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Dual Modification of Chayotextle Starch: Effect on Physicochemical, Functional, and Structural Properties

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Chemically modified starches are widely used as food additives to improve the appearance and physicochemical and biological properties of foods. The aim of this work is to chemically modify chayotextle (*Sechium edule*) starch by hydroxypropylation-crosslinking, and evaluate the effects of degree of substitution (DS), swelling power (SP), solubility index (SI), pasting, thermal properties, and morphological and molecular characteristics. Polar-Tex (modified maize starch) is used as control. The modified chayotextle starches (CHSs) exhibit a white color and DS values between 0.34% and 1.19% hydroxypropyl, dependening on propylene oxide concentration-response. In addition, the SP and pasting properties are higher in modified CHS than in native CHS and Polar-Tex. The SI and temperature of gelatinization of modified starches decrease compared to native sample. The morphology of native and modified CHS granules is spherical and oval. Fourier transform infrared and nuclear magnetic resonance studies confirm the structural modifications of CHS by hydroxypropylation-crosslinking. Dual-modified CHS can be potentially used as an additive in the food industry.

1. Introduction

Starch is a raw material with diverse technological and industrial applications.[1] It is widely used as packaging material or food ingredient for various commercial products.[2,3] As a food additive, starch is commonly employed as stabilizing and texturizing agent, enhancing the acceptability of food products.[4] Tubers, cereals, and legumes are the most important crops for starch production; therefore, exploring underutilized and novel starch sources is an active research area.[5] Chayotextle is a tuberized root from the *Sechium edule* crop that belongs to the Cucurbitaceae family. It is typically consumed as a cooked vegetable; however, the tuber is characterized by its polysaccharide content, where starch is the main component and could be used in foods.[2] Chayotextle starch (CHS) has been used in the food industry as a food additive or food packaging material.[2,3]

Studies have demonstrated that starches in their native form exhibit limited uses in several industrial food applications, associated with low thermal stability, high degree of retrogradation, high viscosity, and low shear stress and solubility, among others.[4] In this context, starch modification is a viable and technological alternative to improve their physicochemical and functional properties as well as their potential food uses because of the introduction of new functional groups into the starch structure.[6] Starches can be modified by chemical, physical, enzymatic, and biological routes in single or dual approaches.[6–8] Among the reported methods, esterification, etherification, acetylation, crosslinking, and hydroxypropylation are some of the most commonly used chemical methods in starch modification because they are effective and inexpensive.[9,10]

Studies have found that starch crosslinking increases glass transition temperature, enthalpy of gelatinization, and resistant content,[11] but reduces viscosity properties, swelling index, and solubility.[12,13] Other studies report the use of crosslinking starch in the elaboration of biofilms, finding that it increases the hydrophobic properties of the films since moisture content, water solubility, and water vapor permeability are reduced and mechanical properties are increased.[13,14] In contrast, low gelatinization, enthalpy, and pasting temperatures are observed when hydroxypropylated starches are used. It has also been reported that a reduction in gelatinized starch retrogradation during cold storage promotes the swelling of starch granules and improves hot and cold viscosities.[15,16]

Dual chemical modification has been successfully used to modify starches from diverse food matrixes, including maize, potato, achira, and common beans, among others. The modification of barley starch by hydroxypropylation and crosslinking for its use in soup improves the clarity of the paste, changes the

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viscoelastic properties of the modified starch, and improves the consistency of the soup.[9,15,17] Alfano et al.[18] reported the incorporation of doubly modified waxy corn starch (Polar Tex – 06730) to an emulsion and found a slight increase in indexes *K* and *n* of rheological properties, which is attributable to the higher viscosity of the dispersing phase. The changes in thermal and rheological properties as well as the reduction in retrogradation rate of starches modified by hydroxypropylation-crosslinking are potentially an excellent alternative to explore and use starches more frequently in food processing.

To date, only a few studies have been performed for characterizing the physicochemical, functional, and molecular structure of native CHS.[19,20] On the other hand, studies on single or dual modifications of CHS are scarce. Therefore, this work aims to evaluate the effect of dual modification by crosslinking-hydroxypropylation on the physicochemical, functional, and structural properties of CHS, using a negative and a positive control

2. Experimental Section

2.1. Materials

Chayote tubers were obtained from a local market in Tulancingo, Hidalgo, Mexico. Polar-Tex (06730) was a double modified (hydroxypropylated and crosslinked) waxy corn starch, which was supplied by Cargill-Mexico. Sodium trimetaphosphate (TMFS) with CAS number: T5508 and propylene oxide (CAS number: 110205) were acquired from Sigma–Aldrich Mexico.

2.2. Starch Isolation

The chayote tubers were peeled and washed with tap water. Subsequently, they were cut into 5-cm cubes. Then, CHS was isolated according to the methodology proposed by Jiménez-Hernández et al.[20] The cubes were macerated in tap water (1:1) in an industrial blender for 2 min. The homogenate was consecutively sieved and washed through 50, 100, 200, 270, and 325 US mesh until the washing water was clean; the mix was pelleted overnight and decanted. The supernatant obtained was dried in a convection oven (APEX, SSE 17 M) at 40 °C for 24 h. The dried powder was ground in a food processor and sieved using a 100 US mesh. The starch was stored in resealable propylene bags at 25 °C prior to analysis.

2.3. Starch Modification

2.3.1. Hydroxypropylation of Starch

Hydroxypropylation of CHS was performed according to Mehfooz et al.[15] The CHS (500 g) was mixed with anhydrous sodium sulfate (100 g) and adjusted to 1 L using distilled water and was then homogenized under magnetic stirring for 30 min. The pH solution was adjusted to 11 using NaOH (1 M). Based on the weight of the starch (dry basis, db), 10%, 25%, and 40% w/v propylene oxide were added to three different batches of the starch slurry. Subsequently, the mixture was left to react (35 °C for 24 h) in a screw-capped flask, and the pH solution was again adjusted to 11 with NaOH (1 M) after the reaction time.

Table 1. Experimental matrix for dual modification of CHS.

Treatment	Hydroxypropylation [% wt.]	Crosslinking [% wt.]
NCHS (C−)		
CDMCS (C+)		
CHS:$H_{10}R_1$	10	1
CHS:$H_{10}R_5$	10	5
CHS:$H_{25}R_1$	25	1
CHS:$H_{25}R_5$	25	5
CHS:$H_{40}R_1$	40	1
CHS:$H_{40}R_5$	40	5

C−, negative control; C+, positive control; wt, weight percent. CDMCS, commercially dual modified corn starch (Polar-Tex); NCHS, native chayotextle starch.

2.3.2. Crosslinking of Starch

TMFS (1% or 5% w/w) was added to each hydroxypropylated CHS batch to promote starch crosslinking, and the mix was then homogenized under magnetic stirring at 35 °C for 3 h. After the reaction, the pH solution was adjusted to 5–6 using 1 M HCl. Briefly, the mixture was centrifuged at 2350 × *g* for 5 min to eliminate the excess chemicals. The starch was oven-dried at 40 °C until a constant weight was reached.[15] Codes for dual-modified CHS are listed in **Table 1**. In this study, a native CHS was used as a negative control, while a commercial dual modified corn starch (Polar-Tex) was used as positive control.

2.4. Degree of Substitution

The degree of substitution (DS) of the modified starch was performed according to the FAO/WHO[21] method established in the Compendium of food additive specifications. The modified starch (50 mg) was mixed with 25 mL sulfuric acid (2 M), placed in a boiling water bath until sample homogenization, and cooled at room temperature. Then, in a cold-water bath, 1 mL of the resulting solution was mixed with 8 mL concentrated sulfuric acid. Subsequently, the sample was boiled in a water bath for 3 min and placed in an ice bath immediately after until the solution was cold. Subsequently, 0.6 mL of ninhydrin was carefully added and homogenized, and samples were placed in a water bath at 25 °C for 100 min. After the volume of each sample was adjusted to 25 mL with concentrated sulfuric acid and homogenized again, the samples were allowed to stand for 5 min and then measured spectrophotometrically (Thermo Fisher Scientific, Model Genesys 10s vis) at 590 nm.

The following equation was used to determine the DS:

$$DS \text{ of hydroxypropyl groups (\% HPS)} = \frac{C \times 0.7763 \times 10 \times F}{W} \quad (1)$$

where *C* is the amount of propylene glycol in the sample solution read from calibration curve (μg mL^{-1}), *F* the dilution factor (if a further dilution has been necessary), and *W* is the weight of sample (mg).

Molar substitution (MS) was determined using the equation:

$$\mathrm{MS} = \frac{162\ (\%\mathrm{HPS})}{(100M) - (M-1)\,W} \quad (2)$$

where %HPS is the DS of hydroxypropyl groups, and M is the molecular weight of C_3H_6O.

2.5. Whiteness Determination (*W*)

The colors of the native and modified starches were evaluated using a chroma meter (Colorimeter YS6060, Benchtop Spectrophotometer, Model NR100, Shenzhen, China), as L^* (lightness), a^*, (redness/greenness), and b^* (yellowness/blueness) values. The degree of whiteness was determined by means of the following equation:

$$W = 100 - \sqrt{(100 - L^2) + a^2 + b^2} \quad (3)$$

2.6. Morphological and Structural Characterization

The morphology of dual-modified CHS granules was determined using a scanning electron microscope (SEM, JEOL, JSM-5800 LV, USA). The CHS sample was prepared according to Sukhija et al.[1] Subsequently, the tape was coated with a 50-nm layer of gold in a JEOL metal ionizer. The procedure was observed in the microscope and registered photographically.

The infrared spectra for the dual-modified CHS were obtained in a Fourier transform infrared spectrometer (Perkin Elmer, Spectrum One, Waltham, MA, USA) equipped with attenuated total reflectance (FTIR-ATR). Sample spectra were recorded at 25 °C, with 24 scans and 4 cm^{-1} resolution in a wavelength range from 4000 to 400 cm^{-1}.

For the nuclear magnetic resonance (carbon-13 NMR [^{13}C NMR] and ^{31}P NMR) analysis, modified CHS (10 mg) was dissolved in deuterated dimethyl sulfoxide under magnetic stirring for 60 min, and the solution was left to stand for 24 h. After this time, the CHS sample was placed in an NMR equipment (VARIAN-NMR 400 MHZ, Germany), where all samples were subjected to 16 scans at 25 °C for 6 h.

2.7. Swelling Power

The SP of modified CHS was quantified following the method by Mehfooz et al.[15] with slight modifications. The starch sample (0.6 g) was mixed with distilled water (30 mL) in a screw-capped centrifuge tube. The sample was heated in a hot water bath at 30, 50, 70, and 90 °C for 30 min and occasionally homogenized. Once the samples were cooled at room temperature, they were centrifuged at 6500 × *g* for 15 min. The SP was calculated using Equation (4):

$$\mathrm{SP} = \frac{(Ws - We)}{W} \quad (4)$$

where *Ws* is the weight of sedimented paste with centrifuge tube (g), *We* the empty tube weight (g), and *W* is the dried starch weight.

2.8. Solubility Index

The solubility of the modified CHS was measured according to the method described by Liu et al.[22] with slight modifications. The modified CHS (0.4 g) was mixed with distilled water (30 mL) in a screw-capped centrifuge tube and homogenized. The resulting solution was heated in a hot water bath at 30, 50, 70, and 90 °C for 30 min and occasionally homogenized. Once samples were cooled at room temperature, they were centrifuged at 6500 × *g* for 15 min. The supernatants were recovered and oven dried at 110 °C for 12 h until they reached a constant weight. The starch solubility was calculated according to Equation (5):

$$SI = \frac{\text{weight of dissolved solids in supernatant}}{\text{sample weight}} * 100 \quad (5)$$

2.9. Pasting Properties

The pasting properties of modified CHS were evaluated with a Rapid Visco Analyzer (RVA-4, Newport Scientific, Sydney, Australia) following the AACC method[23] 76-21.02. The starch sample (3 g, 14% moisture, db) was weighed directly in an aluminum RVA-sample canister, and distilled water was added to a constant weight sample of 28 g. The slurry was then manually homogenized using a plastic paddle to avoid lump formation before the RVA run. A programmed heating and cooling cycle was set for 23 min where the samples were held at 30 °C for 1 min, heated to 95 °C in 7.5 min, and further held at 95 °C for 5 min before cooling to 50 °C within 7.5 min, and holding at 50 °C for 2 min. Results were expressed as Pa s. All measurements were replicated three times.

2.10. Thermal Properties

The thermal properties of modified CHS were examined in a TA Instruments calorimeter (Q2000, Newcastle, DE, USA), according to Paredes-López et al.[24] The starch sample (2 mg db) was weighed directly in a DSC aluminum pan, and deionized water (7 µL) was added. After hermetically sealed, the pans were allowed to stand for 30 min at room temperature for even hydration before thermal analysis. The sample was heated from 30 to 120 °C, at a rate of 10 °C min^{-1}. The onset temperature (T_0), peak temperature (T_p), conclusion temperature (T_c), and enthalpy of gelatinization (ΔH) were obtained from data analysis using the TA Instruments software (v. 4.4). An empty pan was used as reference.

2.11. Statistical Analysis

Data were expressed as the mean ± standard deviation obtained from three independent experiments, and each analysis was performed in triplicate. The data was subject to one-way analysis of variance (ANOVA)/Tukey's test at a confidence level of 95%. Additionally, a principal component analysis (PCA) was performed to describe the correlations between variables and estimate the relationship among starch treatments. All analyses were carried out with Statistica software (v. 12.5 Statsoft, Tulsa, OK, USA).

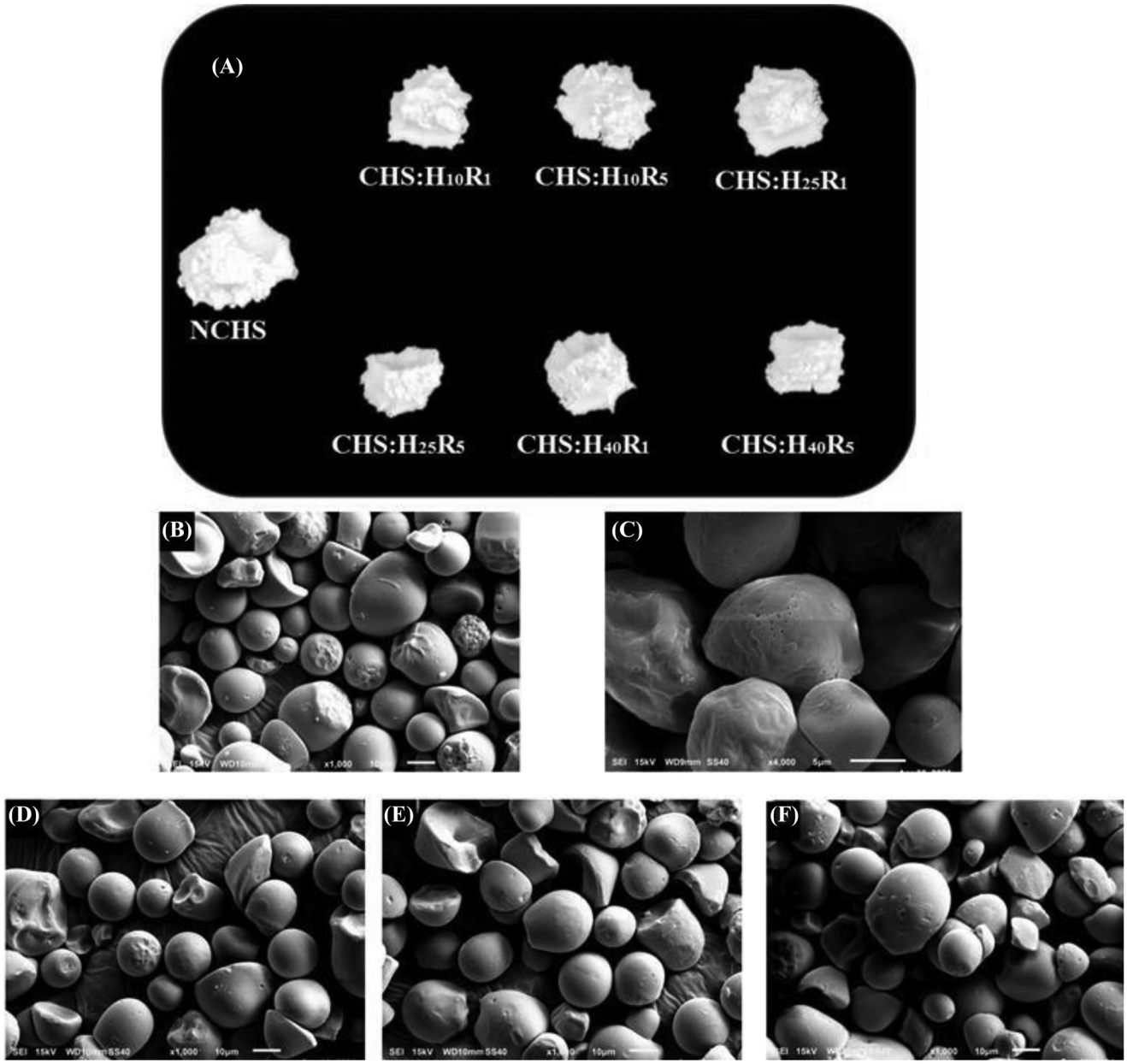

Figure 1. a–f) a) Photographs of the appearance of native and modified CHSs. b–f) Scanning electron micrographs of native chayotextle b), modified corn c), and modified CHSs: d) CHS:$H_{10}R_5$; e) CHS:$H_{25}R_5$; f) CHS:$H_{40}R_5$. For sample identification see Table 1.

3. Results and Discussion

3.1. Determination of Whiteness of Modified Starches

Figure 1a illustrates the visual appearance (whiteness) of native and modified CHS. In general, the native and modified starch samples showed similar whiteness. The whiteness values of CHS were 94.69 ± 0.17, and modified starches showed an interval from 95.93 ± 0.92 to 95.77 ± 0.86. There were no significant statistical differences ($p < 0.05$) between starches, which indicates that the chemical modification did not affect the degree of whiteness of CHS. It has been reported that native CHS exhibits a white-beige color, which may be affected by enzymatic reactions.[2] Furthermore, *Amorphophallus paeoniifolius* starch dually modified by oxidation-crosslinking exhibits a white color.[1]

3.2. Percent Hydroxypropylation (HPS) and Molar Substitution

Incorporating hydroxypropyl groups into the starch chains promotes changes in the physicochemical properties of starch.[25] The DS (% HPS) and MS of CHS and Polar-Tex are shown in **Table 2**. Both CHS:$H_{40}R_1$ (1.18% HPS) and CHS:$H_{40}R_5$ (1.19%

Table 2. Percent hydroxypropylation (%HPS) and molar substitution of dual modified CHS and Polar-Tex.

Treatment	Degree of substitution [%HPS]	Molar substitution
NCHS (C−)	0	0
CDMCS (C+)	2.97 ± 0.13^{e}	0.0856 ± 0.004^{e}
CHS:$H_{10}R_1$	0.34 ± 0.06^{a}	0.0097 ± 0.002^{a}
CHS:$H_{10}R_5$	0.29 ± 0.03^{a}	0.0082 ± 0.001^{a}
CHS:$H_{25}R_1$	0.79 ± 0.02^{c}	0.0223 ± 0.001^{c}
CHS:$H_{25}R_5$	0.64 ± 0.07^{b}	0.0180 ± 0.002^{b}
CHS:$H_{40}R_1$	1.18 ± 0.08^{d}	0.0335 ± 0.002^{d}
CHS:$H_{40}R_5$	1.19 ± 0.03^{d}	0.0337 ± 0.001^{d}

Values are the average of triplicate determinations from the different experiments ($n = 9$) ± standard deviation. Different letters in each column indicate significant differences between treatments ($p < 0.05$). For sample identification see Table 1. CDMCS, commercially dual modified corn starch (Polar-Tex); NCHS, native chayotextle starch.

HPS) exhibited the highest DS by hydroxypropylation; however, the effect on % HPS was dependent on the experimental conditions (from 0.29% to 1.19%). Similar behaviors were reported by Mehfooz et al.[15] in a hydroxypropylated di-starch phosphate from barley starch, finding that the increase in DS depends on the anhydrous sodium sulfate concentration added during the modification process. They found %HPS rose from 1.06% to 1.71% when the chemical reagent content increased from 8% to 12%. Moreover, the extent of %HPS can be presented as MS. The MS in CHS was influenced by the experimental conditions, where the highest values were obtained in CHS:$H_{40}R_1$ (0.0335) and CHS:$H_{40}R_5$ (0.037). Lawal[26] mentioned that the contact of starch molecules with an etherifying agent increased when the chemical reagent levels were higher, promoting a higher MS. This was demonstrated in *Cajanus cajan* starch, where MS increased from 0.06 to 0.17 when the propylene oxide content went from 10% to 40%, and in Canna starch (MS of 0.10 at 10% v/w of propylene oxide).[27] The results presented in this study are lower than those obtained in commercial, dual-modified corn starch (Polar-Tex) used as control (2.97%HPS and MS of 0.0856) and those reported in the literature for modified starch, which may be due to the starch source and experimental conditions.[25] Additionally, it has been reported that the effect of a starch modification process is influenced by the amylose:amylopectin ratio in the sample.[28] However, the modified CHS showed substitution values for hydroxypropyl groups within the ranges allowed by the Food and Drug Administration (FDA), an interval between 7% and 0.2% for MS.[26]

Additionally, DS and MS in some modified CHS were reduced by adding a crosslinking agent (see Table 2). A low concentration of hydroxypropylation reagent (10% and 25%) and higher levels crosslinking agent (5%) lead to a decrease in MS (0.0082 and 0.0180, respectively), but the opposite was observed when low (1%) crosslinking was used (0.0097 and 0.023, respectively). This is likely the result of both modifications taking place in starch glucose molecules and the same carbon atoms (C2, C3, and C6).[29]

3.3. FTIR Characterization of CHS

According to the data analysis from physicochemical and functional properties of native and dual-modified CHSs, CHS:$H_{10}R_5$, CHS:$H_{25}R_5$, and CHS:$H_{40}R_5$ treatments were selected for FTIR and SEM. The FTIR spectra of native and dual-modified CHS are shown in **Figure 2**. The main absorption peaks of CHS were detected around 3269, 2924, 1639, 1419, 1332, 1150, 1062, 995, and 925 cm^{-1} (Figure 2a). The broad band appearing around 3500–3000 cm^{-1} and centered at 3269 and 2924 cm^{-1} was ascribed to the hydrogen bonded O–H stretching vibrations of amylose and amylopectin and the C–H bond symmetric vibrations of glucose units.[1,30] At 3269 cm^{-1}, an increase in the intensity of transmittance values was observed in CHS:$H_{10}R_5$ (90.76%) and CHS:$H_{40}R_5$ (91.28%) against those detected in the native (87.97%) and CHS:$H_{25}R_5$ (87.60%) samples (Figure 2b). This increase may be associated with that in hydroxypropyl groups in the starch molecule during the substitution process.[4] Additionally, a shift displacement at 2928–2930 cm^{-1} and an increase in transmittance intensity from 93.05% to 94.81% were observed in all modified CHS compared to native starch (2924 cm^{-1} and 92% transmittance). The phenomenon is attributed to CH_2 deformation[31] due to the methyl bond formed by hydroxypropylation.[12] A displacement from 7 to 11 cm^{-1} in FTIR spectra of dual-modified starch by crosslinking-esterification was reported by Ren et al.[32] who mentioned that the crosslinking agent reacted with OH groups (at 3382 cm^{-1}) of starch chains.

The peak around 1639 cm^{-1} (Figure 2c) corresponded to adsorbed water (H–O–H bending vibrations), associated with the number of OH groups (scissor vibrations of –OH) in the starch structure, leading to water absorption.[1,22] Moreover, the intensity of transmittance in CHS:$H_{10}R_5$ (96.39%), CHS:$H_{25}R_5$ (96.66%), and CHS:$H_{40}R_5$ (95.26%) was increased when compared to that observed in native starch (94.37%). This effect was probably associated with a starch crystallinity alteration that resulted from amylose hydrolysis caused by the dual-modification. The latter was originated by broke hydrogen bonds, confirming the chemical modification of starch.[8,33] Furthermore, the signal around 1419 cm^{-1} was ascribed to the structural order of starch, while the peaks at 1332 and 1150 cm^{-1} corresponded to CH_2 bending and C–O–C twisting, CO, and C–C stretching of the glycosidic bonds.[9,31] Moreover, an increase in the intensity of transmittance was detected in all dual-modified CHS (CHS:$H_{10}R_5$ = 90.91%; CHS:$H_{25}R_5$ = 91.12%; and CHS:$H_{40}R_5$ = 89.12%) in the peak centered at 1150 cm^{-1} (Figure 2d), compared to native starch (87.87%). It has been reported that changes in this region (1150 cm^{-1}) corresponded to the stretching vibrations of P–O–C bond, indicating a phosphate crosslink effect.[8]

Peaks in the range from 1077 to 995 cm^{-1} (Figure 2d) are associated with the amorphous region (C–O–H, C–O, and C–O–C stretching) of starch molecules[34]; these bands are sensitive to changes in molecular starch structure.[6] An increase in all dual-modified CHS (CHS:$H_{10}R_5$ = 88.09%; CHS:$H_{25}R_5$ = 88.75%; and CHS:$H_{40}R_5$ = 86.24%) was detected in the peak centered at 1077 cm^{-1} (Figure 2d) compared to native starch (84.26%). According to Adhiyamaan and Parimalavalli,[7] any change

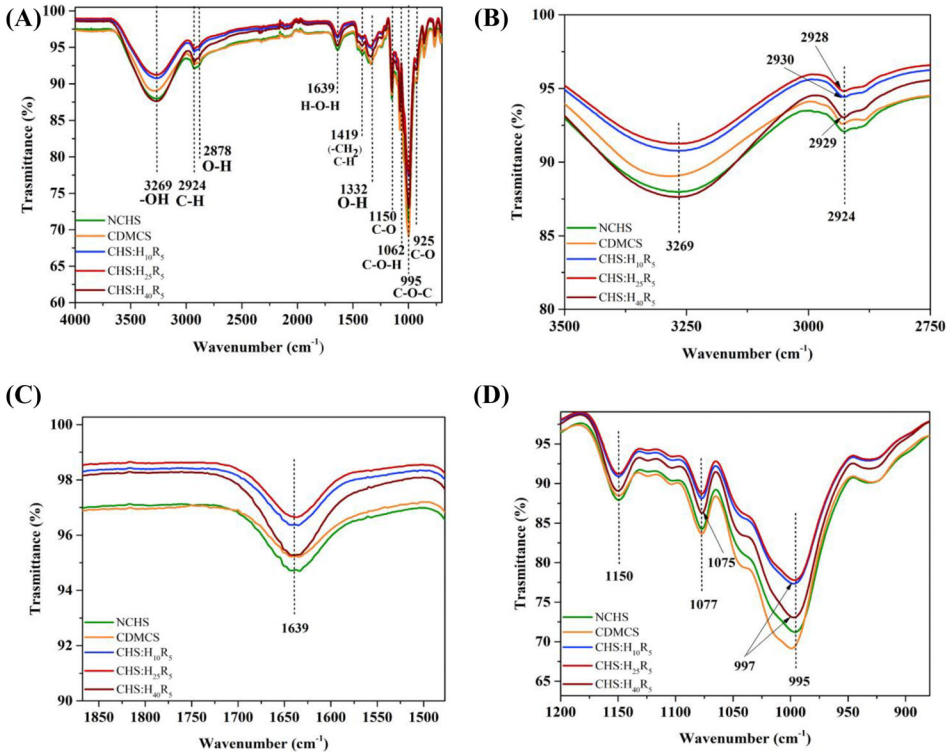

Figure 2. FTIR spectra of native and modified CHSs a) and their changes at 3500–2750 cm^{-1} b), 1639 cm^{-1} c), and 1200–900 cm^{-1} d). See Table 1 for sample identification.

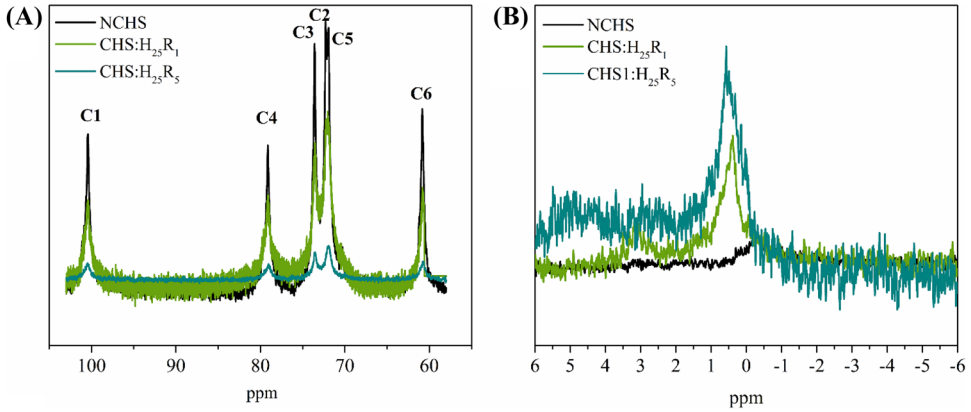

Figure 3. ^{13}C NMR a) and ^{31}P NMR b) spectra of native and modified chayotextle starches. For sample identification see Table 1.

in this region may indicate a decrease/increase in the starch crystallinity structure, associated with a modification process. The peak around 995 cm^{-1} was ascribed to the intramolecular hydrogen bonds of the α-glycosidic bond of the OH group at C-6,[35] and its band was sensitive to hydration.[6] Similar trends in the intensity of transmittance were observed in the peak centered at 995 cm^{-1} (CHS:$H_{10}R_5$ = 77.86%; CHS:$H_{25}R_5$ = 77.33%; CHS:$H_{40}R_5$ = 72.97%; and native starch = 71.12%), which results from bonding in carbohydrate helices due to a structural modification of starch molecules.[30] Furthermore, changes in the peaks around 1077 and 995 cm^{-1} suggested a P–O–C stretching bond by crosslinking effect.[1]

3.4. Nuclear Magnetic Resonance (NMR) Studies

^{13}C NMR is an analytical technique that provides information about the structural identification of diverse materials, including starch.[36] **Figure 3**a shows the ^{13}C NMR spectra of native and modified CHSs (CHS:$H_{25}R_1$ and CHS:$H_{25}R_5$). The native CHS NMR spectra exhibited six characteristic peaks at 100.4, 79.1, 73.5, 72.2, 71.9, and 60.8 ppm, corresponding to C1, C4, C3, C2, C5, and C6 of the anhydrous glucose unit, respectively.[37] Regarding modified CHS starch, the intensity of all signals decreased as their width and degree of crosslinking increased because of the cross-linking that modified the interaction

Table 3. Effect of temperature on swelling power and solubility index of native and dual-modified CHSs.

Treatment	Swelling power [g g^{-1}]				Solubility index [%]			
	30 °C	50 °C	70 °C	90 °C	30 °C	50 °C	70 °C	90 °C
NCHS (C−)	$2.53 \pm 0.21^{a,X}$	$2.78 \pm 0.07^{a,X}$	$7.20 \pm 0.36^{a,Y}$	$7.32 \pm 0.08^{a,Y}$	$0.16 \pm 0.36^{a,X}$	$0.46 \pm 0.25^{a,X}$	$15.24 \pm 0.94^{c,Y}$	$45.63 \pm 0.46^{e,Z}$
CDMCS (C+)	$2.50 \pm 0.05^{a,X}$	$3.13 \pm 0.07^{bc,Y}$	$12.61 \pm 0.05^{b,Z}$	$12.68 \pm 0.14^{b,Z}$	$0.61 \pm 0.02^{f,X}$	$0.68 \pm 0.98^{b,X}$	$0.89 \pm 0.41^{a,Y}$	$0.99 \pm 0.89^{a,Y}$
CHS:$H_{10}R_1$	$2.96 \pm 0.07^{b,X}$	$3.09 \pm 0.16^{abc,X}$	$17.45 \pm 0.76^{e,Y}$	$26.58 \pm 0.56^{f,Z}$	$0.40 \pm 0.01^{de,X}$	$0.39 \pm 0.24^{a,X}$	$2.60 \pm 0.56^{b,Y}$	$5.09 \pm 0.86^{c,Z}$
CHS:$H_{10}R_5$	$2.77 \pm 0.13^{b,X}$	$2.81 \pm 0.11^{ab,X}$	$15.28 \pm 0.44^{d,Y}$	$19.81 \pm 0.28^{e,Z}$	$0.38 \pm 0.10^{cd,X}$	$0.40 \pm 0.13^{a,X}$	$2.16 \pm 0.22^{b,Y}$	$3.67 \pm 0.89^{b,Z}$
CHS:$H_{25}R_1$	$3.76 \pm 0.16^{c,X}$	$3.83 \pm 0.38^{d,X}$	$20.37 \pm 0.34^{g,Y}$	$27.68 \pm 0.10^{g,Z}$	$0.46 \pm 0.23^{de,X}$	$0.50 \pm 0.14^{a,X}$	$2.97 \pm 0.20^{b,Y}$	$6.28 \pm 0.56^{d,Z}$
CHS:$H_{25}R_5$	$2.83 \pm 0.11^{b,X}$	$3.35 \pm 0.18^{c,Y}$	$17.73 \pm 0.22^{e,Z}$	$17.84 \pm 0.47^{d,Z}$	$0.25 \pm 0.11^{b,X}$	$0.39 \pm 0.24^{a,X}$	$2.17 \pm 0.12^{b,Y}$	$4.50 \pm 0.36^{c,Z}$
CHS:$H_{40}R_1$	$2.96 \pm 0.08^{b,X}$	$3.09 \pm 0.08^{abc,X}$	$19.36 \pm 0.30^{f,Y}$	$26.16 \pm 0.23^{f,Z}$	$0.31 \pm 0.03^{bc,X}$	$0.40 \pm 0.26^{a,X}$	$3.29 \pm 0.15^{b,Y}$	$5.90 \pm 0.10^{d,Z}$
CHS:$H_{40}R_5$	$2.89 \pm 0.11^{b,X}$	$3.19 \pm 0.16^{c,Y}$	$14.39 \pm 0.35^{c,Z}$	$16.80 \pm 0.46^{c,Z}$	$0.46 \pm 0.45^{e,X}$	$0.49 \pm 0.22^{a,X}$	$3.16 \pm 0.17^{b,Y}$	$4.66 \pm 0.28^{c,Z}$

Values are the average of triplicate determinations from the different experiments ($n = 9$) ± standard deviation. Lowercase letters in each column indicate significant differences between treatments ($p < 0.05$). Capital letters in each row indicate significant differences between temperature of each treatment ($p < 0.05$). For sample identification see Table 1. CDMCS, commercially dual modified corn starch (Polar-Tex); NCHS, native chayotextle starch.

between starch and solvent, reducing starch solubility.[36] On the other hand, ^{31}P NMR studies identified the product of the crosslinking reaction.[38]Studies in ^{31}P NMR were performed in native and modified CHSs (CHS:$H_{25}R_1$ and CHS:$H_{25}R_5$), as shown in Figure 3b. In general, the intensity of signals between δ 0 and 1 ppm of modified starches were higher than in the native sample, associated with the presence of mono-di-starch mono-phosphate promoted by a higher degree of crosslinking in the sample. During crosslinking modification, TMFS can interact with starch to form different phosphate molecules. Firstly, the TMFS ring opens to form tripolyphosphate, which interacts with starch to form mono-starch triphosphate that, in turn, can give rise to mono-di-starch mono-phosphate or mono-mono-starch mono-phosphate.[39]

3.5. SEM of Modified Starches

SEM analysis has been widely used to identify structural changes in starch granules by single or dual modifications.[30] Figure 1b–f show the SEM images of native and dual-modified CHS and commercial dual-modified corn starch. The morphology of native CHS (Figure 1b) was spherical and oval, according to Jiménez-Hernández et al.[20] It should be noted that the presence of truncated granules in CHS was minimal, indicating that the extraction process was adequate, and the granules were not damaged during this process. Moreover, the commercial dual-modified corn starch (Figure 1c) showed polyhedral shapes in accordance with reported corn starch morphologies.[40] Regarding dual-modified CHS granules (Figure 1d–f), they did not suffer changes in the external structure, independently of the experimental conditions evaluated. It has been reported that chemical modifications in single or dual approaches with low DS does not cause detectable changes in starch granules from various starch sources.[30,32,34,41]

3.6. Swelling Power

Table 3 shows the SP of the native and dual-modified CHS in an interval of temperature from 30 to 90 °C. The SP tends to increase at a higher temperature ($p < 0.05$). Moreover, at all temperatures, the SP of dual-modified CHS was higher than those of native starch and commercial dual-modified corn starch ($p < 0.05$). The highest SP values were observed in dual-modified CHS at 90 °C, at an interval of 16.80–27.68 g g^{-1}. This behavior was dependent on the DS by hydroxypropylation and crosslinking in CHS, and it has been previously reported in various dual-modified starches, such as barley by hydroxypropylated-distarch phosphate, elephant foot yam by oxidation-crosslinking, achira by acid hydrolysis-succination, and banana by crosslinking-microwave.[1,8,9,15] Therefore, the increase in temperature promoted water absorption mainly in the amorphous region of starch molecules, related to the intramolecular weakening of hydrogen bonds, promoting the swelling of starch granules.[26,31]

Regarding dual-modified CHS, similar SP values (26.58, 27.68, and 26.16 g g^{-1}) were observed at 90 °C when they were modified using 1% crosslinking agent, regardless of the increase in the DS, showing values of 0.34, 0.79, and 1.18 for CHS:$H_{10}R_1$, CHS:$H_{25}R_1$, and CHS:$H_{40}R_1$, respectively. Conversely, a decrease in SP values (19.81, 17.84, and 16.80 g g^{-1}) was observed when using a higher content of crosslinking agent (5%), compared to those obtained using 1% of crosslinking agent. Similar trends were reported in dual-modified banana starch by microwave irradiation-crosslinking.[8] It has been found that a higher crosslinking in the starch chain causes increased resistance of the starch granules, leading to a fall in the SP capacity of the modified starch. The latter is attributed to the formation of additional covalent bonds via phosphate groups, hampering granule swelling.[1,8] According to Dey and Sit,[42] when more than one type of starch modification is applied, a disruption of the starch molecule can occur, reducing the water binding capacity and resulting in reduced SP.[1]

3.7. Solubility Index

The SI of the native and dual-modified CHS in the temperature range 30–90 °C is shown in Table 3. The SI tended to increase along with temperature ($p < 0.05$), associated with a structural weakening in starch granules and possibly amylose

Table 4. Pasting and thermal properties of native and dual-modified CHSs.

Treatment	Pasting properties [Pa s]				Thermal properties			
	PV	BV	FV	SV	T_i °C	T_p °C	T_f °C	ΔH J g^{-1}
NCHS (C−)	13.3 ± 0.08^{a}	10.2 ± 0.08^{a}	4.2 ± 0.04^{a}	1.1 ± 0.03^{a}	62.9 ± 0.18^{d}	65.8 ± 0.15^{d}	71.6 ± 0.09^{c}	11.3 ± 0.19^{b}
CDMCS (C+)	11.9 ± 0.12^{a}	8.7 ± 0.54^{a}	12.3 ± 0.01^{ab}	9.1 ± 0.42^{ab}	64.6 ± 0.08^{e}	68.5 ± 0.12^{e}	74.0 ± 0.57^{d}	8.0 ± 1.55^{a}
CHS:$H_{10}R_1$	16.4 ± 0.16^{b}	12.8 ± 0.58^{ab}	10.5 ± 0.12^{ab}	6.9 ± 0.29^{ab}	62.2 ± 0.24^{cd}	64.9 ± 0.25^{c}	70.8 ± 0.87^{bc}	11.3 ± 1.46^{b}
CHS:$H_{10}R_5$	23.1 ± 0.39^{d}	17.7 ± 0.56^{cd}	10.5 ± 0.78^{ab}	5.1 ± 0.62^{ab}	61.7 ± 0.20^{bc}	64.5 ± 0.29^{bc}	70.7 ± 0.13^{bc}	11.2 ± 0.39^{b}
CHS:$H_{25}R_1$	16.8 ± 0.19^{b}	12.5 ± 1.69^{ab}	10.1 ± 1.00^{ab}	5.9 ± 2.51^{ab}	61.8 ± 0.11^{bc}	64.4 ± 0.07^{b}	70.1 ± 0.30^{ab}	10.4 ± 1.12^{ab}
CHS:$H_{25}R_5$	25.3 ± 0.95^{e}	20.5 ± 2.11^{d}	16.7 ± 0.14^{b}	12.0 ± 1.17^{b}	60.8 ± 0.07^{a}	63.5 ± 0.12^{a}	69.3 ± 0.55^{a}	9.8 ± 1.70^{ab}
CHS:$H_{40}R_1$	20.2 ± 0.01^{c}	15.5 ± 0.86^{bc}	9.3 ± 2.81^{ab}	4.6 ± 1.97^{ab}	61.6 ± 0.41^{bc}	63.8 ± 0.31^{a}	69.7 ± 0.88^{ab}	11.5 ± 0.82^{b}
CHS:$H_{40}R_5$	20.5 ± 0.24^{c}	16.1 ± 0.71^{bc}	15.1 ± 5.35^{b}	10.7 ± 5.82^{b}	61.1 ± 0.70^{ab}	63.5 ± 0.08^{a}	69.8 ± 0.03^{ab}	10.9 ± 0.36^{b}

Values are the average of triplicate determinations from the different experiments ($n = 9$) ± standard deviation. Different letters in each column indicate significant differences between treatments ($p < 0.05$). For sample identification see Table 1. T_i: initial temperature, T_p: peak temperature, T_f: final temperature, ΔH: enthalpy of gelatinization. BV, breakdown viscosity; CDMCS, commercially dual modified corn starch (Polar-Tex); FV, final viscosity; NCHS, native chayotextle starch; PV, peak viscosity; SV, setback viscosity.

leaching from starch chains.[1] Moreover, at higher temperatures of 70 and 90 °C, the SI of dual-modified CHS, from 2.16% to 6.28%, was lower than that of native starch, showing values from 15.24% to 45.63% that were higher when compared against those of commercial dual-modified corn starch (0.89%–0.99%).[1,31] It has been reported that CHS gelatinization starts approximately at 69 °C.[20] For this reason, the lowest solubility of native and modified starches has been observed at 30 and 50 °C.[17] On the other hand, it is commonly reported that modified starch (single or dual-modified) exhibits higher solubility than native starch,[1,15,17] mainly in hydroxypropylated modified starches, due to the introduction of hydrophilic groups (hydroxypropyl groups) into the starch molecule by breaking hydrogen bonds.[1,15,26,43] In some cases, the solubility of the modified starches is lower than that of the native starches due to the cross-linking reaction,[15] as reported in this study. Additionally, the solubility of the modified CHS was reduced ($p < 0.05$) with the increasing crosslinking agent content (from 1% to 5%), independently of temperature and hydroxypropylated reagent content.[44] Surendra Babu et al.[8] reported a solubility of 15.56% in native banana starch, higher than in the microwave-crosslinking dual-modified starch from 8.33% to 13.34% at 90 °C, regardless of the experimental conditions. Similar trends have been reported in crosslinked maize starch.[22] These results show that the crosslinking strengthened the structure of starch molecules, preventing the disintegration of the internal starch structure and amylose depolymerization. It also enhanced the bonding between starch chains and helped retain the integrity of starch granules, which is attributed to the formation of covalent bonds by introducing phosphate groups.[8,22] Additionally, there was no increase in the SI of modified foxtail millet starch by combining physical and chemical methods when compared to native starch.[42]

3.8. Pasting Properties

The pasting properties of peak viscosity (PV), breakdown viscosity (BV), final viscosity (FV), and setback viscosity (SV) of native and dual-modified CHS are shown in **Table 4**. The PV of native CHS showed values of 13.3 Pa s, lower than previously reported (14.75 Pa s).[20] Furthermore, modified CHS exhibited a significant increase in PV values between 16.4 and 25.3 Pa s, compared to native starch. Similar trends have been reported in low-substituted hydroxypropylated canna starch[27] and Carioca bean starch modified by acetylation-hydroxypropylation.[10] This increase is related to that in water absorption capacity of starch molecules when hydroxypropyl groups are introduced; however, this effect depends on the substitution level.[27] Furthermore, it must be noted that the CHS modified with a DS of 0.34 and 0.79 (see Table 2), and 1% crosslinking agent exhibited lower PV values (16.4 and 16.8 Pa s) than those with 5% of crosslinking agent content (23.1 and 25.3 Pa s). Nevertheless, no differences were observed in the PV values of the modified CHS when the DS by hydroxypropylation reached percentages of 1.18 and 1.19, independently of the crosslinking agent content (1% = 20.2 Pa s; 5% = 20.5 Pa s). The crosslinked starch chains bonded, increasing their mechanical strength, keeping the swollen granules intact, and preventing viscosity loss.[8] In this context, lower PV has been reported in crosslinked starches when the crosslinking degree is higher but the crosslinking agent content is not necessarily increased.[34] Mehfooz et al.[15] reported that PV of hydroxypropylated-distarch phosphate of barley starch is similar to that of native starch, associated with a balanced of both modification methods.

Additionally, the dual-modified CHS showed increases in BV from 12.8 to 20.5 Pa s, FV from 9.3 to 16.7 Pa s, and SV from 4.6 to 12 Pa s, in comparison to the same values in native CHS of 10.2, 4.2, and 1.1 Pa s, respectively. However, these effects depended on the experimental conditions, where the highest BV, FV, and SV values were obtained in CHS:$H_{25}R_5$. Lawal[26] found an increase in BV in hydroxypropylated pigeon pea starch, suggesting a possible structural reorganization in the amorphous component and crystalline region of starch molecules. An increase in this parameter indicates that CHS shows higher water retention capacity. Surendra Babu et al.[8] reported an increase in FV in dual-modified foxtail millet starch compared to native starch, and they observed that increased FV was the result of starch chain reorientation, increasing the bonded forces. Moreover, there are reports on an increase in

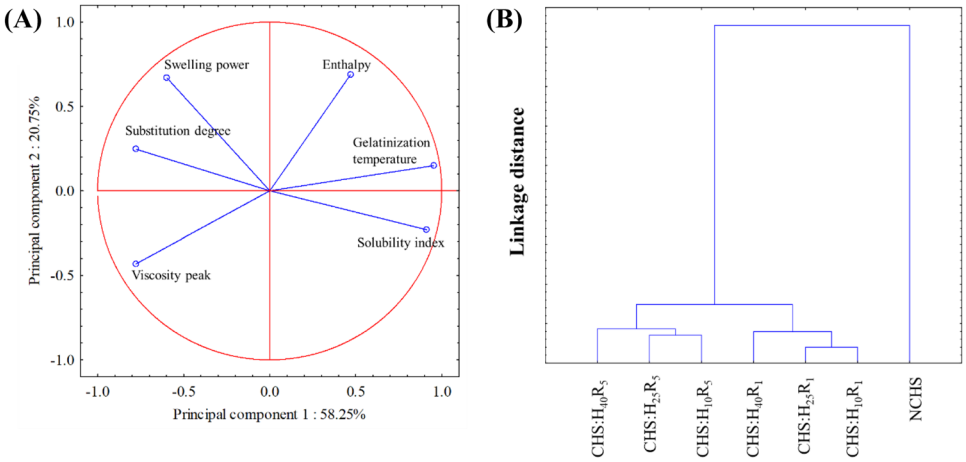

Figure 4. Principal component plot location of different physicochemical and thermal properties a) and hierarchical cluster b) of CHS treatments. See Table 1 for sample identification.

setback parameters in dual-modified achira starch compared to their native part; this phenomenon is associated to the incorporation of new functional groups to the starch structure, which may promote granular integrity and prevent retrogradation due to crosslinking.[9,15] According to these data, dual-modified CHS can resist the breakage of starch chains associated to mechanical stress.[31]

3.9. Thermal Properties

Differential scanning calorimetry (DSC) studies were used to investigate the effect of hydroxypropylation-crosslinking effect on CHS. The thermal properties (T_0, T_p, and T_c) and ΔH of native and dual-modified CHS are shown in Table 4. Native CHS showed T_0 of 62.9°C, T_p of 65.8 °C, and T_c of 71.6 °C, similar to those reported in the literature.[20] On the other hand, all modified CHS exhibited a decreasing pattern ($p < 0.05$) in all temperatures: T_0 from 60.8 to 62.2 °C), T_p from 63.5 to 64.9 °C, and T_c from 69.3 to 70.8 °C against those observed in native starch and dual-modified commercial starch of 64.6, 68.5, and 74 °C, respectively. The pattern was more evident as the degree of hydroxypropylation (substitution) increased.[26] These behaviors align with those reported by Mehfooz et al.[15] who found a decrease in the same thermal parameters in dual-modified barley starch under similar experimental conditions of starch modification. Moreover, similar trends were reported in low-substituted hydroxypropylated canna starch.[27] Additionally, López et al.[40] reported a decrease in the gelatinization temperature and enthalpy in modified corn starch under conditions similar to those proposed in this study. This phenomenon is mainly attributed to starch modification by hydroxypropylation substitution, since the crosslinking process is known to increase the gelatinization temperature and enthalpy of the starch molecules. This behavior is due to the inclusion of hydroxypropyl groups which promotes the breaking of inter- and intramolecular bonds in the starch chains (decrease in hydroxyl groups), leading to water percolation into the starch granules and causing changes in the internal starch granular structure.[1] Singh et al.[29] mentioned that the decrease in enthalpy might indicate the breakdown of the double helix in the amorphous region of starch structure, promoting the melting of starch granules.[9]

3.10. Principal Component Analysis

PCA and hierarchical cluster (HC) analysis are advanced statistical tools used for material characterization (**Figure 4**). These tools have been used to study the structural order of native starch granules[45] and evaluate the effect of ultrasound-assisted extraction on morphological and functional properties of yam starch.[46] They have also been applied to evaluate the effect of dual modification on structural and pasting properties of taro starch.[41] PCA has been used to overview the interrelationship between physicochemical and functional properties of starch due to dual-modification by hydroxypropylation-crosslinking. The first two components (PC1 and PC2) explained an accumulative variance of 58.25% (PC1) and 20.75% (PC2). The physicochemical and functional properties of CHS are given in Figure 4a, and the graph location of native and dual-modified starches is shown in Figure 4b. The loading plot in Figure 4a shows the score values of all starch physicochemical and functional properties (projected in PC1 and PC2 planes, from negative to positive), where the DS (−0.775), PV (−0.777), and SP (−0.597) influence SI (0.912) and gelatinization temperature (0.952) of CHS. Additionally, there is a differentiation between native and modified starch samples (Figure 3b). Three clusters are observed: cluster 1 is located in native CHS, cluster 2 is composed of those starch samples with low crosslinking agent content (CHS:$H_{10}R_1$, CHS:$H_{25}R_1$, and CHS:$H_{40}R_1$), and cluster 3 is characterized by containing starch samples with high crosslinking agent content (CHS:$H_{10}R_5$, CHS:$H_{25}R_5$, and CHS:$H_{40}R_5$). These results suggest that dual-modified starches are different from their native counterpart.[46] According to the literature, viscosity and SP increase as DS is higher because the hydroxypropyl group inserted in the starch chains has an acute affinity for water. On the other hand, changes in gelatinization temperature are associated with the hydroxypropyl group being more voluminous than the

hydroxyl group, which causes the breakdown of the inter- and intramolecular bonds of the starch chain.[15]

4. Conclusions

Native CHS was successfully modified through substitution and crosslinking. The modified CHSs show improvements in functional, thermal, and molecular properties compared to native starch. Functional properties of modified samples, such as viscosity and SP, increased proportionally to the degree of modification. Contrastingly, the SI, enthalpy, and temperature of gelatinization were reduced as the level of modification increased. Nonetheless, the chemical modification did not alter the morphology of the starch granules. FTIR and NMR studies confirmed the structural modifications of CHS by hydroxypropylation-crosslinking. Modified CHS showed a two-fold increase in sticking properties than commercially dual modified corn starch (CDMCS, Polar-Tex). The information obtained in this study indicates that modified CHS could potentially be used as a food additive and a thickening agent in a food matrix.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Reasearch data are not shared.

Keywords

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