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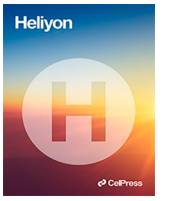

Hydrothermal processes and simultaneous enzymatic hydrolysis in the production of modified cassava starches with porous-surfaces

Figueroa-Flórez Jorge [a,*], Cadena-Chamorro Edith [b], Rodríguez-Sandoval Eduardo [b], Salcedo-Mendoza Jairo [c], Ciro-Velásquez Héctor [b]

[a] Universidad Nacional de Colombia, Facultad de Ciencias, Medellín, Colombia
[b] Universidad Nacional de Colombia, Facultad de Ciencias Agrarias, Medellín, Colombia
[c] Universidad de Sucre, Facultad de Ingeniería, Sincelejo, Colombia

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ABSTRACT

The amylolytic action of α-amylase and amyloglucosidase has been directly implemented in native cassava starches for the formation of cassava microporous granules with unsatisfactory results, however, its incidence in hydrothermally treated granules has never been evaluated. The effect of hydrothermal processes and simultaneous enzymatic hydrolysis on the physicochemical, morphological and structural properties of native cassava starch was evaluated. Native cassava starch presented a rigid, smooth surface, and was exempt from porosities, whereas hydrothermal processes altered the semicrystalline order and increasing the size and number of pores and increasing the size (4.11 ± 0.09 nm) and volume of pores (0.82 ± 0.00 $cm^3/g \times 10^{-3}$). The hydrothermal action followed by enzymatic processes with α-amylase and amyloglucosidase, augmented the processes of internal degradation (endo-erosion) and pore widening (exo-erosion), improving the hydrophilic properties compared to the hydrothermal treatment. Likewise, the hydrothermally process followed by enzymatic hydrolysis for 24 h (HPS + EMS-24) increased the degradation of the amorphous lamellae, consistent with a significant decrease in amylose content. This same dual treatment increased the pore size at 17.68 ± 0.13 nm relative to the native counterpart; therefore, they are considered an effective method in the development of modified cassava starches with porous surfaces.

1. Introduction

Starch is a homopolymer, where α-D-glucopyranose monomer be able to link by α-D-(1,4) and α-D-(1,6) glycosidic bonds to form two polymeric structures: amylose and amylopectin [1]. These linear or branched structures can be packed into a birefringent semi-crystalline complex called granules. The native cassava granules have a lower price in the world market compared with starches from other sources, besides, it deforms under moderate shear stress improving its plasticizing capacity, favoring the formation of tablets during the compression process and its potential use as a binding material [2]. However, native cassava starch has a smooth external surfaces and small pore volumes, which limit their application as encapsulating materials or bioactive substance transporters [3]. To improve the properties of thermo- or photosensitive substances as carrier vehicles for micronutrients or encapsulating materials, it is necessary to increase the adsorption properties through modification processes that promote the development of structures with

* Corresponding author.
E-mail address: joafigueroafl@unal.edu.co (F.-F. Jorge).

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excellent porous characteristics [4].

There are many methods to produce porous starches, which involve physical, chemical, and enzymatic treatments, or a combination thereof. Enzymatic modification is a safe process free of chemical agents, which involves the polymeric degradation of amylose and amylopectin, promoting significant changes both structurally and morphologically [5]. Enzymes with amylolytic activity, such as α-amylase and amyloglucosidase, have been widely used in the development of porous starches from corn, rice, and wheat [4–13]. Likewise, technologies that involve physical processes such as hydrothermal, ultrasound or freeze-thaw treatments have been implemented to increase porosity in number and volume in cereal granules [9,14–19]. However, pore formation has not been significant in tuber starches. The difference is due to intrinsic factors such as granular size, intrinsic pores or cavities, amylose/amylopectin ratio, and semicrystalline packaging [4,20]. Tuber starches with type-A polymorphisms have been reported to have a very dense packing close to the granular surface, which confers molecular resistance to enzymatic hydrolysis [20]. Furthermore, the absence of pores in the native structure seems to limit the enzymatic adsorption process and the consequent biocatalytic action of α-amylase or amyloglucosidase, responsible for the phenomena of internal degradation (endo-erosion) and pore widening (exo-erosion), respectively [4].

Some physical-thermal mechanisms have been studied to promote the enzymatic hydrolysis efficiency in native cassava starches. Researchers evaluated the enzymatic susceptibility of native cassava granules pretreated by annealing under mild conditions very distant from the peak gelatinization temperature [21,22]. The results showed that annealing causes a granular swelling that could promote enzymatic biocatalysis processes. However, the effect of hydrothermal pretreatments closest to gelatinization temperature with citric acid followed by simultaneous enzymatic activity with amylolytic enzymes to obtain microporous cassava starches is unknown. Besides, there are still no reports in the literature about the characteristics of the pores in modified cassava starches in terms of type, size and pore volume. Likewise, previous studies have identified the presence of exo-erosions or small porosities in enzymatically modified cassava granules [23]. In this sense, we hypothesize that hydrothermal process closest to gelatinization temperature with citric acid favor biocatalytic action, altering the granular structure and formation of porous-surfaces on the native cassava starch. This study aimed to evaluate the individual and combined action of hydrothermal processes and enzymatic hydrolysis on the physicochemical, morphological, and structural properties of modified cassava granules with porous-surfaces.

2. Materials and methods

2.1. Materials

Native cassava starch (*Manihot esculenta* cv. M-Tai) supplied by Almidones de Sucre S. A (Induyuca®, Sincelejo, Colombia), amyloglucosidase from *Aspergillus niger* (270 AGU/g, 300 KNU/g, Dextrozyme® GA, Novozymes, Denmark) and α-amylase from *Bacillus licheniformis* (Liquozyme® Supra 2.2X, Novozymes, Denmark) were used.

2.2. Modification process

2.2.1. Hydrothermal pre-treated

Native starch slurry with a water content of 65% w/v was hydrothermally modified, heating in a suspension of citric acid at 0.1 M (pH 4.5) at 60 °C for 24 h under continuous stirring at 200 rpm (Thermo Scientific, MaxQ 4450, USA), followed by cooling to −4.0 °C for 2 h [1,24]. Then, the suspensions were centrifuged at 8200×*g* for 5 min, and the supernatants were decanted. The modified granules were dried to a moisture content between 10 and 11% w/w and subsequently were macerated.

2.2.2. Enzyme modification

The hydrothermally pretreated granules (HPS) were subsequently modified by simultaneous enzymatic hydrolysis processes (EMS). Suspensions at 20% w/v of HPS granules were heated at 60 °C in citric acid buffer (pH = 4.5, 0.1 M), then α-amylase (25 U/g starch) and amyloglucosidase (20 U/g starch) were added simultaneously, the system was kept under constant agitation at 200 rpm for hydrolysis time of 6, 12 and 24 h. Then, the hydrolysates were removed by centrifugation at 8200×*g* for 5 min. The samples were washed with ethanol and distilled water to inactivate residual enzymatic activity. Finally, the starch was dried to a moisture content between 10 and 11% w/w (UFB500, Memmert, Germany). The doubly modified starches were categorized as (HPS + EMS). The doubly modified granules were named HPS + EMS-6, HPS + EMS-12 and HPS + EMS-24, taking into account the hydrolysis time.

Native cassava starch (NCS) was established as a control. Hydrothermal pre-treated starches without hydrolysis (HPS) and enzymatically modified starches without pretreatment (EMS-24) were established as single processes in granular modification. In addition, an attempt was made to simulate the hydrothermal process under conditions of pH 4.5, to be used as a control treatment of the enzymatic treatment (EMS-24).

2.3. Amylose content

The amylose content was determined using the amylose/amylopectin assay kit (Megazyme, Wicklow, Ireland) by the precipitation of amylopectin chains with Concavanalin A.

2.4. Infrared spectroscopy (FTIR-ATR)

Infrared spectra were obtained in the region of 500–4000 cm^{-1} by taking 32 readings at a resolution of 4 cm^{-1} using a spectrometer with a diamond crystal of 1.5 mm in diameter (UATR, PerkinElmer, USA). The ratio of absorbances in bands 1047/1022 cm^{-1} and 925/1022 cm^{-1} was determined to assess the degree of molecular order [25,26].

2.5. Water solubility index and water adsorption index

The water solubility index (WSI) and the water adsorption index (WAI) were determined by adapting the method proposed by Salcedo-Mendoza et al. [23].

2.6. Morphology of the starch

The starch granules were fixed in a sample holder with electroconductive carbon tape and covered with a platinum/gold alloy [27]. The samples were analyzed by scanning electron microscopy (SEM) (JEOL, JSU-5600 LV, Japan) under set conditions at 15 kV, 30 mA, and an amplitude margin of 3000X.

2.7. Degree of crystallinity

Diffraction patterns were obtained with an X-ray diffractometer (X'Pert Pro-MPD, Panalytical, Italy) in the region of 4–40°. The equipment was operated at 1.8 kW, with a current of 40 mA. The degree of crystallinity (DC) was determined as a function of the ratio between the crystalline peaks and the total area, taking as a reference a baseline between the angles $2\theta = 5–35°$. The data were processed using Origin Lab software (OriginLab Corp., USA) [28].

2.8. Determination of particle size

Particle size was determined by light scattering using a Mastersizer Particle Analyzer (Model 3000E, Malvern, UK). Measurements were made in triplicate at room temperature using a refractive index value of 1.53 for starch [29]. The size of the particles was expressed as a function of the mean diameters d-(0.1), d-(0.5) and d-(0.9), where d-(0.9) represents the granular size up to 90% of the total volume of the particles, while d-(0.5) and d-(0.1) correspond to sizes below 50% and 10% of the particle distribution, respectively.

2.9. Specific surface area, diameter and pore volume

Pore characteristics were determined using an automatic specific surface area and porosity analyzer (Quantachrome Inc., NOVA 2000e, USA). Pure nitrogen (>99.99%) was used to determine adsorption-desorption isotherms. The starch samples were dried for 20 h under vacuum at 100 °C, degassed at 125 °C for 24 h and immersed in liquid nitrogen (−196 °C) in the range of relative pressure P/Po ≈ 5–19% [29]. The mean pore diameter and the specific surface area were calculated from the BET method (Brunauer--Emmett-Teller). The total pore volume was estimated with the BJH method (Barrett-Joyner-Halenda) [29].

2.10. Gelatinization properties

Thermal properties were determined using a differential scanning calorimeter (DSC-Q200, TA Instruments, USA) with a starch suspension under the ratio of 2 mg of starch to 6 mg of water in aluminum capsules [9]. The capsules were sealed and stored at 25 °C for 24 h to balance the system. Subsequently, they were heated from 20 to 120 °C at a speed of 10 °C/min. The onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c) were determined from the thermogram. The gelatinization enthalpy (ΔH) was estimated as a function of the endothermic peak area expressed in J/g.

Table 1
Functional properties in native and modified cassava starches.

Treatment	Amylose (%)	WSI (g/g)	WAI (g/g)	DC (%)	$^1OD_1$	$^2OD_2$
NCS	19.55 ± 0.16^{a}	1.27 ± 0.07^{a}	3.30 ± 0.13^{a}	47.30 ± 0.10^{a}	0.93 ± 0.01^{a}	0.49 ± 0.01^{a}
HPS	18.83 ± 0.21^{b}	2.17 ± 0.08^{b}	3.84 ± 0.16^{b}	48.76 ± 0.44^{b}	0.95 ± 0.01^{a}	0.50 ± 0.02^{a}
EMS-24	18.16 ± 0.19^{c}	3.50 ± 0.12^{c}	4.36 ± 0.11^{c}	50.66 ± 0.15^{c}	0.95 ± 0.00^{a}	0.55 ± 0.02^{ab}
HPS + EMS-6	18.29 ± 0.12^{c}	4.59 ± 0.20^{d}	6.77 ± 0.08^{d}	51.03 ± 0.31^{c}	0.98 ± 0.02^{b}	$0.57 \pm 0.00_{ab}$
HPS + EMS-12	17.64 ± 0.16^{d}	4.84 ± 0.19^{de}	6.41 ± 0.07^{e}	52.10 ± 0.17^{d}	0.99 ± 0.02^{b}	0.59 ± 0.00^{bc}
HPS + EMS-24	17.84 ± 0.10^{d}	4.90 ± 0.25^{e}	7.12 ± 0.23^{f}	51.54 ± 0.28^{e}	0.98 ± 0.00^{b}	0.63 ± 0.02^{c}

NCS: native cassava starch; HPS: hydrothermally pretreated starch; EMS-24: enzyme-modified starch; HPS + EMS-(6, 12, 24) dually modified starch. $^1DC_1$ degree of crystallinity determined by X-ray diffraction; ^{2} OD_1 corresponds to the relationship 1047/1022 cm^{-1}; $^3OD_2$ corresponds to the relationship 925/1022 cm^{-1}. Values are shown as mean ± standard deviation. Values followed by different letters within a column denote significant differences ($p < 0.05$).

2.11. Statistic analysis

All experiments and analyses were performed in triplicate at a minimum and reported as mean values ± standard deviations. In addition, results were analyzed using statistical tools such as variance analysis (ANOVA) and the Tukey test for comparison of means with a significance level of 5% using Statgraphics Software (XVI, Statgraphics Inc., USA.).

3. Results and discussion

3.1. Amylose content and physicochemical properties

The single and dual modification processes significantly affected the amylose content ($p < 0.05$) (Table 1). The decrease in amylose content after hydrothermal treatment may be associated with amylose leaching out [30]. The enzymatic action on the amorphous zones could cause the breakdown of α-(1,4) glycosidic bonds, inducing polymeric degradation that resulted in the loss of amylose

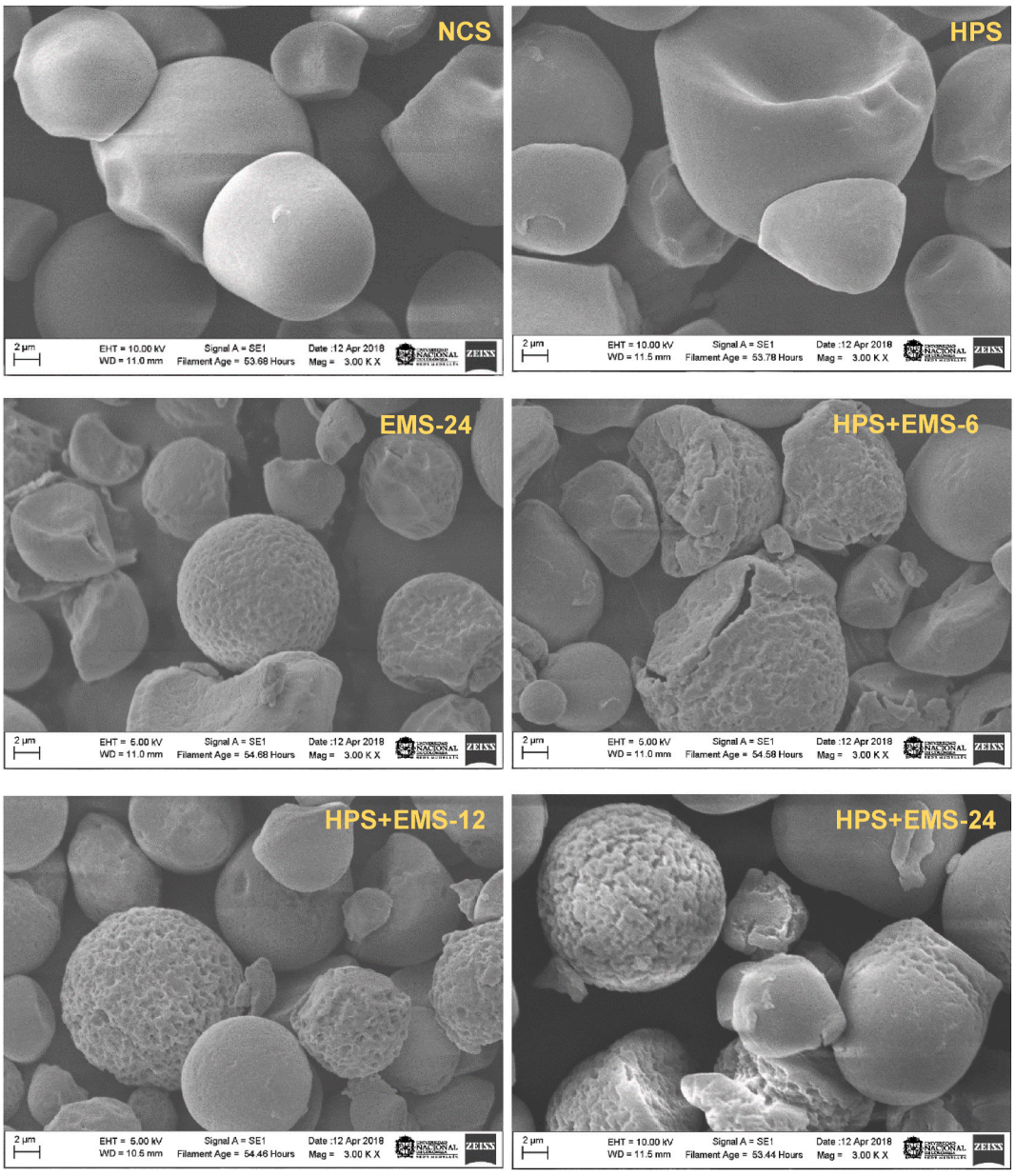

Fig. 1. Micrographs of native and modified cassava starches established by scanning electron microscopy (SEM, 3000X). NCS: native cassava starch; HPS: hydrothermally pretreated starch; EMS-24: enzyme-modified starch; HPS + EMS-(6, 12, 24) dually modified starch.

fractions in EMS-24 and HPS + EMS treatments [8,31]. However, in the HPS + EMS-24 treatment, no changes in the amylose content were evident. This supposes a fast action of the enzymes in the amorphous lamellae affecting the amylose content, whereas a subsequent enzymatic action would affect to a lesser extent the amylose packed with the amylopectin in the most ordered and crystalline zones.

The modification process significantly altered the hydrophilic properties of the modified starches. The WSI and WAI values compared to the native control increased significantly after HPS treatment ($p < 0.05$). Regarding the effect of the enzymatic action in the EMS-24 treatment, an increase in the WSI and WAI values was observed compared to the NCS. These differences were more noticeable in the dual HPS + EMS treatments, increasing significantly with hydrolysis time. Changes in functional properties are possibly due to (1) opening of small pores during swelling induced by hydrothermal treatment [32]; (2) amylose leaching out from amorphous zones during heating [33]; (3) greater availability of OH groups after the breakdown of intermolecular bonds and the depolymerization of amylose and amylopectin [34]; and (4) increase in the size and volume of pores during hydrothermal and enzymatic treatments, which allow extension of the superficial area and promotion of interaction processes between soluble polymeric chains and water molecules [12,35].

3.2. Morphological properties and granular size

The native cassava starch granules presented a spherical or oval morphology characterized by a relatively smooth surface without lacerations or porosities (Fig. 1). Native cassava starch (NCS) revealed minor damage on their surface with truncated ends, associated with the nature or extraction process [23]. Table 2 shows the mean diameter distributions d-(0.10), d-(0.50) and d-(0.90) for the NCS treatment, indicating values of 9.88, 21.89 and 75.10 μm, respectively (Table 2). The HPS did not alter the spherical/oval morphology typical of the native structure. However, slight surface lacerations and modifications in the distribution of mean diameters d-0.10 and d-0.90 are shown. The enzymatic treatment effect is clearly evident in the microstructure of the modified granules. After the enzymatic attack, the appearance of eroded areas on the external granule surface is observed (EMS-24). These changes were more pronounced with the double modification (HPS + EMS) and the increased hydrolysis time, where small cracks, greater porosity, and partial loss of the native morphology are detailed. This result is possibly due to changes in the semicrystalline order of the granules, altering the interaction of the polymer chains, producing granules more susceptible to enzymatic attack [1]. Similar results have been reported in tubers hydrolyzed granules, which presented exo- and endo-erosion from the surface to the central cavity with the consequent alteration of the morphological characteristics [23]. Likewise, the simple and dual modification significantly decreased the distribution of the mean diameters in the following order: NCS < HPS < EMS-24 < HPS + EMS-6 < HPS + EMS-12 < HPS + EMS-24. Changes at the morphological level and in the granular size of the modified starches are possibly due to (1) amylose leaching out during the HPS treatment [25,36,37]; (2) swelling or agglomeration promoted by hydrothermal treatment [15,32,35,38]; (3) formation of surface micropores due to uniform adsorption of enzyme polypeptides [30,39]; (4) phenomenon of exo-erosion on the surface due to the degradation of linear chains in the amorphous zone [1,23,40]; (5) depolymerization of amylopectin and endo-erosion extending from the surface to the hilum promoting the formation of deeper, uniform and homogeneous pores [6,31,41]; and (6) reduction in the linear chain lengths of the polymeric macromolecules [9,29].

3.3. Specific surface area, diameter and pore volume

The single and dual modification processes (Table 2) caused a significant increase in the specific surface area, volume and diameter of the pores ($p < 0.05$). The N_2 desorption isotherms in the modified starches were type IV with a H3 type hysteresis loop (results not shown), characteristic of mesoporous materials [42]. The specific surface area in the NCS samples was significantly lower than that estimated for the modified starches. These results were consistent with the determination of a very small pore volume and the appreciation of a rigid and smooth surface (Fig. 1, Table 2), intrinsic characteristics of native cassava granules. These characteristics

Table 2
Granule size, specific surface area, diameter and pore volume in native and modified cassava starches.

Treatment	d-0.10 (μm)	d-0.50 (μm)	d-0.90 (μm)	Pore volume (cm^3/g x 10^{-3})	Average pore diameter (nm)	Specific Surface area (m^2/g)
NCS	9.88 ± 0.10^{a}	21.89 ± 0.19^{a}	75.10 ± 0.21^{a}	0.16 ± 0.00^{a}	1.22 ± 0.06^{a}	0.57 ± 0.03^{a}
HPS	8.75 ± 0.08^{b}	21.64 ± 0.10^{a}	77.46 ± 0.33^{b}	0.82 ± 0.00^{b}	4.11 ± 0.09^{b}	1.32 ± 0.06^{b}
EMS-24	8.47 ± 0.18^{c}	20.52 ± 0.12^{b}	70.35 ± 0.16^{c}	1.40 ± 0.00^{c}	9.67 ± 0.05^{c}	3.51 ± 0.14^{c}
HPS + EMS-6	7.78 ± 0.07^{d}	19.12 ± 0.21^{c}	68.30 ± 0.40^{d}	2.05 ± 0.00^{d}	9.75 ± 0.10^{c}	3.61 ± 0.10^{c}
HPS + EMS-12	8.05 ± 0.11^{e}	19.55 ± 0.14^{d}	72.87 ± 0.33^{e}	2.09 ± 0.00^{e}	15.77 ± 0.06^{d}	4.66 ± 0.20^{d}
HPS + EMS-24	7.98 ± 0.20^{ef}	18.87 ± 0.05^{c}	67.21 ± 0.14^{f}	2.39 ± 0.00^{f}	17.68 ± 0.13^{e}	4.99 ± 0.19^{d}

NCS: native cassava starch; HPS: hydrothermally pretreated starch; EMS-24: enzyme-modified starch; HPS + EMS-(6, 12, 24) dually modified starch. Values are shown as mean ± standard deviation. Values followed by different letters within a column denote significant differences ($p < 0.05$).

expose the structural and molecular resistance of NCS to the processes of enzymatic degradation and the formation of small porous structures [1,4]. In addition, the increase in surface area after enzymatic treatments could be due to the increase in the number and size of pores induced by the exo- and endo-erosion processes during the synergistic action of amylolytic enzymes. Similar observations have been reported for enzymatically modified cereal starches [7,34].

The diameter and volume of the pores increased with HPS treatment and enzymatic hydrolysis at EMS-24. Shariffa et al. [22] considered that temperatures close to gelatinization can swell the granules during granular swelling, increasing the opening and size of the pores. However, an increase in the size and number of pores during the enzymatic action in the EMS-24 treatment is possibly due to polymer degradation due to exo-erosion on the granular surface. These results were consistent with the SEM analysis, where enzymatic erosion occurred mainly on the surface. Similar behavior was reported in tuber hydrolyzed starches [4,20,29]. Nonetheless, the HPS + EMS-treated granules exhibited a more uniform porosity and larger pores without affecting the native morphology, results consistent with the variation in pore size and volume (Fig. 1, Table 2). This result could be attributed to the enzymatic absorption and subsequent biocatalysis in the amorphous zones near the granular surface causing accentuated exo-erosion [43]. On the other hand, as the pore size widens due to exo-surface erosion with hydrolysis time, it can also induce endo-erosion phenomena from the surface to the center of the granule [5,7,44]. All these changes at the surface or internal level during hydrothermal processes and/or enzymatic biocatalysis could promote the formation of porous-surfaces, altering the morphological and functional properties of the native cassava granules.

3.4. Degree of crystallinity

X-ray diffraction patterns in native and modified cassava starches are shown in Fig. 2. Four crystalline peaks were identified in NCS samples under angles $2\theta = 15$, 17, 17.8 and 23° characteristic of tuber starches with a type-A diffraction pattern, whose amylopectin double helices acquire a configuration in a monoclinic form very well-packaged [24,45]. A small peak was evident at $2\theta = 20°$, related to the interaction of amylose and lipid components after the HPS [46]. The modified starches showed higher intensity in the crystalline peaks after the single and dual treatments, with no evident changes in the type-A diffraction patterns. HPS treatment could promote structural changes, including amylose leaching out and realignment of amylose-amylose or amylose-amylopectin linear chains, affecting the semicrystalline order [45], which could explain the increase in DC after HPS. Similar observations have been reported in waxy corn and common wheat annealed starches [47,48]. Furthermore, an increase in DC during dual HPS + EMS treatment without changes in type-A polymorphism suggests polymeric degradation mainly in the amorphous zone. Shariffa et al. [22] found that amylolytic action especially affected the amorphous regions of the starch granules, extending the area of the crystalline peaks. Analogous results have been reported in hydrolyzed corn and cassava starches [43,44,49].

3.5. Infrared spectroscopy

FT-IR spectra indicate changes in absorption bands characteristic of starchy materials after enzymatic hydrolysis, possibly associated with molecular and structural changes in the starch granules (Fig. 3). In the absorption bands of 3350 to 2930 cm^{-1} characteristic of the OH bond present in glucose units, a slight narrowing and a marked flattening of the absorption peak in the spectra of hydrolyzed starches was observed, probably attributed to changes in the molecular granule order [7,25]. The absorption bands at 1130 and 1160 cm^{-1} related to the vibration of –CO–, C–C or OH groups present in the amylose and amylopectin molecules increased the signal intensity, perhaps due to the depolymerization and breakdown of glycosidic bonds with the enzymatic treatment [23]. Furthermore, a characteristic starch peak is the band length at 995 cm^{-1} associated with the presence of bound water in the structure, whose band possibly shifted to a greater availability of OH groups after depolymerization, which improved the starch granule capacity

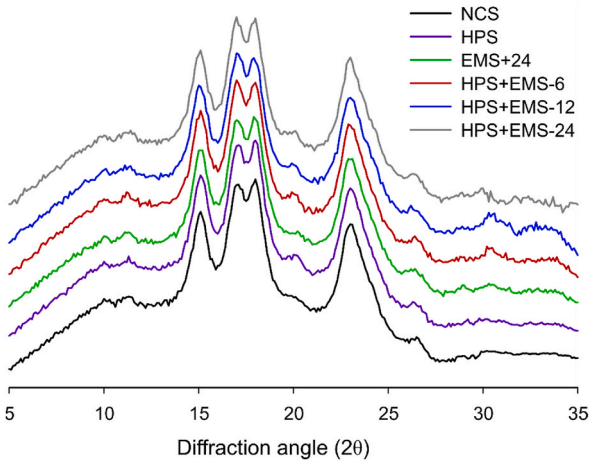

Fig. 2. X-ray diffraction patterns in native and modified cassava starches. NCS: native cassava starch; HPS: hydrothermally pretreated starch; EMS-24: enzyme-modified starch; HPS + EMS-(6, 12, 24) dually modified starch.

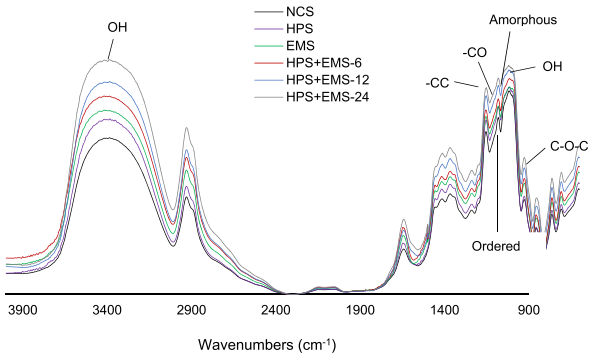

Fig. 3. Infrared spectra (FT-IR) in native and modified cassava starches. NCS: native cassava starch; HPS: hydrothermally pretreated starch; EMS-24: enzyme-modified starch; HPS + EMS-(6, 12, 24) dually modified starch.

to retain and absorb water [9,26].

The FT-IR spectra depend on the changes in the structure of the starch in a short-range order, defined as the double helix order, which reflects the ratio of the number of double helices (ordered domains) over single helices (amorphous domains) [12,25]. The absorbance bands in the 1047/1022 cm^{-1} region allow the estimation of the degree of semicrystalline ordering of the granule, where the 1047 cm^{-1} band is associated with the crystalline structure, while the 1022 cm^{-1} band is associated with the amorphous structure [14]. Additionally, the band in the peak at 925 cm^{-1} has been assigned to the vibrations of the skeletal mode of the α-(1,4) glycosidic bond (C–O–C). Meanwhile, the sharp peak at 1022 cm^{-1} has been correlated to the stretching of C–O and C–O–C groups in the α-(1,6) glycosidic bonds [50]. Therefore, the relationship between the bands was determined at 1047/1022 cm^{-1} to quantify the degree of molecular order (OD_1) in starch granules. Likewise, the 925/1022 cm^{-1} ratio was determined as an approximation to evaluate the intensity of the enzymatic attack on the glycosidic bonds (OD_2) (Table 1). No significant differences in OD_1 and OD_2 values were detected between NCS and HPS ($p < 0.05$); that is, the HPS treatment did not cause significant changes in the semicrystalline granule order. However, a significant increase in OD_1 was noted in the modified starches EMS-24 and HPS + EMS compared to the control ($p < 0.05$). This suggests that there was higher polymeric degradation on the amorphous domains, i.e., loss of single helices, during the enzymatic treatments, consistent with the decrease in amylose content and increase in DC determined by XDR. In addition, a significant increase in OD_2 values was observed in EMS-24 starches with respect to NCS and HPS treatments, which indicates that the synergistic activity of amylolytic enzymes initially attacks α-(1,4) glycosidic bonds in the linear structures; however, this synergistic activity can also attack α-(1,6) branching points with increasing hydrolysis time and possibly cause the loss of double helices. These results could be related to the morphological changes, where exo-erosion phenomena initially predominate, and with increased hydrolysis time, endo-erosion effects were observed, increasing the mesoporous characteristics in the modified cassava starches.

3.6. Gelatinization properties

The gelatinization properties of native starches underwent significant changes after the modification processes (Table 3). HPS treatment promotes double helix stability through realignment of amylose-amylopectin linear chains. The double helix stability improves the starch crystallinity, reducing the swelling power that governs the gelatinization phenomenon [45,51]. This could explain the increase in T_o, T_p and T_c temperatures in samples modified by HPS and dually treated with HPS + EMS. Likewise, changes in transition temperatures are related to the increase in crystallinity degree (DC), an indicator of structural stability and molecular resistance to thermal stress, slowing down the gelatinization process [9,14], which agrees with the semicrystalline order data analyzed by FTIR and X-ray diffraction. These results are in accordance with previous reports in barley and wheat hydrolyzed starches [37,52].

The T_o-T_c temperature range showed a marked difference with the implementation of the physical and enzymatic modification with respect to the NCS treatment. This behavior is supported by that hydrothermal and enzymatic treatments can induce both

Table 3
Gelatinization properties evaluated by differential scanning calorimetry (DSC) in native and modified cassava starches.

Treatment	T_o (°C)	T_p (°C)	T_c (°C)	$[T_c$-$T_o]$ (°C)	ΔH (J/g)
NCS	64.39 ± 0.11^{a}	68.62 ± 0.08^{a}	86.59 ± 0.20^{a}	22.24 ± 0.17^{a}	19.60 ± 0.24^{a}
HPS	65.26 ± 0.11^{b}	70.03 ± 0.19^{b}	84.72 ± 0.27^{b}	19.46 ± 0.11^{b}	15.15 ± 0.41^{b}
EMS-24	70.24 ± 0.08^{c}	72.87 ± 0.28^{c}	86.41 ± 0.39^{bc}	16.17 ± 0.14^{c}	14.33 ± 0.19^{c}
HPS + EMS-6	72.96 ± 0.10^{d}	75.38 ± 0.15^{d}	86.13 ± 0.12^{c}	13.16 ± 0.15^{d}	15.79 ± 0.35^{b}
HPS + EMS-12	71.84 ± 0.06^{e}	74.85 ± 0.30^{e}	86.47 ± 0.09^{bc}	13.63 ± 0.20^{d}	13.80 ± 0.40^{d}
HPS + EMS-24	74.28 ± 0.16^{f}	76.66 ± 0.21^{f}	87.74 ± 0.34^{d}	13.47 ± 0.07^{e}	11.93 ± 0.13^{e}

NCS: native cassava starch; HPS: hydrothermally pretreated starch; EMS-24: enzyme-modified starch; HPS + EMS-(6, 12, 24) dually modified starch. T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, H = gelatinization enthalpy. Values are shown as mean ± standard deviation. Values followed by different letters within a column denote significant differences ($p < 0.05$).

morphological and structural changes that delimit the thermal behavior of starch granules [43,53]. Additionally, it has been shown that the increase in gelatinization temperature is more pronounced for T_o (fusion of weaker crystallites) and less pronounced for T_c (fusion of stable and more perfect crystals) during modification [45,54]. For this reason, the decrease in the T_o-T_c range is possibly due to higher homogeneity, cooperative fusion and more perfect crystals, which allows us to understand the most notable changes in the increase in crystallinity without affecting the type-A polymorphism. However, the decrease in ΔH in modified starches HPS + EMS can be correlated with depolymerization of amylopectin and the loss of double helices or unstable crystallites, which could be destroyed or degraded during the physical-enzymatic processes. The loss of the imperfect crystal affects the value behavior of ΔH in hydrolyzed starches from cereals and tubers, which is related to the crystalline and helical structure [4,6]. Additionally, structural changes in amylopectin and a high proportion of double helices in the amylose structure have been associated with increased gelatinization temperatures and decreased enthalpy in rice hydrolyzed starches [8].

4. Conclusions

Annealing and enzymatic biocatalysis processes allowed the development of modified cassava starches with porous surfaces. The individual treatments of annealing (HPS) followed by enzymatic modification reduced the amylose content, altered the semicrystalline order, and changed the granular surface in native cassava starch. In addition, increasing the pore diameter, pore volume, and specific surface area of modified cassava starches, improve the solubility and adsorption properties of water. The dual modification HPS + EMS corroborated the hypothesis that hydrothermal treatments could swell the granules and enlarge the pores or cavities, which improves the adsorption processes and enzymatic endo-erosion towards the interior of the granules. The HPS + EMS-24 treatment significantly increased the microporous characteristics and the degree of crystallinity and improved the thermal stability of the modified granules; therefore, this treatment could be an effective method for the production of porous starches with application potential as an encapsulating material.

Author contribution statement

Figueroa-Flórez Jorge: Performed the experiments; analyzed and interpreted the data; wrote the paper.

Cadena-Chamorro Edith and Rodriguez-Sandoval Eduardo: Conceived and designed the experiments; contributed reagents, materials, analysis tools or data.

Salcedo-Mendoza Jairo: Conceived and designed the experiments; analyzed and interpreted the data; wrote the paper.

Ciro-Velasquez Héctor: Contributed reagents, materials; analyzed and interpreted the data.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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