Double chemical modification in rice starch: acid hydrolysis optimization process and phosphating

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ABSTRACT
The objective of this work was to carry out a double chemical modification in rice starch: the first modification was acid hydrolysis optimization as a preparatory step for the second modification, which consisted of phosphating. For the optimization of the acid hydrolysis process, the independent variables hydrolysis time (h) and acid concentration (N) were evaluated. We obtained the estimated coefficients of the adjusted model and significance for the responses of viscosity profile (Vmax), water solubility index (WSI), water absorption index (WAI), onset temperature (To), peak temperature (Tp) and ending temperature (Td). The values predicted by the model for the hydrolysis optimization process were 2 h and 1.2 N for HCl concentration. The starch obtained after the hydrolysis process was modified with sodium tripolyphosphate and characterized. Phosphoryl starches were obtained with degrees of substitution of 0.012 to 0.019 and showed good functional and physicochemical properties.

Doble modificación química en almidón de arroz: optimización del proceso de hidrólisis ácida y fosfatación

RESUMEN
El objetivo de este trabajo fue llevar a cabo una doble modificación química en el almidón de arroz: la primera modificación fue la optimización de la hidrólisis ácida como paso preparativo para la segunda modificación, que consistió en la fosfatación. Para la optimización del proceso de hidrólisis ácida, se evaluaron las variables independientes tiempo de hidrólisis (h) y concentración de ácido (N). Fueron obtenidos los coeficientes estimados del modelo ajustado y la significancia de las respuestas del perfil de viscosidad (Vmax), el índice de solubilidad en agua (WSI), el índice de absorción de agua (WAI), la temperatura de inicio (To), la temperatura máxima (Tp) y la temperatura final (Td). Las variables dependientes para la optimización de la hidrólisis fueron Vmax y WSI. Los valores predichos por el modelo para el tiempo de hidrólisis fueron 2 h y 1.2 N para la concentración de HCl. Las condiciones predichas se validaron experimentalmente para obtener 588.57 cP Vmax y WSI de 4.6%. El almidón obtenido después del proceso de hidrólisis se modificó con tripolifosfato de sodio y se caracterizó. Los almidones fosfatados se obtuvieron con grados de sustitución de 0.012 a 0.019 y mostraron buenas propiedades funcionales y fisicoquímicas.

1. Introduction
Rice (Oryza sativa L.) is one of the most produced cereals in the world. It is an annual herbaceous plant of the grass family, which has been consumed for over 5000 years. Currently, its cultivation has spread around the world, Asian countries being the main consumers (Bao & Bergman, 2018). Among commercial starches, rice starch shows special applica-

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Han & Hamaker, 2001; Vandeputte et al., 2003; Wang & Wang, 2004). However, native starches present limitations that reduce their usefulness; therefore, modified starches are normally used to expand the application of starch in several industries (Thirathamthavorn & Charoenrein, 2005).

Acid hydrolysis has been used to modify the starch granule structure and produce soluble starch (Hoover, 2000). The typical procedure to manufacture acid-thinned starch involves treating a concentrated starch slurry (36–40% solids) at a temperature below the gelatinization temperature of the starch (40–60°C) with mineral acid (Wurzburg, 1986). When the desired viscosity or degree of conversion is reached, acid is neutralized and starch is recovered. Hydrolysis can be controlled by acid concentration, reaction time, and temperature (Hoover, 2000). Furthermore, starches from corn, arrowroot, potato, rice, and tapioca have been modified by acid hydrolysis and characterized; however they have not been subsequently modified (Atichokudomchais, Varavinit, & Chinachoti, 2002; John, Raja, Rani, Moorthy, & Eliasso, 2002; Sandhu, Singh, & Lim, 2007; Thirathamthavorn & Charoenrein, 2005; Wang, Truong, & Wang, 2003; Wang & Wang, 2001). The industrial use of acid-hydrolyzed starches is a pre-modification step to produce cationic and amphoteric starches. At present there are several reports on the use of acid hydrolysis, as a stage of previous modification for the preparation of acetylated, phosphated and succinylated starches from different sources such as corn, amaranth, and achira (Cortés et al., 2014; Gómez-Aldapa et al, 2019; Murúa, Beristain, & Martínez, 2009).

Phosphorylation is a chemical modification (derivatization) involving the introduction of functional groups into the starch molecule, which results in markedly altered physicochemical properties. Starch phosphates produced by conventional oven-heating methods and extrusion have been reported for several food applications as microencapsulation of bioactive compounds (Murúa-Pagola et al., 2009). Starch phosphates may be divided into two categories: starch phosphate monoesters in which a starch hydroxyl group is esterified with only one of the three acidic groups of phosphoric acid, and starch phosphate multiesters in which more than one of the acidic groups of phosphoric acid is esterified (CHANG & LII, 1992). The resultant starch phosphate produces a clear paste with increased solubility, swelling power, and freeze-thaw stability (Lim & Seib, 1993). The objective of this work was to perform a double chemical modification in rice starch. The first modification was an acid hydrolysis optimization as a preparatory step for the second modification, phosphating. Based on the above, we studied changes in the physicochemical and functional properties of modified starches.

2. Materials and methods

2.1. Extraction of rice starch

Rice (Oryza sativa) was purchased from a local supermarket in Pachuca, Hidalgo, Mexico. The rice starch (RS) was extracted by wet milling, following the methodology described by Wang and Wang (2004). The clean rice grain was placed in a 1:2 ratio (g/mL) in a 0.1% NaOH solution for 18 h, after which time the basic solution was removed and rinsed. The clean grain was milled with distilled water in a cyclonic mill (BW-J20, Nutribullet, China), obtaining a suspension that was sieved at a particle size of 150 μm (100 mesh, Tyler, USA). The suspension obtained was neutralized with a 5% HCl solution and centrifuged (Centrifuge IEC Centra, GP8R, UK), at 6000 rpm for 15 min, the supernatant was decanted and washed 3 times, eliminating the residues accumulated on the surface. Finally, the starch was dried in an oven with air recirculation (Thermolyne 9000, Thermo Fisher, Scientific, USA) at 40°C for 24 h, dry milled and sieved to a particle size of 150 μm, and stored in an airtight container.

2.2. Acid hydrolysis

The acid hydrolysis was carried out using a central composite rotatable experimental design, with two factors and α = 1.41. The factors tested were hydrolysis time (h) and hydrochloric acid concentration (N), each consisting of 5 levels. The combination of the levels from both factors resulted in 8 different treatments and 5 central points (Table 1). For the acid hydrolysis, 100 g starch were placed in a 1-L beaker and 300 mL of a HCl solution (1:3 w/v) were added (Table 1). The suspension was maintained in constant agitation on a stirring rack at a constant temperature 39 ± 1°C and different times of hydrolysis, according to the experimental design. Once the hydrolysis time was complete, the solution was neutralized (pH = 7) with a 5N NaOH solution. The starch was recovered by centrifugation and washed 3 times with distilled water. Once washed, the starch was dried at 40°C for 24 h in an oven providing air recirculation (Thermolyne 9000, Thermo Fisher, Scientific, USA), milled in a cyclonic mill (BW-J20, Nutribullet, China), and sieved to a particle size of 150 μm (100 mesh, Tyler, USA).

2.3. Characterization of hydrolyzed starches

2.3.1. Water solubility index and water absorption index

Water solubility index (WSI) and water absorption index (WAI) were determined according to Anderson, Conway, Pfefier, and Griffin (1969). Starch (2.5 g db) was placed in a container (50 mL) and 30 mL of distilled water were added. The tubes were maintained at 30 °C with constant agitation for 30 min. The samples were centrifuged at 2012 × g for 10 min. The gel at the bottom of the tubes was weighed to determine WAI (g of gel/g of dry sample), and the dried supernatant weight was expressed as WSI (%) (g of dry solids/g of dry sample).

| Table 1 | Factors and levels of variation of the experimental design for the independent variables. |
|---------|------------------------------------------------------------------------------------------|
| Independent variable | Levels of variation |
| Time (h) | Concentration (N) |
| 1.37 | 0.4 | 2 | 3.5 | 2 | 1 | 5 | 1.6 |

Where y is the response variable, X_1 is time (h) and X_2 is concentration of HCl (N), α = 1.414. Donde y es la variable respuesta, X_1 es el tiempo (h) y X_2 la concentración de HCl (N), α = 1.414.

| Donde y es la variable respuesta, X_1 es el tiempo (h) y X_2 la concentración de HCl (N), α = 1.414. |
2.3.2. Thermal properties
The starch sample onset (T₀), peak (Tₚ), and ending (Tₑ) temperatures and gelatinization enthalpy (ΔH) were measured with a differential scanning calorimeter (DSC822e, Mettler Toledo, Switzerland) according to (Gómez-Aldapa et al. 2019). The sample (4 mg) was placed in a standard 40-µL aluminum crucible (Mettler Toledo, Switzerland), and a Hamilton microsyringe was used to add distilled water at a 1:4 (starch:water) ratio. The samples were hermetically sealed and later evaluated within a 25°C to 95°C interval at a 5°C/min heating rate. Measurements were performed in duplicate.

2.3.3. Pasting property
The viscosity profiles of the native and modified starches were measured with a Rapid Visco Analyzer (RVA-3C, Newport Scientific, Warriewood, Australia) according to (Gómez-Aldapa et al. 2019). The heating and cooling cycles were programmed in the following manner: the samples (3 g db with 25 mL distilled water) were held at 50 °C for 1 min, heated to 92°C at a heating rate of 5.6°C/min, held at 92°C for 5 min, cooled to 50°C at a cooling rate of 5.6°C/min, and held at a final temperature of 50°C for 2 min. The total time of analysis was 23 min.

2.4. Derivatization with sodium tripolyphosphate
The starch resulting from the process optimization was phosphated with sodium tripolyphosphate (STPP) (Sigma-Aldrich, Germany), according to the method described by Paschall (1964), with some modifications. Two different concentrations of STPP were used: 12.6 g/100 g (PS1), established by the author, and 6.3 g/100g (PS2). The phosphating process was carried out in triplicate.

2.4.1. Phosphorus concentration in native, hydrolyzed, and phosphate starch
Phosphorus concentration was quantified according to the official method 995.11 of the AOAC (1995) in native, hydrolyzed, and phosphate starch. The degree of substitution was calculated using the phosphorus concentration (P) and the Paschall (1964) equation:

\[
\text{Degree of substitution} = \frac{(162 \times P)}{(3100 - (102 \times P))}
\]

Each determination was made by triplicate. The indices of absorption and solubility in water (2.3.1), thermal properties (2.3.2), and viscosity profile (2.3.3) were determined for the phosphate starches.

2.4.1.1. Scanning electron microscopy
The modified and native starches were observed with a scanning electron microscope (SEM) (Jeol JSM-6300, Japan). The dried samples were placed in a sample holder covered with double-sided carbon electroconductive tape and were then covered for 3 min with a layer of gold (0.5–1 nm thick). The conditions under which the samples were observed were 15 A and 20 kV. Semiquantitative analysis of phosphorus by X-ray energy dispersion (EDS) was carried out in native and hydrolyzed starches under optimal conditions, as well as in hydrolyzed and phosphate starches.

2.5. Experimental design and data analyses
A central composite rotatable design model composed of two factors (X₁ and X₂) was used for hydrolysis (Table 1). All the trials were performed randomly with a variable number of repetitions. The data were then analyzed using response surface methodology (RSM) with Design-Expert v 7.1.5. The experimental data were adjusted to a second-order polynomial model:

\[
y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2
\]

Where Yᵢ is the response variable, X₁ is time (h), and X₂ is concentration of HCl (N). The significance of the models was tested using variance analysis (F test).

2.5.1. Optimization of acid hydrolysis
The optimization of the acid hydrolysis process was carried out with Design Expert v 7.1.6 (Stat-Ease, MN, USA), using the numerical optimization option. The selected responses for optimization were WSI (%) and Vₘₐₓₚ starch showing the lowest viscosity and the highest solubility index as desirable criteria. The results of the characterization of native and modified phosphate starches (2.5) were expressed as mean ± standard deviation (SD). An analysis of variance (ANOVA) was used to compare the results (p ≤ 0.05) using Statistica v 8.0 (StatSoft, 2007, USA).

3. Results and discussion
3.1. Characterization of hydrolyzed starches
In Table 2, the estimated coefficients of the adjusted model and the level of significance are described. The hydrolysis...
time and the HCl concentration had a significant effect on the linear and quadratic terms of all the responses, while the interaction hydrolysis time-HCl concentration had a significant effect on $V_{\text{max}}$. The adjusted $R^2$ for the different responses was 0.92–0.96. Therefore, the adjusted models are adequate to explain the behavior of the experimental data from the different responses.

### 3.1.1. Water solubility index and water absorption index

The highest value of WSI (%) was obtained at hydrolysis time of 3.5 h and a concentration of 1N HCl (5.4 ± 0.49%). A lowest value of WSI was obtained at 5 h of hydrolysis and a concentration of 0.6N HCl (2.3 ± 0.164%) (Table 3). With respect to WAI (g/g), the different hydrolysis treatments showed values between 2.3 ± 0.047 to 3.5 ± 0.069 g/db. The maximum value of WAI was obtained after treatment for 1.37 h and a concentration of 1N HCl, while the lowest value was obtained at a hydrolysis time of 3.5 h and HCl concentration of 1.6 N (Table 3). The increase in solubility percentage is proportional to the hydrolysis conditions due to the formation of low-molecular weight products generated by the rupture of hydrogen bonds caused in turn by the acid. Said products are more soluble in water, decreasing the viscosity and increasing the amount of soluble starch (Nakazawa & Wang, 2003; Wurzburg, 1989). In addition to the changes in the intramolecular structure of the starch when subjected to acid hydrolysis, functional properties, as water absorption capacity, are also modified due to a decrease in the degree of packaging of starch granules (BeMiller & Whistler, 2009; Singh, Singh, Kaur, Singh-Sodi, & Singh-Gill, 2003).

### 3.1.2. Thermal properties

After acid hydrolysis treatment at different times and concentrations of the hydrolytic agent (HCl), the starches showed the following values: gelatinization $T_p$ between 69.4 ± 0.434 and 83.9 ± 0.441°C, gelatinization $T_e$ between 75.5 ± 0.376 and 89.7 ± 0.330°C, and $T_g$ of gelatinization between 80.8 ± 0.428 and 94.4 ± 0.350°C (Table 3). According to these results, the starches that underwent the acid hydrolysis exhibited a shift in the three temperatures of gelatinization transition with respect to the temperatures of the native starch (Table 4). The displacement of these temperatures is more evident in the hydrolyzed starches with higher concentrations of HCl and longer treatment times. Although this behavior is not clear, it is believed to be the result of a decrease in the amorphous zones of the granule and the prevalence of crystalline areas caused by hydrolysis. The latter generates a rearrangement of amylopectin within the arrangement of double helices, decreasing the permeability of the water inside the granule (Thirathamthavorn & Charoenrein, 2005). Similar results have been reported by Ahmed and Auras (2011) in lentil starch subjected to acid hydrolysis. With respect to the enthalpy of gelatinization, hydrolyzed starch showed a decrease from 11.4 to 10.0 (J/g) in comparison with native starch; similar results were reported by Thirathamthavorn and Charoenrein (2005).

### 3.1.3. Viscosity profile

The starches subjected to hydrolysis treatment showed $V_{\text{max}}$ values between 207.10 ± 11.29 and 995.06 ± 43.425 cP (Table 3), lower values in comparison with the $V_{\text{max}}$ of native starch 2683.3. The attack of the acid is carried out more quickly in the amorphous region of the granules, promoting the fracturing of the granule and leading to a decrease in viscosity. Peak viscosity ($V_{\text{peak}}$) was a measure of the swelling power of the starch in terms of the resistance of swollen granules to shear (Tao et al., 2018). Figure 1 shows the response surface for the variable $V_{\text{max}}$ according to the estimated coefficients of the model. Considering this variable, the interaction between the hydrolysis time and the concentration of hydrochloric acid ($p < .05$) affects the maximum viscosity: the lowest $V_{\text{max}}$ was obtained at longer times of hydrolysis and higher concentrations of acid.

### 3.2. Optimization of acid hydrolysis

Acid hydrolysis was used as a previous step for a second chemical modification (phosphating). The aim of acid hydrolysis was the generation of the largest number of spaces available for the union of phosphate groups. Thus decreasing the viscosity of the native starch, which increases with the addition of the phosphate groups. The numerical optimization was carried out to combine variables that allowed for the obtention of a hydrolyzed starch with the highest WSI and the lowest $V_{\text{max}}$. According to the objective set for the response of $V_{\text{max}}$ and WSI, the values predicted by the model were 2 h for the hydrolysis time and 1.2N for the HCl concentration, with predicted values expressed as the mean±standard deviation (n = 3).

### Table 3. Water solubility index (WSI), water absorption index (WAI), onset temperature ($T_o$), peak temperature ($T_p$), ending temperature ($T_e$) and viscosity profile ($V_{\text{max}}$) of hydrolyzed rice starch.

| Test | Time (h) | HCl (N) | WSI (%) | WAI (g/g) | $T_o$ (°C) | $T_p$ (°C) | $T_e$ (°C) | $V_{\text{max}}$ (cP) |
|------|----------|---------|---------|-----------|------------|------------|------------|------------------|
| 1    | 2        | 0.6     | 4.1 ± 0.81 | 3.1 ± 0.07 | 69.4 ± 0.43 | 75.5 ± 0.38 | 80.4 ± 0.33 | 920.20 ± 6.98   |
| 2    | 2        | 1.4     | 4.9 ± 0.13 | 2.9 ± 0.07 | 72.3 ± 0.31 | 77.8 ± 0.21 | 82.9 ± 0.21 | 797.23 ± 26.63  |
| 3    | 3        | 0.6     | 2.3 ± 0.16 | 3.0 ± 0.63 | 80.2 ± 0.24 | 87.4 ± 0.26 | 92.8 ± 0.08 | 995.06 ± 43.42  |
| 4    | 5        | 1.4     | 2.3 ± 0.23 | 2.4 ± 0.01 | 81.2 ± 0.19 | 87.9 ± 0.06 | 93.1 ± 0.24 | 239.20 ± 15.02  |
| 5    | 1.37     | 1       | 2.6 ± 0.24 | 3.5 ± 0.05 | 73.4 ± 0.96 | 79.0 ± 0.64 | 83.6 ± 0.31 | 893.05 ± 16.04  |
| 6    | 5.62     | 1       | 3.3 ± 0.95 | 2.4 ± 0.10 | 82.3 ± 0.53 | 88.8 ± 0.47 | 93.8 ± 0.39 | 207.10 ± 11.29  |
| 7    | 3.5      | 0.4     | 2.5 ± 0.28 | 2.8 ± 0.01 | 74.4 ± 0.55 | 79.8 ± 0.23 | 86.6 ± 0.33 | 1518.3 ± 34.08  |
| 8    | 3.5      | 1.6     | 4.4 ± 0.17 | 2.3 ± 0.05 | 83.9 ± 0.44 | 89.7 ± 0.33 | 94.4 ± 0.35 | 268.06 ± 2.46   |
| 9    | 3.5      | 1       | 3.7 ± 0.07 | 2.6 ± 0.04 | 82.5 ± 0.39 | 88.7 ± 0.33 | 93.7 ± 0.24 | 732.36 ± 19.15  |
| 10   | 3.5      | 1       | 2.5 ± 0.16 | 2.5 ± 0.02 | 80.9 ± 0.28 | 88.0 ± 0.66 | 93.3 ± 0.79 | 760.64 ± 14.19  |
| 11   | 3.5      | 1       | 5.4 ± 0.49 | 2.7 ± 0.03 | 71.6 ± 0.28 | 77.5 ± 0.36 | 82.5 ± 0.41 | 796.95 ± 16.14  |
| 12   | 3.5      | 1       | 2.1 ± 0.76 | 2.6 ± 0.04 | 80.9 ± 0.89 | 87.3 ± 0.36 | 92.7 ± 0.59 | 795.50 ± 12.16  |
| 13   | 3.5      | 1       | 4.3 ± 0.07 | 2.6 ± 0.02 | 74.4 ± 0.77 | 79.9 ± 0.04 | 85.9 ± 0.36 | 803.73 ± 12.02  |

Values are expressed as the mean±standard deviation (n = 3).

Valores expresados como la media ± desviación estándar (n = 3).
| Starch (Treatment) | Phosphorus content | Solubility and absorption water index | Thermal properties | Viscosity |
|-------------------|--------------------|--------------------------------------|--------------------|----------|
|                   | % Phosphorus       | Degree of substitution               | WSI (%)            | WAI (g/g) | T<sub>s</sub> (°C) | T<sub>p</sub> (°C) | T<sub>e</sub> (°C) | ΔH (J/g) | V<sub>max</sub> (cP) |
| Native            | 0.037 ± 0.002<sup>a</sup> | 0.002 ± 0.0001<sup>a</sup> | 0.4 ± 0.10<sup>a</sup> | 2.3 ± 0.030<sup>a</sup> | 67.0 ± 0.552<sup>b</sup> | 72.8 ± 0.693<sup>c</sup> | 78.1 ± 0.686<sup>a</sup> | 11.4 ± 0.172<sup>a</sup> | 2683.3 ± 664.977<sup>d</sup> |
| Hydrolyzed        | 0.023 ± 0.003<sup>a</sup> | 0.001 ± 0.0001<sup>a</sup> | 4.6 ± 0.22<sup>a</sup> | 2.4 ± 0.048<sup>a</sup> | 76.4 ± 0.730<sup>c</sup> | 80.1 ± 0.453<sup>c</sup> | 84.6 ± 0.681<sup>c</sup> | 10.0 ± 0.111<sup>a</sup> | 588.5 ± 5.858<sup>c</sup> |
| PS1               | 0.360 ± 0.062<sup>c</sup> | 0.019 ± 0.008<sup>b</sup> | 3.7 ± 0.94<sup>c</sup> | 2.9 ± 0.216<sup>c</sup> | 64.1 ± 0.599<sup>c</sup> | 71.5 ± 0.516<sup>c</sup> | 77.9 ± 0.107<sup>b</sup> | 49 ± 1.147<sup>c</sup> | 1107.5 ± 277.638<sup>c</sup> |
| PS2               | 0.229 ± 0.021<sup>b</sup> | 0.012 ± 0.001<sup>b</sup> | 2.2 ± 0.40<sup>b</sup> | 2.7 ± 0.236<sup>b</sup> | 67.8 ± 0.309<sup>b</sup> | 74.6 ± 0.664<sup>b</sup> | 79.1 ± 0.3 25<sup>b</sup> | 5.9 ± 0.258<sup>b</sup> | 920.1 ± 436.510<sup>b</sup> |

Values are expressed as the mean ± standard deviation (n = 3).

Values in the same column with different letters show significant differences (p < 0.05).

PS1 = phosphating starch with 12.6 g of TPS/100 g and PS2 = 6.3 g of TPS/100 g.

Valores expresados como la media ± desviación estándar (n = 3).

Valores en la misma columna con letras diferentes presentan diferencia significativa (p < 0.05).

PS1 = almidón fosfatado con 12.6 g de TPS/100 g y PS2 = 6.3 g de TPS/100 g.
values of 101.5 cP for $V_{\text{max}}$ and 5.09% WSI. The conditions predicted by the optimization model were validated experimentally by triplicate, obtaining $V_{\text{max}}$ of 588.57 ± 5.86 cP and WSI of 4.65 ± 0.224%. The rice starch obtained under the optimal conditions of hydrolysis showed a WAI of 2.40 ± 0.048 g/g, a gelatinization start temperature of 76.4 ± 0.7°C, a gelatinization peak temperature of 80.1 ± 0.4°C, and a final temperature of gelatinization of 84.6 ± 0.6ºC, while the energy required to carry out the gelatinization was 10.0 J/g (Table 4).

### 3.3. Characterization of phosphating starches

The phosphorus content in the different starches was quantified as shown in Table 4. Rice starch in its native form has significant concentrations of phospholipids, so the presence of phosphorus is evident. In this case, native starch had a concentration of 0.037%, with a degree of substitution of 0.002. Vandeputte et al. (2003) reported a similar percentage of phosphorus (0.046 to 0.056%) in native rice starch, with a degree of substitution of 0.001. After phosphating with 12.6 g/100 g of STPP (PS1), we determined a phosphorus concentration of 0.36% with a degree of substitution of 0.019. When reducing the concentration of STPP to 6.3 g/100 g (PS2), the phosphorus concentration was 0.22% with a degree of substitution of 0.012. The FDA has established a limit of 0.4% total phosphorus in modified starch with STPP (Code of Federal Regulation, 2015); both phosphate starches are within the limits of phosphorus allowed by the FDA. Lim and Seib (1993) reported that, at a concentration of 0.4% phosphorus, starches obtained show solubility, high swelling capacity, freeze-thaw stability, and clear pastes. After EDS analysis, phosphorus concentrations were: 0.04% in native starch, 0.02% in hydrolysate, 0.43% in PS1, and 0.24% in PS2.

#### 3.3.1. Water solubility index and water absorption index

Table 4 shows the values of WSI (%) for phosphate starches (PS1 and PS2). These starches showed a significant difference with respect to native starch ($p < 0.05$). Regarding hydrolyzed starch, the phosphate starches in both concentrations showed lower values. Sitohy & Ramadan (2001) obtained phosphate starches from corn, rice, and potato with different degrees of substitution. They established that highly water-soluble starches are obtained at low degrees of substitution. They attributed this effect to the repulsion between the negative charge of the phosphate groups in the starch molecule that reduces the intermolecular attractive forces. A subsequent increase in the degree of substitution could decrease the solubility by the formation of cross-links between starch polymers.

The highest WAI was obtained in PS1 (2.9 ± 0.216 g/g), the starch with the highest phosphorus concentration. This effect could be related to the formation of cross-links, which are generated at higher phosphorus concentrations. Phosphating is one of the most commonly used methods in chemical modification of starches. It produces a repulsion between the adjacent chains in the granule, caused by the introduction of negatively charged phosphate groups, leading to a decrease in the number of associations between the starch chains and an increase in hydration capacity, as well as stability against freezing. This enhances their use as thickeners and stabilizers in food (Lim & Seib, 1993; Liu, Ramsden, & Corke, 1999).

#### 3.3.2. Thermal properties

As for the gelatinization temperature, phosphate starch granules had modifications with respect to native and hydrolyzed starch, decreasing their enthalpy of gelatinization of 11.4 ± 0.172 (native starch) to 4.9 ± 1.147 for PS1 and 5.9 ± 0.258 for PS2 (Table 4). (Tao et al., 2018) mentioned that higher enthalpy indicated highly ordered crystalline regions, which shows the structural change in phosphate starches. CHANG and LI (1992) reported that cassava and corn phosphate starches obtained by conventional method or extrusion exhibited lower gelatinization temperatures with respect to native starches because the modifications make them easily penetrable by water. In addition, it has been reported that amylose concentration reduces the melting point of crystalline regions (Singh, Isono, Srichuwong, Noda, & Nishinari, 2008; Singh et al., 2003; Tao et al., 2018), modifying in turn its enthalpy of gelatinization.
3.3.3. Viscosity profile
The increase in the viscosity profile when adding phosphate groups depends on the phosphorus concentration. Then, $V_{\text{max}}$ was $1107.6 \pm 277.64$ cP for PS1 and $920.2 \pm 43.65$ cP for PS2. The phosphate starches had an increase in viscosity of more than 36% with respect to the hydrolyzed starch under the optimum conditions. However, hydrolyzed starch and PS1 and PS2 starches showed lower $V_{\text{max}}$ with respect to native starch. The decrease in viscosity in the modified starches is due to the dissociation of the double helices of the amylopectin caused by the temperature reached during the heating. In consequence, the stability of the granule decreases when it breaks and generates a drop in viscosity (BeMiller & Whistler, 2009).

3.3.4. Scanning electron microscopy
Figure 2 shows the micrographs taken from native rice starch (A), starch obtained under optimum hydrolysis conditions (B), starch PS1 (C), and of starch PS2 (D). The micrograph of the native starch presents granules of polyhedral shape and of variable size ranging from 0.46 to 2.15 μm, with an average size of 0.85 μm, and a smooth surface. Contrastingly, the hydrolyzed starch and the phosphates exhibit a rough surface due to the chemical modification that generates damages in the native structure. Furthermore, surface erosion in certain regions of granules is evident due to the hydrolysis effect that can cause pores or fissures, depending on the degree. The hydrolyzed-phosphate starches underwent greater degradation and agglomeration with respect to the native and the hydrolysate. Changes in the structure of rice starch granules have been reported by Singh et al. (2003) and Singh, Sodhi, and Singh (2009). Acid hydrolysis at low acid concentrations causes pitting or cracking, which indicates the formation of pores or channels (Wang, Blazek, Gilbert, & Copeland, 2012), becoming more evident at a higher concentration of the hydrolytic agent or longer exposure time. When the hydrolysis conditions are very strong, the complete destruction of the starch granule can be promoted, generating fragments of amylose and amylopectin (Campanha & Franco, 2011).

4. Conclusions
The concentration of the hydrolytic agent and the time of exposure to this agent modify the molecular structure of the granules to a lesser or greater degree, altering their physicochemical, thermal, and paste properties, due to the attack of the amorphous zones of the granule. The hydrolysis was a preparative stage for phosphating since its viscosity was reduced and its solubility increased, leaving in turn a greater number of regions for the interaction and incorporation of phosphate groups. The concentration of phosphorus and the degree of substitution generated a new modification in the properties already modified by acid hydrolysis. Starches were obtained with low viscosity, high solubility, and phosphorus concentrations within the limits established by the FDA. Therefore, the starches obtained can be used as thickeners in low viscosity foods and the formation of food coatings, or as wall material for the encapsulation of flavorings and active compounds.

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