%, 2%, and 3% (w/w) to the aqueous starch suspension.

2. Characterization of native and modified starch: proximate chemical composition of corn starch:

Moisture, ash, crude fiber, lipid, and protein contents of the starch samples were determined using proximate analysis according to the methods of the American Association of Cereal Chemists (AACC, 2012).

Phosphorus Content

The phosphorus content in starch samples was quantified using UV-Vis spectroscopy at 385 nm wavelength according to Passauer *et al.* (2010). For sample preparation, 0.2 g of starch was subjected to acid digestion in a micro-Kjeldahl apparatus, initially treated with 2 mL concentrated nitric acid (HNO₃) with heating until cessation of brown fume evolution. After cooling, the digestion was continued with 1.5 mL each of 60% perchloric acid and additional HNO₃, heating until white fumes emerged. The cooled digest was diluted with 3 mL distilled water, briefly reheated, and finally brought to 10 mL volume with distilled water. Phosphorus quantification employed the vanadomolybdate colorimetric method, where 2 mL of digest was reacted with 0.5 mL 60% perchloric acid, 1.5 mL 20 mM ammonium vanadate (in 2.4% perchloric acid), and 3 mL 3.53% ammonium molybdate solution in a final volume adjusted to 10 mL with distilled water. Color development proceeded for 30 minutes before absorbance measurement at 385 nm.

Water Absorption Capacity (WAC):

Water absorption capacity was measured following the method of Zhang *et al.* (2005). Pre-weighed, clean, dry centrifuge tubes (W) were filled with 0.5 g of starch (W1), followed by the addition of 7 mL of distilled water. The samples were incubated in a water bath at 60 °C for 30

minutes, then cooled in cold water for another 30 minutes. After centrifugation at 2683 × g for 15 minutes, the supernatant was discarded, and the tubes containing the sediment were reweighed (W2).

$$\text{WAC (g/g)} = (\text{W2} - \text{W})/\text{W1}$$

Oil Absorption Capacity (OAC):

Oil Absorption Capacity was determined according to the method described by Abdul-Hamid and Luan (2000). A 4 g sample (M) was placed into a 50 mL graduated centrifuge tube containing 20 mL of maize oil (V1). The contents were agitated for 30 seconds at 5-minute and 30-minute intervals. The tube was then centrifuged at 1500 × g for 25 minutes. The volume of unabsorbed (free) oil (V2) was recorded, and the OAC was calculated using the following formula:

$$\text{OAC (mL/g)} = (\text{V1} - \text{V2})/\text{M}.$$

Degree of Gelatinization (DG):

The gelatinization extent was quantified using the iodine-binding spectrophotometric method (Wootton *et al.*, 1971). For analysis, 200 mg starch aliquots were separately suspended in either 100 mL distilled water or 10 M KOH solution, each mixed for 5 minutes. Following centrifugation at 1500 × g for 25 minutes, 1 mL aliquots of supernatant from each treatment were diluted tenfold with distilled water. After adding 100 µL iodine reagent (0.5% I₂ in 1% KI), the solutions' absorbance was recorded at 600 nm using a Cecil Aquarius 7400 UV-visible spectrophotometer (Cambridge, UK). The degree of gelatinization (DG%) was subsequently calculated from the ratio of absorbances obtained from the aqueous and alkaline treatments, where complete gelatinization in 10 M KOH served as the 100% reference value.

$$\text{DG (\%)} = (\text{Absorbance of fresh solution} / \text{Absorbance of alkali solubilized solution}) \times 100$$

Apparent Viscosity using Brookfield Viscometer:

Apparent viscosity was measured using a Brookfield viscometer (AMETEK, Model DV1 ML VTJO). The starch sample was poured into a 250 mL beaker (7 cm in diameter and 9 cm in height) and placed in a TC500 water bath (Brookfield Engineering Inc.). The temperature was raised to 20 °C. Two minutes after immersing spindle No. 6 at 30 RPM into the sample, measurements were taken to eliminate the effects of immediate time dependency and to allow thermal equilibrium within the sample.

Microstructure of Starch Samples:

Microstructural characterization, starch specimens were mounted on conductive carbon tape and sputter-coated with a 10-nm gold-palladium layer to ensure surface conductivity. Imaging was conducted using a field-emission scanning electron microscope (FE-SEM; JEOL JSM-5200, Tokyo, Japan) operating at an acceleration voltage of 15 kV under high vacuum conditions.

3. Statistical Analysis

All experimental data are presented as mean values ± standard deviation (where applicable). Statistical significance was evaluated using one-way analysis of variance (ANOVA) in SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA), followed by post-hoc Duncan's multiple range test for pairwise comparisons. The Differences between treatment means were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

1. Chemical composition of native and modified corn starch:

Corn starch was chosen as a benchmark for comparison due to its well-established physicochemical properties. The proximate composition of native and cross-linked modified starches, treated with varying concentrations of TKPP, is presented in Table 1. The results revealed a significant difference ($P \leq 0.05$) in the composition of the four starch samples. As detailed in Table 1, modification with TKPP led to a significant ($P \leq 0.05$) reduction in the moisture content of the starch. The lowest moisture content, 6.06%, was observed in the starch modified with 1% TKPP, compared to 11.43% in the native corn starch. This reduction is attributed to the interaction between the hydroxyl ($-OH$) groups of the starch's glucose units and the bi- or poly-functional chemical reagent used in the modification. This interaction decreases the number of available $-OH$ groups for binding with water molecules, thus reducing the starch's water-binding capacity.

Table 1. Proximate physio-chemical analysis of Native corn starch compared with Chemical Modification by TKPP

Component (%)	Native Corn starch	1% TKPP	2% TKPP*	3% TKPP
Moisture	11.43 ^a ± 0.153	6.06 ^d ± 0.115	6.26 ^c ± 0.058	7.2 ^b ± 0.100
Acidity	2.17 ^a ± 0.058	1.78 ^b ± 0.076	1.6 ^c ± 0.076	1.28 ^d ± 0.076
pH	5.4 ^d ± 0.020	6.75 ^c ± 0.050	7.2 ^b ± 0.031	7.82 ^a ± 0.029
Protein	0.4 ^c ± 0.015	0.92 ^b ± 0.025	0.95 ^b ± 0.031	1.05 ^a ± 0.050
Nitrogen content	0.06 ^c ± 0.002	0.15 ^b ± 0.004	0.15 ^b ± 0.005	0.17 ^a ± 0.008
Fats	0.30 ^a ± 0.006	0.27 ^b ± 0.010	0.29 ^a ± 0.010	0.27 ^b ± 0.006
Fiber	0.27 ^{ab} ± 0.006	0.29 ^a ± 0.006	0.27 ^{ab} ± 0.015	0.26 ^b ± 0.006
Ash	0.26 ^d ± 0.010	0.3 ^c ± 0.010	0.35 ^b ± 0.015	0.46 ^a ± 0.015
Phosphorus	0 ^d ± 0.00	0.0158 ^c ± 0.000	0.164 ^b ± 0.001	0.333 ^a ± 0.001

Data are mean ± SD, n=3, means with the same letter in the same raw are not significantly different at 5%

*TKPP: Tetra potassium pyrophosphate

The ash content in the cross-linked starch samples increased with higher TKPP concentrations, with values of 0.30, 0.35, and 0.46% for starch modified with 1, 2, and 3% TKPP, respectively. This increase is due to the incorporation of phosphate groups into the amylopectin structure during modification, a similar trend observed in potato starch, which naturally contains such phosphate groups. The highest ash content was found in the starch modified with 3% TKPP, followed by those modified with 2% and 1% TKPP.

The increase in ash content may be due to the retention of by products from the reaction of potassium (K) and phosphorus (P) in the modified starch. Notably, the ash content of the modified starch remained within the permissible limits established by the British Pharmacopoeia, which stipulates that starch samples should contain less than 0.6% ash (Adebowale and Lawal, 2003).

Fat content in the samples ranged between 0.27% and 0.30%, with the native corn starch exhibiting the highest value. The slight reduction in fat content following chemical

modification suggests that TKPP-treated starches may be more resistant to rapid rancidity. This could enhance the storage stability of the modified starch.

Protein content increased in the cross-linked starches compared to the native sample. However, it is recommended to minimize both protein and lipid contents in starch, as these components can interfere with their physicochemical and functional properties (Leonel *et al.*, 2021).

Phosphorus content in the modified corn starch ranged from 0.0158% to 0.333% (w/w), whereas the native starch showed no detectable phosphorus. These values are consistent with those reported in previous studies on the phosphorylation of sago, arrowroot, and sweet potato starch (0.065–0.25% w/w) [Sugih *et al.*, 2013; 2015; 2016], as well as wheat, sago, and corn starch (0.052–0.320% w/w) [Muhammad *et al.*, 2000]. Importantly, the phosphorus content in all TKPP-modified starch samples remained below the maximum level authorized for food use (0.4%) as specified by the Food Chemicals Codex (Stahl *et al.*, 2007).

Phosphorus content serves as an indirect indicator of the degree of cross-linking, with higher phosphorus levels signifying a greater extent of cross-linking.

2. Physical analysis of Native corn starch compared with Chemical Modification by TKPP

Table 2 displays the physical properties of native and modified corn starch. The phosphorylated starch showed an increase in viscosity with higher TKPP and phosphorus content. Corn starch modified with 3% TKPP had the highest viscosity at 58,000 cP, followed by 2% TKPP with 57,230 cP, and 1% TKPP with 53,517 cP, compared to the native corn starch, which had a viscosity of 43,560 cP. These results closely align with those obtained by Chantaro *et al.* (2013) and Liu *et al.* (2022). The presence of hydrophilic phosphate groups in phosphorylated starch facilitates water penetration into the amorphous regions of the granules, resulting in higher viscosity (Nathania *et al.*, 2017).

The water and oil absorption capacities (WAC and OAC) of native corn starch were 1.51 g/g and 0.060 g/g, respectively, as shown in Table 2. Phosphorylation of corn starch, through the introduction of phosphate groups, increased both WAC and OAC. The highest values were observed in starch modified with 3% TKPP, which recorded WAC and OAC values of 1.68 g/g and 0.08 g/g, respectively. This enhancement in absorption capacity is attributed to the increased hydrophilicity and reduced molecular bonding of starch molecules in the presence of phosphate groups (Codex, 2003).

Gelatinization leads to substantial changes in the physical, chemical, and biological properties of starch. The gelatinization characteristics of oxidized and cross-linked starch are influenced by the extent of modification (Ai and Jane, 2015). Starches modified with chemical derivatives, including acetyl (Sodhi and Singh, 2005), cationic, hydroxypropyl, and phosphate (monoester) groups (Liu *et al.*, 1999), generally show a lower ΔH and gelatinization temperature compared to control starch. This is because the incorporation of chemical derivatives disrupts the crystalline structure of the starch granules, causing destabilization (Ai and Jane, 2015). Based on these findings, the results in Table 2 show a significant decrease in gelatinization values for the modified starch compared to the control, with an increase in gelatinization values corresponding to the higher percentage of TKPP used in the modification process.

Table 2. Physical analysis of Native corn starch compared with Chemical Modification by TKPP

Properties	Native Corn starch	1%TKPP	2%TKPP	3%TKPP
Particle size % (180 μ)	99.90 ^a ± 0.000	99.73 ^a ± 0.010	99.60 ^a ± 0.010	99.56 ^a ± 0.020
Apparent Viscosity	43560 ^d ± 79.37	53517 ^c ± 76.38	57230 ^b ± 220.68	58000 ^a ± 0.00
OHC	1.51 ^d ± 0.010	1.54 ^c ± 0.010	1.60 ^b ± 0.000	1.68 ^a ± 0.020
WHC	0.060 ^d ± 0.000	0.065 ^c ± 0.005	0.070 ^b ± 0.000	0.080 ^a ± 0.000
DG (C°)	70 ^a ± 1.00	68 ^a ± 1.00	68 ^{ab} ± 1.00	67 ^b ± 0.00

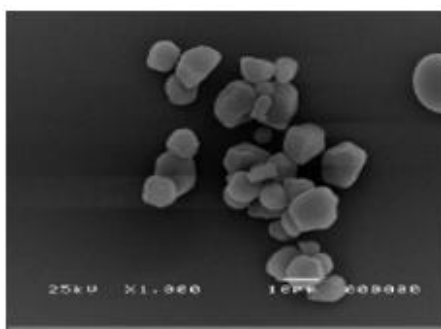
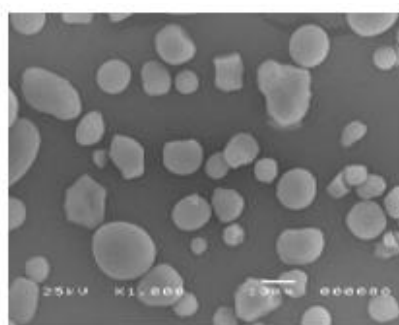
* TKPP: Tetra potassium pyrophosphate

Data are mean ± SD, n=3, means with the same letter in the same row are not significantly different at 5%

2. Scanning Electron Microscopy (SEM)

Figure 1 presents scanning electron micrographs of native and chemically modified starch samples. The scanning electron microscopy images showed that the starch granules of native corn starch were smaller compared to those of the chemically modified samples, with noticeable morphological

differences. This suggests that the cross-linking treatments used in this study significantly affected the size of the corn starch granules. Van Hung and Morita (2005) noted that the morphological characteristics of starch granules are closely related to their size.

**Fig. 1a. native corn starch****Fig. 1b. chemically modified starch****Fig. 1. (a and b). Micrographs with approximations of the samples (a) native corn starch and (b) chemically modified starch.**

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الصفات الوظيفية والكيميائية للنشا المعدل الغذائي بطريقة ربط النشا

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الملخص

النشا هو مكون من المكونات الهامة في مجال الصناعات الغذائية وغيرها. إن خواص النشا المعدل أعلى من تلك الموجودة في النشا الأصلي. تعتبر عملية الفسفرة هي أحد التعديلات الشائعة المستخدمة لتعزيز الخواص الوظيفية للنشا وتعديل سلوكه المميز (الجلتنة، اللصق، التراجع). تم تعديل الصفات الطبيعية لنشا الذرة الأصلي عن طريق الارتباط المتبادل مع ملح رباعي بيرو فوسفات البوتاسيوم (TKPP) بثلاثة تركيزات مختلفة 1، 2، 3، لإنتاج نشا ثنائي الفوسفات. وتميزت أنواع النشا المعدلة الناتجة بالخصائص الكيميائية والفيزيائية. بالتالي أدى تعديل صفات نشا الذرة باستخدام TKPP إلى انخفاض ملحوظ ($P \geq 0.05$) في محتوى الرطوبة والحموضة والدهون في عينات النشا المعدلة وزيادة محتواها من البروتين والرماد مقارنة بالنشا الأصلي. أدى تعديل النشا إلى زيادة معنوية ($P \geq 0.05$) في قيم اللزوجة وامتصاص الماء وامتصاص الزيت لعينات النشا المعدلة مع زيادة نسبة TKPP المستخدمة في التعديل. ولكن أدى ذلك إلى انخفاض واضح ($P \geq 0.05$) في درجة حرارة الجلتنة. أظهرت نتائج الفحص المجهر الإلكتروني أن حبيبات النشا في عينة نشا الذرة الأصلية كانت أصغر من مثيلاتها الموجودة في نشا الذرة المعدلة كيميائياً، وكانت هناك تغييرات مورفولوجية واضحة لحبيبات النشا الأصلية والمعدلة كيميائياً.