



Scientific Research

Optimization of the production of acetylated distarch phosphate (E1414) and evaluation of its properties for use in canned products

Hamed Saberian^{1*}, Ali Forouhar²

1-Assistant Professor of Department of Agro-Industrial Waste Processing, Academic Center for Education, Culture and Research (ACECR) at IUT, Isfahan, Iran

2- Assistant Professor, Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan, Iran.

ARTICLE INFO	ABSTRACT
<p>Article History:</p> <p>Received: 2025/10/25</p> <p>Review: 2025/11/10</p> <p>Accepted: 2025/11/26</p> <hr/> <p>Keywords:</p> <p>Starch, Dual modification, Acetylation, Cross-linking, RVA</p>	<p>Starch, as one of the most widely used natural polysaccharides in the food industry, is of interest due to its properties such as swelling, viscosity and gel formation ability; however, limitations such as thermal instability, retrogradation and syneresis reduce its efficiency in industrial processes. To overcome these limitations, chemical modification of starch, especially in a dual form (acetylation and phosphorylation), is an effective method to improve its performance. In this study, the production and optimization of acetylated distarch phosphate (E1414) were investigated using response surface modeling (RSM) and central composite design (CCD). The effective factors included the percentage of cross-linking agent (STMP/STPP) in the range of 1 to 10% and the concentration of acetic anhydride in the same range. Physicochemical properties, including percentage of acetyl groups and degree of substitution (DS), swelling power, syneresis, transparency and thermal properties (RVA), were evaluated as the main responses. The results showed that increasing the concentration of acetic anhydride significantly increased the degree of acetylation (up to 2.04%) and improved swelling capacity, reduced syneresis and increased transparency, while increasing the cross-linking factor reduced swelling and increased stability against heat and shear stress. Finally, the optimal concentration for the production of modified starch was determined to be 10% acetic anhydride and 1% cross-linking agent (STMP/STPP). This combination resulted in a starch with high thermal and shear stability, suitable transparency and minimal syneresis. Therefore, E1414 starch is recommended as an effective stabilizer and thickener for use in canned products and food products with intense heat processing.</p>
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1- Introduction

Starch is a key carbohydrate component of fruits, seeds, and tubers. It is the second most available natural biopolymer after cellulose and serves many purposes in the food and other industries. However, the industrial use of native starches is limited due to drawbacks regarding stability against shear stress, thermal resistance under processing conditions, and high sensitivity towards syneresis and Retrogradation (Rincón-Aguirre et al., 2018). In general, starch modification can be classified into three groups, which are chemical, physical and enzymatic modifications, in order to overcome the drawbacks mentioned above. Starch can be chemically modified by the incorporation of phosphate and acetyl in its molecular structure. These changes may comprise solubility, thermal stability, viscosity, shear resistance, etc. (Mali et al., 2001).

Native starch can be acetylated with acetic anhydride. The catalysts for this reaction are alkaline compounds that are added following the addition of starch and acetic anhydride. Three -OH groups at C2, C3, and C6 are substituted with acetyl groups. The allowable range of acetylation of starch is 2.5%, resulting in a degree of substitution (DS) of 0.1 (Bello-Pérez et al., 2010). The reasons for carrying out such acetylation are to enhance the starch in terms of its thickening ability, stability, resistance to Retrogradation, increased freeze-thaw stability, clarity, gelling properties and solubility of the granulated starch (Sodhi & Singh, 2005). The progress of the acetylation reaction is influenced by the reaction conditions, such as the use of acetic anhydride, vinyl acetate, temperature, catalysts, reaction time, starch source, and raw starch that is granulated with a more specific arrangement (Huang et al., 2010). Phosphate salts may cause cross-linking between amylopectin molecules with linear or branched configurations. The modified starches with this kind of configuration result from the formation of ether or ester bonds with the hydroxyl groups of the starch molecular entities. Within this category of starch, the strength of the starch granule is improved by the formation of a three-dimensional network (Woo & Seib, 2002). The cross-linking through hydrogen bonds increases granule strength. As a result, the resistance of the granules to break down is increased and the viscosity of starch paste is improved in the presence of acids and under high shear conditions. This type of starch is also heat-

stable and undergoes low Retrogradation during the thawing process; however, the gelatinization temperature of the starch is high. The negative effects of cross-linking, such as reduced paste transparency and low stability at low-temperature streams, can be mitigated by other modifications such as etherification and esterification (Mali et al., 2001).

It is known that single modifications, like cross-linking, improve some characteristics of a polymer but may also create some adverse effects (e.g., gelling power may be improved, but the polymer may show a stronger tendency to retrograde). Then, the dual modification of starches has been suggested. This approach stabilizes the structure of starch and makes it suitable for use in food products subjected to harsh processing (Bello-Pérez et al., 2010). Acetylated distarch phosphate (E1414) is one of the many types of modified starch. To produce E1414, starch is first reacted with phosphate compounds (sodium trimetaphosphate) to introduce cross-linking, after which the starch is known as E1412, which has a reduced swelling capacity. Separately, the acetylation of starch with acetic anhydride leads to the production of a modified starch (E1420) with improved swelling capacity. The combination of the two methods of modification leads to a product that has intermediate properties between the two previous starch types. This starch is known to be E1414 (Lewandowicz et al., 2022). Acetylated distarch phosphate is commonly utilized in food products, such as puddings, salad dressings, and canned food. The acetylated distarch phosphate has a residual phosphorus content of 0.14% for wheat and potato starch, while for other starches it is 0.04%, as well as a maximum allowed acetyl group content of 2.5 (Cui et al., 2014).

Previously, research has been conducted on the production and characterization of acetylated distarch phosphate, some of which are mentioned below. Liu et al. (2022) investigated the effects of phosphorylation and acetylation on chestnut starch. These authors found that the phosphorylation improved the solubility, swelling power, and paste clarity of starches, and the effect of the phosphate group depended on the degree of substitution (DS) (Liu et al., 2022). Furthermore, the acetylation is considered a modification that enhances starch paste clarity and reduces the formation of tight structures, thus yielding a softer paste. Aghili et al. (2018) showed that the influence

of these modifications is source and conditions-dependent (Aghili Dehnavi et al., 2018). Heydarian et al. (2024) reported that modified starch can be used in sauces as it has thermal stability and shear-resistant properties that are developed by the phosphorylation process. These products will withstand the high temperature and severe stress of pasteurization and homogenizing treatment (Heydarian et al., 2025). Ascheri et al. (2014) suggested that phosphorylation modification of *S. lycocarpum* starch led to an increase in its water absorption capacity, peak viscosity, and a decrease in pasting temperature (Ascheri et al., 2014). Moreover, starch modification by phosphorylation alters the swelling and pasting characteristics of the samples. More specifically, higher amounts of phosphorus in the starch will decrease the paste viscosity and change the granule morphology (Joseph et al., 2024). Chemical modification of starch by phosphorylation improves the functional characteristics and makes them suitable for various food applications (Rahim et al., 2021).

There is little information in the literature regarding the production of acetylated distarch phosphate. Optimizing the production process of this type of starch, especially in terms of the type and concentration of the cross-linking and acetylation agents, can yield specific functionalities and applications. Therefore, our study aimed to optimize the production of acetylated distarch phosphate (E1414) across a wide range of cross-linking agent (1-10%) and acetylation agent (1-10%) concentrations to generate distinct product variants. The dually modified starches were characterized and compared to each other to determine their most likely food applications.

2- Materials and Methods

Materials

Acetic anhydride (98% purity) was procured from Pars Shimi Co. Sodium hydroxide, potassium hydroxide, hydrochloric acid, sodium sulfate, phenolphthalein, and ethanol were purchased from Dr. Mojalli Co. STPP (sodium tripolyphosphate) and STMP (sodium trimetaphosphate) were obtained from Merck Co. Potato starch was supplied by Talachin Co.

Optimization of Acetylated Distarch Phosphate (E1414) Production

A dual modification process was employed to produce E1414 starch. The initial step involved cross-linking with STMP, followed by a subsequent acetylation step using acetic anhydride. Production was guided by a Response Surface Methodology (RSM)-based experimental design with two independent variables: (1) the concentration of the cross-linking agent (1-10%) and (2) the percentage of acetic anhydride (1-10%), as detailed in Table 1.

Step 1: Cross-linking of Starch

Potato starch was cross-linked as described by Shi et al. (2013). In brief, 100 g of potato starch was placed in a beaker. A cross-linking agent [a mixture of STMP (99%), and STPP (1%)] was added in concentrations ranging from 1 to 10% (w/w on a starch basis). Then, 140 ml of distilled water was added, and the pH was adjusted to 11. Then, the suspension was stirred for 3 h at 45°C. The cross-linking of starch with STMP requires a pH higher than 9, so the pH value was adjusted to 11. The pH level was monitored and adjusted as necessary throughout this time. The reaction was followed by neutralizing the suspension, where the pH value was adjusted to 6 using 0.1 N HCl. The Cross-linked starch was washed four times with distilled water and dried in an oven for 24 hours at 40 °C (Shi et al., 2013).

Step 2: Starch Acetylation

The potato starch (100 g) with water (225 ml) was stirred for 1 hour, and then, the pH was adjusted to 8.0 using sodium hydroxide solution (3%). Acetic anhydride was dropped slowly into the well-stirred suspension. At the same time, the pH was strictly maintained between 8 and 8.5 by simultaneous addition of 3% NaOH solution. After the reagent had been added in its entirety, stirring was continued for another 60 min. The pH value of the mixture was set to 4.5 using 0.5 M HCl, then washed three times with distilled water. The resulting starch was finally dried at 40 °C. Different concentrations of acetic anhydride from 2.5% to 15% (w/w) were tested to find the optimal conditions. Then the cross-linking starch produced in an earlier step was acetylated to make E1414,

entirely based on the values indicated in Table 1 (Sodhi & Singh, 2005).

Determination of Physicochemical and Thermal Properties of Starch

Acetyl Percentage and Degree of Substitution (DS)

One gram of the starch sample was added to 50 mL of 75% ethanol solution. The suspension was

$$\text{Acetyl (\%)} = \frac{\text{Blank (ml)} \times \text{Sample (ml)} \times \text{Molarity of HCl} \times 0.043 \times 100}{\text{sample weight (gr)}} \quad (1)$$

$$\text{DS} = \frac{162 \times 162 \times \text{Acetyl (\%)}}{4300 - (42 \times 162 \times \text{Acetyl (\%)})} \quad (2)$$

Where:

V_{blank} = Volume (mL) of HCl used for the blank titration

V_{sample} = Volume (mL) of HCl used for the sample titration

M_{HCl} = Molarity of the HCl solution

Determination of Solubility and Swelling Power

2% (w/v) starch slurry was made up and heated at 95°C for 30 minutes with continued stirring to maintain uniform consistency throughout the

$$\text{WSI (\%)} = \frac{W_1}{0.1} * 100\% \quad (3)$$

$$\text{SP} \left(\frac{\text{g}}{\text{g}} \right) = \frac{W_s}{0.1 (100\% - \text{WSI})} \quad (4)$$

Where:

WSI = Water Solubility Index (%)

W₁ = Dry weight of the supernatant (g)

W_s = Weight of the sediment (gel) (g)

0.1 = Dry weight of additional starting suspended starch sample (g) for 2% suspension (i.e., 2% of 5g total suspension weight is a standard assumption

placed in a 50°C water bath for 30 minutes, then taken up and stirred for 30 min, followed by adding 40 mL of 0.5 M KOH. Then it was titrated with 0.5 M HCl (phenolphthalein as indicator). A blank titration (using native, non-acetylated starch) was carried out using the same procedure. The acetyl percentage and degree of substitution (DS) were then computed according to Equations 1 and 2 (Mirmoghtadaie et al., 2009).

mixture. Then, the slurry was slowly cooled down to a temperature of 25°C and then centrifuged at 2500 × g for 15 min. The supernatant liquid was decanted off with particular care not to disturb settled material on the precipitating part, and finally, the gel pellet was collected. The dry weight of supernatant and gel was determined using an oven at 105°C. Then the Water Solubility Index (WSI) and Swelling Power (SP) were calculated using the following relationships (Ačkar et al., 2010):

Determination of Acetyl Percentage and Degree of Substitution (DS)

also used here, but the exact dry weight of starting material should be used).

Syneresis

The starch suspension was prepared in a 5% (w/v) solution, heated up for 30 minutes in a water bath at 90°C, and then cooled to room temperature. The prepared samples were stored at 4°C and syneresis was determined after 5 days. Gel solids were tested by centrifuging the gels at 3000 × g for 15 min and

measuring the water released in volume units. The syneresis value after 5 days is reported (Mirmoghtadaie et al., 2009).

Determination of Starch Paste Clarity

Starch paste clarity was determined by using the method proposed by Mahmood et al. (2000). After it had been heated in a boiling water bath for 30 min and then cooled to room temperature, 1% (w/w) starch solution (pH 6.5) was measured in percentage of light transmittance (%T) at a wavelength of 650 nm (Sitohy et al., 2000).

Analysis of Pasting Properties by Rapid Visco Analyzer:

A Rapid Visco Analyzer (RVA, Petren, Australia) was used to test the pasting behavior of the selected starch samples as a function of temperature according to the method of Zhang et al. (2020). Briefly, 3 g (at 12% moisture basis) of each starch sample was suspended in 25 ml of distilled water in an RVA aluminum canister. Temperature profiles were as follows: Initial equilibration at 50°C, heating to 95°C and holding at 95°C, then cooling it down again to 50 °C. Key parameters, Peak Viscosity (maximum viscosity during heating), Final Viscosity (viscosity after cooling to 50°C), Breakdown (Peak Viscosity minus Trough Viscosity, indicating thermal stability (Fan et al., 2025)), and Setback (Final Viscosity minus Trough Viscosity, indicating the tendency for Retrogradation) were recorded in centipoise (cP) (Zhang et al., 2020).

Statistical Analysis

The results of acetylated starch (E1420) production were analyzed using a One-Factor approach. Analysis of variance (ANOVA) and the Duncan test were used for mean comparisons. Response Surface Methodology (RSM) with a Central Composite Design (CCD) was used to optimize production of acetylated distarch phosphate (E1414). The data were analyzed using Design Expert 7. The experimental range for each factor was selected based on preliminary test results. After optimization, confirmatory tests were conducted under the optimal conditions, and the mean experimental values were compared with the predicted ones of the model equation. The results

presented are the mean values from at least two replicates.

3-Results and Discussion

Optimization of Acetylated Starch (E1420) Production

Acetylated starch is widely used in food and related products, such as in packaging. Based on the degree of substitution, High-DS acetylated starch contains more than 2.5% acetyl groups (DS > 0.1). Based on the degree of substitution, modified starches are classified into two categories: (i) low-DS acetylated starch (DS < 0.1), for which the content of acetyl groups is less than 2.5%. This type is widely utilized in the food industry as it can create viscosity, texture, and stability (Bello-Pérez et al., 2010). The first steps of modification employed various quantities of acetic anhydride (2.5-15%) to acetylate potato starch. The resulting acetyl content and extent of substitution for each sample were then established (results not given here). With the concentration of acetic anhydride increasing from 2.5% to 12.5%, the percentage of substituted acetyl groups also increased considerably (from 0.38% to 2.58%). However, there was no statistical difference between samples acetylated using 12.5% or 15% acetic anhydride. Since the highest permissible acetyl group content in edible acetylated starches is 2.5%, and considering the costs of using acetic anhydride on the final product, 10% acetic anhydride was used. In this condition, the highest level of acetyl content was 2.09% and a DS was 0.081. It was also found that no more than 1% could be used as the lowest level.

Optimization of Acetylated Distarch Phosphate (E1414) Production

A Response Surface Methodology (RSM)-based experimental design was employed, utilizing two independent variables: the concentration of the cross-linking agent STMP (1-10%) and the percentage of acetic anhydride (1-10%). The acetyl group content and various physicochemical properties of the resulting E1414 starches, including syneresis, water retention capacity, swelling power, paste clarity, and thermal properties, under different experimental conditions are presented in Table 1

Table 1. Effect of acetic anhydride and STMP concentration on the different responses in E1414

No.	B: Acetic anhydride Con. (%)	A: Cross-linking agent (%)	Acetyl group (%)	DS (%)	Swelling Power (%)	Syneresis (day5) (%)	T (%)
1	1	1	0.32	0.012	28.19	14.64	41.59
2	1	10	0.32	0.012	24.55	3.67	37.10
3	10	1	2.04	0.078	38.76	0	61.05
4	10	10	1.83	0.070	36.99	1.51	49.98
5	5.5	1	0.75	0.028	35.46	13.77	40.73
6	5.5	10	0.75	0.028	27.72	2.86	44.08
7	1	5.5	0.32	0.012	35.45	9.00	39.40
8	10	5.5	1.82	0.070	41.85	3.70	48.08
9	5.5	5.5	0.97	0.037	33.09	12.80	42.40
10	5.5	5.5	0.75	0.028	37.77	10.52	45.51
11	5.5	5.5	0.75	0.028	37.28	11.00	43.90

Results indicated that the quadratic model for the acetyl group of the starches was significant. The high value of the R^2 of this model indicates that 98.8% of the experimental data of acetyl content could be explained by the quadratic model. Furthermore, the adjusted R^2 value ($R^2 = 0.976$) was very similar to the R^2 value. This result showed that an appropriate model was selected for the correlation between the experimental data and the values predicted by the model. The Lack-of-Fit test indicates the model's failure to represent data points not included in the regression model range. This parameter was not significant. Therefore, all results confirmed that the quadratic regression model reliably predicts the acetyl group percentage

of the samples under varying conditions of cross-linking agent and acetic anhydride concentration. A quadratic regression analysis was performed on the experimental data, yielding Equation (6) for the predicted acetyl group percentage in terms of actual factors:

$$\text{Acetyl group (\%)} = 0.26 + 0.010 A + 0.018 B - 0.003 AB - 0.0003 A^2 + 0.0156 B^2 \quad (6)$$

Table 2. Examination of the coefficients of the quadratic model for evaluating the percentage of starch acetyl groups (%) in E1414

Source	Squares	df	Square	Value	Prob > F	
Model	4.019345	5	0.803869	82.94979	< 0.0001	significant
A-crosslink	0.007704	1	0.007704	0.794979	0.4134	
B-ac	3.728817	1	3.728817	384.7699	< 0.0001	
AB	0.011556	1	0.011556	1.192469	0.3246	
A ²	8.11E-05	1	8.11E-05	0.008368	0.9307	
B ²	0.254319	1	0.254319	26.24268	0.0037	
Residual	0.048455	5	0.009691			
Lack of Fit	0.017638	3	0.005879	0.381579	0.7804	not significant
Pure Error	0.030817	2	0.015408			
Total	4.0678	10				

In equation 6, the terms A represent the concentration of the cross-linking agent (STMP), and B represents the acetic anhydride. As can be seen in Table 2, only the linear effect of variable B and its quadratic term were statistically significant ($p < 0.05$). This indicates that the acetic anhydride concentration was the main factor significantly affecting the acetyl group content of the starches. However, the concentration of the cross-linking agent (STMP) had no significant influence on this parameter. Aghili Dehnavi et al. (2017) showed that the acetylated chickpea starch with 6% acetic anhydride contained 1.79% to 1.98% of acetyl, at DS values between 0.069 and 0.076. However, they found no significant difference between these two concentrations (Aghili Dehnavi et al., 2018). Also, Mirmoghtadaie et al. (2009) reported a different result in the acetylation of oat starches. These authors reported that acetylation with 6% and 8% acetic anhydride yielded significantly different acetyl contents of 1.54% and 2.92%, respectively (Mirmoghtadaie et al., 2009). Singh Sodhi et al. (2005) reported that the acetyl content of rice starch ranged from 2.26% to 3.68. Singh and Sodhi (2004) also noted that larger granules tend to have a higher degree of acetylation than smaller ones (Sodhi & Singh, 2005).

Swelling Power

The analysis of variance indicated that the quadratic model describing the Swelling Power capacity of the starches was statistically significant. This result is actually a fairly strong effect. Using this equation and doing multiple regression analysis, we would be able to grasp these fundamentally important issues.

$$\text{Swelling Power (\%)} = 27.85 + 2.39 A + 0.14 B + 0.023 AB - 0.274 A^2 + 0.074 B^2 \quad (7)$$

The concentrations of the cross-linking agent sodium trimetaphosphate (STMP) and acetic anhydride are represented respectively by A and B in Eq (7). It was found that the interaction between AB is not significant ($p \geq 0.05$). Furthermore, the actual value shown for factor B was its linear effect only, whereas the experimental value of A² was for the intermediate cross-linking agent. The results showed that both acetic anhydride concentration and the concentration of the cross-linking agent, STMP, significantly affected the swelling power of starches. But at a low concentration of STMP, the swelling power is not significantly different from zero. So, a low concentration of STMP did not significantly affect the swelling power; however, at higher concentrations of the cross-linking agent, the swelling power decreased.

Table 3. Examination of the coefficients of the quadratic model for evaluating the swelling power of E1414 starches

Source	Squares	df	Square	Value	Prob > F	
Model	251.68	5	50.34	7.6	0.022	significant
A-crosslink	28.79	1	28.79	4.35	0.0915	
B-ac	144.16	1	144.16	21.77	0.0055	
AB	0.88	1	0.88	0.13	0.7305	
A ²	77.85	1	77.85	11.76	0.0187	
B ²	5.8	1	5.8	0.88	0.3922	
Residual	33.11	5	6.62			
Lack of Fit	19.86	3	6.62	1	0.5354	not significant
Pure Error	13.25	2	6.62			
Cor Total	284.79	10				

Pérez et al. (2010) reported that the swelling power of barley starch granules was increased by acetylation to a level of 6% (from 14.21 to 19.35 g/g). The swelling power is generally increased as a result of steric hindrance caused by bulky, hydrophilic acetyl groups being introduced into the starch molecules, as well as repulsion between starch chains (Bello-Pérez et al., 2010). This in turn leads to penetration of water into the amorphous zones of the starch.

In a study conducted by Singh et al. (2016), it was shown that the high concentrations of fast-reacting cross-linkers like phosphorus oxychloride (POCl_3) would dramatically reduce the swelling capacity compared to slow-reacting substances such as Epichlorohydrin (EPI). As POCl_3 concentration increases, swelling power decreases because it reinforces the granola structure. Therefore, cross-linked starch is more stable to lose viscosity, heat, acid, and other stimuli that cause decomposition compared to native starch. Acetyl groups in starch granules promote access to the amorphous regions for water by disrupting hydrogen bonds (Mirmoghtadaie et al., 2009). Aghili Dehnavi et al. (2017) found that acetylation of chickpea starch, at 6% and 8% of its original weight, didn't alter the swelling power compared to native starch (Aghili Dehnavi et al., 2018). Ku et al. (2010) found that the swelling power is highly correlated with the extent of cross-linking, but X-ray diffraction patterns showed no significant changes in crystallinity of corn starch. The SEM measurements confirmed this, the dark area on the surface of cross-linked starch granules being absent compared to the native starch (Koo et al., 2010). Pang et al. (2019) reported that the lowest swelling power of E1414 was at acidic pH 3, while the native starch granules would swell significantly even in acid environments. These authors also confirmed that cross-linking increases the stability and strength of starch granules, making them less prone to breakage in severe thermal or acidic conditions.

The results indicated that among the modified starches, the sample with the maximum cross-linking (89.5%) and maximum acetyl group content (1.6% to 2%) showed the lowest swelling power. However, the highest swelling power was observed in the moderately cross-linked starch (with 1.5% to 2% acetyl groups and 28.8% cross-linking). The reason for this observation is likely related to the relatively high level of acetyl groups, which have a greater effect on swelling power than crosslinking (Pang et al., 2019).

Syneresis

The syneresis phenomenon occurs due to increased intermolecular associations and bonding between amylose and amylopectin in the starch paste during storage, leading to the expulsion of trapped water. Syneresis reflects the potential and tendency of a starch gel to retrograde and, in a way, indicates the gel's stability (Zhang et al., 2020). The syneresis of the samples under different experimental conditions (varying concentrations of the cross-linking agent and acetic anhydride) was measured from day 1 to day 5, with the cumulative value for the fifth day reported (Table 4). Analysis of variance (ANOVA) indicated that the quadratic model for determining the syneresis of the starches on the fifth day was significant. The lack of fit for this model was also not significant. A quadratic model in terms of actual factors, is presented as follows:

$$\text{Syneresis (day 5) (\%)} = 14.84 - 0.40 A + 0.60 B + 0.15 AB - 0.11 A^2 - 0.21 B^2 \quad (8)$$

In this equation, A and B represent the concentration of the cross-linking agent (STMP) and the concentration of acetic anhydride, respectively. As can be seen in Table 4, the effects of the cross-linking agent concentration, the acetic anhydride concentration, and their interaction on the syneresis of the samples on the fifth day were all statistically significant.

Table 4. Examination of the coefficients of the quadratic model for evaluating E1414 syneresis on the 5th day

Source	Squares	df	Square	Value	Prob > F
Model	264.03	5	52.81	12.76	0.0071 significant
A-Crosslink	69.18	1	69.18	16.72	0.0095
B-ac	81.54	1	81.54	19.7	0.0068

AB	38.89	1	38.89	9.4	0.0279	
A ²	12.41	1	12.41	3	0.1439	
B ²	44.23	1	44.23	10.69	0.0222	
Residual	20.69	5	4.14			
Lack of Fit	17.81	3	5.94	4.12	0.2014	not significant
Pure Error	2.88	2	1.44			
Total	284.73	10				

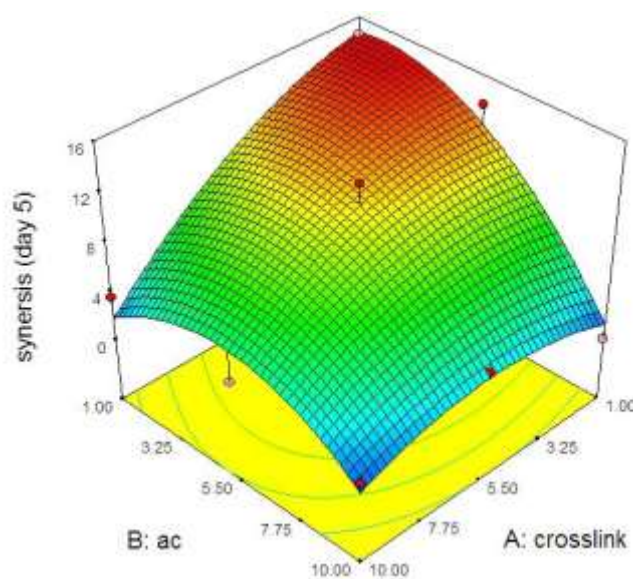


Figure 1. Interaction between acetic anhydride concentration and crosslinking agent on the syneresis of E1414 on the fifth day of storage

According to Figure 1, both the concentration of STMP and the concentration of acetic anhydride had a negative effect on the syneresis of the starches, although the effect of acetic anhydride concentration was more pronounced at lower concentrations of the cross-linking agent. For instance, at a 1% STMP concentration, the highest syneresis occurred at a 1% acetic anhydride concentration. Mirmoghtadaie et al. (2009) stated that the syneresis phenomenon in starch gels is caused by increased molecular associations between starch chains at low temperatures, which expels water from the gel structure (Mirmoghtadaie et al., 2009). Aghili Dehnavi et al. (2017) reported that the presence of cross-linking agents in starch granules leads to increased

syneresis, while the presence of acetyl groups in chickpea starch molecules decreased it. They reported that the syneresis values of 27.5%, 26%, and 28% for the native starch, acetylated starch at 8%, and cross-linked starch with 0.1% phosphorus oxychloride, respectively (Aghili Dehnavi et al., 2018). Singh Sodhi and Singh (2005) also showed that acetylation of rice starch significantly reduced syneresis (Sodhi & Singh, 2005). They attributed this to the presence of acetyl groups, which enhance the water-holding capacity of the gels during refrigerated storage.

The increase in syneresis over storage time is due to the leaching of amylose and amylopectin chains. Amylose crystallizes during the initial hours of

storage, while amylopectin crystallizes in the later stages. This can explain the significant difference in syneresis between the first day and subsequent days in high-amylose potato starch while most researchers have reported that cross-linking agents increase syneresis, the results of this study indicated that the lowest syneresis occurred at the highest concentration of STMP. The likely reason for this observation is the reduced water absorption and swelling of the starches at higher STMP concentrations (as confirmed in Table 3). When water absorption and swelling are lower, the potential for syneresis can also be reduced.

Measurement of Paste Clarity in Optimized E1414 Starches

The clarity of a starch paste is highly important for its application in food products. The clarity of starch paste is a positive attribute that can be

challenging. Both cross-linking and acetylation influence the clarity and stability of starch paste during storage at low temperatures (Mali et al., 2001). As shown in Table 5, only variable B (acetic anhydride concentration) had a significant linear effect. It is evident from Table 5 that only the concentration of acetic anhydride affected the clarity of the starch ($P < 0.05$); while the crosslinking agent's concentration (STMP) had no influence on this parameter. A multiple regression analysis (quadratic model) was performed on the experimental data, yielding Equation (9) for the predicted paste clarity in terms of actual factors:

$$T (\%) = 39.29 - 0.0054 A + 0.404 B - 0.081 AB + 0.142 B^2 \quad (9)$$

Table 5. Examination of the coefficients of the quadratic model for evaluating T (%) in E1414

Source	Squares	df	Square	Value	Prob > F	
Model	338.67	4	84.67	5.92	0.0280	significant
A-crosslink	24.85	1	24.85	1.74	0.2355	
B-ac	280.44	1	280.44	19.61	0.0044	
AB	10.82	1	10.82	0.76	0.4177	
B ²	22.56	1	22.56	1.58	0.2558	
Residual	85.80	6	14.30			
Lack of Fit	80.96	4	20.24	8.37	0.1096	not significant
Pure Error	4.84	2	2.42			
Total	424.47	10				

Analysis of the Thermal Pasting Properties of E1414 Starches

For the RVA test, three modified samples were selected. The acetic anhydride level was fixed at 10% for all samples, as increasing its concentration led to higher degrees of acetylation, swelling power, and paste clarity, while reducing syneresis. However, three different STMP concentrations were chosen, minimum (1%), maximum (10%), and average (5.5%), because both the lowest and highest concentrations resulted in reduced syneresis. In the initial phase of the RVA test, water absorption by the starch granules occurs rapidly as the temperature increases. The starch

granules absorb water, and swell when heated. The water absorbed into the granules helps to melt the crystalline regions in the granules. As a result, this allows water to enter the granules still more quickly. These things bring about a sudden jump in the viscosity of the water-starch mixture. At the same time, granule disintegration begins. The interplay of swelling and disintegration ultimately determines the peak viscosity (Kumar & Khatkar, 2017). Figure 2 and Table 6 indicate that the time and temperature required to reach peak viscosity were higher for the E1414 samples compared to the native starch control. Furthermore, the modified samples exhibited increased resistance to temperature and mechanical shear. The presence of cations in the starch also influences these

properties. It has previously been established that a longer time to reach peak viscosity can be attributed to higher concentrations of divalent cations, such as calcium and magnesium, and/or a higher amylose content in the starch (Higley et al., 2003). Therefore, although the final viscosity of the modified E1414 samples and the unmodified

native starch was approximately similar, the modified starches' ability to withstand longer and more severe thermal processing makes them preferable for applications involving intense heat treatment

Table 6. Comparison of viscosity indices of dual modified starch paste (E1414)

samples	Peak Viscosity	Holding strength	Final Viscosity	Breakdown	Setback
10% Acetyl + 1% C.R	3022	1665	5278	1357	3613
10% Acetyl + 5.5% C.R	2911	1404	4788	1507	3384
10% Acetyl + 10% C.R	3626	1862	4836	1764	2974
CONTROL	6717	2135	4496	4582	2361

C.R= cross-link agent

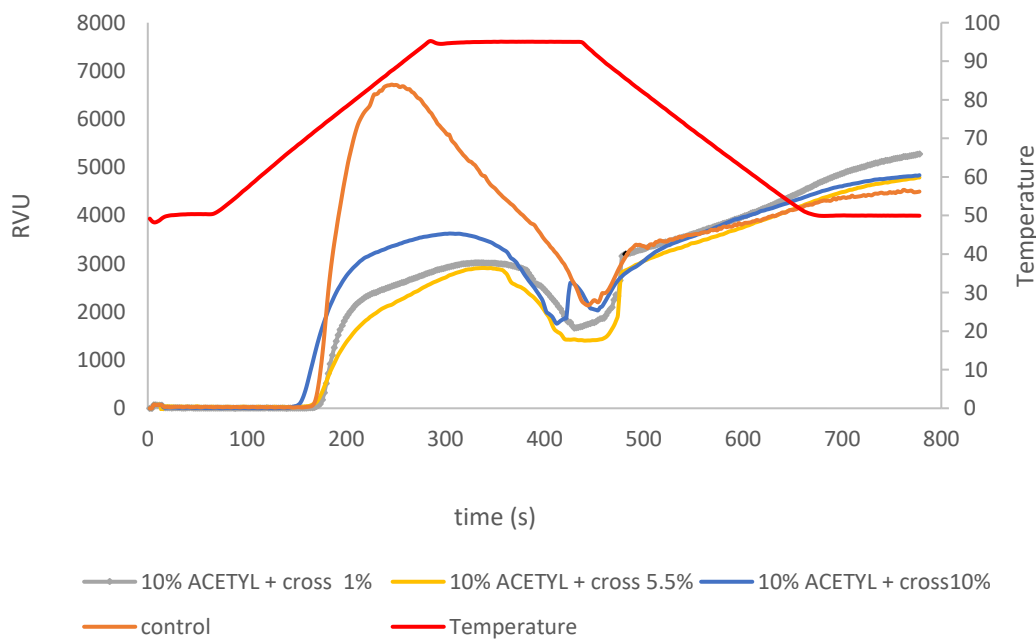


Figure 2. Comparison of viscosity changes of dual modified starch paste (E1414)

Paste viscosity is a crucial parameter for sensory evaluation, starch quality assessment, and the design of food processing equipment. Peak viscosity in a starch paste is influenced by the extent of amylose leaching, the formation of

amylose-lipid complexes, friction between swollen granules, and the degree of granule swelling. Sodhi et al. (2005) demonstrated that acetylation lowered the pasting temperature of starch and also increased its peak viscosity (Sodhi & Singh, 2005).

In contrast, as shown in Figure 2, the peak viscosity temperature increased in all modified E1414 samples. The gelatinization temperature is a function of the degree of cross-linking within the starch. This suggests that this category of modified samples could be used as stabilizers in products requiring high processing temperatures but low viscosity during the process itself (Ai & Jane, 2015). When compared to the control sample, the peak viscosity decreased in all E1414 starch samples. However, a comparison among the E1414 samples revealed that the sample with 10% cross-linking agent exhibited the highest peak viscosity within this group. As mentioned earlier, peak viscosity at this stage is governed by the balance between granule swelling and structural disintegration. Therefore, using a 10% cross-linking agent appears to strike a balance where the structure of the granules is maintained while allowing for swelling, resulting in a higher peak viscosity compared to the other E1414 samples.

For the samples with 1% and 5.5% cross-linking agent, no significant difference in peak viscosity is observed. While the acetyl groups weaken the granule structure, they increase swelling and consequently elevate viscosity. Prieto Garcia et al. (2012) also demonstrated that acetylating barley starch with acetic anhydride and vinyl acetate increased the molecular size from 19 μm in native starch to 22 μm and 104 μm for the vinyl acetate and acetic anhydride acetylated starches, respectively (Garcia et al., 2012). On the contrary crosslinking increases granule strength but lowers viscosity. The sample's final viscosity is determined by this pair of opposing forces. For example, Gonzalez and Perez (2002) reported that the peak viscosity of acetylated rice starch was greater than that of native starch. They attributed this to solubility and increased water absorptivity resulting from acetylating groups on the molecule's periphery being opened up. Researchers suggested that the rising viscosity when a starch suspension is cooled indicates its tendency towards Retrogradation; native rice starch (Gonzalez & Pérez, 2002).

According to Figure 2 and Table 6, the breakdown viscosity of the modified samples decreased compared to the control sample. This is likely due to the greater structural integrity imparted by the cross-linking bonds in the modified starch. Higher breakdown values indicate that starch granules are less resistant to heat and mechanical shear. Lower

breakdown values may result from reduced granule swelling due to lower hydration capacity. It can also be attributed to the movement of amylose molecules within the amorphous regions; amylose molecules act as swelling inhibitors, reducing the disintegration of the swollen starch granules (Yan et al., 2021).

In starch paste analysis, one of the most critical parameters is the final viscosity, which shows the sample's performance in industrial applications. After crosslinking, the stability of the starch increases, but after acetylation it falls off sharply (Lewandowicz et al., 2022). The E1414 starches under test were found to have a higher final viscosity than the control sample (Figure 2). Looking at the viscosity patterns of modified E1414 starches, in addition to this, modified starches can be seen to belong to a swelling type between intermediate and small granules. This means that during the heating phase, paste viscosity gradually increases, good stability is displayed in the holding period, and there is a significant drop in viscosity during the cooling phase.

Previous studies demonstrated that acetylated starches exhibit higher paste viscosity than their native starch. This observation is likely due to the increased swelling power and solubility of the sample. Another reason that has been previously reported is the weakening of associative forces within the amorphous regions of the granules (Sodhi & Singh, 2005). Similarly, Jyothi et al. (2012) demonstrated that cross-linking led to a reduction in breakdown viscosity. This was maybe due to the limited water binding and reduced swelling of the starch sample (Omojola et al., 2012).

The degree of acetylation and the source of starch affect the results of the starch crosslinking process. Furthermore, the degree of crosslinking affects the stabilization of granule structure, allowing the modified starch to achieve a higher degree of granule swelling upon heating compared to the native, unmodified starch. In such cases, higher swelling power and higher peak paste viscosities can be observed. In contrast, high levels of cross-linking progressively lead to reduced granule swelling, solubility, amylose leaching, and peak viscosity. Therefore, cross-linked starches generally possess a higher pasting temperature and greater resistance to acid-thermal conditions. The

increase in the enthalpy and temperature of gelatinization in starch is attributed to reduced molecular mobility in the amorphous regions of the granule, resulting from the increased number of intermolecular cross-links (Bonomi et al., 2017).

Based on Table 6, the setback viscosity of the E1414 starch samples was higher than that of the native sample. Furthermore, with the increase in the percentage of crosslinking agent, the recovery viscosity decreases. The effect of the acetyl groups becomes more pronounced, as a lower level of the cross-linking agent was used. This is leading to an increase in setback viscosity of samples. A similar effect was observed for the syneresis characteristic (Figure 1). The setback viscosity value indicates the degree of short-term Retrogradation of the starch paste during the cooling storage. This property primarily reflects the recrystallization of amylose. A higher setback viscosity is attributed to a greater amount of amylose leaching from the starch (Yan et al., 2021). Sodhi et al. (2005) demonstrated that the acetylation of starch results in higher setback viscosity (Sodhi & Singh, 2005). It can be concluded that the investigated E1414 starches are suitable candidates for use in products that require low viscosity during processing to facilitate thermal processing, pumping, and filling, based on the higher final viscosity and superior thermal resistance observed in the E1414 starch. Based on these characteristics, the starch modified with 10% acetic anhydride and 1% STMP is preferable. This specific sample exhibits a higher final viscosity and, simultaneously, achieves its peak viscosity later in the process, indicating its higher thermal resistance. Consequently, the application of this food ingredient is recommended for canned products containing solid ingredients suspended in a sauce.

4-Conclusion

The dual modification of potato starch through phosphorylation and acetylation resulted in a starch with significantly improved starch characteristics compared to its native starch. This optimizes modification, establishing an optimal balance between swelling power, thermal stability, and reduced syneresis in modified starch. RVA analysis revealed that the modified E1414 starches exhibited lower peak viscosity but higher thermal and shear stability than the native sample. These characteristics make them very suitable for use in

food products that require prolonged thermal processing. The E1414 starch formulation with 1% cross-linking agent (STMP/STPP) and 10% acetic anhydride was identified as the optimal combination, demonstrating desirable rheological behavior and gel stability during storage. Furthermore, as its high thermal stability, and reduced syneresis, the modified E1414 starch can be effectively utilized as a stabilizer and thickener in canned products containing suspended solid ingredients.

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Author Contributions

All activities were carried out by the author.

Competing Interests

The author confirms that he / she has no financial conflicts of interest or competing interests in this study.

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بهبودسازی تولید فسفات دو نشاسته‌ای استیله (E1414) و ارزیابی ویژگی‌های آن جهت کاربرد در

محصولات کنسروی

حامد صابریان^{۱*}، علی فروهر^۲

۱- دکتری، استادیار گروه پژوهشی فرآوری پسماند و ضایعات کشاورزی، جهاد دانشگاهی واحد صنعتی اصفهان، اصفهان

۲- دکتری، استادیار گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه صنعتی اصفهان، اصفهان، ایران

اطلاعات مقاله	چکیده
تاریخ های مقاله :	نشاسته به عنوان یکی از پرکاربردترین پلی‌ساکاریدهای طبیعی در صنایع غذایی، به دلیل ویژگی‌هایی چون تورم، گرانبوی و توانایی تشکیل ژل مورد توجه است؛ با این حال، محدودیت‌هایی نظیر ناپایداری حرارتی، پس‌روی و آب‌اندازی موجب کاهش کارایی آن در فرآیندهای صنعتی می‌شود. به منظور رفع این محدودیت‌ها، اصلاح شیمیایی نشاسته به ویژه به صورت دوگانه (استیلاسیون و فسفریلاسیون) روشی مؤثر برای بهبود عملکرد آن است. در این پژوهش، تولید و بهینه‌سازی فسفات دو نشاسته‌ای استیله (E1414) با استفاده از طرح سطح پاسخ (RSM) و طراحی مرکب مرکزی (CCD) مورد بررسی قرار گرفت. عوامل مؤثر شامل درصد عامل ایجادکننده اتصالات عرضی (STMP/STPP) در بازه ۱ تا ۱۰ درصد و غلظت استیک انیدرید در بازه مشابه بود. خواص فیزیکوشیمیایی، از جمله درصد گروه‌های استیل و درجه جانشینی (DS)، قدرت تورم، آب‌اندازی، شفافیت و خواص حرارتی (RVA)، به‌عنوان پاسخ‌های اصلی ارزیابی شدند. نتایج نشان داد که افزایش غلظت استیک انیدرید موجب افزایش معنی‌دار درجه استیلاسیون (تا ۲/۰۴٪) و بهبود ظرفیت تورم، کاهش آب‌اندازی و افزایش شفافیت شد، در حالی که افزایش عامل اتصالات عرضی موجب کاهش تورم و پایداری بیشتر در برابر حرارت و تنش برشی گردید. در نهایت، غلظت بهینه برای تولید نشاسته اصلاح‌شده، ۱۰٪ استیک انیدرید و ۱٪ عامل ایجادکننده اتصالات عرضی (STMP/STPP) تعیین شد. این ترکیب سبب ایجاد نشاسته‌ای با پایداری حرارتی و برشی بالا، شفافیت مناسب و حداقل آب‌اندازی گردید. بنابراین، نشاسته E1414 به‌عنوان یک پایدارکننده و قوام‌دهنده مؤثر برای کاربرد در محصولات کنسروی و فرآورده‌های غذایی با فرآیند حرارتی شدید پیشنهاد می‌شود.
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* مسئول مکاتبات:	
Saberian@acecr.ac.ir	