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Optimizing reaction conditions for the production of phosphorylated wheat starch by sodium trimetaphosphate (STMP) in order to be used in the formulation of sauces

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ABSTRACT

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Wheat starch is the most common type of starch in many countries and can be modified to produce a variety of starches. The widespread use of starch offers many opportunities in various industries due to its affordability, high safety and biodegradability. However, characteristics such as insolubility in cold water, heat intolerance, low resistance to applied stresses, and low freeze-thaw stability limit the use of natural starch for various industrial uses. One of the most efficient techniques for starch modification is chemical modification, but it raises concerns about environmental pollution and high chemical costs. For this reason, in this study, in order to reduce the consumption of chemicals, the reaction conditions for the production of phosphorylated starch with sodium trimetaphosphate (STMP) using three independent variables: pH (9.5, 10.5 and 11.5), temperature (110, 130 and 150 degrees Celsius) and reactant concentration (1.5, 3 and 4.5%) were optimized. According to the results of the physicochemical tests, the optimization criteria of starch used in all kinds of sauces with higher swelling and solubility index, higher dough transparency, less gel syneresis and higher freeze-thaw stability were selected. Then, in order to check the structural, thermal and pasting properties, additional tests were performed on selected and natural starch samples; The results showed that the starch produced under optimal conditions is suitable for use in the formulation of all kinds of sauces due to improved textural properties, higher viscosity and increased heat tolerance.

1-Introduction

Wheat is one of the most common varieties used in the human diet. In addition to the production of flour, bread and pasta, this crop is also widely used in the production of starch [1]. In many countries (such as Iran and many European countries), wheat starch is the most common type of starch that can be used to produce various types of modified starch [2].

Starch, as the main polysaccharide in plants, has wide applications in many food and non-food products. It is mainly used as an energy source for humans and affects the physicochemical properties of foods by acting as thickeners, colloidal stabilizers, bulking agents, water retention and gelling agents [3]. Native starch is a suitable stabilizer and texture regulator in food systems [4], but limitations such as low shear and thermal resistance, thermal decomposition and high tendency towards retrogradation, low solubility, transparency and poor stability limit its application in some food products [5, 6]. To overcome the limitations of natural starch and to develop new capabilities, starch modification is an essential task that has been carried out for many years. Various modified starches are generally produced through physical, chemical, genetic or enzymatic methods [7, 8]. Starch modification has a long history and has been used to introduce functional groups into starch molecules. [9].

Chemical modification of starch can be achieved by a variety of chemical reactions such as acid hydrolysis, oxidation, etherification, esterification, and cross-linking [10]. Among these methods, cross-linking is commonly used to modify native starch, in which different cross-linking agents such as sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), epichlorohydrin (EPI), and phosphoryl chloride (POCl_3) have been used [11]. By using this method, intermolecular and intramolecular bonds are formed at random locations in starch granules, which stabilize and strengthen the granules [12]. In addition, starch crosslinking is affected by various factors including starch source, concentration and composition of cross-linking agent, degree of substitution, pH, reaction time,

and temperature [13]. In the present study, STMP was used as a cross-linking agent for wheat starch due to its non-toxic nature, lower penetration rate, and better cross-linking ability [14].

Starch used in sauce formulation should be able to withstand the temperature of the pasteurization process and achieve the desired stability and texture in the final product. Studies show that cross-linking modification of starch increases rheological properties, thermal stability, shear resistance, paste transparency and freeze-thaw stability, all of which collectively contribute to the thickening properties of cross-linked starch in various food products such as soups and sauces [15]. Therefore, the aim of this study was to optimize the production method of phosphorylated wheat starch with sodium trimetaphosphate (STMP) for use in sauce formulation. In this study, we optimized the production conditions for phosphorylated wheat starch and examined the resulting changes by investigating its structural, thermal, gelling, pasting, and morphological properties. Additionally, we compared the modified wheat starch with native wheat starch.

2- Materials and Methods

2-1- Materials

High purity wheat starch was obtained from Khoshe Zarrin Starch Company. The chemical compounds used were obtained from Merck, Germany. Pure amylopectin extracted from waxy corn starch (110120) and pure amylose from amylose potato starch (A0512) were obtained from Sigma.

2-2- Measurement of moisture, protein, fat and ash

Measurement of moisture by oven method, protein by Kjeldahl method, fat by Soxhlet method and ash by furnace combustion method were performed according to standard methods (AOAC, 2000) and in triplicate [16].

2-3- Production of phosphorylated starches by sodium trimetaphosphate method

Raw starch (60 g) was dissolved in 90 ml of distilled water at 25°C using a magnetic stirrer, and 5% sodium sulfate based on the weight of starch was added to it, and the pH of the suspension was adjusted with 1M sodium hydroxide at 9.5, 10.5, and 11.5. Then, the STMP/STPP mixture was added at a ratio of 99:1 at concentrations of 1.5, 3, and 4.5%. The reaction medium was maintained at the mentioned pH for 1 hour with stirring, and then dried to about 10-15% moisture at 40°C using an air-flow oven. The dried mixture was transferred to a closed container and heated at 110, 130, and 150°C for 2 hours. After cooling to room temperature, the starch was dissolved in 120mL of distilled starch and its pH was adjusted to 6.5 with hydrochloric acid. Finally, the resulting modified starch was recovered by centrifugation (3000g, 10 min), washed four times with distilled water, washed twice by ethanol-filtration, and dried in a fume hood [17].

2-4- Measurement of amylose content

0.1 g of starch was dissolved in 8 ml of 90% dimethyl sulfoxide solution at 85°C for 15 min and after making up to 25 ml, one ml of this solution was transferred to a 50ml falcon and 40ml of deionized water and 5 ml of iodine solution (0.0025 mol/L iodine in 0.0065 mol/L potassium iodide) were added and made up to volume. The absorbance of the solution was determined after 15 min at a wavelength of 600 nm. The solution containing all the reactants without starch sample was used as an absorbance control. A standard diagram was drawn using purified amylose and amylopectin [18].

2-5- Determination of phosphorus content and degree of phosphorus substitution in phosphorylated starch

The phosphorus content in modified starch was determined using a colorimetric method, as a result of the reaction with ammonium molybdate according to the method of Jackson (1967) [19]. Initially, 0.1 of the starch samples were digested with 15 ml of a mixture of three acids: nitric acid, perchloric acid and sulfuric acid (with ratios of 10:4:1 from left to right,

respectively). After the color became clear, the samples were transferred to a standard 50 ml flask and diluted with 10 ml of distilled water. Then, 5 ml of ammonium molybdate reagent was added to the flasks and made up to volume with distilled water. The control sample was also prepared using 5 ml of ammonium molybdate reagent. The absorbance of the samples at 490 nm was determined using a spectrophotometer. A standard curve was prepared using the absorbance of different dilutions of potassium dihydrogen orthophosphate. Finally, the degree of phosphorus substitution was determined using the following equation:

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

where DS is the degree of substitution, P is the percentage of phosphorus content (based on dry weight) of the phosphorylated starch.

2-6- Measurement of swelling power and solubility percentage

A certain amount of starch sample was weighed and poured into a falcon, and then 1.5% w/w deionized water was added to it to prepare a suspension. The samples were homogenized by vortexing and heated in a water bath at temperatures of 60, 75, and 90°C for 30 minutes. During this time, the samples were homogenized by vortexing every 5 minutes. After cooling, the tubes were centrifuged at 3000 g for 15 minutes. The supernatant was carefully poured into a pre-weighed plate, drained, and dried in an oven at 110°C until constant weight was reached. The weight of the deposited layer was calculated, and then the solubility and swelling power were determined based on the following relationships [20, 21].

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

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

m₁: weight of the starch prototype, m₂: dried supernatant, and m₃: deposited layer

2-7- Measurement of starch gel syneresis

To determine the amount of starch gel syneresis, a 5% starch suspension in deionized

water containing 200 ppm sodium azide was prepared in a screw-capped tube and, after homogenization with a vortex, was placed in a boiling water bath for 30 minutes with stirring. After the cooking time, the tube was quickly cooled with a mixture of water and ice and stored in the refrigerator. Then the tubes were removed from the refrigerator and centrifuged for 15 minutes at 2500 g. The supernatant was removed and the weight difference was measured. The percentage of syneresis was calculated by calculating the percentage of water removed relative to the initial weight of the gel [22].

2.8- Freeze-thaw stability

In order to determine the stability of starch gels against freezing and thawing, a 5% suspension of starch in deionized water containing 200 ppm sodium azide was prepared in a screw-capped tube. This mixture was homogenized for 15 minutes at room temperature by vortexing and then cooked in a boiling water bath for 20 minutes with mechanical stirring at 160 rpm. The paste was cooled to room temperature and frozen at -20°C for 24 hours. Thawing was performed at 30°C for two hours in an incubator. The centrifuge tubes were centrifuged at 8000g for 10 minutes. Gel water content was determined as the percentage of water separated relative to the initial weight of the gel [23].

2-9- Transparency of starch pastes

The transparency of pastes obtained from natural and modified starches was determined according to the method of Reddy and Seib (1999) [24]. First, 0.05 g of each starch was suspended in five milliliters of distilled water and poured into a screw-capped test tube and placed in a 95°C water bath for 30 minutes, shaking several times every five minutes. After cooling, the transparency of the starches was measured using a spectrophotometer at a wavelength of 650 nm against a water control sample.

2-10- Fourier Transform Infrared Spectroscopy (FTIR)

The infrared spectra of the native starch samples and the optimized modified sample were performed using an FTIR spectrometer. Initially, each of the samples was mixed well with potassium bromide powder and pressed into a tablet to be placed in the device. 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 cm⁻¹ [25].

2-11- Determination of X-ray diffraction (XRD) patterns

The native starch samples and the optimized modified sample were examined in an X-ray diffractometer at a voltage of 35 kV and an amperage of 30 mA. The recorded differences were between 2 theta angles of 4 to 70 degrees, with a step of 0.04 degrees. The percentage of crystallinity of each starch sample was determined using the following:

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

where A_c and A_a were the areas of crystalline and amorphous regions, respectively, obtained based on X-ray diffractometry of the samples [25].

2-12- Determination of thermal properties (DSC)

In order to investigate the thermal properties of starch gelatinization, 5 mg of starch (based on dry weight) was weighed into a 40 µL aluminum container and after adding 20 µL of deionized water and sealing, it was equilibrated at refrigerator temperature for 24 hours. After the container was equilibrated with the environment, it was heated from 20 to 120°C at a rate of 10°C/min using a differential scanning calorimetry device. The data were analyzed by software to determine the onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinization (ΔH) [26].

2-13- Morphological study of starch granules (SEM)

A 1% starch suspension in ethanol was prepared and after homogenization with a vortex mixer, a drop was transferred to an aluminum base and after drying, it was coated using a gold-palladium coating device with a current intensity of 9 mA and a vacuum of 3-10 Pa, then imaging was performed with a scanning electron microscope with an accelerating voltage of 20 kV [27].

2-14- Determination of starch pasting behavior

The starch pasting behavior was determined using a rapid viscosity analyzer (RVA). A 14% starch suspension (28 grams) was prepared in the device container and, after preliminary stirring with the device stirrer, was placed in the device chamber. The stirring speed was set to 960 rpm for the first ten seconds of the test and then 160 rpm. The changes in the sample viscosity during the test were measured based on the sample temperature change program in five stages. 1- Holding at 50°C for one minute; 2- Increasing the temperature from 50°C to 95°C at a rate of 12°C/min; 3- Holding at 95°C for 2.5 minutes; 4- Reducing the temperature from 95°C to 50°C at a rate of 12°C/min; and

5- Holding at 50°C for two minutes. The indicators of pasting temperature, peak viscosity, trough viscosity and final viscosity were determined by the software based on the graph of viscosity changes against temperature [28].

2-15- Measurement of starch gel firmness

In order to examine the firmness of starch gel, first a 10% suspension of starch in deionized water was prepared and stirred for 15 minutes by a mechanical stirrer. The sample was placed in a boiling water bath for 15 minutes at a stirring speed of 160 rpm for cooking. The resulting hot dough was transferred to a cylindrical mold with a diameter and height of 10 mm and kept in the refrigerator for 24 hours to form and develop the gel. The texture profile analysis test of the gel sample was performed using a texture analyzer with a 50 N load cell at ambient temperature, under conditions of two compressions at a speed of 2 mm/s, a percentage change of 50% and an initial loading force of 10 g. In this test, the sample was subjected to a 50% axial tension to prevent gel degradation during the test, as suggested by Pons and Fiszman [29]. Based on the force-time data, the firmness index will be calculated.

Table 1. Box-Benken design matrix for optimization of parametrs affecting phosphorylated starch production.

Sample	pH	Temperature (C)	Concentration (%)
1	11.5	130	4.5
2	10.5	150	1.5
3	9.5	110	3
4	10.5	110	4.5
5	11.5	110	3
6	11.5	130	1.5
7	10.5	130	3
8	10.5	150	4.5
9	11.5	150	3
10	9.5	130	4.5
11	10.5	130	3
12	10.5	110	1.5
13	10.5	130	3
14	9.5	150	3
15	10.5	130	3
16	9.5	130	1.5
17	10.5	130	3

3- Results and Discussion

3-1- Chemical Composition

Table 2 shows the chemical composition of native wheat starch. The very low levels of protein and fat indicate that the purchased wheat starch was of adequate purity and that most of the remaining protein was removed

during the separation process. Consequently, it can be claimed that the differences in the functional properties of the starch were not influenced by impurities [30].

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 rate at 60°C of phosphorylated wheat starches was in the range of 15.28-19.4%. This was while this index was obtained in the initial starch used as 2.00%. A linear model with interaction effects to describe the effect of temperature, pH and reactant concentration on the dissolution at 60°C of the produced phosphorylated starch was significant (p<0.05).

Analysis of variance showed that the effect of temperature was insignificant, but the effect of pH and reactant concentration was significant (p<0.05). Among the interaction effects, only the pH-concentration effect was significant

(p<0.05). As shown in Figure 1, increasing pH reduced the solubility rate at 60°C of phosphorylated starches. On the other hand, increasing the concentration increased the solubility rate at 60°C. Regarding the pH-reagent concentration interaction, an antagonistic effect was also observed; so that at low levels of reagent concentration, increasing the pH increased the starch dissolution rate at 60°C, while increasing the reagent concentration reversed this effect (Figure 1). In addition, the solubility rate at 60°C had a significant correlation with the phosphorylation rate, equal to 0.720 (p<0.05).

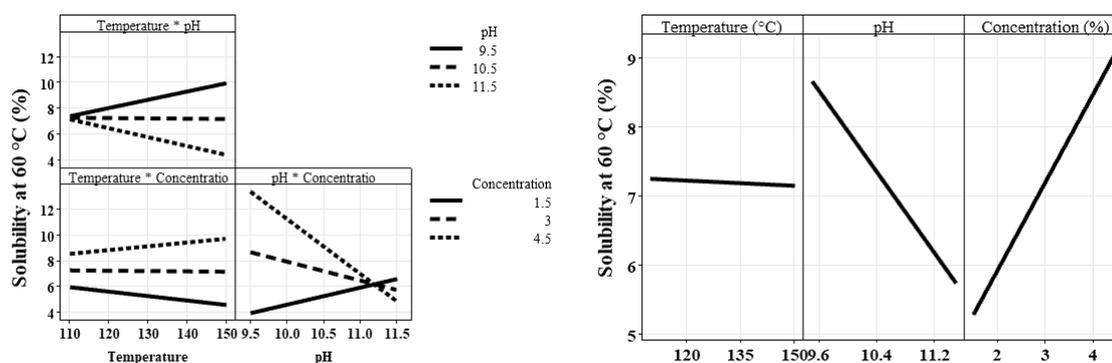


Fig 1. Simple and reciprocal effects of temperature, pH and reactant concentration on the solubility rate of phosphorylated wheat starch at 60°C.

On the other hand, the solubility of native starch at 75°C was estimated to be 3.57%. This parameter was 1.39-22.75% in phosphorylated starches; also, in order to describe the effect of temperature, pH, and reagent concentration on the solubility of phosphorylated starch at 75°C,

a linear model with interaction effects was significant (p<0.05).

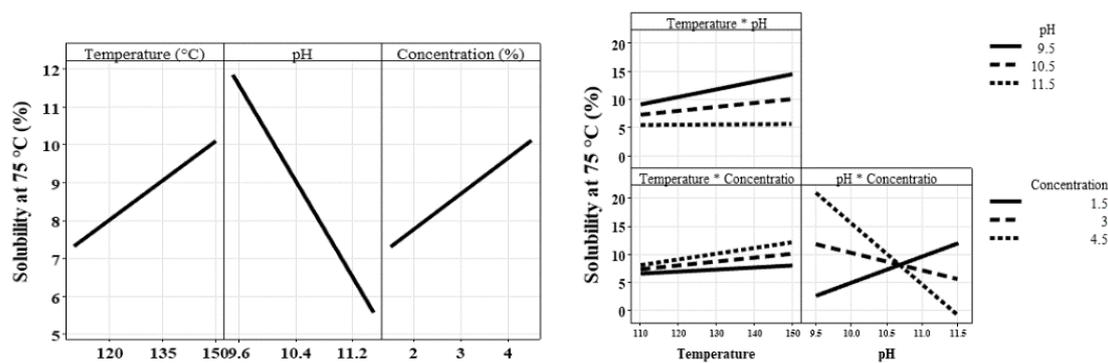


Fig 2. Simple and reciprocal effects of temperature, pH and reactant concentration on the solubility rate of phosphorylated wheat starch at 75°C.

According to the analysis of variance of the data, only the linear effect of pH and the interaction effect of pH - reactant concentration on the solubility at 75°C of phosphorylated starch were significant ($p < 0.05$). The linear effect of pH was such that increasing pH caused a decrease in the solubility at 75°C of phosphorylated starch. According to Figure 2 and the negative coefficient of the equation, the interaction effect of pH - reactant concentration can be concluded that this effect is antagonistic. The results showed that there was no significant correlation between the solubility rate at 75°C and the phosphorylation rate.

$$\text{Solubility at } 75^{\circ}\text{C} (\%) = -215.0 + 0.687 T + 21.00 \text{ pH} + 52.93 C - 0.0651 T \cdot \text{pH} + 0.0221 T \cdot C - 5.225 \text{ pH} \cdot C$$

On the other hand, the range of solubility changes at 90°C for phosphorylated starches was 24.60-4.13%, while this value was 4.98%

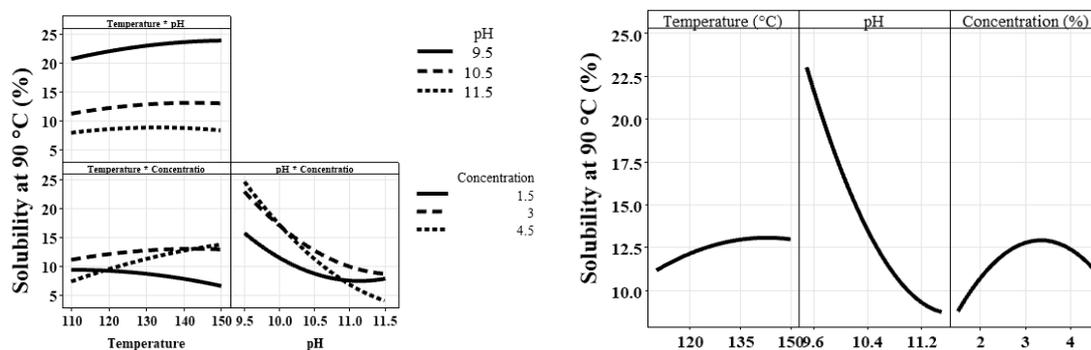


Fig 3. Simple and reciprocal effects of temperature, pH and reactant concentration on the solubility rate at 90°C of phosphorylated wheat starch.

In general, the correlation between the solubility rate of phosphorylated starch samples and the degree of starch phosphorylation showed that the solubility of phosphorylated

starch is directly correlated with the degree of phosphorylation and that the solubility rate increases with increasing phosphorylation (Figure 4). The researchers also stated that the effect of the variables studied on the solubility rate at 90°C for phosphorylated samples, linear models with interaction and quadratic effects were significant ($p < 0.05$). Analysis of variance showed that the linear effect of pH, the square of pH and the concentration of the reactant, as well as the interaction effect of pH and the concentration of the reactant on the dissolution rate at 90°C were significant ($p < 0.05$). The results of the effect of pH on the solubility rate at 90°C showed that increasing pH decreased the dissolution rate at 90°C for phosphorylated starches. The negative pH coefficient of the term pH and reactant concentration in the fitted relationship showed that this interaction was antagonistic (Figure 3). The correlation coefficient between the phosphorylation rate of the samples and the solubility rate at 90°C was significant ($p < 0.05$) and was 0.503.

starch is directly correlated with the degree of phosphorylation and that the solubility rate increases with increasing phosphorylation (Figure 4). The researchers also stated that the

presence of hydrophilic phosphorus groups is responsible for increasing the storage capacity of STMP/STPP cross-linked starch and the placement of these groups on the starch molecule contributes to the electrostatic repulsion between starch chains, thereby facilitating further water absorption and swelling, leading to an increase in the solubility percentage [31]. Olawoye et al. (2021) investigated the effect of a cross-linking agent (sodium hexametaphosphate) on the properties of banana starch. They found that cross-linking

increased the water absorption capacity and oil absorption capacity due to the increased space between starch chains due to the steric hindrance of this compound added to the starch molecule [32]. In contrast, Radi et al. (2022) reported that cross-linking of potato starch with POCl_3 significantly reduced the water absorption capacity compared to native starch due to the formation of a covalent bond and resistance to the mobility of the amorphous region of the starch granule [31].

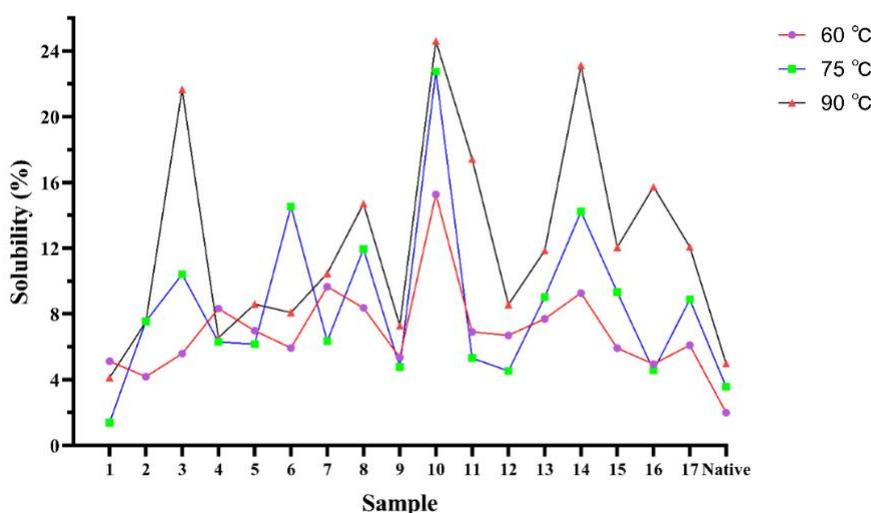


Fig 4. Solubility of native and crosslinked starch samples.

3-3- Swelling power

The results showed that the swelling power at 60°C for phosphorylated starches ranged from

12.79 to 4.85 g/g, while this value was estimated to be 5.54 g/g for the original raw starch. Regarding the effect of temperature, pH, and reagent concentration changes on the swelling power at 60°C, the linear model with interaction effects was significant ($p < 0.05$).

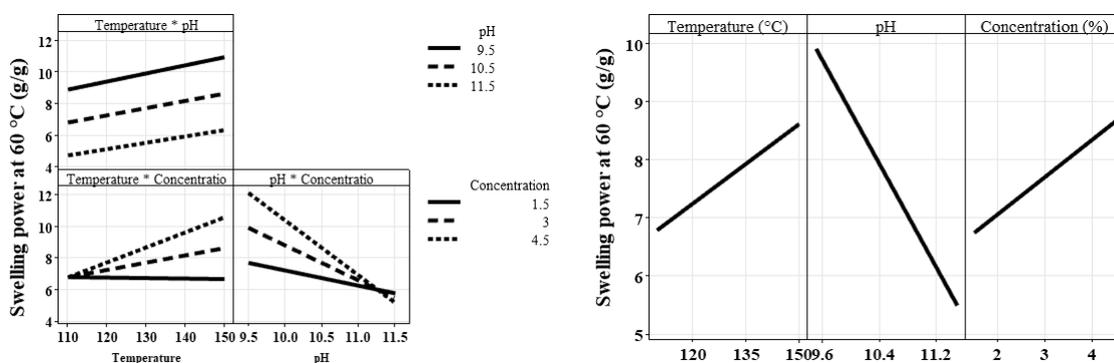


Fig 5. Simple and reciprocal effects of temperature, pH and reactant concentration on swelling power at 60°C temperature of phosphorylated wheat starch

The results of the analysis of variance showed that the interaction effects of temperature-

reactant concentration and pH-reactant concentration were significant, while the

interaction effect of temperature-pH was not ($p < 0.05$). Temperature and reactant concentration had an increasing effect, and in contrast, pH had a decreasing effect on the swelling power at 60°C. The interaction effect of temperature-reactant concentration was synergistic, and the interaction effect of pH-reactant concentration was antagonistic (Figure 5). On the other hand, the correlation coefficient between the swelling power at 60°C and the phosphorylation level was 0.720.

The swelling power of natural starch at 60°C was 59.7%, while this index for phosphorylated starches was in the range of 14.14–6.04 g/g. On the other hand, the linear model was significant to describe the effect of temperature, pH and reagent concentration on swelling power at 75°C. As shown in Figure 6, swelling power at 75°C decreased with increasing pH ($p < 0.05$). The correlation coefficient between swelling power at 75°C and phosphorylation level was 0.543.

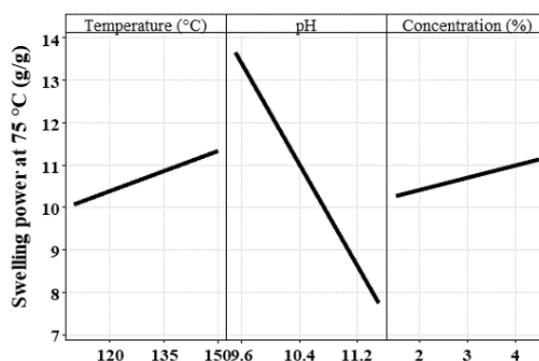


Fig 6. Simple effects of temperature, pH and reactant concentration on swelling power of phosphorylated wheat starch at 75°C.

On the other hand, the results showed that the range of changes in the swelling power of phosphorylated starches at 90°C was in the range of 18.73 - 6.69 g/g, while this amount was 9.81 g/g in the original raw starch. On the other hand, in order to investigate the effects of reaction conditions on the swelling power at 90°C, a linear model with interaction effects was significant ($p < 0.05$). The results of the analysis of variance showed that the linear and interaction effect of pH value and reagent

concentration on the swelling power at 90°C was significant. These two factors acted oppositely so that increasing the pH value and reagent concentration caused a decrease and an increase in the swelling power at this temperature, respectively (Figure 7). Thus, the antagonistic effect observed between this pH value and reagent concentration is expected. On the other hand, the swelling power at 90°C correlated with the degree of starch phosphorylation with a coefficient of 0.498.

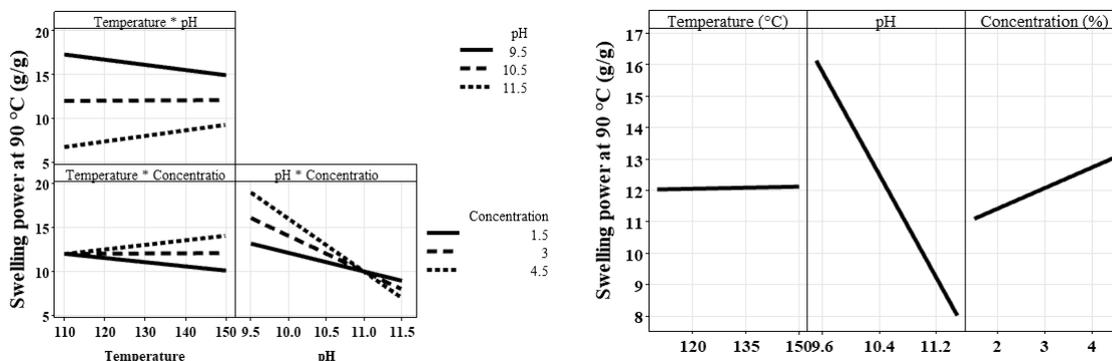


Fig 7. Simple and reciprocal effects of temperature, pH and reactant concentration on swelling power of phosphorylated wheat starch at 90°C.

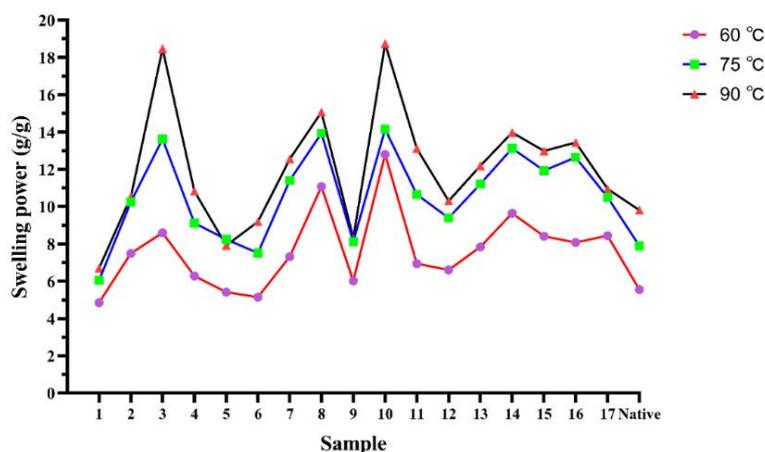


Fig 8. Swelling power of native and crosslinked starch samples.

Overall, the results of the correlation analysis between the swelling power at different temperatures and the degree of phosphorylation of the produced starch showed a positive correlation. It was found that with the increase in the degree of phosphorylation, the swelling power of starches also increases (Figure 8). Therefore, it can be stated that there is a direct relationship between the amount of phosphorylation and the swelling power of starches. In addition, it is believed that the introduction of phosphate groups into starch granules helps to increase the swelling power; because the repulsion between adjacent starch molecules caused by the negatively charged phosphate groups reduces the intra-chain linkages and ultimately leads to an increase in the swelling power [33].

According to Iweajunwa et al. (2023), cross-linking of starch extracted from two tubers (*Icacina senegalensis* and *Cyrtosperma senegalense*) increased the swelling power compared to natural starch [34]. While Singh et al. (2009) reported that the swelling power of Moth bean starch decreased with increasing degree of cross-linking due to formation of intermolecular bridges in starch chains by cross-linking reagent [35]. Hirsch et al. (2002) investigated the effect of the type of cross-linking compound on the swelling power behavior of waxy maize starch and observed that both POCl_3 and STMP compounds reduced the swelling of starch granules and there was an inverse correlation between the concentration of these compounds and the swelling rate, but

the magnitude of this inverse correlation between the concentration and swelling rate was much stronger in starches treated with POCl_3 than STMP. The researchers attributed this to the mechanical difference in the cross-linking reaction and stated that the effect of cross-linking on the granule structure by POCl_3 , which is a very fast reactant, is different compared to STMP, which is a slower reactant. The researchers also stated that POCl_3 cross-links appeared to be more effective in preventing starch swelling than STMP. The researchers explained this phenomenon by claiming that there was a high concentration of POCl_3 cross-links on the granule surface, which resulted in a hard shell on the outer layers of the granule, but STMP, which is a slower reactant, penetrated the granules and therefore the cross-links from this material had a much more dispersed effect because they were more evenly distributed throughout the granule volume [36].

3-4- Transparency

The transparency of natural wheat starch paste was determined to be 79.3%; while this value varied from 1.15 to 10.55 for phosphorylated starches. On the other hand, in order to investigate the effect of reaction variables (temperature, pH value and reagent concentration) on the transparency of phosphorylated starch pastes, a linear model with interaction effects was significant. The results of analysis of variance showed that only the linear effect of pH value and the interaction effect of pH value-reagent concentration were significant ($p < 0.05$). So that the pH value

showed a decreasing effect on the transparency of the samples. The intensity of the effect of pH value was affected by the reagent concentration, and with increasing reagent concentration, the slope of the graph and consequently the effect of pH value increased (Figure 9). According to the correlation coefficient obtained between the degree of phosphorylation and starch gel transparency (0.562), the degree of gel transparency increased with increasing phosphorylation.

The increase in gel transparency with increasing phosphorylation can be attributed to the presence of charged phosphate groups, which help in the hydration of starch molecules and reduce the rate of their recrystallization. In addition, these phosphate groups can strengthen the bonds between starch molecules and water, leading to increased swelling power and transparency of the paste [37]. On the other hand, Kaur et al. (2006) have attributed the decrease in transparency of cross-linked starch gels to a partial decrease in swelling power [24,

33]. In this study, the increase in gel transparency with increasing phosphorylation can be related to the increase in swelling power of granules. The correlation coefficients of gel transparency and starch swelling power at temperatures of 90, 75, and 60 are 0.882, 0.821, and 0.864, respectively ($p < 0.05$), which confirms the increase in gel transparency due to the increase in swelling power (as mentioned above, the swelling power increased with increasing phosphorylation).

Mehfooz et al. (2020) reported that barley starch cross-linked with STMP/STPP showed higher gel clarity and lower retrogradation tendency than native starch due to higher swelling power (highly swollen granules allow better light transmission) [38]. Similarly, Chandak et al. (2022) found that lotus seed starch cross-linked with different concentrations of STMP showed higher gel clarity and lower retrogradation rate than native starch [39].

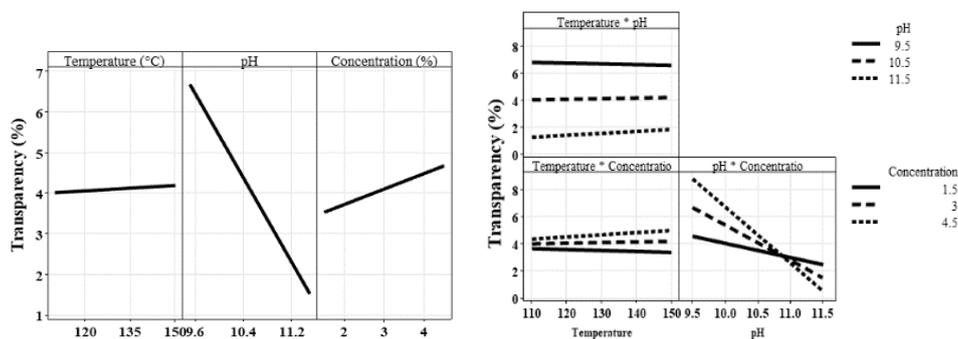


Fig 9. Simple and reciprocal effects of temperature, pH and reactant concentration on the transparency of phosphorylated wheat starch paste.

3-5- Syneresis

As shown in Figure 10, the syneresis of the native wheat starch sample was 33.64%; phosphorylation modification with different reaction conditions shifted this value to the range of 29.56–71.62. The quadratic model was also significant ($p < 0.05$) in order to interpret the effect of reaction conditions (effect of temperature, pH value and reactant concentration) on the syneresis of phosphorylated starch samples. On the other hand, the results of variance analysis showed that only the linear and square effect of pH value on syneresis was significant ($p < 0.05$). So

that with increasing pH value, the syneresis of the samples increased (Figure 11).

Sripabloom et al. (2023) reported that cross-linking of cassava starch with lower concentrations of STMP/STPP significantly reduced the retrogradation rate and syneresis of starch gel. However, higher concentrations of STMP/STPP did not show a significant change compared to native starch. Low-level cross-linking limits the mobility of starch chains and can delay retrogradation. However, higher concentrations provide a more closed structure that increases the hydrogen bonding ability

between starch chains and increases the rate of retrogradation [40].

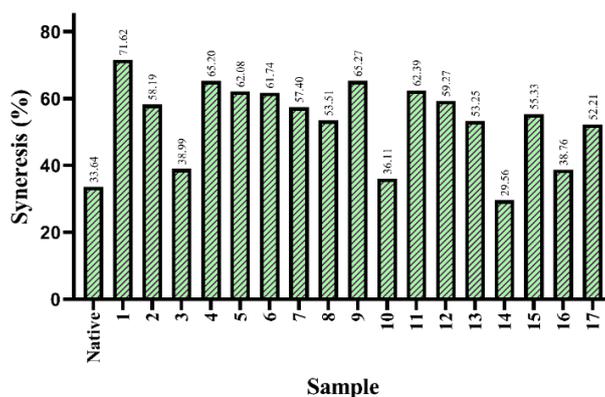


Fig 10. Syneresis of native and crosslinked starch samples.

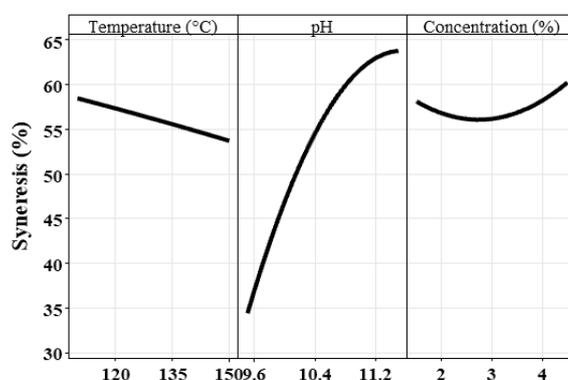


Fig 11. Effects of temperature, pH and reactant concentration on the syneresis of phosphorylated wheat starch.

3-6- Freeze-thaw stability

The stability of starch-containing food products during the freezing and thawing process is an important property that affects their textural properties. During freezing, the water in the starch turns into ice, and during thawing, the resulting water easily leaves the matrix, which is known as syneresis. Phase separation leads to re-linking and recrystallization of starch chains. Hence, repeated freeze-thaw causes phase separation and syneresis and retrogradation [41]. The results showed that phosphorylation modification changed the water release rate after freeze-thaw from 26.31% for native starch to the range of 76.65–10.05%. In addition, in order to interpret the reaction conditions (temperature, pH value and reagent concentration) on the waterlogging rate after freezing and thawing conditions of phosphorylated starch samples, a linear model

was significantly ($p < 0.05$). The results of the analysis of variance showed that only the effect of pH value on the syneresis rate after freezing-thawing was significant ($p < 0.05$) and as shown in Figure 12, it showed its effect in an increasing manner. On the other hand, there was no correlation between the syneresis rate after freezing-thawing and the phosphorylation rate ($p < 0.05$).

Xiaofan et al. (2022) found that the introduction of phosphate groups into the tiger nut starch molecule by forming a three-dimensional network and creating steric hindrance made the system more stable [42]. In contrast, Oh et al. (2019) reported that cross-linking chestnut starch with STMP/STPP reduced its freeze-thaw stability. The higher syneresis of cross-linked starch could be due to the limited swelling of cross-linked starch, which results in the release of a relatively large amount of water

[43]. Nakkala et al. (2022) reported that phosphorylated turmeric starch showed higher freeze-thaw stability than native starch because the phosphate groups are positively charged and

hydrophilic, and thus form a three-dimensional gel after gelatinization and subsequent freezing [44].

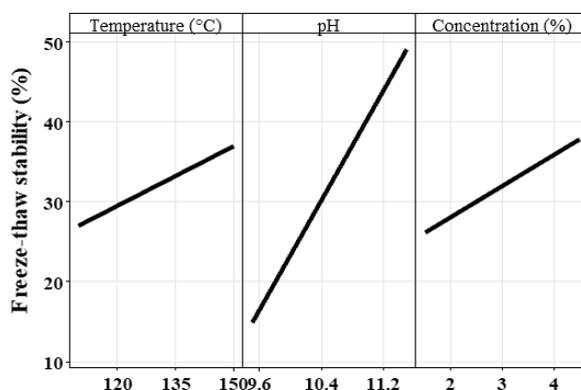


Fig 12. The effect of temperature, pH and reactant concentration on the Freeze-thaw stability of phosphorylated wheat starch.

3-7- Phosphorus content

The properties of modified starches are significantly affected by the amount of each chemical substitution applied, and adjusting the percentage of specific chemical substitution on a particular type of starch can result in different properties [8]. The efficiency of the cross-linking reaction is indicated by the degree of substitution, which indicates the number of moles of cross-linked phosphate present in anhydrous glucose (glucose anhydride). The degree of substitution of modified starch is calculated by subtracting the percentage of intrinsic phosphorus from that of native starch, since native starch contains certain amounts of phosphate monoesters. In this study, the range of variation of the phosphorylation amount was estimated to be 0.27–1.34%, and the intrinsic phosphorus content of the wheat starch used was 0.047% (Figure 13). The results of data analysis showed that the linear model for investigating the effects of temperature, pH and reagent concentration on the phosphorylation

rate of wheat starch was significant ($p < 0.05$). On the other hand, the results of variance analysis showed that the effect of all three factors under study was significant ($p < 0.05$), so that, according to Figure 13, with increasing temperature and reagent concentration, the phosphorylation rate increased and with increasing pH, this rate decreased.

Kim et al. (1999) investigated the effect of temperature on the phosphorylation of rice starch by extrusion and stated that the phosphorylation rate increased with increasing extrusion temperature from 120 to 180°C [45]. Muhammad et al. (2000) investigated the effect of pH on the phosphorylation of sago starch using STMP and STPP separately or in combination. These researchers stated that when using STMP alone, increasing the pH from 6 to 10 increased the phosphorylation rate, but using the combination of STMP and STPP in the same pH range resulted in a decrease in the phosphorylation rate [46].

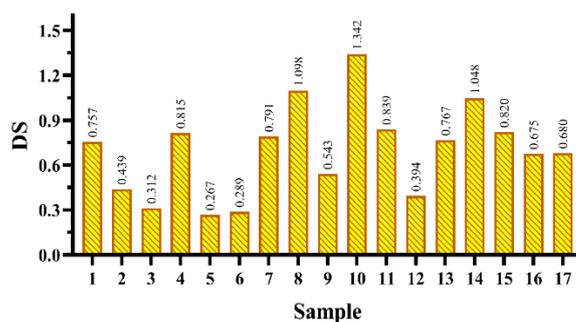


Fig 13. Degree of substitution of cross-linked starches

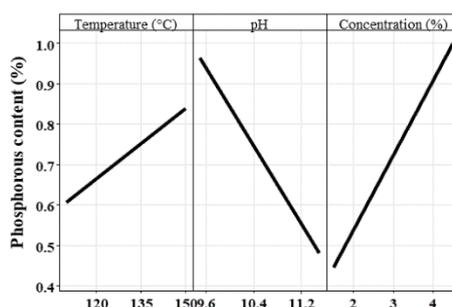


Fig 14. The effect of temperature, pH and reagent concentration on the degree of phosphorylation of wheat starch.

According to the results of physicochemical tests, the characteristics of the starch used in the sauce formulation were used as optimization criteria; phosphorylated starch with higher swelling power, greater paste transparency, less gel syneresis, and higher freeze-thaw stability was selected; then, in order to investigate the morphological, structural, thermal, gelation, and paste-forming properties, supplementary tests were conducted on the optimized sample and natural starch.

3-8- Amylose content and gel firmness

The results showed that starch phosphorylation reduced the amylose content of natural starch from 25.78 percent to 22.43 percent; because the presence of phosphodiester bonds in starch chains reduced the formation of amylose iodine complexes and consequently reduced the amylose content. In addition, the decrease in amylose content can be attributed to the formation of cross-links between amylose and amylopectin chains, which prevents the interaction of the structure with iodine [47]. Ačkar et al. (2010) reported that the amylose content of native Golubica wheat starch was

20.27 and that of native Srpanjka starch was 22.49, and this value varied for different wheat starch genotypes [48].

Chandak et al. (2022) found that the amylose content of lotus starch decreased significantly with increasing STMP concentration from 1 to 5% and ranged between 13.45 and 25.16%. These researchers stated that the decrease in amylose content of cross-linked starches was due to intramolecular and intermolecular interactions in amylose molecules or between amylose and amylopectin molecules [39]. In addition, Sharma et al. (2020) study showed that cross-linking (STMP) caused a decrease in amylose content of fava bean starch [49].

The concept of firmness refers to the ability of food materials to resist compressive force. As the strength of the gel increases, the amount of force required to break its structure also increases [50]. The results showed that cross-linking modification increased the firmness of the phosphorylated starch gel (0.84 N) compared to native starch (0.21 N) (Table 3). The increased firmness of the phosphorylated starch gel compared to native starch can be attributed to the fact that the reduction in the amount of soluble amylose results in a softer

gel, while more amylose leaks from the starch granules into the intergranular space and forms a stronger gel network, ultimately resulting in a harder gel than native starch.

On the other hand, gel firmness is mainly due to starch gel retrogradation, which is accompanied by amylopectin recrystallization, resulting in

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

Sampl	Amylose (%)	Firmness of the gel (N)
Native starch	25.78 ± 1.31 ^a	0.21 ± 0.41 ^b
Phosphorylated Starch	22.43 ± 0.72 ^b	0.84 ± 0.34 ^a

3-9- X-ray diffraction and crystallinity percentage

The A-type diffraction patterns with peaks at 15, 17, 18 and 23° for native and cross-linked starches are shown in Figure 15. The diffraction pattern of cross-linked starches was similar to that of native starch. These results indicated that cross-linking did not change the crystalline pattern of starch. According to Chen et al. (2017), cross-linking normally occurs in the amorphous parts of starch granules without affecting the crystalline pattern [53].

The findings showed that cross-linked starches exhibited lower crystallinity (RC) compared to native starch. This was attributed to the reduction of hydrogen bonding in the crystalline structure of wheat starch granules (Table 4). Majzoubi et al. (2009) stated that the cross-linking agent, which acts as a bridge, can reduce the distance between starch molecules

harder gels [51]. According to Mua and Jackson (1997), starches with longer amylopectin chains form harder gels [52]. The higher gel firmness of phosphorylated starch compared to native starch confirms the increased tendency of this starch to form gels, as well as higher values of ultimate viscosity and viscosity recovery.

without changing the crystalline structure of the starch inside the granules. Furthermore, cross-linking may occur mainly in the amorphous regions of the starch granules because these regions are easily accessible to cross-linking reagents [2]. Chandak et al. (2022) investigated the effect of different concentrations of cross-linking agent on lotus starch. They found that the crystallinity values decreased slightly with increasing levels of cross-linking agent, due to chemical modification and changes in the crystal structure that occur during the cross-linking process [39]. In contrast, the study by Karmvir et al. (2018) showed that cross-linked sorghum starch has higher crystallinity than its native starch. These researchers attributed this increase to the introduction of a functional group after chemical modification, which mainly occurred in the amorphous region of the starch granule and caused the destruction of starch molecules [54].

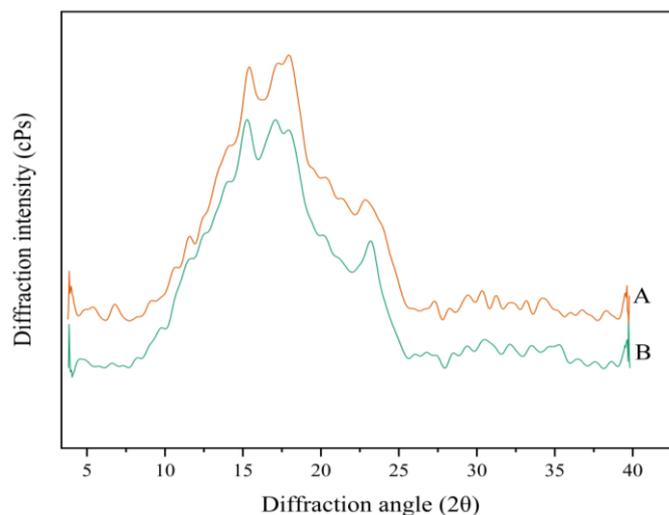


Fig 15. X-ray diffraction pattern of native (A) and phosphorylated wheat starch (B).

3-10- FTIR

The FTIR spectra of the samples are presented to investigate the changes in the molecular structure of cross-linked starch compared to native starch. As shown in Figure 16, the absorption spectra of native and phosphorylated starch were similar; indicating that cross-linking modification does not introduce new chemical groups into the starch structure, but only enhances the absorption of certain peaks. In order to investigate the changes in the intensity of the peaks, the following main peaks in the FTIR spectra of wheat starch samples were considered:

1- wavelength 3427cm^{-1} , stretching vibrations of internal hydroxyl groups (O-H); 2- wavelength 2926cm^{-1} , asymmetric stretching of carbon-hydrogen bond (C-H); 3- wavelength 1640cm^{-1} , bending vibrations H-O-H in the amorphous regions of starch (related to the adsorbed water in the amorphous region of starch granules); 4- Wavelengths 1363cm^{-1} and 1338cm^{-1} , C-H stretching; 5- 1242cm^{-1} , C-OH, CH₂OH stretching; 6- The range $1100\text{-}1160\text{cm}^{-1}$ is asymmetric C-O-C stretching; 7- Peaks $928\text{-}1077\text{ cm}^{-1}$ are assigned to 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. This lack of difference was attributed to the lack of change in the chemical structure of the molecules compared to native starch [56]. However, phosphorylation caused a decrease in the intensity of the peaks at 3264cm^{-1} , 1640cm^{-1} and 994cm^{-1} compared to native starch, which could be attributed to the disruption of hydrogen bonds and the introduction of phosphate groups among the hydroxyl groups of the starch samples. Similarly, Shalviri et al. (2010) reported a decrease in the peak intensity in cross-linked starch compared to native starch [57]. In contrast, Jyothi et al. (2006) observed that the O-H stretching band in the region of $3000\text{-}3600\text{cm}^{-1}$ was broadened and sharper in the spectrum of cross-linked starches compared to native starch [58].

On the other hand, the intensity of the peaks at 997cm^{-1} and 1298cm^{-1} increased in phosphorylated starch compared to native starch, the reason for the increase in the intensity of these peaks in cross-linked starch is due to the bonds forming the P-O-C bond. Also, the slight increase in the intensity of the peaks of phosphorylated starch in the region of $1150\text{-}1300\text{cm}^{-1}$ compared to native starch was attributed to the increase in the symmetric stretching of the phosphorus-oxygen (P=O) bond; Therefore, the decrease in the intensity of the peak at 3264cm^{-1} (–OH stretching vibrations) and the increase in the intensity of the peak at $1150\text{-}1300\text{cm}^{-1}$ (symmetric stretching of the phosphorus-oxygen bond (P=O)) indicate that the starch has reacted with STMP [59].

On the other hand, the absorption intensity of the peaks at 994cm^{-1} and 1082cm^{-1} can be attributed to the changes in the crystal structure and crystallinity of starch polymers, while the absorption intensity of the peak at 1018cm^{-1} is related to the structure of the amorphous regions of starch granules [60]. Therefore, the absorption ratios of 1082 to 1018cm^{-1} and 994 to 1018cm^{-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 1018 to 994cm^{-1} is related to the internal changes in the amorphous regions of starch granules [61].

The absorption intensity of the peaks at 994cm^{-1} and 1082cm^{-1} is related to the changes in the crystallinity of starch polymers, while the absorption intensity of the peak at 1018cm^{-1} is related to the amorphous structure of the starch granule regions. Consequently, the absorption ratios of 1082 to 1018cm^{-1} and 994 to 1018cm^{-1} are used to characterize the degree of ordering of the crystalline regions of starch granules (degree of ordering; DO) and the double helix content (DD), respectively. However, the absorption ratio of 1018 to 994cm^{-1} is related to the internal changes in the amorphous regions of starch granules [62].

The present study compared the peak intensity ratio of phosphorylated starch with that of

native starch and showed that phosphorylation led to a decrease in the ratio of DD and DO to starch by reducing hydrogen bonds in the crystal structure of wheat starch granules. On the other hand, phosphorylation led to an increase in the ratio from 1018 to 994 cm^{-1} by reducing the order in the crystal structure of wheat starch granules, which was confirmed by X-ray diffraction and crystallinity percentage analysis. While the study by Shi et al. (2013)

showed that the amorphous regions of starch granules were reduced by crosslinking. These researchers attributed the reason for the reduction of these regions to the alignment and association of amylose-amylose or amylose-amylopectin in the amorphous regions of starch; because the modification of cross-linking changed the structure of starch molecules by making them more compact compared to native starch molecules [2].

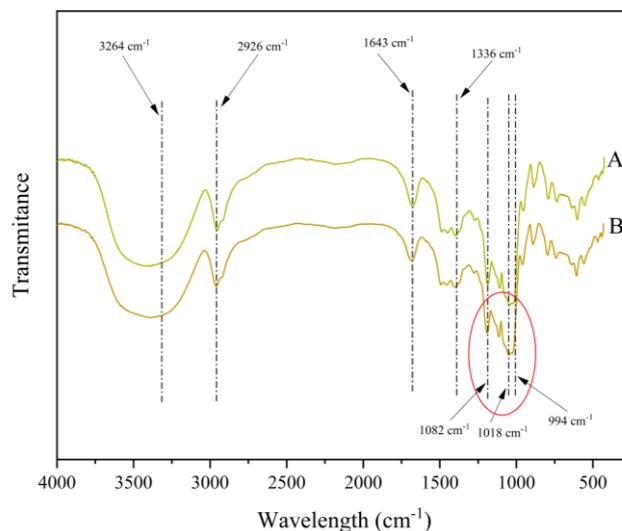


Fig 16. FT-IR spectra of native (A) and phosphorylated wheat starch (B).

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	1.38 ± 0.04^a	1.29 ± 0.02^a	0.83 ± 0.07^b	44.52 ± 0.57^a
Phosphorylated Starch	1.34 ± 0.09^b	1.20 ± 0.06^b	0.87 ± 0.08^a	41.22 ± 0.35^b

3-11- DSC

Thermal properties of the onset (T_o), peak (T_p), and conclusion (T_c) temperatures of gelatinization and the enthalpy of gelatinization (ΔH) of native and cross-linked starch were evaluated by differential scanning calorimetry (DSC) (Table 5). The results showed that the modification of starch phosphorylation significantly increased T_o and T_p compared to native starch, which can be attributed to the introduction of phosphate groups to starch molecules and the strengthening of intermolecular interactions, which inhibited starch gelatinization [63]. During this modification, the strengthening of the

amorphous structure of starch granules through covalent bonding led to the formation of a highly amorphous and complex internal structure. This resulted in a decrease in the final temperature (T_c) and the enthalpy of gelatinization (ΔH). The results of the peak intensity ratio in Fourier transform spectroscopy and the percentage of crystallinity confirmed this relationship and showed a positive correlation with the experimental results [53].

Sharma et al. (2021) studied the thermal properties of Barnyard millet starch cross-linked with (STMP) at different levels (1, 3 and 5%). They found that cross-linking increased the T_o and T_p values, while decreasing the T_c and ΔH values. They attributed the increase in

T_o and T_p values to the strengthening of the molecular connectivity of starch granules after cross-linking, while they attributed the decrease in T_c and ΔH to the formation of a highly amorphous and complex internal structure [64].

In contrast, Olayemi et al. (2021) found that the T_o , T_p , T_c , and ΔH values increased with increasing citric acid concentration in Cyprus starch. This indicated that cross-linking by citric acid resulted in higher values for these parameters. Furthermore, the researchers noted that cross-linked starches exhibited higher gelatinization temperatures, indicating better cross-linking ability compared to native starch [65]. Dong and Vasanthan (2020) studied the

effect of cross-linking modification with $POCl_3$ and STMP in aqueous and semi-dry forms on corn, chickpea, and fava bean starch. They reported that the cross-linked starches showed potential changes in their gelatinization properties. Cross-linking with $POCl_3$ shifted the endothermic peak to higher temperatures for all three starch samples. Similarly, STMP treatment in a semi-dry form significantly reduced the T_o value while increasing the T_p and T_c values of all starches compared to their native starch. As a result, this study showed that cross-linking agents and crosslinking methods have different effects on the linkages of starch molecules [66].

Table 5. Thermal properties of native and phosphorylated wheat starch.

Sampel	T_o	T_p	T_c	ΔH
Native starch	52.90±0.13 ^b	58.12±11 ^b	64.37±0.06 ^a	8.48±0.11 ^a
Phosphorylated Starch	53.40±0.16 ^a	58.20±0.24 ^a	62.58±0.11 ^b	5.47±0.10 ^b

3-12- Pasting

The pasting parameters of native and cross-linked starch are shown in Table 6. During heating and gelatinization, the molecular arrangement of starch undergoes significant changes. The pasting temperature (PT) is the minimum temperature required to cook starch. The peak viscosity (PV) represents the highest viscosity of the starch at the equilibrium point between swelling and leakage of the polymer due to the increase in temperature, while the breakdown viscosity (BD) indicates the stability of the starch granules against the increase in temperature and shear forces. During cooling, the aggregation of amylose molecules, which leads to the increase in viscosity, is indicated by the final viscosity (FV), while the set-back viscosity (SB) indicates the ability of the starch to gel or retrograde [67].

The results showed that the pasteurization temperature of cross-linked starch increased compared to native starch; the reason for this increase can be attributed to the prevention of amylose release from starch molecules, which

leads to molecular reinforcement and limited swelling during the gelatinization process [46]. Similarly, Sharma et al. (2021) reported that crosslinking modification increased the pasting temperature of litchi kernel starch compared to native starch [68]. Jyothi et al. (2006) stated that cross-linking modification increases the energy required for swelling of starch granules by creating bonds between starch molecular chains [58].

On the other hand, the results showed that the peak viscosity and breakdown viscosity of cross-linked starches increased compared to native starch, which was attributed to the increase in intra- and intermolecular interactions of starch granules, as the increase in these interactions reduced the degradation of starch granules during heating and resulted in increased viscosity [69].

Cross-linking modification through the formation of covalent bonds and cross-linking between amylose and amylopectin strengthened the swollen starch granules, and as a result, reduced the breakdown viscosity and fracture of starch paste under mechanical and thermal shear, which was proportional to the increase in the resistance of starch granules to

mechanical and thermal shear [33]. Chandak et al. (2022) also reported that crosslinking modification due to the stiffening of starch molecules reduced the viscosity fracture of kitol starch compared to native starch [39].

On the other hand, cross-linking by increasing the water absorption of starch granules resulted in larger starch granules compared to native starch, resulting in higher final viscosity of the modified starch during cooling [70]. Setback viscosity (SB) is an indicator of the ability of starch paste to form a gel or retrograde [71]. Cross-linking modification slightly increased the amount of viscosity reversal compared to native starch. According to Sudheesh et al. (2020), phosphorylation modification increases viscosity reversal due to increased rearrangement of starch chains that leak out of the starch granules through surface damage that occurs during modification [72].

The results of Jia et al. (2022) showed that the cross-linking agent STMP reduced the pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), breaking viscosity (BV), final viscosity (FV), and setback viscosity of corn starch [73]. Similarly, the cross-linking agent sodium hexametaphosphate reduced the pasting properties of banana starch [32]. While in the study of Sudheesh et al. (2021), the cross-linking agent phosphorus oxychloride increased the PV, TV, SB, and FV parameters of kitol starch [72].

The cross-linking modification of starch varies depending on the starch source, cross-linking factor, its concentration, and reaction conditions. Lower reagent concentration and lower degree of cross-linking can lead to increased swelling and maximum viscosity during heating of starch with water, while higher degree of cross-linking increases granule strength and leads to lower maximum viscosity [74].

Table 6. Pasting property of native and phosphorylated wheat starch

Sampel	Viscosity (cP)					
	PT (°C)	PV	TV	BD	FV	SB
Native starch	65.90±0.64 ^b	1774.00±19.50 ^b	1541±18.38 ^b	233.00±18.50 ^a	1869.00±11.46 ^b	464.00±17.10 ^b
Phosphorylated Starch	66.71±0.97 ^a	1902.00±17.41 ^a	1674±11.40 ^a	228.00±10.66 ^b	2487.00±12.45 ^a	813.00±22.50 ^a

3-13- Morphology of starch granules

Scanning electron microscope (SEM) images of native and cross-linked starch at 3000x magnification are shown in Figure 17. Wheat starch granules have round, elliptical, and disc-like shapes ranging in size and shape from small to large.

The results showed that cross-linking modification caused holes and cracks on the

surface of starch granules. According to Van Hung and Morita (2005), starch granules are affected by external erosion caused by chemical modification processes [75]. Siroha and Sandhu (2018) and Koo et al. (2010) observed slight changes in the surface of pearl millet starch and cross-linked corn starch granules compared to their native starches. These researchers stated that cross-linking modification affects the morphology of starch granules with respect to granule size. So that larger granules have undergone more minor changes compared to smaller sized granules [76, 77].

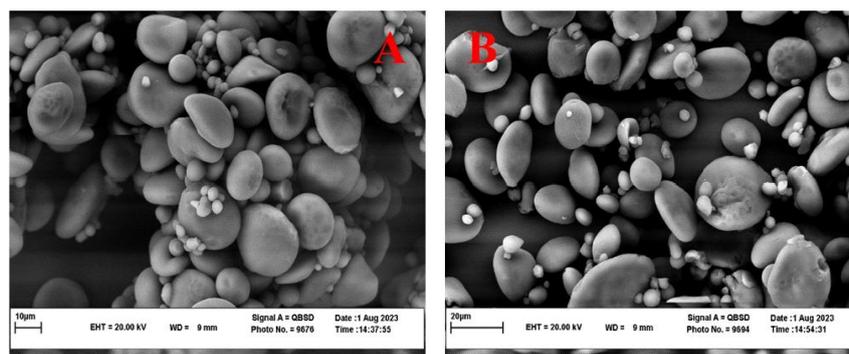


Fig 17. Scanning electron micrography of native (A) and phosphorylated wheat starch (b).

4- Conclusion

The results showed that 17 types of treatments performed in this study, according to different reaction conditions (pH, temperature and reactant concentration), could effectively affect the properties of wheat starch. Meanwhile, considering the required characteristics of starch used in the formulation of various sauces, a starch with higher swelling power, higher solubility, greater paste transparency, less syneresis and greater freeze-thaw stability was selected as the optimal sample and all supplementary tests were performed and compared with the natural starch sample.

The findings showed that phosphorylation of wheat starch led to a decrease in the crystallinity of natural starch from 44.52% to 41.22%. This decrease 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 increased the pasting temperature, peak viscosity, breakdown viscosity, final viscosity and setback viscosity compared to natural starch, while it reduced the breakdown viscosity. The results of the comparison of thermal behavior showed that phosphorylation, by introducing phosphate groups to starch molecules and strengthening intermolecular interactions, increased the onset T_0 and peak T_p gelatinization temperatures compared to the natural starch sample; while the same treatment reduced the conclusion temperature (T_c) and the enthalpy of gelatinization (ΔH). 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 could withstand pasteurization conditions and high tension during homogenization, making this type of modified starch suitable for use in sauce formulations.

5- References

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بهینه سازی شرایط واکنش تولید نشاسته گندم فسفریله توسط تری متافسفات سدیم (STMP) به منظور استفاده در فرمولاسیون انواع سس ها

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نشاسته به دلیل مقرون به صرفه بودن، ایمنی بالا و زیست تخریب پذیری، در صنایع مختلف غذایی و غیرغذایی به طور گسترده مورد استفاده قرار می گیرد. اگرچه، ویژگی های ی مانند نامحلول بودن در آب سرد، عدم تحمل گرما، مقاومت کم نسبت به تنش های اعمالی و پایداری انجماد-ذوب کم، کاربرد نشاسته طبیعی برای مصارف مختلف صنعتی را محدود می کند. نشاسته گندم یکی از رایج ترین انواع نشاسته در بسیاری از کشورها به ویژه ایران است و می توان آن را برای کاربرد در محصولات مختلف اصلاح کرد. یکی از کارآمدترین تکنیک ها برای اصلاح نشاسته، اصلاح شیمیایی است، اما نگرانی هایی را در مورد آلودگی محیط زیست و هزینه های بالای مواد شیمیایی ایجاد می کند. به همین دلیل در این مطالعه به منظور کاهش مصرف مواد شیمیایی، شرایط واکنش تولید نشاسته فسفریله با تری-متافسفات سدیم (STMP) با استفاده از سه متغیر مستقل pH (۹/۵، ۱۰/۵ و ۱۱/۵)، دما (۱۱۰، ۱۳۰ و ۱۵۰ درجه سانتی گراد) و غلظت واکنشگر (۱/۵، ۳ و ۴/۵ درصد)، بهینه سازی شد. ملاک بهینه سازی وجود شاخص تورم و حلالیت بالاتر، شفافیت بالاتر خمیر، سینرسیس کمتر ژل و پایداری انجماد-ذوب بالاتر به منظور استفاده در انواع سس انتخاب گردید. سپس به منظور بررسی خصوصیات ساختاری، حرارتی و خمیری شدن، آزمون های تکمیلی بر نمونه نشاسته بهینه و طبیعی انجام شد؛ نتایج نشان داد که نشاسته تولید شده تحت شرایط بهینه به دلیل بهبود ویژگی بافتی، ایجاد ویسکوزیته بالاتر و افزایش تحمل گرما برای استفاده در فرمولاسیون انواع سس ها مناسب می باشد.

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