Abstract

Banana starch has a highly resistant starch (RS) and slow-digested starch (SDS) content, making it attractive as a functional ingredient. Unfortunately, banana starch requires modification processes due to the loss of RS and SDS during gelatinization because of its thermolabile characteristics. This study explores the effect of banana starch modification by enzymatic, heat moisture treatment (HMT) and dual modification (HMT+ enzymatic) on its nutritional (RS, SDS) and functional properties (hydration, structural, gelation, rheological). HMT and dual modifications decrease RS (from 44.62 g/100 g to 16.62 and 26.66 g/100 g, respectively) and increase SDS (from 21.72 g/100 g to 33.91 and 26.95 g/100 g, respectively) in raw starch but induce structural changes that enhance RS (from 3.10 g/100 g to 3.94 and 4.4 g/100 g, respectively) and SDS (from 2.58 g/100 g to 9.58 and 11.48 g/100 g) thermo-resistance in gelled starch. Also, changes in the functional properties of starches were evidenced, such as weaker gels (hardness<41 g), lower water absorption (<12.35 g/g), high starch solubility (>1.77 g/100 g) and increased gelatinization temperature. Improved gelatinization temperature and RS thermostability resulted from modifications that could expand banana starch applications as a beverage and compote thickener agent.

Keywords: Starch modification, HMT, Dual modification, Starch Structure, Banana Starch, Resistant starch, Slow digestive starch.

Highlights

- HMT and Dual modification increase the RS and SDS content of the starch gel.
- HMT modification changes the banana starch structure.
- The enzyme modification increases the starch crystallinity degree
- T+

10he modified banana starch can be used as a thickener agent in beverages and compote.

Exploring the Effects of Enzymatic and Thermal Treatments on Banana Starch Characteristics

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1. Introduction

Native banana starch has high amylose content (> 25%), a high amount of resistant starch (RS) (> 65%), and a slowly digested starch (SDS) [1]. Those properties are desirable for using banana starch as a functional ingredient because SDS is slowly digested in the small intestine, offering gradual glucose release and low glycemic response. At the same time, RS resists digestion, benefitting colonic health [2]. However, the RS and SDS thermolability limits the inclusion of this starch whenever food processing requires an increase in temperature. The RS in a banana starch is mainly type 2 (RS2), which is highly fragile with the cooking and gelatinization process. Indeed, Hoyos-Leyva et al. [3] showed in *Morado* native banana starch a significant RS reduction (85.8%) with the gelation process. Other limiting factors for the industrial use of native banana starch are the high tendency to retrograde, the poor freeze-thaw stability and the cohesiveness of their pastes [4].

Chemical, physical, enzymatic, and biotechnological modification methods, or their combination, have been proposed to modify the physicochemical and nutritional characteristics of native starch. Previous studies have mainly focused on the chemical modification of banana starch to increase the water binding capacity, improve the freeze-thaw stability and increase the RS and SDS content [5,6]. Nevertheless, physical or enzymatic methods have attracted researchers' attention, as they are environmentally favorable and easy to industrialize with no toxic effects on human beings. Physical modifications such as heat moisture treatments (HMT) have been applied to provide banana starches and flours with higher RS and SDS, increasing crystallinity and gelatinization temperature [3,7]. In fact, according to Hoyos-Leyva et al. [3], HMT reduces the amorphous area in the semi-crystalline lamella, which can be associated with the changes in RS and SDS and the increase of the gelatinization temperatures; their results showed a reduction of RS loss (48.7%) in *Morado* banana after gelation process.

On the other hand, enzymatic modification has been applied in other botanical starches to increase the adsorption capacity, being attractive for industrial uses as porous starches. The porous starch undergoes partial enzymatic hydrolysis, leading starch with high adsorption capacity. It has a potential application for flavor delivery and probiotic encapsulation, among other uses. Enzymes such as alfa-amylase, glucoamylase, and amyloglucosidase, among others, and their combination have been used to produce porous starch. In banana starch, an enzymatic modification with amylopullulanase has recently been applied to increase the RS content in banana flour from 38.5% to 68.99% [8].

Currently, combining methods to modify starch is at the forefront of research. Dual modification has been applied to improve the properties and uses of single-modified starches. Accordingly, the combination of autoclaving and pullulanase process has been applied to banana starch and flour to improve the RS content[9]. A previous study in wheat starch demonstrated that a dual modification with HMT and enzymatic hydrolysis with α-amylase and glucoamylase exhibits higher water and oil absorption capacity, higher gelatinization temperature and higher crystallinity than the native and enzymatically modified starch [10]. To the authors' knowledge, a study on enzymatic modification and dual modification, with HTM and enzyme hydrolysis, in native banana starch has not been explored. Furthermore, the impact of these modifications on the banana starch gel digestibility has not been considered, which is an important factor for consumer consumption. The native banana starch has a strong granule structure, making it less feasible for enzymatic modification; HMT in banana starch could be used as a previous treatment for enzymatic hydrolysis due to the alteration in the crystalline and amorphous regions of the starch granule, making it susceptible to enzymatic modifications [10].

Therefore, considering the possible industrial uses and nutritional benefits of modified banana starch, the research goal was to explore the individual effect of physical (HMT) and enzymatic modification of starch banana, besides their combination, to study possible synergistic or antagonistic effects. The physicochemical and nutritional characteristics of the treated banana starches were evaluated, especially the resistant starch content and its thermolability after the gelatinization.

2. Materials and methods

Green bananas with 1.2 to 2.1°Brix were acquired from Guayaquil's (Ecuador) local market. Cellulase (Rohament CL, 900 U/g) provided by AB Enzymes (Darmstadt, Germany) and xylanase (Pentopan, 2.500 U/g) from Novozymes (Copenhagen, Denmark) were used for the starch extraction.

Instead, enzymatic solutions of α-amylase (100 U/ml) from Sigma Aldrich (Darmstadt, Germany) and glucoamylase (100 U/ml) from GRANOTEC (Santiago, Chile) were used for enzymatic modification.

2.1. Starch isolation

Bananas were peeled and sliced into 2 cm discs. Then, the slices were soaked in 1% citric acid solution for 10 min. Wet milling was carried out considering a banana: water ratio of 2:3. The slurry was mixed with 800 U/g of cellulase and xylanase and kept at 35°C for 3 h. Subsequently, the slurry passed through two sieves (No. 100 and 200 mesh), and after that, it was washed twice with distilled water. Then, the cake retained in the sieves was discarded, and the resulting solution was sedimented for one hour. Finally, the sediment was collected and dried at 50°C for 24 h. The starch was milled and screened through a No. 70 mesh. The protein and ash content of the native banana starch was measured following the AOAC methodology 920.87 and 923.03, respectively. The results were protein: 1.39±0.02% and ash: 0.13±0.04%.

2.2. Starch modification

2.2.1. Heat moisture treatment

The HMT was performed in two heating cycles. In the first cycle, the banana starch moisture content was adjusted to 30% with distilled water, allowing it to equilibrate for 20 h at room temperature. Then, tempered starch was heated in an oven at 100°C for five hours. After the heating stage, the starch was dried at 50°C for 24 h, milled, and screened through a No. 70 mesh. Subsequently, the second cycle was performed similarly to the previous process. The HMT process was carried out by duplicate.

2.2.2. Enzymatic treatment

Preliminary experiments of enzymatic treatment were carried out to determine the incubation time needed to reach 30% of starch hydrolysis. The degree of hydrolysis was quantified by the methodology described in Cornejo et al. [11], using the equation:

$$Hydrolysis\ degree\ (\%) = (G_1/G_2) \times f_1 \times 100$$

Where G_1 is the free glucose (g) after hydrolysis, G_2 is the total glucose in the raw starch sample (g) at the beginning of hydrolysis, and f_1 is the conversion factor of free glucose to anhydroglucose, as occurs in starch equal to 0.9.

Regarding the enzymatic treatment, 10% w/v (DB) starch suspension in sodium phosphate buffer (pH 6.0) was prepared. Immediately, a mixture of α-amylase and glucoamylase (1:3) was incorporated into the solution, considering the enzymatic activity of 8 U/g starch for α-amylase and 24 U/g for glucoamylase. The suspension was stirred at 175 rpm and heated at 50°C for 16 h to reach 30% hydrolysis. The suspension was centrifugated at 2000 rpm for 5 min, and the supernatant was discarded. Finally, the sediment was dried at 50°C for 24 h, and then the sample was screened through a No. 70 mesh and stored at room temperature for further analysis. The enzymatic modification was carried out by duplicate.

2.2.3. Dual modification: HMT+ Enzymatic

As mentioned above, banana starch has a strong granule structure; therefore, for the combined treatment, banana starch was first treated with the HMT and then with the enzymatic treatment method. The hydrolysis was performed for nine hours to achieve 30% of hydrolysis. The modified starch was dried and milled as described above. Dual modification was carried out by duplicate.

2.3. Structural properties

X-ray diffraction analysis was measured with an X'Pert PRO diffractometer (PANalytical, Boulder, United States) operating at 30 mA and a voltage of 40 kV. Starch samples were placed in a closed system with 100% moisture for 24 hours. Analysis was performed under the following parameters: angle range of 5° a 40° (2θ). Each sample was replicated three times. The software OriginPro ®Version 2023 (OriginLab Corporation, Northampton, MA, USA) was used to measure the crystalline area (Ac) and the amorphous area (Aa) of the X-ray diffractograms. The crystallinity (C) was determined through the equation C (%) = (Ac/(Ac+Aa))*100.

The changes in the granule morphology of native and modified banana starches were observed using a scanning electron microscope (Inspect S50, Thermo Fisher Scientific, Hillsboro, Oregon) operating at an acceleration voltage of 10.50 kV. The starch samples were covered with a thin layer of gold (Emitech, SC760) and observed at 5000X magnification.

2.4. Starch composition

Total and resistant starch content was determined using Megazyme Total Starch and Resistant Starch Kits (Neogen, Lansing, Michigan, USA). Digestible starch properties (slow and rapid digestible starch) were analyzed following the procedure reported by Cornejo et al. [12] Two samples were

evaluated for these analyses: the starch granule (raw starch) and the gelatinized starch. For the gelatinization process, 10% (w/v) banana starch was mixed with distilled water and heated at 90°C for 10 min; then, the gel was cooled in an ice-water bath for 10 minutes.

Amylose content was quantified according to the Hoover and Ratnayake [13] method using defatted starch. Values were the average from three replicates.

2.5. Functional properties of starches and gels

Hydration and thermal properties were evaluated to characterize the starches. The water absorption index (WAI) and water solubility index (WSI) were performed following the method of Cornejo and Rosell [14] Water holding capacity (WHC) and water binding capacity (WBC) were performed following the AACC 56-30.01. For Oil Absorption Capacity (OAC), 0.1 mg starch samples were mixed with 10 ml of refined oil. The mixture was agitated on a vortex for 30 min at 21°C. Then, the suspension was centrifuged at 3000 rpm for 10 min at 4°C. The supernatant was discarded, and the pellet was weighted. OAC was calculated as the amount of oil absorbed by a gram of flour sample. Eight replicates were made for each determination.

A differential scanning calorimeter (DSC) Q 200 (TA Instruments, Newcastle, USA) was employed to analyze banana starch thermal properties. 8 mg of starch: water mixture at a 1:3 ratio (w:w dry weight basis) was weighed into the hermetically aluminum pan. Samples were heated from 25°C to 100°C at 10°C/min. Gelatinization temperatures (To, Tp, Tc) and gelatinization enthalpy (ΔH) were calculated by TA Instruments Universal Analysis 2000 Software Version 4.5 (Waters Corporation, Milford, Massachusetts). Measurements were performed in triplicate.

The rheological properties, gel strength and syneresis were evaluated in starch gel by triplicate. Gels were prepared as previously indicated in the starch composition analyses.

For rheological analysis, the storage modulus (G') and loss modulus (G'') were measured using a Kinexus PRO rheometer (Malvern Instruments, Worcestershire, UK) at 25°C with a cone-plate geometry (diameter of 4 mm; angle of 4°). The linear viscoelastic region was estimated at 0.1%.

For gel strength analysis, the samples were prepared in glass bottles and kept at room temperature for 30 minutes. The samples were stored at 4°C for 24 hours. After this time, the measurement was done in a CT3 Brookfield Texture Analyzer (AMETEK Brookfield, Middleboro, MA, USA) with a 1.27 mm probe and considering an activation load of 4.5 g, speed: 0.5 mm/s and an objective distance of 4 mm.

Starches gels syneresis were evaluated by repeated freeze-thawed cycles. The gels were frozen at -22°C for 22 h and thawed at 30°C for 1.5 h. Subsequent freezing and thawing cycles were repeated up to five times. The syneresis was determined after the 1st, 3rd, and 5th cycles. For assessment, the tubes were centrifugated at 9000x*g* for 15 min, and the free water was discarded. Syneresis was expressed as the percentage of water released from the gel.

2.6. Statistical analysis

The Statgraphic Centurion XVII Software Version 16.1.03 (StatPoint Technologies, Inc., The Plains, Virginia, USA) was used for statistical analysis. Data that followed a normal distribution were subjected to ANOVA analysis, and the comparison of the treatments was performed with Fisher's least significant differences (LSD) test ($p<0.05$). The Kruskal-Wallis test was used to evaluate the non-parametric data, and Bonferroni's multiple range test was used to compare treatments with ($p<0.05$). The relationship between response variables was analyzed through the Pearson correlation coefficient.

3. Results and discussion

3.1. Structural properties

Figure 1 illustrates the micrographs of the native and modified starches obtained in the scanning electron microscope. As shown in Figure 1A, the native banana starch granules had irregular, flat, and elongated shapes. Regarding their surface area, the appearance was smooth and without cracks, and the granule size ranged between 35 to 46 μm. Similar observations were reported in other banana varieties [1,15]. Figure 1B displays the banana starch treated with HMT, which showed slight superficial damage and a rough appearance. Besides, this modification increased the proportion of small starch fragments agglomerated with the intact starch granules. The rupture and changes in the surface of the starch granules resulted from the partial gelatinization of the granules due to the temperature and humidity conditions of the thermal treatment [16]. With the enzymatic modification (Figure 1C), the surface of the starch granules displayed small, circular and shallow depressions. Despite the packed structure of the banana starch, the applied enzymatic treatment could modify the granules. Some damaged starch granules showed large grooves, which is reasonable since the damaged structures with fissures, cracks, or pores are more susceptible to enzymatic hydrolysis. Starches from the dual treatment (Figure 1D) exhibited deeper pores on the granules than those obtained with the enzymatic method, and the surface was slightly melted.

Previous research reported that HMT produces an increment of the enzyme's binding site that promotes higher enzymatic susceptibility in wheat starch [10], which is consistent with the results obtained in this study. Likewise, it is essential to point out that starches previously treated with HMT reached 30% hydrolysis in almost half the time (9h) than the native starch (16h). This effect occurred due to the molecular rearrangement caused by the HMT treatment, which facilitated the access of the enzymes to the amorphous zones of the starch [17].

Diffractograms of native and modified starches are represented in Figure 2. Native banana starch shows strong peaks near 17° and 20°, which agrees with a type B crystallinity, as reported previously by Yang et al. [15]. No significant change in crystallinity pattern is observed with the enzymatic modification, probably due to the hexagonal packing characteristic of this type of crystallinity [18], making it more resistant to enzyme activity [15]. This result is consistent with the low-depth cavities observed on the surface of the starch granules treated with enzymes. In contrast, HMT and dual modification show a peak intensity reduction at 17° and 20°. Indeed, the B-type pattern switches to the A-type pattern, and the peak at 5° disappears. Wu et al. [1] also described a transformation of the B-type to the A-type pattern in banana starch granules after HMT in the presence of citric acid at 90°C, stating that the B-type pattern is prone to transformation. Therefore, the HMT could alter the structure of banana starch. The present results agree with previous research that reported changes in crystallization structure from C to A and from B to A type pattern after an HMT process, e.i. in bean [19] and waxy potato starch starches [20].

Figure 2 also shows the degree of crystallinity of banana starches. Starches modified by enzymes (enzymatic and dual modification) show a significant increment of crystallinity compared with native starch. In dual-modified starches, the increment of crystallinity could be associated with the structural rearrangement of starches caused by HMT, which facilitates the enzymatic hydrolysis in amorphous regions of the starch granule, concentrating the crystalline fraction [21]. In contrast, the starch treatment only with HMT shows no significant change in crystallinity, even though previous findings have reported an increment in other banana varieties [1].

3.2. Starch composition

Table 1 shows the results of native and modified banana starch composition. In native starch, no significant changes were observed in total starch content for HMT and enzymatic starches, except for

dual modification, which was slightly reduced due to enzymatic hydrolysis that breaks down the starch, forming less complex polysaccharides. Moreover, the amylase endo-action significantly reduced amylose content due to changes in the amorphous domain mainly formed by amylose, which is less dense and more susceptible to enzymatic attack [22]. In the case of dual modification, the HMT process promotes enzyme accessibility and allows further degradation of the amylose chains. In addition, according to Cahyana et al. [7], partial gelatinization with HMT displays a less compact granule surface, making it more susceptible to enzyme attack. In fact, the HMT process affects not only the total starch and the amylose content but also the RS content.

Table 1 reveals that enzymatic modification does not significantly change the RS content of the starch granule. On the other hand, HMT and dual modification starches present a significant reduction of RS in the starch granule. Despite native and modified banana starches having substantial RS content, these values decline significantly after gelatinization. In general, starch gels (10%) from native and modified starches experienced a reduction of more than 80% in the RS content due to the swelling and burst of the starch granules that increased the enzyme binding, leading to the rise of digestion [23]. This reduction is caused by the RS type II (RS2) present in the native starch. As mentioned above, banana native and enzymatic starches show a B pattern, and thus more resistance to digestion, but their poor thermal stability allows easy degradation. Even though granule starches treated with HMT and dual modification have significantly low RS content in the starch granule, the results show that this structure is more thermostable than native and enzymatic starches. HMT and mainly dual-modified starch gels present higher RS contents in the starch gels (Table 1). The improvement could result from the change in RS type and crystallinity. After gelatinization, RS3 is formed by recrystallization of the starch [24]. Ma et al. [25] point out that the moisture content during the HMT process could increase the starch chain mobility for molecular rearrangement and facilitate the retrogradation of HMT starches, leading to the increased formation of RS3.

Concerning the RDS and SDS content of starch granules, all starch modifications increase RDS and SDS, except enzyme modifications that significantly reduce SDS. The HMT transforms RS into SDS and RDS in the starch granule. On the other hand, when the banana starch granule forms a gel, the starches treated with HMT present an increase in SDS and a reduction in RDS compared to the native starch gel. When starch modified with HMT is gelatinized, the RS and SDS are more thermostable. The HMT could disrupt the crystalline structure and form molecular rearrangements of the starch granule,

generating a dense starch matrix [16] and improving its thermoresistance. Curiously, amylose content is correlated significantly with both parameters (RDS and SDS). RDS has a positive correlation ($r=0.69$; $p<0.05$), while SDS has a negative correlation ($r=-0.65$; $p<0.05$). These correlations can be associated with modifying methods that degrade amylose molecules and concentrate the crystalline fraction composed mainly of amylopectin [22].

In contrast to some reports in the literature, our results in enzymatic and dual modification showed an increase in RDS with an increase in crystallinity. As we mentioned above, the increment of crystallinity in these samples responded to enzymatic hydrolysis in the amorphous region that also produced grooves in the starch granule, as was observed in Figure 1. This damage in the structure makes it more suitable for enzymatic hydrolysis during digestion.

3.3. Functional properties

Table 2 details the hydration properties of native and modified starch granules. In general, the modified starches increase WHC, WBC and OAC. In the case of WBC, the dual modification shows a reduction. The partial gelatinization of HMT-modified banana starches increases their water/oil binding capacity [26]. Regarding the enzymatic modification, the enhancement of hydration properties is a consequence of the porosity that improves the access of oil/water molecules to the pore channel [27]. Additionally, the presence of hydroxyl groups between the starch chains provides it with better hydrophilic properties and allows the formation of covalent and hydrogen bonds with water molecules [28]. The dual modification reduces the WBC, presumably due to the excessive damage of starch caused by the thermal process and ruptures of the starch structure by enzymes [26,27].

Table 2 also summarizes the absorption properties of banana starch gels. Except for HMT, all modification methods decreased the water absorption index (WAI) and increased the solubility index (WSI). Between them, enzymatic starches have the lowest reduction in the WAI compared to native starch (only 6%). Enzymatic treatment modified the granular integrity due to an inner molecular rearrangement of the granule, creating a more ordered double-helix segment of the branched starch side chains, affecting its water absorption [27,29]. Likewise, the HTM process considerably reduces the WAI of banana starch. This behavior has been attributed to the dissociation of the double helical structure in the amorphous region due to internal rearrangement of the starch granules caused by interaction amongst starch polymer chains [30] reducing the swelling capacity. A strong negative correlation was found between WAI with the RS and SDS content of the starch gel ($r=0.94$; $p<0.05$) and a positive correlation

with the RDS content of the starch gel ($r=0.93$; $p<0.05$). These correlations support the idea of Gani and Ashwar [31], who reported that limited swelling of starch caused by starch component interactions influences the formation of SDS and RS. Also, the correlations corroborate that the HMT and enzymatic starch modifications produce a stronger molecular interaction that restricts water retention and protects from enzymatic hydrolysis during digestion.

Regarding WSI, only starches treated with enzymes increased this property, especially with the dual modification. Enzymes produce small molecular and soluble starch fragments, which are released quickly from starch granules during swelling [27]. In the case of dual modification, performing HMT treatment before enzyme modification increased the vulnerability of starch granules to enzymatic attack, allowing a higher concentration of small fragments that leached outside the granules [30], which might lead to remnant starch that is more enzymatically resistant. A positive correlation was found between the WSI and RS of the starch gel ($r=0.73$; $p< 0.05$) and a negative correlation with the RDS content of the starch gel ($r=-0.67$; $p<0.05$).

Figure 3 presents the endothermic curves of native and modified starches. An increment of gelatinization temperatures was noticed with the modification methods. The level of increase of the gelatinization temperatures followed the order enzyme-modified starch <HMT-modified starch < dual modification. Also, the figure indicates a change in the enthalpy and widening of the curves when enzymatic treatment was included. Table 2 details the gelatinization temperatures (To, Tp, Tc), the enthalpy (ΔH) and the temperature range (Ig) obtained from the thermal endothermic curves of native and modified starches. Data confirm significantly increased gelatinization temperatures (To, Tp and Tc) of modified starches. According to previous studies, the HMT suppresses the mobility of the starch chains in the amorphous regions by an increment of amylose-amylose and amylose-amylopectin interactions that increase the gelatinization temperatures of the starch [32]. Instead, with the enzymatic modification, the gelatinization temperatures are affected by the hydrolysis of specific starch structures, which depends on the enzymes used. Ai and Jane [33] suggested that enzymes like α-amylase, α-1, 4-glucanotransferase or bacterial glucoamylase prefer hydrolyzing starch crystallites with shorter branched chains that produce higher gelatinization temperatures. In the case of the dual modification (HMT + enzymatic), the synergy between both treatments caused a significant increase in the gelatinization temperatures. It proved that HMT modifies the starch structure, allowing the enzyme a more profound hydrolysis of the banana starch.

Additionally, the increase in banana starch gelatinization temperature with the modification treatments could be related to changes in the starch structure. During the modification treatment, irregular patterns in the starch granules and long amylopectin branched chains could form stable crystallites [33], making the starch granule more resistant to gelatinization. Indeed, the correlation analysis shows a strong positive correlation of gelatinization temperatures (To, Tp, Tc) with RS ($0.87< r < 0,98$; $p< 0.05$) and SDS ($0.84< r < 0,95$; $p< 0.05$) and a strong negative correlation with RDS ($-0,88< r < -0,96$; $p< 0.05$) of the starch's gels. This result confirms the statement of Hoyos-Leyva et al. [3], who indicate that HMT reduces the amorphous area in the semi-crystalline lamella, which can be associated with the changes in RS and SDS and the increase of the gelatinization temperatures.

Regarding ΔH, enzymatic-modified starch displays higher values than native, HMT and dual banana starches. This result reveals that enzymatic processes increase the number of double helices from the crystalline regions; thus, more energy is required to disrupt the starch granular structure. On the other hand, the HMT modification significantly reduced the ΔH compared to native starch, as observed in HMT and dual modification. This result reveals that enzymatic processes increase the number of double helices from the crystalline regions; thus, more energy is required to disrupt the starch granular structure. On the other hand, the HMT modification significantly reduced the ΔH compared to native starch, which suggested easy destruction of double helices and occasioned a weaker starch granule structure despite their higher gelatinization temperatures. HMT promotes water molecules entering the crystalline region from the amorphous region and destroying the hydrogen bond in the starch, leading to the double helix's disintegration, thus decreasing enthalpy [34,35]. This behavior caused by the HMT process has been reported previously by Wu et al. [1] in the banana starch variety Pei Chiao. Interestingly, the dual modification presents a decrease in enthalpy and an increase in crystallinity. This result was also reported in wheat starch's dual modification (HTM and enzymatic modification) [10]. The dual effect of HTM and enzymatic modification could explain this behavior. As reported in previous research, the HMT process weakens the granule structure, making the destruction of double helices easy and decreasing the enthalpy value [34,35]. On the other hand, the subsequent enzymatic modification increases crystallinity, as mentioned above.

The gelatinization range (Ig) corresponding to the degree of heterogeneity of crystallites in starch granules [10] showed completely different results with the different modification treatments. As shown in Table 2, HMT starches have the lowest value compared with the other treatments, which suggests a

reduction of the crystallite heterogeneity, whose behavior was also observed in HMT wheat starch [36], while enzymatic hydrolysis had the contrary effect. The Ig did not reduce significantly in the dual modification compared with native starch despite the opposite impact of each modification treatment (HMT and Enzymatic).

Table 2 presents the freeze-thaw stability of starch gels, measured as syneresis percentage, after the first, third and fifth freeze-thaw cycles (FTC). The HMT modification process improved the syneresis resistance even though the first FTC presented no significant difference with the native starch. This behavior in the first FTC is similar to the results obtained by Cahyana et al. [7], which reported a unique cycle of FTC in banana flour gels (*Musa acuminata colla*). However, when continuing the freeze-thaw cycles, HMT starches behaved better than native starch. In the third and fifth FTC, the increment percentage of syneresis between FTC was much lower in the HMT starches compared to the native starch. These results indicate a change in the structure of the starch, which allows these structures to acquire a much more ordered form, thus allowing greater resistance to syneresis. The HMT modification reduced the amylose released by increasing the bond between molecules in the amorphous region, and therefore, the amount of water that comes out when thawing was low [37].

All modified starches form weaker gels than native starch, probably caused by a decline in amylose content after modification (Table 2). After cooking, amylose gelation provides the gel's hardness [33] because amylopectin does not offer a strong network, but the small amounts and lower ability to form a gel of amylose cannot create a consistent network [33,38]. This hypothesis is more clearly evidenced in enzymatic and dual-modified starches, in which the amylose content reduction could influence the decrease in gel strength. In fact, a correlation between gel hardness and RDS content of starch gel was found ($r \approx 0.75$; $p<0.05$) and a negative correlation between gel hardness and RS content of the starch gel ($r \approx -0.65$; $p<0.05$). Thus, these results show that in starch gel, RS is made primarily by amylopectin and forms fewer rigid gels with less WAI.

Figure 4A-C shows the viscoelastic behavior of the modified starches. All gels show a viscoelastic solid behavior because of $G' > G''$ (Figura 4A and 4B respectively), and the phase angle (tan δ) approached 0°. Likewise, the reduction of tan δ in all modified starches (Figure 4C) suggested a weaker structure and a less gel-like behavior of the suspensions [39], evidenced by the correlation with the gel's hardness with G' and G'' ($r>0,81$; $p<0.01$). The results show that the modification produces a marked reduction in the viscoelasticity properties of the starch. The variation of elastic modulus (G'_{max}) and the viscous modulus (G''_{max}) (table 2), with the modification reveals a change in the starch structure that affects the particle-particle interaction. In fact, a strong negative correlation of G'_{max} and G''_{max} with the RS and the SDS content in the starch gel ($r<-0,89$; $p<0.001$) showing the formation of imperfect crystallites as mention before. Also, the decrease in amylose concentration by the modification could influence the decrease in G' and G'' of the starches. In addition, the deformability of starch granules during the HMT process [39] probably caused a remarkable G' and G'' reduction in HMT and dual-modified starches. The rheological behavior exposes that the modified banana starch can be used as a thickener or stabilizer in beverages.

4. Conclusion

The research demonstrated significant changes caused by the modification processes on the banana starch structure. The evidence suggests an improvement in the nutritional features of banana starch gels, especially with the HMT treatment. Although the HMT process significantly reduced the RS and SDS content in raw starch, this treatment enhanced the starch structure resistance, increasing the RS and SDS in the gelled starch. This improvement is also observed in the dual modification. Concerning the functional properties, HMT and dual modification produce weak gels with WAI, but these modifications also increased their WSI, WHC, OAC and gelatinization temperature. These results suggest that HMT or dual-modified banana starches can be used as functional ingredients in beverages and compotes. In general, this study allows the use of environmentally favorable and suitable for industrial use technologies such as HTM and enzymatic individual and dual modification to increase the nutritional properties of banana starch. Further research is required to improve the starch modification with other enzymes like pullulanase and amyloglucosidase, among others, to evaluate the effect of the type enzyme in the dual modification.

5. Credit Author Statement

Garofalo Ma. Angeles: Writing- Original draft preparation, Investigation, Formal Analysis; Villon P.: Investigation; Cornejo F: Conceptualization, Supervision, Funding acquisition, Writing-Reviewing and Editing; Rosell C.M.: Conceptualization, Writing- Reviewing and Editing

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Tables

Table 1. Starch composition of native and modified banana starches

Starch	Type of Modification	Native	HMT	Enzymatic	Dual
Granule	**Total starch (g/100 g)**	91.16±2.05[a]	90.31±1.62[a]	91.64±1.31[a]	89.76±2.71[b]
	Amylose content (%)	21.40±0.53[a]	21.01±0.51[ab]	20.47±0.76[b]	17.34±0.36[c]
	RS (g/100 g)	44.62±0.76[a]	16.62±0.94[c]	44.10±1.01[a]	26.66±0.50[b]
	RDS (g/100 g)	23.43±0.53[c]	40.45±3.58[a]	37.61±1.70[ab]	35.53±0.50[b]
	SDS (g/100 g)	21.72±0.16[c]	33.91±0.19[a]	11.16±0.46[d]	26.95±0.59[b]
Gel	**RS (g/100 g)**	3.10±0.07[d]	3.94±0.23[b]	3.44±0.10[c]	4.34±0.12[a]
	RDS (g/100 g)	87.92±1.71[a]	77.04±0.42[c]	83.17±0.57[b]	73.96±3.00[c]
	SDS (g/100 g)	2.58±0.47[c]	9.58±1.12[b]	3.40±0.90[c]	11.48±1.22[a]

RS: resistant starch; RDS: rapidly digestive starch; SDS: slowly digestive starch. Values in the same row with different letters differ significantly ($p<0.05$) (n=3). All results are given per 100 g of dry matter.

Table 2. Functional properties of native and modified banana starches

Type of Modification	Native	HMT	Enzymatic	Dual
***Hydration Properties**				
WHC (g/g)	1.64 ± 0.09[b]	1.90 ± 0.11[a]	1.90 ± 0.13[a]	1.85 ± 0.12[a]
WBC (g/g)	1.59±0.01[b]	1.74±0.09[a]	1.68±0.02[a]	1.31±0.03[c]
OAC (g/g)	2.13±0.02[c]	2.38±0.06[a]	2.30±0.05[b]	2.25±0.04[b]
WAI (g /g)	12.35±0.10[a]	6.96±0.13[c]	11.57±0.39[b]	5.72±0.18[d]
WSI (g /100g)	1.77±0.45[c]	2.35±0.20[c]	6.12±0.92[b]	24.18±1.35[a]
Gelation temperatures				
T_i (°C)	70.62±0.10[d]	80.58±0.98[b]	73.70±0.91[c]	83.66±0.42[a]

T_p (°C)	73.58±0.16^{d}	84.79±2.21^{b}	77.21±0.48^{c}	89.57±0.22^{a}
T_c (°C)	80.72±0.27^{c}	88.30±3.12^{b}	87.37±0.43^{b}	95.04±0.56^{a}
ΔH (J/g)	3.36±0.01^{b}	1.01±0.07^{d}	3.54±0.12^{a}	1.19±0.10^{c}
Ig (°C)	10.10±0.28^{bc}	7.72±2.14^{c}	13.52±1.14^{a}	11.37±0.98^{ab}
Freeze/thaw stability				
Cycle 1 (%)	56.57±4.37^{c}	58.13±3.31^{c}	63.20±3.88^{b}	74.37±0.85^{a}
Cycle 3 (%)	70.93±3.67^{b}	63.23±1.37^{c}	74.42±2.93^{a}	76.73±1.09^{a}
Cycle 5 (%)	76.03±2.63^{b}	65.68±1.00^{c}	77.23±3.20^{ab}	78.97±0.89^{a}
Gel Hardness (g4)	83.67±11.09^{a}	40.67±7.25^{b}	12.67±1.04^{c}	10.0±0.50^{c}
Rheological properties				
$G'_{máx}$ (Pa)	4963.5±25.5^{a}	2544.5±11.5^{c}	3501.00±49.00^{b}	2357.33±29.14^{d}
$G''_{máx}$ (Pa)	248.05±1.25^{a}	71.02±7.30^{c}	116.13±8.46^{b}	27.56±6.13^{d}

WHC: water holding capacity; WBC: water binding capacity; OAC: oil absorption capacity; WAI: water absorption index; WSI: water solubility index; ΔH: enthalpy; Ig: gelatinization range; G': storage modulus; G": loss modulus. Values in the same row with different letters differ significantly ($p<0.05$) (n=3) except for hydration properties (n=8). *Hydration properties are given in dry matter.

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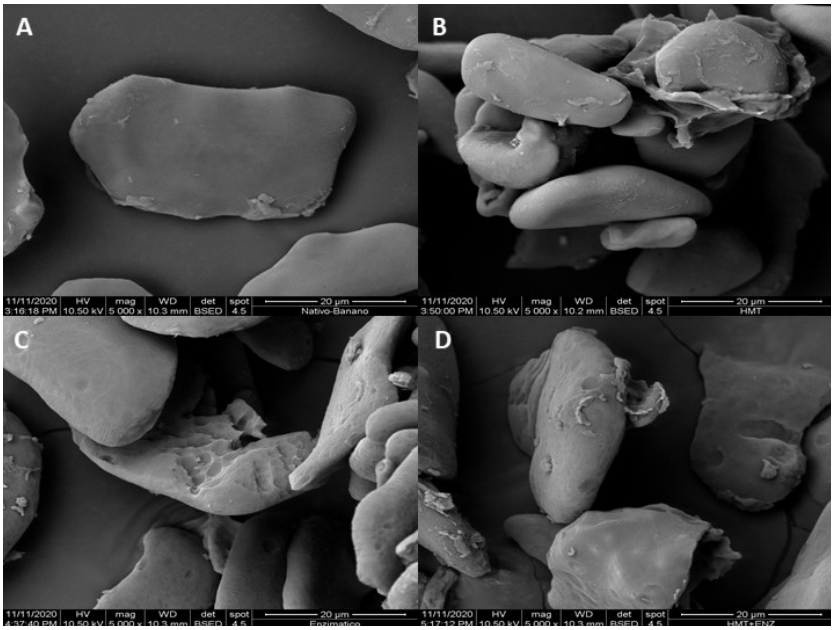

Figure 1. SEM images of native and modified banana starches. A: Native Starch; B: HMT; C: Enzymatic; D: HMT + Enzymatic.

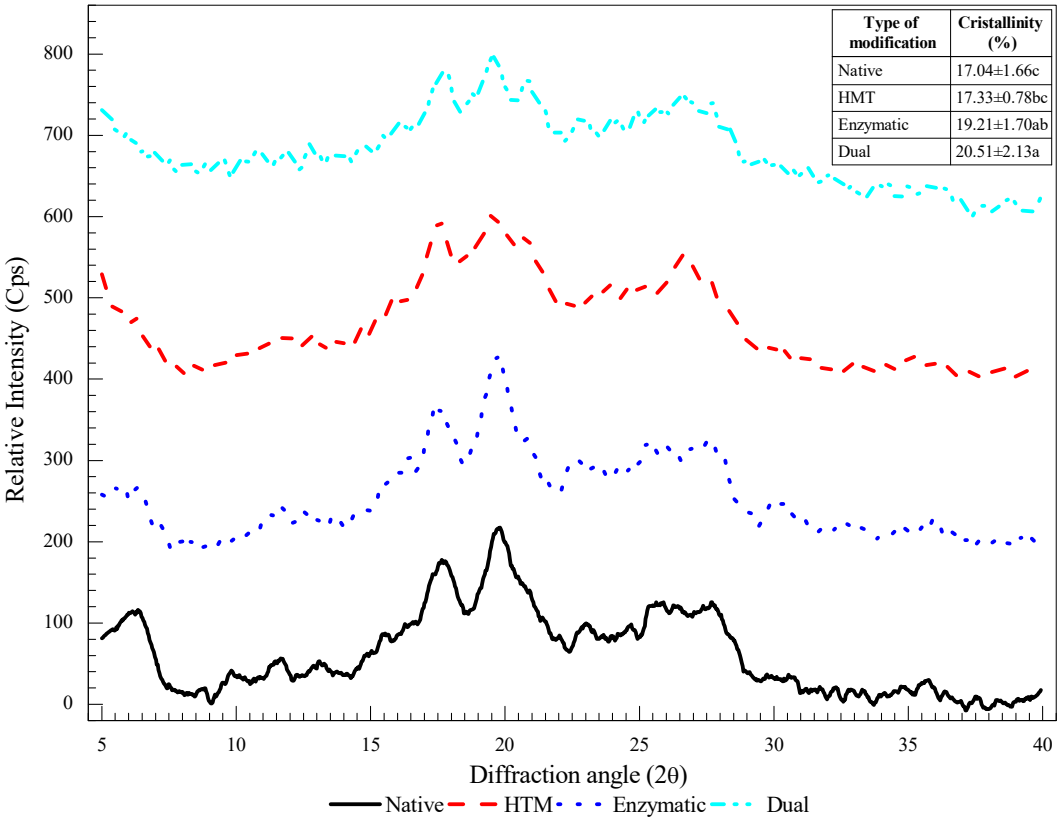

Figure 2. Diffractograms of native and modified banana starches.

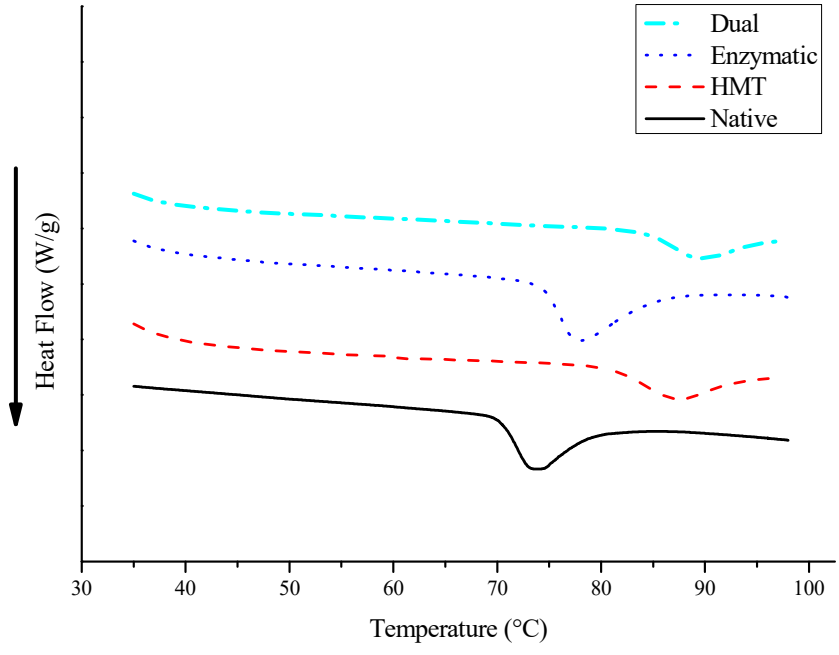

Figure 3. Endothermic curves of native and modified banana starches