



## Scientific Research

### Optimizing reaction conditions for the production of phosphorylated wheat starch in order to be used in the formulation of canned foods

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## ABSTRACT

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Wheat is one of the main crops of Iran, most of which consists of starch and protein. The widespread use of starch provides many possibilities in various industries due to its reasonable price, high safety and biodegradability. However, natural starch needs to be modified for various industrial applications due to limitations such as insolubility in cold water and heat intolerance. Therefore, it is necessary to modify wheat starch in order to improve its properties and reduce the dependence on the import of modified starches. Chemical modification is one of the most efficient starch modification techniques, but it is associated with concerns such as environmental pollution and high costs of chemicals. In this study, the reaction conditions for the production of phosphorylated starch with oxychloride (Poc13) using three independent variables of pH (9.5, 10.5 and 11.5), temperature (25, 35 and 45 degrees Celsius) and reactant concentration (0.03, 0.075 and 0.12 percent), were optimized. According to the results of the physicochemical tests, the optimization criteria of starch used in canned products with higher swelling and solubility index, high transparency of dough, less gel syneresis, and higher freeze-thaw stability were selected, then additional tests were carried out in order to check structural and thermal properties. and pulping was done on selected and natural starch sample; The results showed that phosphorylation made the resulting modified starch suitable for use in the formulation of canned products by improving textural characteristics, creating higher viscosity and increasing heat tolerance.

## 1- Introduction

Wheat is one of the most important agricultural products in Iran, which has led to the establishment of multiple processing factories, including starch production facilities, in recent years. However, due to the inability to utilize native starch in various industries, it seems essential to modify wheat starch to enhance its properties and prevent the extensive import of modified starches into the country. The application of natural starch in industry is limited due to its unstable nature against changes in temperature, pH, and shear forces (high viscosity breakdown) [1]. Furthermore, natural starch is impermeable to water at ambient temperatures and is insoluble in cold water due to intermolecular and intramolecular hydrogen bonds and its semi-crystalline structure [2]. On the other hand, starch easily retrogrades; therefore, modifications are necessary to increase solubility, improve texture characteristics, and enhance heat tolerance for diverse industrial applications [3]. Various modification methods have been applied over the past decades to stabilize starch and adapt its functional properties for industrial use [4]. Any alteration in the structure of starch molecules caused by various environmental, operational, and processing factors is referred to as modification [5]. In the industry, physical, chemical, and enzymatic treatments are used to change the properties of natural starch. The differences in the physicochemical properties of starch determine its application in industry [6, 7].

Chemical modification includes oxidation, succinylation, esterification, etherification, and phosphorylation [8]. In this method of starch modification, substituting various functional groups onto the starch molecule enhances its functional properties and causes changes in gelatinization temperature, pH, rheological properties, and pasting characteristics of the starch [9]. Although chemical modification techniques are widely used and highly effective, there are significant concerns regarding environmental pollution, wastewater treatment, food safety, and the high costs associated with existing chemical modification methods [10]. For this reason, optimizing test

conditions for higher reaction efficiency and lower chemical consumption for starch modification was conducted in this research.

Chemical modification primarily relies on attaching functional groups to starch granules without any physical change in their shape. Each glucose unit in amylose and amylopectin contains three active hydroxyl groups, which are the main sites for chemical modification in starch [11]. The properties of chemically modified starch depend on treatment conditions such as pH, temperature, reactant concentration, reaction time, and the presence of a catalyst. Additionally, the source of the starch, the type of substituent, and the degree of substitution (DS) also influence the physicochemical properties of modified starches [12].

The process of producing cross-linked starches uses chemicals to bond amylose and amylopectin branches through ester linkages, thereby enhancing the strength and integrity of the granule structure. The aim of cross-linking is to create modified starch that, compared to natural starch, has a firmer gel, higher solubility, increased swelling power, and the ability to withstand processing conditions (acid, temperature, and shear) without degradation (low viscosity breakdown). Common cross-linking agents include sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), epichlorohydrin (ECH), and phosphorus oxychloride ( $\text{POCl}_3$ ). The starch used in the formulation of canned foods must be able to withstand the sterilization process temperature and maintain the desired stability and texture in the final product.

Therefore, the objective of this study is to optimize the production method of phosphorylated wheat starch using phosphorus oxychloride ( $\text{POCl}_3$ ) for use in various canned food formulations. In addition to optimizing the production conditions of phosphorylated wheat starch, this study compares the structural, thermal, gelation, pasting, and morphological changes in the optimized modified wheat starch to those of natural wheat starch.

## 2- Materials and Methods

### 2-1- Materials

High-purity wheat starch was obtained from Khoshe Zarin Starch Company. The chemicals used were provided from Merck, Germany. Pure amylopectin extracted from waxy corn starch (110120) and pure amylose derived from amylose potato starch (A0512) were acquired from Sigma Company.

### 2-2- Measurement of Moisture, Protein, Fat, and Ash

Moisture was measured using the oven drying method, protein using the Kjeldahl method, fat using the Soxhlet method, and ash using the furnace method, according to standard methods (AOAC, 2000).

### 2-3- Production of Phosphorylated Starches Using Phosphorous Oxichloride (POCl<sub>3</sub>)

Raw starch (60 g) was dissolved in 90ml of distilled water while stirring at temperatures of 25, 35, and 45°C. Then, sodium sulfate (10%) based on the weight of the starch was added. The pH of the resulting suspension was adjusted to 9.5, 10.5, and 11.5 using one molar sodium hydroxide. Phosphorous oxichloride was then added to the suspension in three concentrations of 0.05, 0.1, and 0.2% (based on the weight of the starch). The reaction environment was maintained at the specified pH levels for one hour while stirring, after which one molar hydrochloric acid was added to bring the pH to 6.5. The resulting modified starch was recovered by centrifugation (3000 g, 10 minutes) and washed four times with distilled water. It was then washed twice with ethanol-filtration and dried in a fume hood.

### 2-4- Measurement of Amylose Content

0.1 gram of starch was dissolved in 8ml of dimethyl sulfoxide (90%) at 85°C for 15 minutes. After reaching volume in a 25ml flask, one milliliter of this solution was transferred to a 50ml flask. To this, 40ml of deionized water and five milliliters of iodine solution (0.0025 mol/L iodine in 0.0065 mol/L potassium iodide) were added and brought to volume. The absorption of the solution was determined at a

wavelength of 600nm after 15 minutes. A control for absorption was prepared using the solution containing all reagents without the starch sample. The standard curve was plotted using purified amylose and amylopectin.

### 2-5- Determination of Phosphorus Content and Degree of Phosphorus Substitution in Phosphorylated Starch

The phosphorus content in the modified starch was determined using a colorimetric method based on the reaction with ammonium molybdate, following the Jackson method (1967) [18]. Initially, 0.1g of starch samples were digested with 15ml of a mixed acid solution of nitric acid, perchloric acid, and sulfuric acid in the proportions of 10:4:1 respectively. After the color became clear, the samples were transferred to a 50ml standard flask and diluted with 10ml of distilled water. Then, 5ml of ammonium molybdate reagent were added to the flasks, which were filled to the mark with distilled water. The control sample was prepared using 5ml of ammonium molybdate reagent as well. The absorbance of the samples was measured at 490nm using a spectrophotometer. The standard curve was created using absorbance of various concentrations of potassium dihydrogen phosphate. Finally, the degree of phosphorus substitution was determined using the following equation:

$$\text{Equation 1) } DS = \frac{162 \times P}{3100 - (102 \times P)}$$

Where DS is the degree of substitution, and P is the percentage of phosphorus (based on dry weight) in phosphorylated starch.

### 2-6- Measurement of Swelling Power and Solubility Percentage

A specific amount of starch sample was weighed and distilled water was added to prepare a 1.5% (w/w) suspension. The samples were mixed uniformly using a magnetic stirrer and heated in a water bath at temperatures of 60, 75, and 90°C for 30 minutes. During this time, the samples were mixed every 5 minutes. After cooling, the samples were centrifuged at 3000g for 15 minutes. The supernatant liquid was

carefully decanted into a pre-weighed dish and dried in an oven at 110°C until a constant weight was achieved. The weight of the sediment layer was calculated, and then solubility and swelling power were determined based on the following equations [19, 20]:

$$\text{Solubility} = (m_2 * 100) / m_1$$

$$\text{Swelling Power} = m_3 / (m_1 - m_2)$$

Where  $m_1$  is the weight of the initial starch sample,  $m_2$  is the weight of the dried supernatant liquid, and  $m_3$  is the weight of the sedimented layer.

### 2-7- Measurement of syneresis of Starch Gels

To determine the water retention of starch gel, a 5% starch suspension in deionized water containing 200 ppm sodium azide was prepared in a centrifuge tube. After homogenization, the mixture was placed in a boiling water bath for 30 minutes with continuous stirring. After cooking, the tubes were rapidly cooled with an ice-water mixture and stored in the refrigerator. The tubes were then removed from the refrigerator and centrifuged at 2500g for 15 minutes. The floating layer was discarded, and the weight difference was measured. The percentage of water retention was calculated based on the percentage of water separated compared to the initial weight of the gel [21].

### 2-8- Measurement of Freeze-Thaw Stability

To assess the stability of starch gels against freezing and thawing, a 5% starch suspension in deionized water containing 200 ppm sodium azide was prepared in a centrifuge tube. This mixture was maintained at room temperature for 15 minutes and then heated in a boiling water bath for 20 minutes with mechanical stirring at a speed of 160rpm. The resulting paste was cooled to room temperature and then frozen at -20°C in a freezer for 24 hours. Thawing was carried out at 30°C for two hours in an incubator. The centrifuge tubes were then centrifuged at 8000g for 10 minutes. Water retention of the gel was determined based on the percentage of water separated compared to the initial weight of the gel [22].

### 2-9- Clarity of Starch Pastes

The clarity of the paste obtained from natural and modified starches was measured based on the method described by Reddy and Seib (1999) [23]. First, 0.05 grams of each starch was suspended in 5ml of distilled water and placed in a centrifuge tube, which was then maintained in a water bath at 95°C for 30 minutes, with stirring every five minutes. After cooling, the clarity of the starches was measured using a spectrophotometer at a wavelength of 650nm against a water (control sample).

### 2-10- Fourier transform infrared spectroscopy (FTIR)

The infrared spectrum of natural starch samples and optimized modified sample was performed using FTIR spectrometer. At first, each of the samples was mixed well with potassium bromide powder and made into a pressed tablet to be placed in the machine. The infrared spectra of the samples were recorded based on the spectral transmittance and with a resolution of 1.93/cm in the wavelength range of 400 to 4000  $\text{cm}^{-1}$  [24].

### 2-11- Determination of X-ray diffraction pattern (XRD)

The natural starch and optimized modified samples were analyzed using an X-ray diffractometer at 35 kV and 30 mA. The 2-theta angles recorded ranged from 4 to 70 degrees, with increments of 0.04 degrees. The crystallinity percentage of each starch sample was calculated using the following equation:

$$\text{Crystallinity}\% = \frac{A_c}{(A_c + A_a)}$$

$A_c$  and  $A_a$  represent the areas of the crystalline and amorphous regions, respectively, obtained via X-ray diffractometry of the samples [24].

### 2-12- Determination of thermal characteristics (DSC)

To assess the thermal properties of starch, 5 mg of starch (dry weight) was placed in a 40  $\mu\text{L}$  aluminum container, and 20  $\mu\text{L}$  of deionized water was added. After sealing, the mixture was equilibrated at 4°C for 24 hours. The sample was then heated from 20 to 120°C at a rate of

10 °C/min using a differential scanning calorimetry device. Software analysis determined the starting temperature ( $T_o$ ), peak temperature ( $T_p$ ), final temperature ( $T_c$ ), and gelatinization enthalpy ( $\Delta H$ ) [25].

### 2-13- Examining the morphology of starch granules (SEM)

A 1% starch suspension in ethanol was prepared, homogenized using a vortex mixer, and a drop was placed on an aluminum base. After drying, it was coated with gold-palladium using a coating machine (9 mA, vacuum of 3-10 Pa). Imaging was performed using a scanning electron microscope at an accelerating voltage of 20 kV [26].

### 2-14- Determining starch pasting behavior

The pasting behavior of starch was analyzed using a Rapid Viscosity Analyzer (RVA). A 14% starch suspension (28 grams) was prepared in the machine's container and mixed before being placed in the chamber. The stirring speed was initially set to 960 rpm, then 160 rpm for the first ten seconds of the test. Viscosity changes were measured throughout five temperature stages: 1) holding at 50°C for one minute; 2) raising temperature from 50°C to 95°C at 12°C/min; 3) maintaining 95°C for 2.5 minutes; 4) cooling from 95°C to 50°C at 12°C/min; and 5) holding at 50°C for two minutes. The software calculated pasting temperature, peak viscosity, trough viscosity, and final viscosity based on the viscosity-temperature graph [27].

### 2-15- Measuring the hardness of starch gel

To evaluate the hardness of the starch gel, a 10% starch suspension was prepared in deionized water and stirred for 15 minutes. The sample was heated in a boiling water bath for 15 minutes at 160 rpm. The resulting hot mixture was transferred to a cylindrical mold (10 mm diameter and height) and refrigerated for 24 hours to form the gel. A texture profile analysis was conducted using a texture analyzer with a 50 Newton load cell at room temperature, under double compression conditions at 2 mm/s, with a 50% strain and an initial load of 10 grams. This approach prevented gel destruction during testing, as suggested by Pons and Fiszman [28]. The hardness index was calculated from the force-time data.

### 2-16- Statistical analysis

To optimize the conditions for producing phosphorylated starch with oxochloride, this research employed a surface method using box Benken's design (Table 1). Three independent variables—pH (9.5, 10.5, and 11.5), temperature (25, 35, and 45°C), and reagent concentration (0.03, 0.075, and 0.12%)—were optimized at three levels. The criteria for optimization included hydration characteristics, swelling power, paste transparency, and freeze-thaw stability. Additional tests were conducted on the optimal sample and native starch. One-way ANOVA and Duncan's mean comparison test were used for significance testing, with data processed and graphs created using Minitab and Origin 2022 software.

**Table 1. Box-Benken's statistical design treatments used to optimize reaction conditions for the production of phosphorylated starch with oxochloride**

Sample	pH	Temperature (C)	Concentration (%)
1	11.5	35	0.12
2	10.5	45	0.03
3	9.5	25	0.075
4	10.5	25	0.12
5	11.5	25	0.075
6	11.5	35	0.03
7	10.5	35	0.075
8	10.5	45	0.12
9	11.5	45	0.075
10	9.5	35	0.12
11	10.5	35	0.075
12	10.5	25	0.03

13	10.5	35	0.075
14	9.5	45	0.075
15	10.5	35	0.075
16	9.5	35	0.03
17	10.5	35	0.075

### 3- Results and Discussion

#### 3-1- Chemical composition

Table 2 presents the chemical composition of native wheat starch, revealing low levels of protein and fat, indicating high purity of native starch. The low protein content suggests that impurities do not significantly affect the functional properties of the starch [29].

**Table 2. Chemical composition of commercial wheat starch**

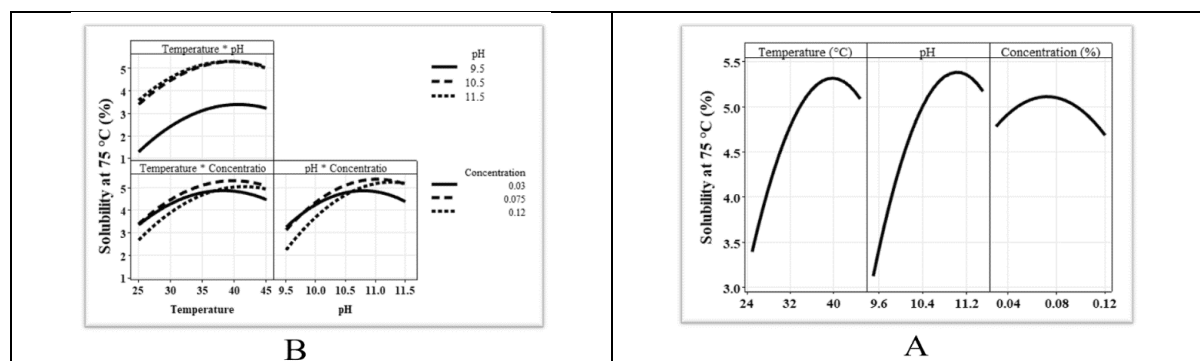
Chemical composition	Content (%)
Moisture	11.00 ± 0.40
Protein*	0.77 ± 0.06
Fat*	0.09 ± 0.02
Ash*	0.30 ± 0.01

\* The content of protein, fat and ash is calculated based on dry matter.

#### 3-2- Solubility

The solubility of phosphorylated wheat starches at 60°C ranged from 1.12% to 3.98%, with the initial starch solubility of 2.00%. However, no significant model emerged linking process variables to the solubility rate at this

temperature. In contrast, the solubility of native starch at 75°C was 3.57%, while solubility of phosphorylated starches varied from 1.12% to 5.71%. A significant quadratic model ( $p < 0.05$ ) was established to describe the effects of temperature, pH, and reactant concentration on dissolution at 75°C.



**Fig 1. Simple and reciprocal effects of temperature, pH and reactant concentration on the solubility rate of phosphorylated wheat starch at 75°C.**

Variance analysis confirmed that temperature and pH significantly influenced solubility ( $p < 0.05$ ). As illustrated in Figure 1, the effect of temperature peaked around 40°C, while pH effects heightened up to around 11 before declining. A significant inverse correlation ( $p < 0.05$ ,  $r = -0.530$ ) was observed between the degree of dissolution at 75°C and the degree of phosphorylation.

At 90°C, the solubility of phosphorylated starches ranged from 11.28% to 5.58%, compared to 4.98% for raw starch. Linear models assessing the solubility rate at this temperature were also significant ( $p < 0.05$ ), indicating that temperature and pH positively influenced solubility (Figure 2). An inverse correlation ( $p < 0.05$ ,  $r = -0.652$ ) between phosphorylation degree and solubility at 90°C

was observed, suggesting the solubility of phosphorylated starches decreases with increasing phosphorylation.

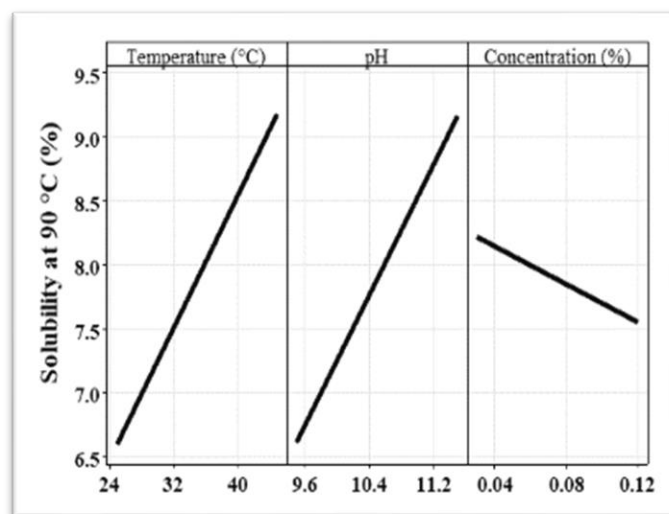


Fig 2. Simple and reciprocal effects of temperature, pH and reactant concentration on the solubility rate of phosphorylated wheat starch at 90°C.

These findings indicate that the phosphorylation reaction conditions—temperature, pH, and reactant type—affect starch characteristics, leading to variances in solubility among phosphorylated starches. Supporting this, Wongsagonsup et al. (2014)

reported that increasing concentrations of STMP/STPP in cross-linking tapioca starch enhanced dissolution rates up to a point before causing a decline compared to natural starch [30].

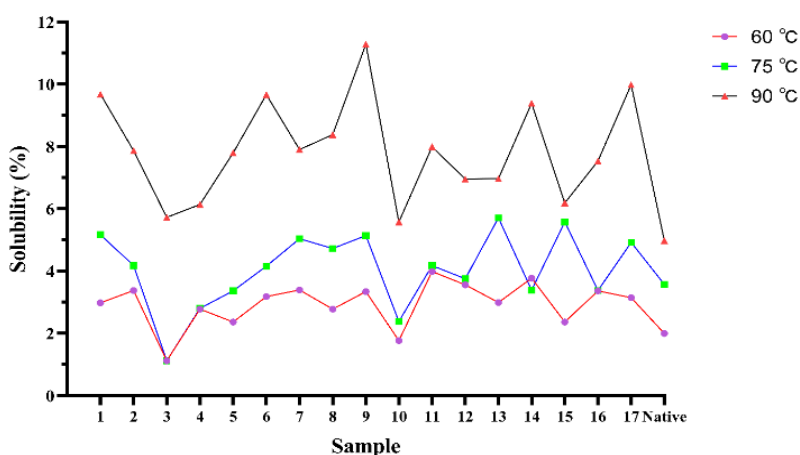


Fig 3. Solubility of native and crosslinked starch samples at 60, 75 and 90°C.

### 3-3- Swelling power

The swelling power of phosphorylated starches at 60°C ranged from 6.50 to 7.48 g/g, compared to 5.54 g/g for raw starch. The quadratic model

indicated significant effects of temperature, pH, and reactant concentration on the swelling power of the phosphorylated starch at 60°C ( $p < 0.05$ ).

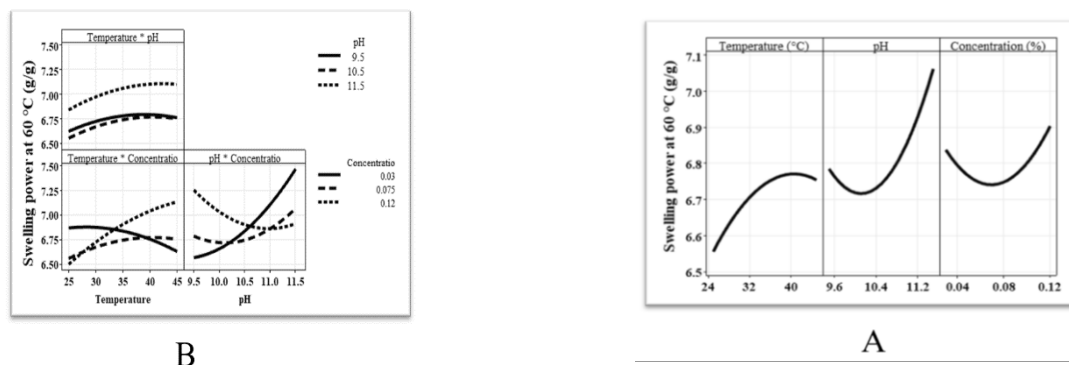


Fig 4. Simple and reciprocal effects of temperature, pH and reactant concentration on swelling power of phosphorylated wheat starch at 60°C.

Analysis of variance revealed significant linear and quadratic effects of pH, as well as interaction effects between temperature-reagent concentration and pH-reagent concentration ( $p < 0.05$ ). Increasing the pH to approximately 10.2 initially decreased the swelling power of phosphorylated starch at 60°C, after that, it increased the swelling power (Figure 4). The temperature-reactant concentration interaction showed a synergistic effect, while the pH-reagent concentration interaction was antagonistic. Additionally, a significant correlation was found between swelling power at 60°C and phosphorylation level ( $p < 0.05$ ), with a correlation coefficient of -0.544.

At 75°C, the swelling power of phosphorylated starches ranged from 6.53 to 9.78 g/g, compared to 7.59 g/g for native starch. No significant model was found for the effects of

temperature, pH, and reagent concentration on swelling power at this temperature, nor was the correlation between swelling power at 75°C and degree of phosphorylation significant.

For phosphorylated starches at 90°C, swelling power ranged from 10.51 to 13.79 g/g, while raw starch exhibited 9.81 g/g. A significant linear model, along with interaction effects, was identified for temperature, pH, and reagent concentration at this temperature ( $p < 0.05$ ). The results indicated a linear increase in swelling power with temperature and pH, as well as synergistic effects between temperature-pH and temperature-reagent concentration ( $p < 0.05$ ). The correlation coefficient between swelling power at 90°C and wheat starch phosphorylation was also significant ( $p < 0.05$ ), and it was equal to -0.655.



Fig 5. Simple and reciprocal effects of temperature, pH and reactant concentration on swelling power of phosphorylated wheat starch at 90°C.



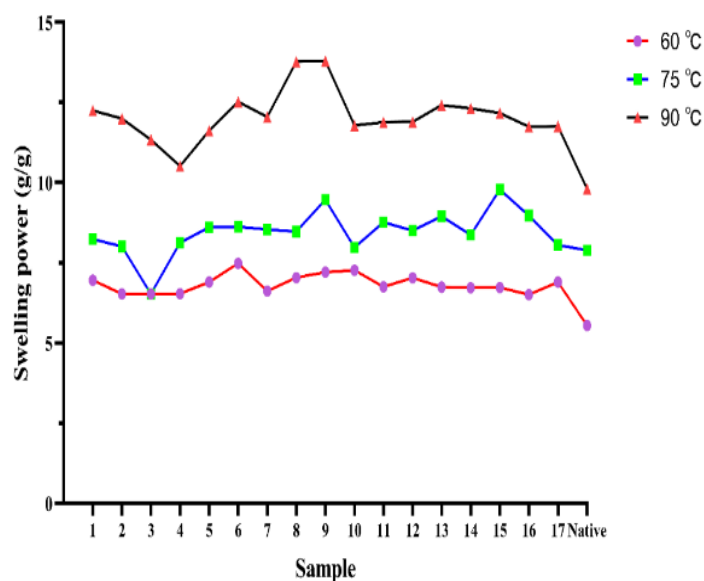


Fig 6. Swelling power of native and crosslinked starch samples at 60, 75 and 90°C.

The correlation analysis between swelling power at various temperatures and the degree of phosphorylation of produced starches indicated a negative correlation; as phosphorylation increased, swelling power decreased (Figure 6). Cross-linked starches exhibit lower swelling power than native starch due to the formation of internal bridges that restrict granule swelling [31]. However, the incorporation of phosphate groups can enhance swelling; the repulsion between negatively charged phosphate groups reduces intra-chain connections and increases the hydration of swollen molecules, thereby elevating swelling power [32].

Wang et al. (2003) found that phosphorylated corn starch exhibited improved physicochemical, thermal, and pasting properties. Their findings suggested that the negative charges of phosphate groups repelled each other, facilitating water absorption into starch granules, which increased swelling power and solubility. Additionally, swelling power and solubility rose with higher phosphorylation percentages [33].

Conversely, Hwang et al. (2009) studied corn starch cross-linked with  $\text{POCl}_3$  and observed that an initial concentration of 0.01% increased swelling compared to native starch, but higher concentrations led to decreased swelling power [31].

### 3-4- Transparency

The transparency of phosphorylated starch pastes ranged from 2.62% to 4.66%, compared to 3.79% for raw starch. Temperature, pH, and reagent concentration affected gel transparency through significant interactions within a binomial model ( $p < 0.05$ ). Analysis of variance revealed significant linear effects of temperature, pH, and reagent concentration, as well as squared effects of temperature and pH, indicating that increased temperature and pH enhanced transparency, while higher reagent concentrations reduced it (Fig 7). Moreover, increased pH intensified the temperature's effect on gel transparency. A significant correlation coefficient of  $-0.856$  ( $p < 0.05$ ) showed that gel transparency decreased with rising phosphorylation levels.

Variations in starch characteristics post-phosphorylation can arise from differences in phosphorylation amounts, distribution of phosphate groups between amylose and amylopectin, and the types of phosphorylation (monophosphate or diphosphate crosslinks) [34]. Heo et al. (2017) reported a reduction in starch gel transparency due to phosphate cross-linking compared to natural starch [35]. Similar findings have been noted in corn [36] and tapioca [30] starches, consistent with the present study; increased cross-link numbers

likely enhance compactness and molecular weight of starch granules [37]. Granules bound by cross-links refract light instead of allowing it to pass through, resulting in decreased transparency [32]. Kaur et al. (2006) and Reddy and Seib (1999) suggested that reduced transparency in cross-linked starch pastes is partly due to decreased swelling power [32,23].

The increase in gel transparency with higher phosphorylation levels can be attributed to the charged phosphate groups, which help rehydration of starch molecules and reduce their recrystallization. These groups enhance the bonds between starch and water, thus increasing swelling power and transparency [38].

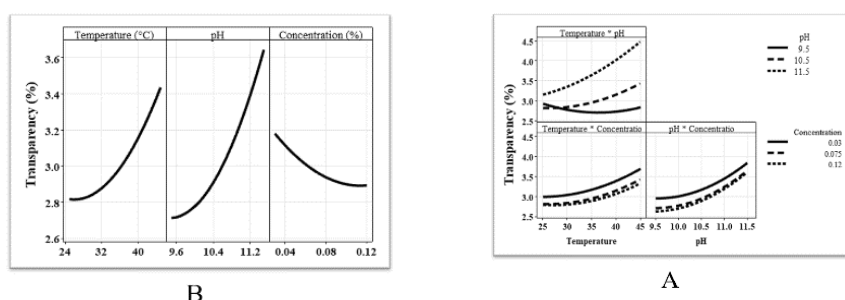


Fig 7. Simple and reciprocal effects of temperature, pH and reactant concentration on the transparency of phosphorylated wheat starch paste.

### 3-5- Syneresis

Phosphorylated wheat starch samples had a syneresis between 21.42% and 41.59%, compared to 33.64% for raw starch. The linear model was significant ( $p < 0.05$ ) for the effect of temperature, pH value and reagent

concentration on the syneresis of phosphorylated starch samples. On the other hand, the results of analysis of variance showed that, among the process variables, only the effect of temperature was significant ( $p < 0.05$ ), so that with its increase, the syneresis of the samples decreased (Fig 8).

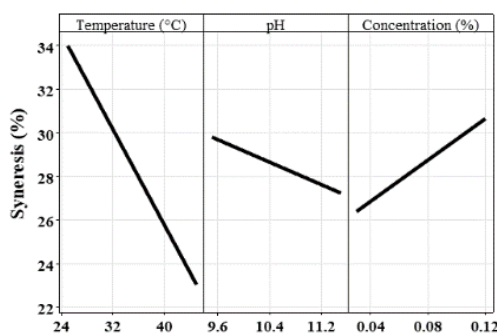


Fig 8. Effects of temperature, pH and reactant concentration on phosphorylated wheat starch syneresis.

### 3-6- Freeze-thaw stability

The syneresis degree after freeze-thaw cycles reflects starch retrogradation behavior [39]. syneresis changes for phosphorylated starch samples ranged from 12.85% to 36.86%, while raw starch showed 31.26% (Figure 9). The linear model for interpreting the effect of temperature, pH value and reactant concentration on the amount of syneresis after freezing and thawing of phosphorylated starch

samples was significant ( $p < 0.05$ ). The results showed that only the effect of pH value on syneresis after freeze-thaw was significant ( $p < 0.05$ ), and as shown in Figure 10, with its effect diminishing. The level of syneresis after freeze-thaw cycles had a significant negative correlation ( $p < 0.05$ ) with the level of phosphorylation, so that the correlation coefficient was equal to -0.692.

The results indicated that the freeze-thaw stability in phosphorylated samples decreased with increasing levels of crosslinking. This is because the repulsive forces between phosphate groups within the phosphorylated starch molecules can prevent the re-association of

these molecules, enhancing their interaction with water. Consequently, this may lead to reduced water loss and increased freeze-thaw stability [40].

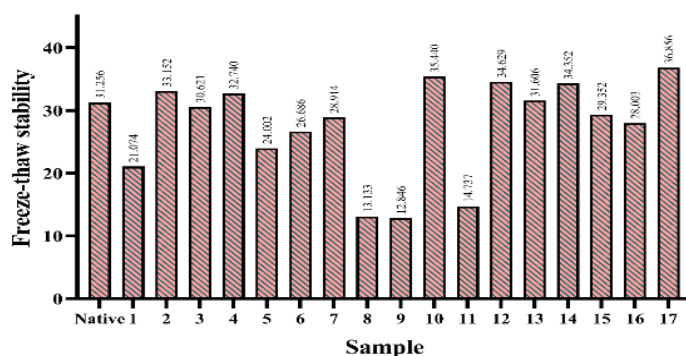


Fig 9. Freeze-thaw stability of native and crosslinked starch samples.

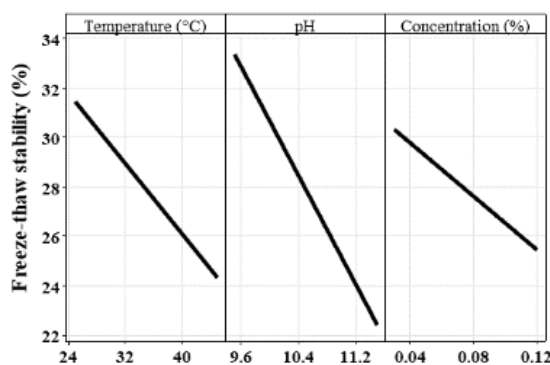


Fig 10. The effect of temperature, pH and reactant concentration on the Freeze-thaw stability of phosphorylated wheat starch.

### 3-7- Phosphorus content

The level of each type of chemical substitution applied to modified starch has a significant impact on its properties. Changing the percentage of a specific chemical substituent on a type of starch may lead to different characteristics being observed [41]. The efficiency of the cross-linking reaction is expressed by the degree of substitution, which indicates the number of phosphate groups that have cross-linked glucose anhydride. Native starch contains specific amounts of monoester phosphate, and therefore, the degree of substitution of modified starch is calculated after subtracting the intrinsic phosphorus percentage in native starch. In the current study,

the range of phosphorylated amounts was estimated to be between 0.14% and 0.46%, and the inherent phosphorus content in the wheat starch used was 0.047% (Figure 11). Data analysis results indicated that the best model for examining the effects of temperature, pH, and reactant concentration on the degree of phosphorylation of wheat starch was the linear model ( $p < 0.05$ ). On the other hand, the analysis of variance showed that only the effect of pH was significant ( $p < 0.05$ ), with an increase in pH leading to a decrease in phosphorylation (Figure 12).

Research by Sang et al. (2010) found that at pH levels 9-12, increased pH decreased phosphorus content in phosphorylated wheat starch, with

the lowest observed at pH 12 [42]. Lin et al. (2009) noted that rice starch phosphorylation

initially increased with temperature, peaking at 140°C before declining [43].

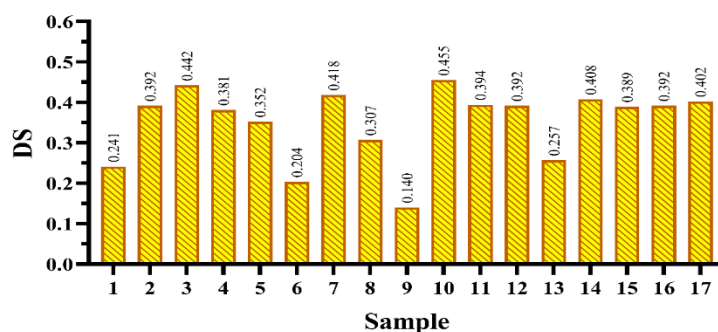


Fig 11. Degree of substitution of cross-linked starches.

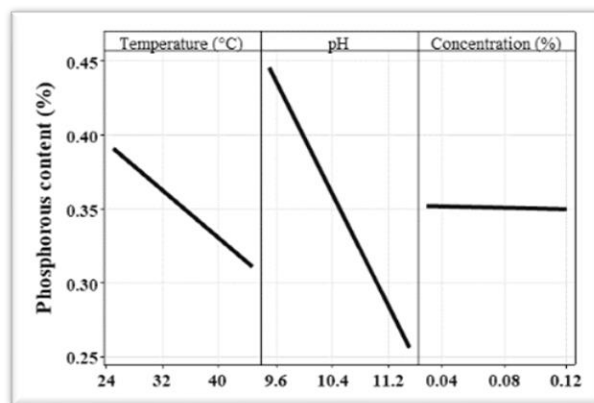


Fig 12. The effect of temperature, pH and reagent concentration on the degree of phosphorylation of wheat starch.

The results of physicochemical tests identified the optimal characteristics of starch for canned product formulation. Phosphorylated starch was selected for its higher swelling power, solubility, paste transparency and freeze-thaw stability, and reduced gel syneresis. Consequently, further tests were conducted on the optimal sample, assessing its morphological, structural, thermal, gelling, and pasting properties in comparison to native starch.

### 3-8- Amylose content and gel firmness

Wheat starch naturally contained 25.78% amylose and 74.22% amylopectin (Table 3). According to Sasaki et al. (2002), the amylose content of wheat starch varies from 18.5% to 28.6% depending on the starch genotypes [29]. Majzoobi et al. (2012) also reported the amylose content of wheat starch as 26.35%

[44]. On the other hand, results indicated that phosphorylation significantly reduced amylose content ( $p < 0.05$ ), with phosphorylated starch having an amylose level of 12.62%. This phenomenon can be attributed to the high pH during the phosphorylation process. The use of high pH values during the cross-linking reaction (phosphorylation) leads to the release of amylose chains from starch granules, decreasing amylose content [45]. Furthermore, the introduction of phosphodiester bonds within the starch chains reduces the formation of amylose-iodine complexes [46]. Additionally, the decrease in amylose can also be attributed to cross-linking between amylose and amylopectin chains, such that the resulting structure does not allow for iodine binding, interfering with the determination of amylose levels [47]. Similarly, Sudheesh et al. (2021) investigated the effect of cross-linking modification on the properties of chitin starch

and reported that this modification resulted in a reduced amylose content in the modified starch compared to native starch [48]. Conversely, Wei et al. (2011) reported that the addition of 11% tripolyphosphate to potato starch led to a reduction in amylose content from 28.79% in raw starch to 14.05% in modified starch [14].

Firmness is a measure of a food's resistance to compressive force, and as gel firmness increases, more force is required to break down the gel structure [49]. The results of the gel firmness assessment for both natural and phosphorylated starches in this study are presented in Table 3. According to the results, the gel firmness of phosphorylated starch (4.84 Newtons) was significantly higher than that of the control sample ( $p < 0.05$ ). The reduction in soluble amylose leads to a softer gel, allowing more amylose to leach from the starch granules, resulting in a more robust gel network [29].

On the other hand, long-term textural changes associated with retrogradation or the recrystallization of the amylopectin component occur, while short-term changes relate to the amylose fraction, which leads to the formation of the initial gel network. The recrystallization of amylopectin involves the recrystallization of the outer branches of amylopectin and the increased stiffness of swollen granules, thereby reinforcing the continuous amylose phase. Consequently, it can be stated that the re-association of amylopectin chains plays a significant role in the retrogradation of starch gel [50]. The higher gel strength of phosphorylated starch compared to native starch indicates a greater tendency of this starch to form a gel and results in higher final viscosity and setback viscosity.

**Table 3. Amylose content and gel hardness of native and phosphorylated wheat starch.**

Sample	Amylose	Firmness of the gel (N)
Native starch	25.78 ± 1.31 <sup>a</sup>	0.21 ± 0.41 <sup>b</sup>
Phosphorylated Starch	12.61 ± 0.84 <sup>b</sup>	4.83 ± 0.23 <sup>a</sup>

### 3-9- X-ray diffraction and crystallinity percentage (RC%)

The XRD pattern of normal and cross-linked wheat starch is shown in Figure 13. Natural wheat starch has an A-type crystallinity pattern with strong diffraction peaks at around 15, 17, 18 and 23° [51]. As shown in the figure, the crystallinity patterns of phosphorylated wheat starch were similar to those of natural wheat starch, so it can be stated that this modification method did not change the crystallinity pattern of the starch [52].

On the other hand, the results showed that the phosphorus-containing and cross-linked starches showed less crystallinity compared to natural starch due to the reduction of hydrogen bonds in the crystal structure of wheat starch granules (Table 4). According to Kou et al. (2010), phosphate cross-linking occurs in the amorphous regions of starch granules [53]. In the present study, the decrease in crystallinity after phosphorylation indicated that the order in the crystal structure of wheat starch granules after cross-linking was reduced. On the other hand, Shukri et al. (2015) reported that phosphorylated waxy maize starch showed a significant decrease in crystallinity compared to native starch [54].

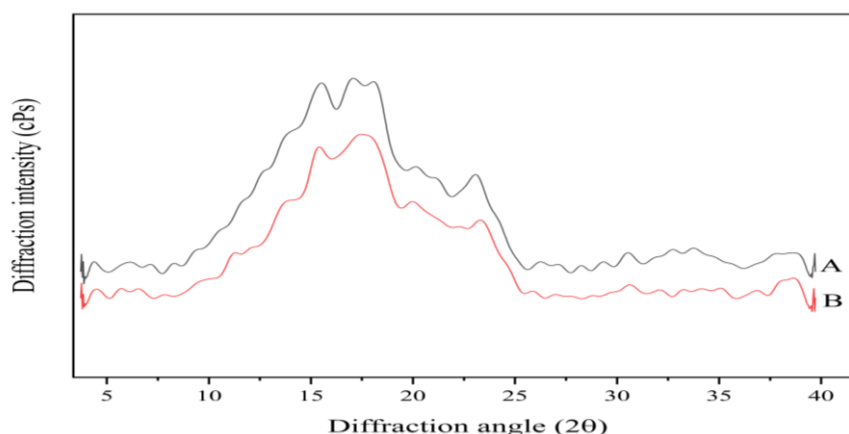


Fig 13. X-ray diffraction pattern of native (A) and phosphorylated wheat starch (B).

### 3-10- FTIR

In order to understand the changes in the molecular structure of cross-linked starch compared to native starch, the FTIR spectra of these samples are shown in Figure 14. As can be seen, native and phosphorylated starch had similar absorption spectra, which indicates that the method used to produce phosphorylated starch did not create any new chemical groups in the starch structure and only changed the absorption intensity of some peaks. In order to investigate the changes in the peak intensity, the following five main peaks in the FTIR spectra of wheat starch samples were considered:

The wavelength of  $13427\text{cm}^{-1}$  is attributed to the stretching vibrations of the internal hydroxyl groups (O-H). Also, the asymmetric stretching of the carbon-hydrogen bond (C-H) can be attributed to the wavelength of  $12926\text{cm}^{-1}$ . On the other hand, the wavelength of  $11640\text{cm}^{-1}$  is related to the adsorbed water of the amorphous region, and the bending vibrations of H-O-H in the amorphous regions of starch are an absorption band of starch (104). The bands around  $11363\text{cm}^{-1}$  and  $11338\text{cm}^{-1}$  were assigned to the C-H stretching in alkanes, and  $11242\text{cm}^{-1}$  is attributed to the C-OH, CH<sub>2</sub>OH stretching. On the other hand, the peaks in the region of  $11160\text{-}1100\text{cm}^{-1}$  are mainly attributed to the asymmetric C-O-C stretching. While the peaks at  $11077\text{-}928\text{cm}^{-1}$  are assigned to the C-O, C-O-H stretching in starch [55].

The results showed that there was no significant difference between the O-H, C-H and C-O vibrational bands in native and phosphorylated starches, due to the lack of change in the chemical structure of the molecules compared to native starch. However, phosphorylation caused a slight decrease in the intensity of the peaks at  $13264\text{cm}^{-1}$  (associated with hydroxyl),  $11640\text{cm}^{-1}$  (associated with intramolecular hydrogen bonding), and  $1994\text{cm}^{-1}$  (associated with intramolecular hydrogen bonding of hydroxyl groups at C-6) for phosphorylated starch [56]. Similarly, Ashwar et al. (2017) investigated the effect of STMP/STPP combination on rice starch. The results of this report showed that STMP/STPP reacts with the OH groups of the starch molecule and results in a decrease in the intensity of the peak at  $13290\text{cm}^{-1}$  due to the reduction of OH stretching vibrations [57].

On the other hand, phosphorylation caused a slight increase in the intensity of the peaks at  $836\text{cm}^{-1}$  and  $889\text{cm}^{-1}$  of the cross-linked starches compared to the native starch due to the formation of the P-O-C bond. In addition, a slight increase in the intensity of the peaks of phosphorylated starch in the region of  $1150\text{-}1300\text{cm}^{-1}$  compared to the native starch sample confirmed the increase in the symmetrical stretching of the phosphorus-oxygen bond (P=O) [56]. According to Sang et al. (2007), in the starch cross-linked with STMP/STPP, the starch structure is not broken, but phosphate groups are formed after cross-linking [58].

On the other hand, the absorption intensity of the peaks at  $994\text{cm}^{-1}$  and  $1082\text{cm}^{-1}$  can be attributed to the changes in the crystal structure and crystallinity of starch polymers, while the absorption intensity of the peak at  $1018\text{cm}^{-1}$  is related to the structure of the amorphous regions of starch granules [59]. Therefore, the absorption ratios of  $1018$  to  $1082\text{cm}^{-1}$  and  $994$  to  $1018\text{cm}^{-1}$  are used to describe the degree of order of the crystalline regions of starch granules (degree of order; DO) and the double helix content (DD), respectively. However, the absorption ratio of  $994$  to  $1018\text{cm}^{-1}$  is related to the internal changes in the amorphous regions of starch granules [60].

In the present study, comparing the peak intensity ratio of phosphorylated starch with that of native starch showed that phosphorylation, through the reduction of hydrogen bonds in the crystal structure of wheat starch granules, caused a decrease in the DD and DO ratio compared to the control starch, while phosphorylation, through the reduction of order in the crystal structure of wheat starch granules, increased the ratio from  $994$  to  $1018\text{cm}^{-1}$ , which was confirmed by the results of X-ray diffraction and percentage crystallinity tests.

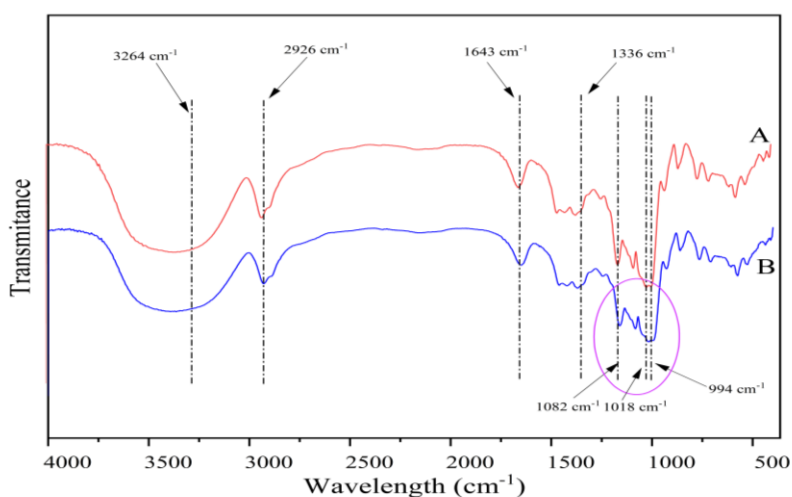


Fig 14. FT-IR spectra of native (A) and phosphorylated wheat starch (B).

Table 4. XRD and FTIR parameters of native and phosphorylated wheat starch.

Sampel	1082/1018 (DO)	994/1018 (DD)	1018/994 (Amorphous)	RC%
Native starch	$1.34 \pm 0.06^a$	$1.29 \pm 0.04^a$	$0.77 \pm 0.03^b$	$44.52 \pm 0.57^a$
Phosphorylated Starch	$1.28 \pm 0.08^b$	$1.21 \pm 0.05^b$	$0.82 \pm 0.07^a$	$37.93 \pm 0.46^b$

### 3-11- DSC

Thermal properties of natural and phosphorylated starches are shown in Table 5. As can be seen, phosphorylation, by introducing phosphate groups to cross-linked starch molecules, enhanced intermolecular interactions, resulting in inhibition of gelatinization and an increase in the initial gelatinization temperatures  $T_o$ , peak  $T_p$  compared to natural starch [61]. While the same

modification treatment, due to the strengthening of the amorphous structure of starch granules through covalent bonding, resulted in the creation of a very amorphous and complex internal structure, which led to a decrease in the final temperature  $T_c$  and the gelatinization enthalpy  $\Delta H$  of phosphorylated starch compared to natural starch [62]. The results of the ratio of peak intensities in the Fourier spectroscopy test and the percentage of

crystallinity confirm this claim and showed a positive correlation with the results of this test.

Gonenc et al., (2019) studied the thermal properties of corn starch cross-linked with glutaraldehyde GLU in both acidic and alkaline environments. The results of this study showed that the peak gelatinization temperature of cross-linked starches in acidic environment due to the introduction of hemiacetal crosslinks into starch by GLU, stiffened the molecular structure of starch and increased the gelatinization temperature, while the gelatinization enthalpy decreased significantly. However, in alkaline environment, both the peak gelatinization temperature and the gelatinization enthalpy decreased compared to native starch [63].

**Table 5. Thermal properties of native, phosphorylated wheat starch.**

Sampel	T <sub>o</sub>	T <sub>p</sub>	T <sub>c</sub>	ΔH
Native starch	52.90±0.13 <sup>b</sup>	58.12±11 <sup>b</sup>	64.37±0.06 <sup>a</sup>	8.48±0.11 <sup>a</sup>
Phosphorylated Starch	53.81±0.10 <sup>a</sup>	59.80±0.12 <sup>a</sup>	62.30±0.08 <sup>b</sup>	8.03±0.11 <sup>b</sup>

### 3-12- Pasting

The pasting characteristics of natural and phosphorylated starch are presented in Table 6. The results showed that the pasting temperature (PT) of phosphorylated starches decreased somewhat compared to natural starch; while the final viscosity (FV) and setback viscosity (SB) of phosphorylated starch increased significantly compared to natural starch. This indicates a higher tendency to retrogradation (backward phenomenon) in this starch. The reason for the increased tendency of this type of modified starch to retrogradation can be attributed to morphological changes during modification that cause leakage of amylose chains from starch granules and the linking of these chains. From another perspective, cross-linking with low concentration limits the mobility of starch chains and can delay retrogradation. However, higher concentrations of cross-linking agent create a more compact structure that increases the hydrogen bonding ability between starch chains and increases the retrogradation rate [66].

Similarly, Sudheesh et al. (2020) reported that phosphorylation of kithul starch with POCl<sub>3</sub>

Other studies have shown that the formation of starch monoesters with phosphates leads to a decrease in gelatinization temperatures and enthalpy of gelatinization [64]. Therefore, the decrease in gelatinization temperature is likely due to changes in starch granules such as the opening of amylopectin double helices during phosphorylation [38].

Kaur et al. (2006), by investigating the effect of crosslinking with POCl<sub>3</sub> in potato starch, observed that at low levels of POCl<sub>3</sub>, T<sub>o</sub>, T<sub>p</sub>, T<sub>c</sub> and enthalpy of gelatinization values decreased significantly, and with increasing reactant concentration, these thermal properties increased significantly in cross-linked starches [32]. Similar results in this regard have been reported by Yoneya et al. (2003) [65].

significantly increased the retrogradation rate; this increase was attributed to the rearrangement of starch chains that had leaked out of the starch granules through surface damage that occurred during modification [46], while Sudheesh et al. (2021) reported that phosphorylation of sorghum starch with EPI significantly reduced the retrogradation rate [67].

On the other hand, the results showed that the peak viscosity (PV) of phosphorylated starch was reduced compared to native starch due to the high reactivity of POCl<sub>3</sub> and the cross-linking of the outer layer or internal channels of starch granules, resulting in a stiffer granule surface compared to native starch [68]. Aaliya et al. (2022) investigated the effect of the crosslinking agent sodium hexametaphosphate on the pasting properties of talipot starch. The results of this report showed that this modification resulted in a decrease in pasting parameters including pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BD), final viscosity (FV), and setback viscosity (SB) [68].

The results of the study by Aaliya et al. (2022) showed that crosslinking, through increasing



the intra- and intermolecular interactions of starch granules, reduces the degradation of starch granules during heating and leads to an increase in viscosity [69]. Similarly, crosslinking of kitol starch with phosphorus oxychloride also increased PV, TV, SB and FV [69]. As a result, it can be stated that the modification of starch crosslinking on the pasting behavior of starch varies depending on

the starch source, the crosslinking agent, its concentration and the reaction conditions. Lower reagent concentration and lower degree of crosslinking can increase swelling and maximum viscosity during heating of starch with water, while higher degree of crosslinking increases granule strength and leads to a decrease in maximum viscosity [52].

**Table 6. Pasting property of native and phosphorylated wheat starch.**

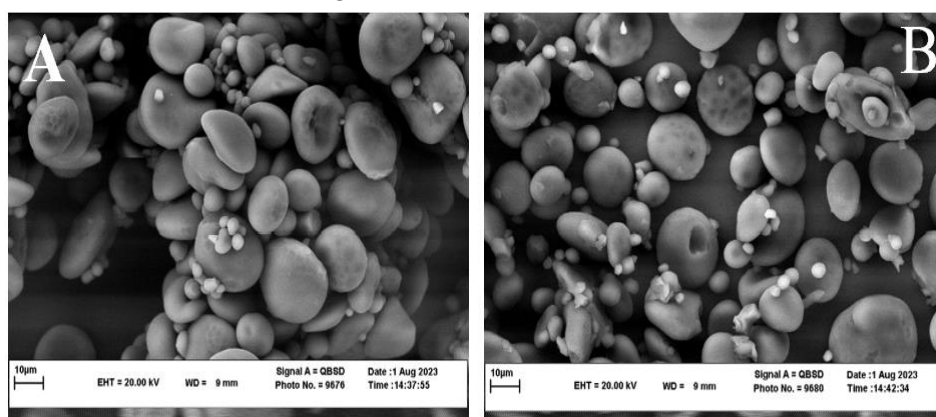
Sampel	PT (°C)	PV	Viscosity (cP)			
			TV	BD	FV	SB
Native starch	65.90±0.64 <sup>a</sup>	1774.00±19.50 <sup>a</sup>	1541±18.38 <sup>a</sup>	233.00±18.50 <sup>a</sup>	1869.00±11.46 <sup>b</sup>	464.00±17.10 <sup>b</sup>
Phosphorylated Starch	64.77±0.57 <sup>b</sup>	1587.00±16.94 <sup>b</sup>	1460±10.32 <sup>b</sup>	127.00±13.70 <sup>b</sup>	3147.00±21.20 <sup>a</sup>	1606.00±22.80 <sup>a</sup>

### 3-13- Morphology of Starch Granules

Electron microscope images of natural and phosphorylated wheat starches at 3000x magnification are shown in Figure 15. In these images, large type A starch granules with dimensions of about 12 to 20  $\mu\text{m}$  and small type B granules with dimensions of about 2 to 8  $\mu\text{m}$  are visible, and the granules have round, elliptical, disc-like and also irregular shapes.

There was a slight difference in surface morphological characteristics between the natural and phosphorylated starch granules studied. So that in natural wheat starch, pores are observed on the surface of the granules

(Figure A), but it seems that the number and depth of these pores in the granules of phosphorylated starches (Figure B) show a slight increase. Therefore, it seems that the starch phosphorylation reactions in this study had little effect on the appearance of the granules. There are reports that the surface properties of starch granules did not change by some researchers such as Kaur et al. (2006) regarding the crosslinking of potato starch and Sun et al. (2017) regarding the crosslinking of wheat starch [32, 70]. However, the study by Majzoubi et al. (2009) observed that blister-like spots were formed on the surface of starch granules due to the crosslinking of wheat starch by  $\text{POCl}_3$  [71].



**Fig 15. Scanning electron micrography of native (A) and phosphorylated wheat starch (b).**

### 4- Conclusion

The results presented in the present study showed that the reaction conditions (pH,

temperature, reagent concentration) can effectively affect the properties of wheat starch. For this reason, under all changes in reaction conditions, 17 different types of modified

starch were produced and their physicochemical properties were investigated. In the meantime, considering the required properties of starch used in canned products, a starch with higher swelling power, higher solubility, greater dough transparency, less syneresis and greater freeze-thaw stability was selected as the optimal sample and all supplementary tests were performed on it and compared with the natural starch sample. The results showed that the phosphorylation of wheat starch reduced the crystallinity of natural starch from 44.52 to 37.93 percent, which showed a positive correlation with the double helix content and degree of order in the FTIR test results. On the other hand, this modification treatment reduced the amount of PT, PV, TV and BD compared to natural starch and increased the amount of SB and FV. As a result, it can be stated that starch phosphorylation caused the starch to become resistant to high temperatures and shear forces and created a starch that can withstand harsh processing conditions in food production, making this type of modified starch suitable for use in the formulation of canned products. In addition, the optimized starch can be used to create heat-stable gels in ready-to-eat foods or as a fat substitute in low-fat products.

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مقاله علمی-پژوهشی

بهینه سازی شرایط واکنش برای تولید نشاسته فسفریله گندم برای استفاده در فرمولاسیون مواد غذایی کنسرو شده

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گندم یکی از محصولات زراعی اصلی ایران است که بخش اعظم این غله از نشاسته و پروتئین تشکیل شده است. استفاده گسترده از نشاسته به دلیل قیمت مناسب، ایمنی بالا و قابلیت زیست تخریب پذیری، در صنایع مختلف امکانات بسیاری را فراهم می کند. با این حال، نشاسته طبیعی به دلیل محدودیت هایی مانند نامحلول بودن در آب سرد و عدم تحمل گرما برای کاربردهای صنعتی مختلف نیاز به اصلاح دارد. از این رو، اصلاح نشاسته گندم به منظور بهبود ویژگی ها و کاهش وابستگی به واردات نشاسته های اصلاح شده ضروری است. اصلاح شیمیایی یکی از تکنیک های اصلاح نشاسته است که بیشترین کارایی را از خود نشان داده است، اما با دغدغه هایی نظیر آلودگی محیط زیست و هزینه های بالای مواد شیمیایی همراه است. در این مطالعه شرایط واکنش تولید نشاسته فسفریله با اکسی کلراید ( $POCl_3$ ) با استفاده از سه متغیر مستقل pH (۹/۵، ۱۰/۵ و ۱۱/۵)، دما (۲۵، ۳۵ و ۴۵ درجه سانتی گراد) و غلظت واکنشگر (۰/۰۳، ۰/۰۷۵ و ۰/۱۲ درصد)، بهینه سازی شد. با توجه به نتایج آزمون های فیزیکوشیمیایی، ملاک بهینه سازی نشاسته مورد استفاده در محصولات کنسروی وجود شاخص تورم و حلالیت، شفافیت خمیر و پایداری انجماد-ذوب بالاتر و سینرسیس کمتر ژل انتخاب گردید. سپس آزمون های تکمیلی به منظور بررسی خصوصیات ساختاری، حرارتی و خمیری شدن بر نمونه نشاسته انتخاب شده و طبیعی انجام شد؛ نتایج نشان داد که فسفریلاسیون از طریق بهبود ویژگی های بافتی، ایجاد ویسکوزیته بالاتر و افزایش تحمل گرما، نشاسته اصلاح شده حاصل را برای استفاده در فرمولاسیون محصولات کنسروی مناسب ساخت.