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Open Access

J. Mex. Chem. Soc. **2026**, 70(1):e2416

Received December 27th, 2024
Accepted April 30th, 2025

<http://dx.doi.org/10.29356/jmcs.v70i1.2416>
e-location ID: 2416

Keywords:

Acid hydrolysis, *Manihot esculenta*,
modified starch, phosphorylation,
physicochemical properties

Palabras clave:

Hidrólisis ácida, *Manihot esculenta*,
almidón modificado, fosfatación,
propiedades fisicoquímicas

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Química de México

ISSN-e 2594-0317

Optimization of Laboratory-scale Operating Conditions for the Modification of Cassava Starch (*Manihot esculenta*) Via Acid Hydrolysis and Phosphation

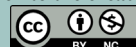
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Abstract. This study explores the chemical modification of natural cassava starch through acid hydrolysis and phosphorylation, evaluating the impact of these treatments on key physicochemical properties such as viscosity, gelatinization temperature, water solubility, and acidity index. The reaction parameters for each process were optimized, including HCl concentration, sodium tripolyphosphate (TPS) concentration, and reaction time, achieving optimal conditions of 0.5 N HCl for 1 h for acid hydrolysis and 0.1 % TPS for 1 h for phosphorylation. These parameters were selected for their efficiency and cost-effectiveness in terms of reagent consumption and processing time, resulting in a significant improvement in the physicochemical properties of the starch. Acid hydrolysis reduced the viscosity of the starch, indicating a change in its molecular structure, while the gelatinization temperature increased, suggesting enhanced thermal stability. Water solubility showed a substantial increase, indicating greater availability for different applications. Phosphorylation with sodium tripolyphosphate also improved solubility and the acidity index due to the introduction of phosphate groups. Together, these results demonstrate that both treatments are highly effective in modifying cassava starch, expanding its potential for use in industries such as food, bioplastics, and pharmaceuticals, where the improved properties can offer new opportunities for the development of innovative products.

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Resumen. Este estudio explora la modificación química del almidón de yuca natural mediante hidrólisis ácida y fosfatación, evaluando el impacto de estos tratamientos sobre sus propiedades fisicoquímicas clave, como la viscosidad, la temperatura de gelatinización, la solubilidad en agua y el índice de acidez. Se optimizaron los parámetros de reacción para cada proceso, incluyendo la concentración de HCl, la concentración de tripolifosfato de sodio (TPS) y el tiempo de tratamiento, alcanzando condiciones óptimas de 0.5 N de HCl durante 1 h para la hidrólisis ácida y 0.1 % de TPS durante 1 h para la fosfatación. Estos parámetros fueron seleccionados por su eficiencia y bajo costo en términos de consumo de reactivos y tiempo de procesamiento, lo que resultó en una mejora significativa de las propiedades fisicoquímicas del almidón. La hidrólisis ácida redujo la viscosidad del almidón, evidenciando una alteración en su estructura molecular, mientras que la temperatura de gelatinización aumentó, indicando una mayor estabilidad térmica. La solubilidad en agua mostró un incremento sustancial, lo que sugiere una mayor disponibilidad para diversas aplicaciones. Por su parte, la fosfatación con tripolifosfato de sodio también mejoró la solubilidad y el índice de acidez, debido a la introducción de grupos fosfato. En conjunto, estos resultados demuestran que ambos tratamientos son altamente efectivos para modificar el almidón de yuca, ampliando su potencial de uso en industrias como la alimentaria, la de bioplásticos y la farmacéutica, donde las propiedades mejoradas pueden ofrecer nuevas oportunidades para el desarrollo de productos innovadores.

Introduction

Starch is a crucial functional component in many food systems, serving as the primary energy source in the diet and contributing to the structure, texture, consistency, and appearance of the products in which it is incorporated [1,2]. In the industry, starch is used in both its natural and modified forms [3–5]. However, despite its numerous functional properties, natural starches have been limited in use due to the undesirable characteristics they develop upon cooking [6,7].

Cassava starch (*Manihot esculenta*), in particular, has garnered considerable attention due to its abundance, low cost, and excellent functional properties [8–11]. However, to overcome the limitations of natural starches, various chemical modification techniques have been developed to yield derivatives with specific properties tailored to particular industrial processes [12,13].

Among these techniques, modification through derivatization (substitution) and conversion (degradation) are widely used in the food industry for their ability to provide stability and meet specific functional needs [12,14]. Acid hydrolysis, a conversion technique, breaks the glycosidic bonds of the amylose and amylopectin chains in starch, reducing the molecules to smaller sizes and altering their functional properties [15,16]. On the other hand, phosphorylation, as a derivatization technique, esterifies the hydroxyl groups of starch using phosphate agents, which decreases retrogradation and enhances its functionality [17,18].

Despite the growing interest in these techniques, there remains a gap in the scientific literature regarding the optimization of specific reaction conditions for cassava starch. This study aims to fill this gap by evaluating how operational parameters such as acid concentration, phosphating agent, and reaction time affect key starch properties, including viscosity, gelatinization temperature, water solubility index (WSI), water absorption index (WAI), and acidity index.

This approach provides an innovative methodology by directly correlating modification conditions with the final functional properties of starch. Furthermore, it emphasizes the importance of cassava starch as a sustainable and competitive raw material compared to more traditional sources like corn or potato. The detailed optimization of operational parameters also seeks to maximize reaction efficiency, improve the quality of the final products, and reduce production costs.

In this context, this work not only advances scientific knowledge on cassava starch modification but also provides a solid foundation for the development of more efficient and sustainable industrial processes. The potential applications of these modified starches are vast, particularly in the food industry, where the demand for functional and stable materials continues to rise.

Materials and methods

Natural cassava starch was used, extracted from roots of various varieties (INTA Amarilla, INTA Reyna, INTA Tololar, Blanca Criolla, Ceiba, Valencia, and Perú), provided by the company ALCASA from Nicaragua. All reagents used were of analytical grade, including hydrochloric acid (HCl), sodium hydroxide (NaOH), and sodium tripolyphosphate (TPS). For the standardization of the titrant solution, a buffer solution was prepared using potassium hydrogen phthalate (KHP), suitable for standardizing bases like NaOH, and 1 % phenolphthalein was used as the indicator.

Chemical modification of natural starch by acid hydrolysis method

The methodology employed was adapted from the work of Vargas et al. (2019) with some modifications [19]. A concentrated starch solution was prepared with a ratio of 1.2 L of water per kilogram of natural starch. The mixture was heated with constant stirring at 40 °C for the specified time (t_H) according to the experimental design for each of the four treatments performed (Table 1). Upon reaching the established conditions, the required amount of HCl was added to achieve the desired acid concentration (C_A), at which point the hydrolysis reaction commenced. During the process, time, temperature, and constant stirring (580 rpm) were strictly controlled. Once the reaction time was completed, the reaction was halted by neutralizing the solution with 3 % NaOH. The resulting starch was washed with distilled water and filtered under vacuum to maximize recovery. Subsequently, it was placed in aluminum trays and dried using a hot air gun. Finally, the dried starch was pulverized and sieved using 60 and 230 mesh screens. The moisture content was adjusted to 12-14 % using a thermobalance before proceeding to packaging and storage.

Table 1. Treatments applied for the modification of cassava starch by the acid hydrolysis method.

Treatment	C_A (N)	t_H (h)
T1-H	0.5	1
T2-H	0.5	2
T3-H	1.0	1
T4-H	1.0	2

Chemical modification of natural starch by phosphation method

The phosphation process for modifying natural cassava starch was carried out based on principles established in the literature on starch modification, with specific adaptations for this study [20]. The procedure began with the preparation of a concentrated aqueous solution, using a ratio of 1.2 L of water per kilogram of natural starch. The mixture was heated to 40 °C before adding the appropriate amount of sodium tripolyphosphate (TPS), expressed as a weight percentage as indicated in the experimental design (Table 2). The reaction was performed under basic pH conditions, adjusting the pH to 8.5 by adding NaOH at the start of the process, ensuring strict control of reaction variables: time (TP), temperature, and constant stirring at 580 rpm. At the end of the reaction time, the solution was neutralized with HCl to stop the phosphation process, and the water was removed by vacuum filtration. The drying, sieving, and storage processes were carried out following the same procedure used in the acid hydrolysis method.

Table 2. Treatments applied for the modification of cassava starch by the phosphation method.

Treatment	C_{TPS} (% p/p)	T_P (h)
T1-P	0.1	0.5
T2-P	0.1	1.0
T3-P	0.5	0.5
T4-P	0.5	1.0

Physicochemical characterization of natural and modified starch

Water solubility index (WSI) and Water absorption index (WAI)

The WSI and WAI indices were evaluated following the procedures described in the Technical Guide for the Production and Analysis of Cassava Starch [21]. For this, 1.25 g of starch (dry basis) was mixed with 30 mL of distilled water preheated to 60 °C, and the mixture was vigorously stirred for 1 minute. The samples were then maintained in a water bath at 60 °C for 30 min. After this process, the samples were centrifuged at 4900 rpm for 30 min. The resulting sediment was weighed to determine the WAI, while the supernatant was evaporated at 70 °C for 24 h to calculate the WSI based on the weight of the soluble solids. The obtained values were calculated using Equations 1 and 2, respectively.

$$\text{WAI} = \frac{\text{Gel weight (g)}}{\text{Sample weight (g)}} \quad \text{Equation 1}$$

$$\text{WSI} = \frac{\text{Soluble Weight (g)} * V * 10}{\text{Sample weight (g)}} \quad \text{Equation 2}$$

Gelatinization temperature (GT)

The gelatinization temperature (GT) was determined using a Brabender-E Viscometer, employing a 7 % starch solution prepared from 450 mL of the sample. The dry weight of the sample was calculated using Equation 3 to ensure measurement accuracy. The solution was then analyzed directly in the instrument under the established conditions.

$$\text{Actual sample weight} = \frac{\frac{450 * \% \text{ Solids}}{100}}{\frac{100 - \% \text{ Moisture}}{100}} \quad \text{Equation 3}$$

Brookfield viscosity

The sample previously analyzed (gelatinization temperature) was cooled to 25 °C and its viscosity was determined using a Brookfield viscometer, model B-one Plus, with an L2 spindle at 10 rpm for 50 seconds.

Acidity index

The acidity index was determined by the titration method suggested in the Technical Guide for the Production and Analysis of Cassava Starch [21]. For this, a solution of 20 g of starch (dry basis) in 100 mL of distilled water was prepared, homogenized, and filtered under vacuum. A 50 mL aliquot of the filtrate was then titrated with 0.1 N NaOH, previously standardized with a KHP standard solution, using 1 % phenolphthalein as the indicator. The calculations were performed using Equation 4.

$$\text{Na} * \text{Va} = \text{Nb} * \text{Vb} \quad \text{Equation 4}$$

where;

Na = Normality of the acid

Va = Volume of the acid

Nb = Normality of the base (NaOH)

Vb = Volume of the base

Optimization of operating parameters in the hydrolysis and phosphorylation processes

The optimization of concentration and time parameters in the hydrolysis and phosphorylation processes for the modification of natural cassava starch was conducted through a detailed analysis using main effects plots generated

with Minitab V.14 software. This approach enabled the precise identification of optimal conditions to maximize the desired functional properties of the modified starch.

Results and discussion

The results obtained, shown in Fig. 1, demonstrate that the chemical modification of cassava starch through acid hydrolysis and phosphorylation had a significant impact on its properties, particularly on viscosity. In the case of acid hydrolysis, the viscosity of the starch ranged from 542 to 33.25 mPa·s, representing a notable decrease compared to the natural starch, which had a viscosity of 21,461.5 mPa·s (Fig. 1(a)). This behavior is consistent with previous studies indicating that acid hydrolysis degrades starch molecules, reducing their ability to retain water and form viscous gels [15].

On the other hand, phosphorylated starches also showed a reduction in viscosity compared to the natural starch, although the decrease was not as pronounced as in the case of acid hydrolysis. The viscosities of phosphorylated starches ranged from 18,460 to 19,410 mPa·s (Fig. 1(a)). This behavior is consistent with previous findings for modified cassava starch and rice starch [19,22]. The decrease in viscosity in these starches could be attributed to TPS-induced cross-linking, which causes an increase in the cross-linking of starch chains, improving their resistance to swelling and reducing their ability to form viscous gels. This decrease could be the result of an increase in intermolecular association, which limits the water absorption capacity of starch and consequently reduces its viscosity [23–25].

The GT showed a significant increase in all treatments compared to the native starch, which exhibited a GT of 67 °C (Fig. 1(b)). This increase, reaching a maximum of 70.2 °C, suggests that the structural modifications undergone by the starch induce greater resistance to gelatinization, requiring more energy to initiate this process. In the case of hydrolyzed starches, this increase in GT could be related to a reduction in swelling power, which delays the formation of viscosity, as noted in previous studies [15]. A similar behavior was reported by Salcedo et al. (2017), who observed an increase in the GT of hydrolyzed cassava starch, even under conditions of high acid concentration (0.63 M) and prolonged reaction times (10 h) [26]. This phenomenon can be partly explained by the more branched structure of cassava starch, combined with its low amylose content, which promotes an increase in crystallinity and hydrogen bond density, thereby elevating the GT [27,28]. Thus, modifications in the molecular structure of the starch not only affect its swelling capacity but also its thermal stability, requiring higher temperatures for gelatinization. On the other hand, the observed increase in the gelatinization temperature (TG) of the starch modified by phosphorylation could be attributed to the crosslinking induced by the TPS, which strengthens the interactions between the polysaccharide chains, thereby enhancing the structural stability of the starch [29,30]. This structural reinforcement requires more energy to break the formed bonds, making it more difficult for the gelatinization process to begin [31].

The water solubility index (WSI) of the hydrolyzed starches (Fig. 1(c)) ranged from 3.46 % to 2.21 % (g/100g of sample), values consistent with those reported by Salcedo et al. (2017) for cassava and yam starches [26]. In contrast, the native starch exhibited a WSI of 0.5 %, indicating that acid hydrolysis significantly increases starch solubility. This increase can be attributed to the formation of low molecular weight linear fractions, which leads to a higher number of hydroxyl groups capable of retaining water through hydrogen bonding, thereby facilitating the dissolution of starch granules [32].

In the case of phosphorylated starches (Fig. 1(c)), the WSI values ranged from 1.35 % to 2.36 %, suggesting that phosphorylation also moderately enhances water solubility, although to a lesser extent than hydrolyzed starches. This phenomenon is consistent with the findings of Vargas et al. (2019) in rice starch, who attributed the increase to the repulsion induced by the negatively charged phosphate groups in the starch structure, which reduces intermolecular interactions and facilitates dissolution [19].

Regarding the water absorption index (WAI), the hydrolyzed starches exhibited values ranging from 2.25 to 1.86 g of water/g of starch, reflecting a significant decrease compared to the value of 3.96 recorded for the native starch. This reduction is consistent with results reported by several authors for cassava, yam, sweet potato, and corn starches [26,32,33]. The reduction in the WAI could be attributed to the loss of the amorphous fraction of starch, which promotes an increase in crystallinity and consequently reduces water retention capacity, as crystalline regions are less prone to interact with water molecules. This phenomenon has been widely documented in the literature. Amaraweera et al. (2021) reported an increase in the relative crystallinity (RC) of cassava starch from 24 % to 33 %

following acid hydrolysis with 2.2 M HCl for 90 min, attributed to the selective degradation of amorphous domains and the reorganization of crystalline regions [34]. Similarly, Wang et al. (2022) confirmed this trend, highlighting that the reduction in amorphous content enhances the compact structure of starch granules, thereby limiting their water retention capacity [35]. These findings support our observations and further substantiate the inverse relationship between WAI and starch crystallinity. In contrast, the phosphorylated starches showed a WAI lower than that of the native starch, which differs from the findings of Vargas et al. (2019), who observed an increase in WAI for phosphorylated rice starch. This discrepancy could be explained by differences in the type of starch used [19]. In the study by Vargas et al., rice starch that had been previously modified by acid hydrolysis was employed, using higher concentrations of acid than those used in this research. Phosphation may alter the molecular structure of the starch, modifying the arrangement of amylose and amylopectin chains. This structural alteration could result in greater compactness and reduced branching, thus restricting the formation of spaces available for water interaction, thereby decreasing the water retention capacity of phosphorylated starch [36,37].

Finally, the acid value of the hydrolyzed starches showed a decrease compared to the natural starch (Fig. 1(d)). While the natural starch exhibited a value of 0.0005 meq/g, the hydrolyzed starches ranged between 0.0003 and 0.0002 meq/g. This trend can be attributed to the effects of acid hydrolysis, which fragments the amylose and amylopectin chains, generating lower molecular weight products. The degradation of starch macromolecules reduces the number of functional groups susceptible to acidification, leading to a decrease in the acid value [38].

In contrast, the acid value of the phosphorylated starches (Fig. 1(d)) showed a significant increase compared to the natural and hydrolyzed starches. The phosphorylated starches reached values of up to 0.0011 meq/g (T4-F). This increase can be attributed to the incorporation of phosphate groups from the TPS into the starch structure, which increases the number of acidic groups present in the sample. This effect was more pronounced in treatments with higher concentrations of TPS and longer exposure times (T4-F), suggesting that the degree of modification is directly related to the increase in the acid value.

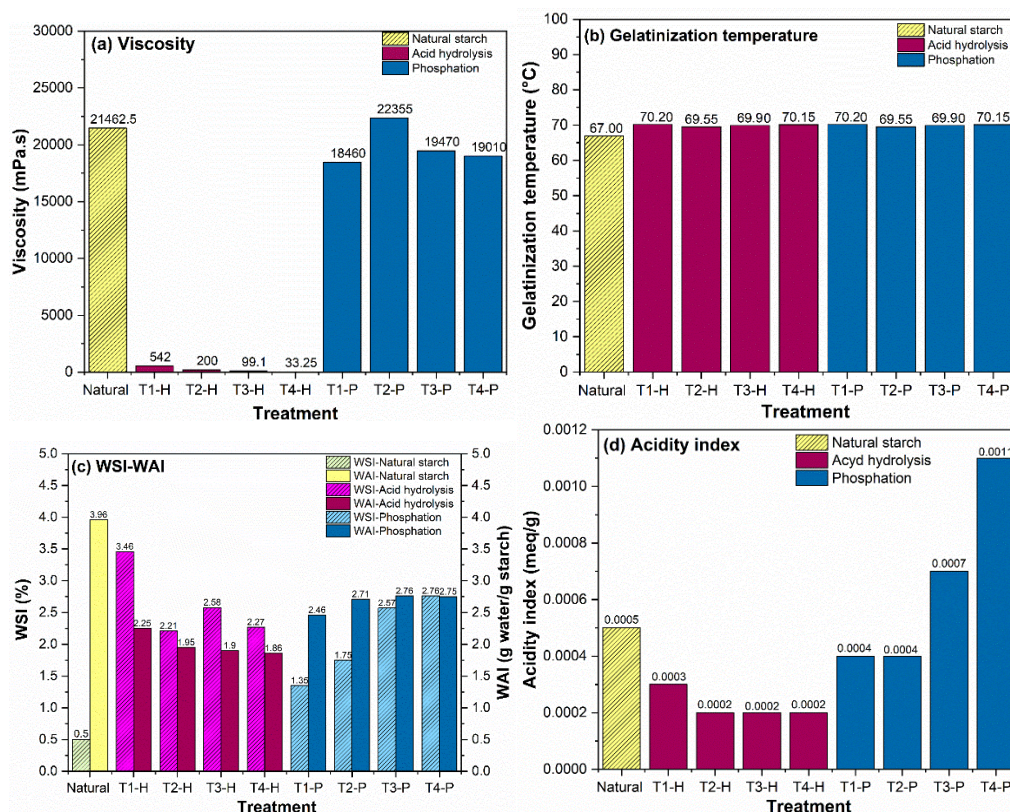


Fig. 1. Effect of modification processes on the properties of natural cassava starch: (a) viscosity, (b) gelatinization temperature, (c) WSI and WAI, and (d) acidity index.

According to the main effects graphs presented in Fig. 2(a), viscosity decreases as both concentration and time increase. This behavior suggests that acid hydrolysis degrades starch chains more rapidly and efficiently under higher values of these parameters. To minimize viscosity, it would be preferable to select high values for both factors; however, using a lower acid concentration could be a more cost-effective alternative if extreme viscosity reduction is not required.

On the other hand, the gelatinization temperature represented in Fig. 2(b) increases with concentration and decreases moderately with time. A lower concentration might suffice if the goal is to simplify the process. Considering that time has less impact on this variable, a reaction time of one hour could be chosen to reduce variability without significantly affecting gelatinization.

Regarding WSI and WAI, both parameters decrease with increasing concentration and reaction time, as shown in Fig. 2(c) and 2(d). However, reaction time has a more significant influence on solubility, while reagent concentration is the predominant factor for water absorption. In this context, it is recommended to use the lowest concentration and reaction time to optimize the process. Similarly, for the acidity index (Fig. 2(e)), given that the obtained values are significantly low, the choice of concentration and time becomes indistinct, with lower values being preferable to simplify the procedure.

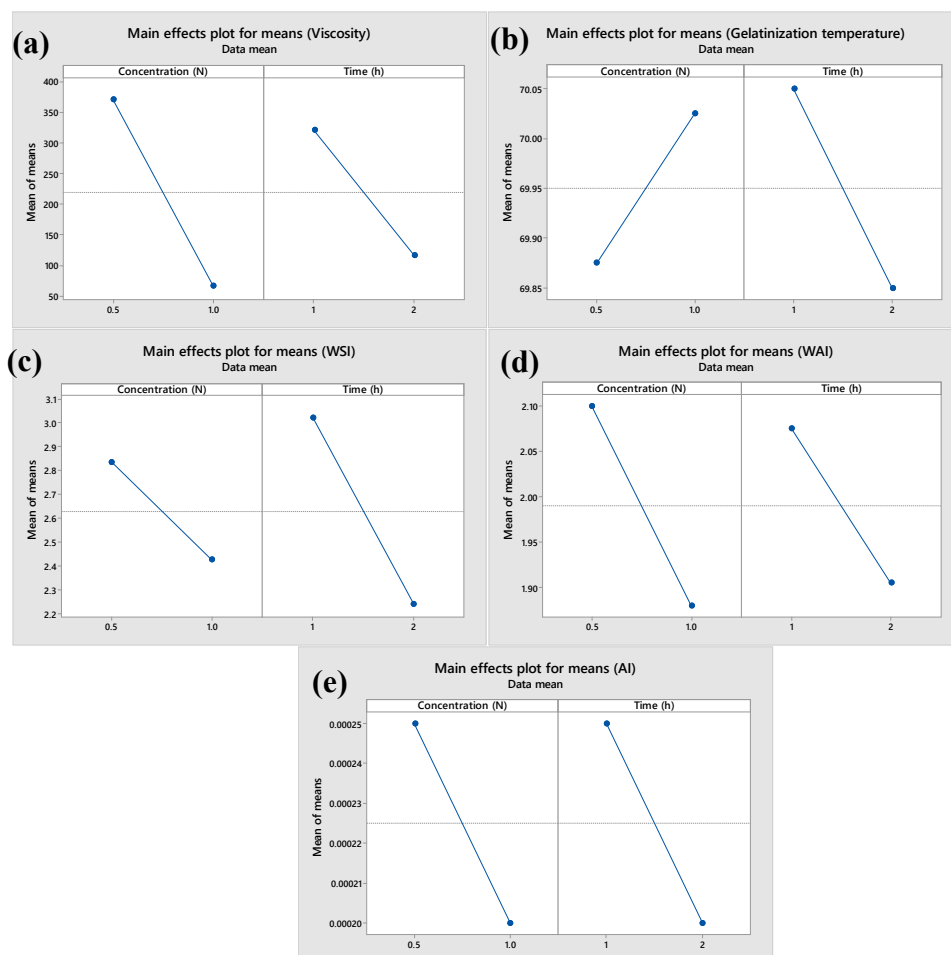


Fig. 2. Main effects of the factors in the acid hydrolysis process on the response variables: (a) viscosity, (b) gelatinization temperature, (c) water solubility index, (d) water absorption index, and (e) acidity index.

Fig. 3 shows the main effects of the concentration and time factors evaluated during the phosphorylation of natural starch on the response variables. It can be observed that viscosity decreases with an increase in concentration

and a shorter reaction time (Fig. 3(a)). From a cost and efficiency perspective, lower viscosity could be beneficial, as it facilitates the handling and processing of starch [39]. Therefore, a high concentration combined with a reduced reaction time seems to be the optimal combination to minimize this property.

Regarding GT, it increases with concentration and decreases with time (Fig. 2(b)). Higher GT can improve the thermal stability of starch, which is beneficial for applications requiring heat resistance, such as the production of food products processed at high temperatures (e.g., fruit purees, instant soups, or ready-to-eat meals), where the preservation of starch structure is essential during heating [40–42]. Additionally, higher GT is favorable in the production of bioplastics or in the pharmaceutical industry, where the thermal stability of starch is crucial for maintaining the product's properties during processing or storage [43,44]. Therefore, if higher thermal stability is required, the combination of a high concentration and reduced time appears to be the optimal choice to ensure the preservation of these properties.

Finally, the WSI tends to increase with both concentration and time, as shown in Fig. 3(c). A high value of this variable could indicate good functionality in applications such as the manufacturing of food products, where water solubility is crucial, such as in instant soups or powdered beverages [45,46]. On the other hand, the WAI (Fig. 3(d)) increases with concentration, while time appears to have little effect on this parameter, as shown in the graph. If water absorption is a desired goal, such as in the production of gels or bakery products, a high concentration seems to be favorable [47]. Finally, the acidity index increases with both concentration and time (Fig. 3(e)). An elevated acidity index could affect the flavor and stability of the product in food applications, such as in canned goods or dairy products, so lower concentration and time could be beneficial.

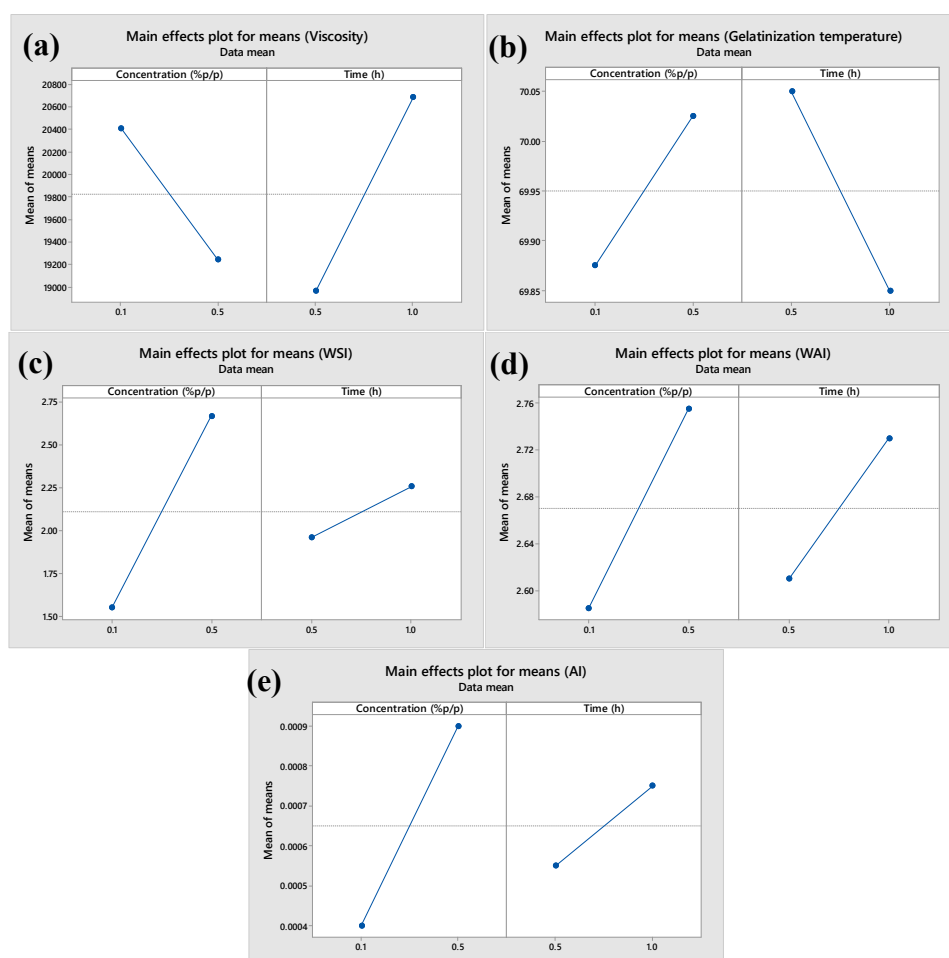


Fig. 3. Main effects of the factors in the phosphation process on the response variables: (a) viscosity, (b) gelatinization temperature, (c) water solubility index, (d) water absorption index, and (e) acidity index.

Conclusions

The chemical modifications of cassava starch through acid hydrolysis and phosphorylation significantly altered its physicochemical properties, suggesting that these treatments may serve as an effective strategy to enhance its functional characteristics for various industrial applications. Acid hydrolysis resulted in a marked reduction in viscosity, water absorption index, and acid value, suggesting the degradation of starch chains and increased crystallinity, factors that limit water retention capacity and the formation of viscous gels. In contrast, phosphorylation increased the gelatinization temperature, improved water solubility, and elevated the acid value due to the incorporation of phosphate groups into the starch structure, reinforcing the interactions between polysaccharide chains and enhancing thermal stability.

Both treatments proved effective in modifying the starch properties, making it suitable for a range of industrial applications. Acid hydrolysis is particularly useful when the goal is to reduce viscosity and water absorption, while phosphorylation is more suitable when enhanced thermal stability and water solubility are required. The results suggest that combining these modifications could be utilized to develop starch-based materials with specific properties for applications in the food, bioplastics, and pharmaceutical industries. The selection of optimal concentration and time conditions will depend on the desired properties, providing flexibility to tailor the process to various industrial needs.

Acknowledgements

The authors would like to express their gratitude to the company ALCASA for the support received by providing us with the space and material necessary to carry out the analyses of this research project.

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