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Effect of Combined Heat-Moisture/Lactic Acid Treatment on the Physicochemical and In Vitro Digestibility Properties of Corn Starch

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Native corn starch is subjected to dual heat moisture (30 g per 100 g d.b.)/lactic acid (10 g per 100 g d.b.) treatment (HMLAx) at different temperatures ($x = 90$, 110, 130, and 150 °C), and is compared with heat moisture (HMx) treatment alone. Neither HMLAx nor HMx change the crystallinity type (A-type) of the corn starch, but the relative crystallinity is lowered by 60–80%. Both treatments reduce the gelatinization enthalpy, but it is higher for HMLAx (≈70%), and induces reductions in rapidly digestible starch (up to ≈70%) and increases in slowly digestible starch (up to ≈35%) and resistant starch (RS) fractions. The variations are temperature dependent. A principal component analysis is carried out (9 formulations, 17 variables), showing that heat moisture and lactic acid act independently ($p < 55\%$) in the modification of starch, giving rise to different physicochemical (solubility, viscoelasticity, relative crystallinity), thermal, and in vitro digestibility properties.

1. Introduction

Starch is the main energy source for human physical activity. It is consumed as diverse food products such as bread, processed cereals, snacks, and many others. However, the increasing prevalence of metabolic syndrome worldwide and the array of adverse health conditions associated to it have called for the design of healthier starchy foods. The modulation of the digestibility via modifications of starch chains is an ongoing research topic.[1] The rationale behind this approach, is to slow down the amylolysis reactions that breakdown the starch molecules into sugars, in order to reduce their availability and the absorption glucose by the organism.[2]

Several approaches have been proposed for modifying the starch structure and, in this way, modulate the digestibility rate, including chemical methods such as oxidation, esterification, and etherification modifications.[3] For instance, 1-octenyl succinic anhydride has proven to increase the content of starch resisting enzymatic hydrolysis (i.e., resistant starch).[4] Mild hydrolysis via mineral acids (e.g., HCl) has led to important reductions in starch digestibility rates.[5] Despite their effectiveness, chemical modifications have become increasingly controversial as the use of chemical agents are under scrutiny by regulation agencies and consumers. Starch modification by organic acids, as an alternative to the use of mineral acids, has emerged in the recent years. In contrast to mineral acids, organic acids (e.g., lactic and citric) are regarded as safe for their use in food products. Furthermore, the underlying processes are sustainable and environmentally friendly as these acids are biodegradable by aerobic and anaerobic methods. Xie and Liu[6] showed that citric acid reacted with corn starch at different temperatures (120–150 °C, 3–9 h) to form starch citrates that resisted amylolytic reactions (type IV resistant starch). The effect of citric and lactic acids on starch digestibility and the link with starch molecular structure have been documented in the recent years.[7,8]

On the other hand, physical methods for modifying starch structure have been increasingly used. Heat-moisture (HM) treatment is one such method, which is deemed as a safe and green. HM treatment is carried out at temperatures above the gelatinization temperature of starch, under relatively low moisture content (≈35%). It has been shown that HM treatment can reduce the enzymatic hydrolysis rate by increasing the levels of slowly digestible starch (SDS) and resistant starch (RS) fractions.[9] Repeated heat-moisture treatment cycles had the ability of producing thermostable starch with reduced digestibility properties.[10] Variations of the basic HM method have been explored, showing that the formation of starch structures

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retarding or resisting amylolytic reactions depend on several factors, including moisture content, temperature, and treatment time.[11]

Van Hung and coworkers[12,13] assessed the combination of HM treatment and esterification on the modification of corn starches with different amylose contents, finding that resistant starch content was improved. The approach was further explored by Shaikh et al.[14] and Singh et al.[15] for starches from different botanical sources, corroborating that the dual treatment produced higher indigestible starch fractions than HM treatment alone.

The combined use of HM and organic acids for starch modification involves complex physical and chemical mechanisms. Organic acids can undertake cross-linking reactions with starch chains to form, that is, starch lactate and citrate. On the other hand, organic acids at temperatures of 100 °C and higher are sufficiently active to induce hydrolysis reactions. How these two mechanisms interact to produce modified starch with reduced contents of rapidly digestible starch (RDS) is an issue that has not been clarified at all. In particular, the role of the treatment temperature should be evaluated in order to design treatment methods oriented to produce starches with specified properties. In this regard, the aim of the present work was to assess the effect of using a combined heat-moisture/lactic acid treatment at different temperatures on the physicochemical and in vitro digestibility of normal corn starch, in contrast to HM treatment alone. Our results pinpointed that if relatively higher slowly digestible and resistant starch fractions are desired in comparison to the RDS fraction, and for achieving reduced starch digestibility, the combined heat moisture/lactic acid treatment should be rather used than the heat moisture treatment alone.

2. Experimental Section

2.1. Materials

Native corn starch (NS; CAS number 9005-25-8, amylose content 25.3%, moisture content 10.5%, pH 4.8; ash 0.5%, protein 0.1%), D-(+)-glucose (CAS 50-99-7, purity N 99.5%), DL-lactic acid (CAS 50-21-5), α-amylase from porcine pancreas (CAS A3176, pH 5.5–8.0, 51–54 kDa, 5 IU mg^{-1}) and amyloglucosidase (CAS A3306, > 300 u mL^{-1}) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents used were analytical grade and purchased from Metroquim (CDMX, Mexico). All water used in the experiments was deionized.

2.2. Heat Moisture/Lactic Acid Treatments

Lactic acid (LA; 20 g) and water (20 g) were mixed and the pH was adjusted to 3.5 with NaOH (10 M), with the help of a pH meter (model HI 8424, Hanna Instruments, Limena, Italy). The final volume was adjusted to 50 mL with water. LA solution (50 mL) was mixed with starch (50 g) in a stainless-steel tray and conditioned for 12 h at room temperature. The tray was then placed in a forced-air oven (SW-17TA, Blue-M, New Columbia, PA, USA) and heated at different treatment temperatures (90, 110, 130, and 150 °C) for 2 h to achieve a moisture level of about 5–10%. Each treatment was carried out in triplicate. The resulting mixture was washed five times with distilled water to remove unreacted LA and products of the treatment (e.g., sugars). The resulting pasta was dispersed in water and centrifuged (Model Z 513 K, Hermle Labortechnik, Wehnigen, Germany) at 6000 × *g* for 10 min. The supernatant was discarded, and the precipitate was dried in a forced air oven for a temperature of 35 °C until achieving a moisture content of 30 g per 100 g db, and finally ground. Lactic acid content in the ground solids was of 10 g per 100 g db. NS without lactic acid addition was prepared using the same procedure as control. Samples were coded as HM-LAx and HMx, respectively, where "x" stands for the treatment temperature.

2.3. Analysis Methods

2.3.1. Reducing Sugars

The total reducing sugars contained in the native and modified starch samples before washing were estimated by the 3,4-dinitrosalicylic acid method[16,17] using a solution of 10 mM glucose as standard.

2.3.2. Solubility

The solubility of native and modified starch samples was determined using the method described by Utrilla-Coello et al.[18] Briefly, starch sample was dispersed in distilled water (1 g per 100 mL), placed in centrifuge tubes and heated to temperatures 30, 40, 60, and 80 °C for 30 min, in a water bath under intermittent shaking. Afterward, the tubes were cooled down to room temperature and centrifuged at 3500 × *g* for 15 min. The supernatant was dried at 95 °C for 8 h. The solubility was estimated as

$$\text{Solubility}\,(\%) = \frac{W_{ds}}{W_s} \times 100 \quad (1)$$

Here, W_{ds} is the weight of the dried supernatant and W_s is the weight of the sample.

2.3.3. Morphology

The morphology of native and modified starch granules was assessed from images obtained by light microscopy. Details of the sample preparation and measurement parameters were done as described by Vernon-Carter et al.[19] To this end, starch sample was dispersed in distilled water (5 g per 100 mL) and allowed to stabilize at room temperature for 1 h. A drop of the dispersion was placed on a glass slide, covered with a coverslip, and observed with an optical microscope (Olympus BX45, Olympus Optical Co., Tokyo, Japan) coupled to an image analyzer system (digital Olympus camera C3030 and Image Pro-Plus version 4.5 software, Media Cybernetics, Inc., Rockville, MD, USA).

Further analysis of starch morphology was carried out by scanning electron microscopy (SEM) as described by Flores-Silva

et al.[20] Starch particles were mounted on carbon sample holders using double-side sticky tape and were observed using a JEOL JMS 7600F scanning electron microscope (Akishima, Japan) with the GB-H mode at 1 kV accelerating voltage. Micrographs at 2500× magnification are presented. Samples were not metalized since the microscopy equipment operates under ultra-vacuum conditions.

2.3.4. Viscoelasticity

The viscoelastic properties of gels formed by native and modified starch samples were obtained as follows. Starch dispersion (5 g per 100 mL) was heated at 95 °C for 20 min in a water bath, followed by cooling down at room conditions. The formed gel was allowed to equilibrate for 1 h prior to measurements in a Physica MCR 300 rheometer (Physica Messtechnik GmbH, Stuttgart, Germany). Strain sweep experiments were conducted isothermally (25 °C). Strain deformations (0.01–1000%) were scanned at 1 Hz. The storage (G′) and loss (G′′) moduli were recorded by means of the (US200/32 V2.50) equipment's software.

2.3.5. X-Ray Diffraction

X-ray diffraction (XRD) measurements of native and modified starch samples were conducted by the procedure described by Flores-Silva et al.[20] Measurements were conducted in an X-ray diffractometer (D8 Advance, Bruker, Germany) operated at 40 kV and 40 mA. Samples were scanned over an angular range of 4° to 40° (2θ) at a scanning speed of 0.05° min^{-1}. Relative crystallinity of the samples was calculated as follows:

$$\mathrm{RC}\,(\%) = \frac{A_c}{A_c + A_a} \times 100 \tag{2}$$

Here, A_c is the crystalline area and A_a is the amorphous area on the X-ray diffractogram.

2.3.6. Fourier-Transform Infrared

Fourier-transform infrared (FTIR) measurements of native and modified starch samples were carried out by following the procedure of Flores-Silva et al.[20] Measurements were obtained from a Perkin Elmer spectrophotometer (Spectrum 100, Perkin Elmer, Waltham, MA, USA) endowed with a crystal diamond universal ATR sampling accessory. The FTIR spectrum was reported as the mean value of five measurements for each sample. A numerical deconvolution procedure with Gaussian functions (half-width of 15 cm^{-1}, resolution enhancement 1.5) was carried out to obtain individual contributions for distinctive bands.

2.3.7. Differential Scanning Calorimetry

The thermal properties of native and modified starch samples were estimated by a differential scanning calorimeter equipment as described by Vernon-Carter et al.[21] Sample and distilled water with ratio 1:3 were weighed and placed in a 40 µL differential scanning calorimetry (DSC) aluminum pan. After sealing, the starch–water mixture was allowed to equilibrate at room temperature for 1 h. The pan was heated from 25 to 100 °C at 10°C min^{-1} to scan the thermal changes using a differential scanning calorimeter equipment (DSC-1 Mettler-Toledo, Columbus, OH, USA). An empty pan was used as reference. The gelatinization parameters (onset temperature T_o, peak temperature T_p, conclusion temperature T_c, and enthalpy ΔH) were estimated by means of the software of the equipment.

2.3.8. Amylose Content

The amylose content of native and modified starch samples was estimated by the method of Vernon-Carter et al.[21] Starch sample (500 mg) was mixed with 1 mL of ethanol and then dispersed in 10 mL of KOH (1 N) for 1 h at 35 °C to achieve complete dissolution. The volume was adjusted to 100 mL with distilled water in a volumetric flask. Subsequently, 2 mL of the resulting suspension were taken and 50 mL of distilled water and 100 µL of alcoholic phenolphthalein solution (1% w/v) were added. HCl (0.1 N) was added dropwise until a slight pink color was expressed. Then, 2 mL of iodine solution (2.0 g of potassium iodide and 0.2 g of iodine in 100 mL of distilled water) were added to the neutralized solution and adjusted to 100 mL in a volumetric flask. The dispersion was allowed to rest for 30 min for fully color development. The amylose content was calculated from the ratio of absorbance measurements at 620/510 nm. Scans were performed by 201 UV–vis Spectrophotometer (Spectronic Genesys 2, Thermo Electron Corporation, Madison, WI, USA).

2.4. In Vitro Digestibility

The procedure followed to conduct the amylolytic hydrolysis of native and modified starch samples is based on the traditional Englyst's test with some modifications as described by Flores-Silva et al.[20] The RDS was the starch hydrolyzed within the first 20 min of incubation, and the SDS the starch digested between 20 and 120 min. Undigested starch after 120 min was considered as the resistant starch (RS) fraction.[22] The kinetics of in vitro digestion was numerically fitted (i.e., least-squares) by the model proposed by Goñi et al.:[23]

$$C\,(t) = C_\infty \left(1 - \exp\left(-k_H t\right)\right) \tag{3}$$

Here, $C(t)$ is the total hydrolyzed starch, C_∞ is the equilibrium concentration, and k_H is the hydrolysis rate constant.

2.5. Statistical Analysis

All the experiments were conducted in triplicate. Experimental data were analyzed by means of analysis of variance (ANOVA) and reported as mean value ± standard deviation. Significant differences were estimated via Tukey's HSD test ($p < 0.05$) with the SPSS 22.0 statistical software for Windows (SPSS, Inc., Chicago, IL, USA). With the aim to evaluate the effects of the different treatment methods and temperature in the physicochemical and digestibility attributes of the modified starches, a principal component analysis (PCA) was conducted.

Table 1. Characteristics of the modified corn starches.

Sample	Reducing sugars [g per 100 g db]	Yield [g per 100 g db]	Amylose content[g per 100 g db]	Relative crystallinity [%]	995/1022 (-)	1047/1022 (-)
NS	2.27 ± 0.06^{g}	-	28.34 ± 0.61^{a}	27.43 ± 0.85^{a}	0.54 ± 0.02^{d}	0.33 ± 0.01^{e}
HM90	5.83 ± 0.07^{f}	96.14 ± 0.87^{a}	28.51 ± 0.57^{a}	22.58 ± 0.76^{b}	0.72 ± 0.02^{b}	0.36 ± 0.01^{d}
HM110	8.95 ± 0.11e	93.65 ± 0.91^{b}	27.62 ± 0.62^{a}	16.72 ± 0.82^{d}	0.78 ± 0.03^{ab}	0.43 ± 0.01^{b}
HM130	9.86 ± 0.12^{de}	92.42 ± 1.01^{bc}	27.03 ± 0.56^{a}	12.32 ± 0.63^{e}	0.71 ± 0.02^{b}	0.33 ± 0.01^{a}
HM150	10.74 ± 0.11^{d}	90.75 ± 0.86^{c}	27.87 ± 0.59^{a}	10.27 ± 0.58^{f}	0.66 ± 0.01^{c}	0.31 ± 0.01^{ef}
HMLA90	11.32 ± 0.12^{d}	92.21 ± 0.93^{bc}	27.01 ± 0.55^{a}	24.91 ± 0.75^{ab}	0.81 ± 0.03^{a}	0.47 ± 0.01^{a}
HMLA110	14.51 ± 0.13c	89.76 ± 0.94^{d}	27.13 ± 0.56^{a}	19.32 ± 0.78^{c}	0.78 ± 0.02^{ab}	0.40 ± 0.01^{b}
HMLA130	15.52 ± 0.21^{b}	84.53 ± 0.98^{e}	26.06 ± 0.37b	18.41 ± 0.85^{c}	0.77 ± 0.02^{ab}	0.39 ± 0.01^{bc}
HMLA150	23.16 ± 0.21^{a}	72.62 ± 1.02^{f}	25.21 ± 0.43^{c}	18.03 ± 0.77^{cd}	0.72 ± 0.02^{b}	0.35 ± 0.01^{d}

Values are means ± standard error, of three replicates; Superscripts with different letters in same column indicate significant differences ($p \leq 0.05$); NS: Native starch, HMX: Heat moisture treatment at "X" temperature, HMLAX: Heat moisture combined with lactic acid treatment at temperature "X".

3. Results and Discussion

3.1. Reducing Sugars and Starch Yield

Table 1 shows the reducing sugars content in the native starch and the modified starches in the treatment pasta (i.e., without washing). The reducing sugars reflect the amount of products released by effect of thermal stress and acidic hydrolysis. The reducing sugars for native starch was about 2.27 g per 100 g db and increased after heat-moisture treatment. However, the effect was more marked for the combined heat-moisture and lactic acid treatment, indicating that lactic acid induced hydrolytic fragmentation of starch chains. The effect was more pronounced as the treatment temperature was increased, probably due an increased hydrolytic activity of lactic acid. The starch yield after treatments is also displayed in Table 1, showing that in line with the production of reducing sugars, the amount of modified starch decreased with the treatment temperature. Interestingly, the starch loss by the combined effect of heat-moisture and lactic acid hydrolysis was as high as 27% for temperature of 150 °C.

3.2. Amylose Content

Table 1 presents the estimated amylose content for the native and modified starches. The native starch showed a content of about 28.34 g per 100 g db. With both treatments, HMx and HMLAx, amylose tended to decrease non-significantly with increasing treatment temperature, with the exception of treatments HMLA130 and HMLA150 that exhibited significant decreases. Decrease in amylose content can be linked to fragmentation of starch chains on the granule surface. Reduction of the apparent amylose content was previously documented for acidic hydrolysis of pea starch[24] and corn starch.[25] The effect was ascribed to the fragmentation of short-size amylose chains. In our case, the effect was more visible in the combined treatment, suggesting that acid hydrolysis took place in the granule bulk.

3.3. Morphology

The morphology of native and treated starches was assessed by light and SEM microscopy (**Figures 1** and 2). Starch granules treated with HMT at 90 and 110 °C retained the shape of native corn starch, with polygonal geometry and showing the birefringence pattern as reflected by the Malta cross (Figure 1a,b,e,f). However, the integrity of the starch granules was disrupted by HMT at 130 and 150 °C. Light microscopy images in Figure 1c and d exhibited starch granules with large cracks and fragments, caused maybe by thermal stress. The effect was more marked at 150 °C (Figure 1d) where starch granules showed extensive fragmentation and loss of birefringence pattern. SEM images in Figure 1g,h show that the granule surface suffered important degradation, with caves and fractures not shown by granules treated at lower temperatures. Kawabata et al.[26] reported that HM treatment produced cracked granules with fractures for potato and corn starches. The combined HMLA treatment had a more visible effect in the morphology of the starch granules (**Figure 2**). Even for low treatment temperature (90 °C, Figure 2a,e), some pasting material on the granule surface can be observed. The granule morphology was not disrupted and showed a clear birefringence pattern (Figure 2a). However, some outer material covering the granule surface was exhibited (Figure 2e). This suggests that, in addition to the thermal stress, the hydrolytic action of lactic acid disrupted the granule surface by eroding the external layers. The effect was more evident as the treatment temperature was increased to 110 °C (Figure 2b,f). The granule integrity was seriously affected, and granule fragmentation is clearly observed (Figure 2b), while only some birefringence pattern was retained. The outer material on the granule surface was significantly increased (Figure 2f). The treatment at higher temperatures led to a profound deterioration of the granule integrity. Figure 2c for 130 °C shows an interesting pattern of granule erosion as reflected by several concentric rings. In turn, this suggests that the hydrolytic action of lactic acid acted primordially at the hilum region of the starch granule, penetrating the granule structure and removing starch chains via fragmentation reactions. After drying, the removed material was deposited on the granule surface as a paste covering

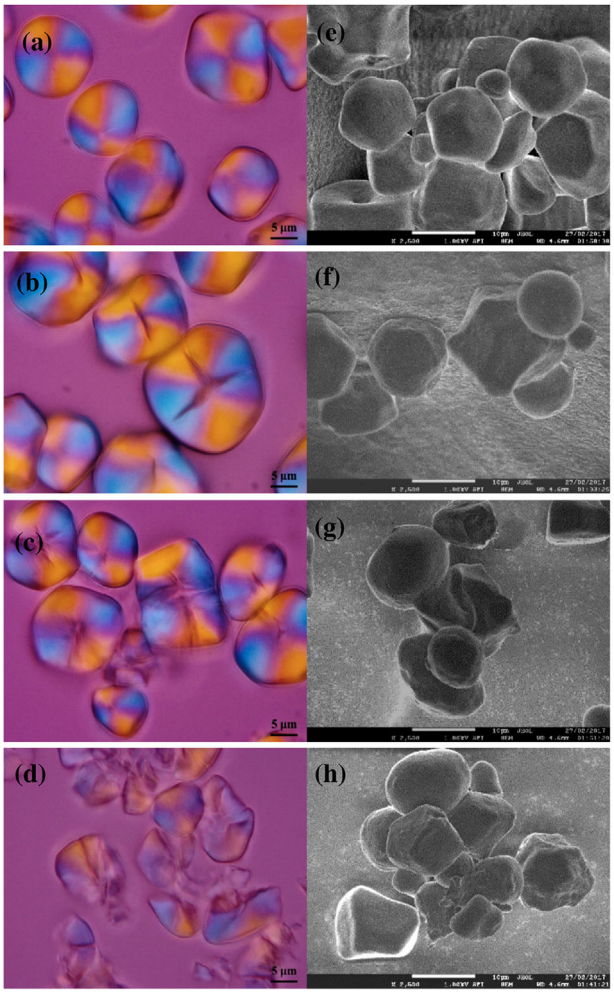

Figure 1. a–d) Light microscopy and e–h) SEM of heat-moisture treated corn starch granules: a,e) (90 °C), b,f) (110 °C), c,g) (130 °C) d,h) (150 °C).

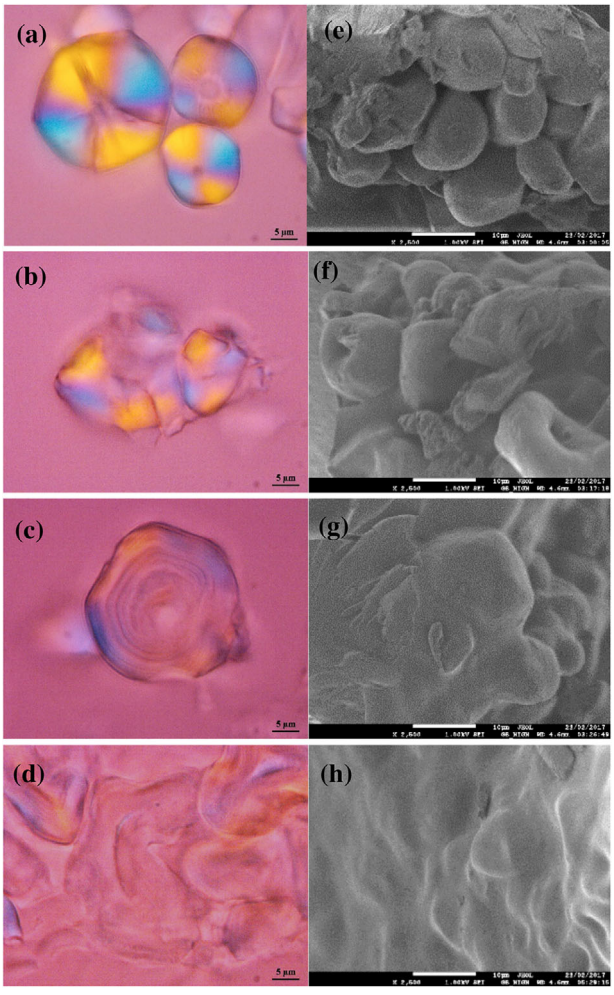

Figure 2. a–d) Light microscopy and e–h) SEM of combined heat-moisture/lactic acid treated corn starch granules: a,e) (90 °C), b,f) (110 °C), c,g) (130 °C) d,h) (150 °C).

the whole granule (Figure 2g). For the highest treating temperature (150 °C), the starch granule was completely disrupted (Figure 2d), leaving only residues of the birefringence pattern. In this case, the dried starch appeared as a paste without vestiges of the original granular structure (Figure 2h). These results are in line with previous reports showing that lactic acid had a strong effect on the breaking down of starch granules into smaller fragments.[9,12]

3.4. Solubility

Figure 3 presents the degree of solubility as function of temperature of the native and treated starches. Starches treated only with HM presented significantly lower degree of solubility as compared to the native starch counterpart. In general, the degree of solubility did not exhibit a well-defined trend respect to the treatment temperature. However, one can observe that the starch treated at 90 °C showed the lowest degree of solubility. In contrast, the starches treated with the combined method presented higher degree of solubility, which increased markedly as the treatment temperature increased. This behavior can be attributed to the high amount of short chain amylose generated by acid hydrolysis, which have high mobility and diffuse out of the granules during swelling.[27]

3.5. Viscoelasticity

The storage modulus (G′) of native starch exhibited a constant region for strain deformation values of up 30% (**Figure 4**a,b). At higher strain %, breakage of swelled starch chains took place with the consequent weakening of the gel structure and decrease in the modulus values. The loss modulus (G″) was about tenfold smaller than the storage modulus, indicative of the strong solid-like character of the starch gel. The loss modulus also exhibited a plateau region for strain deformation values of up 10%. However, the behavior was not monotonous decreasing as in the case of G′. In fact, a loss modulus overshoot region was exhibited for strain deformations from about 10% to 300%. This effect has been attributed to the jamming of insoluble remnants contained in the starch gel bulk.[28] Figure 4c (for HMx) and Figure 4d (for HMLAx) display the storage and loss moduli behavior in the

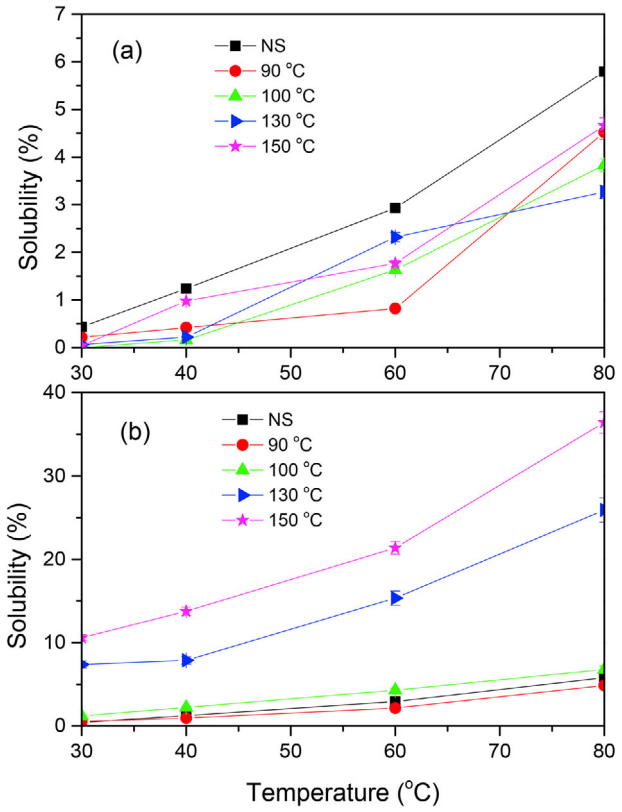

Figure 3. Solubility of native and modified starches as function of the heating temperature: a) heat-moisture and b) combined heat-moisture/lactic acid treatments.

strain independent region (i.e., plateau regions) as function of the treatment temperature. Both moduli tended to decrease with the treatment temperature for HMx, but a clear trend was not exhibited by the moduli for HMLAx (Figure 4d). While the gap between the viscoelastic moduli values tended to decrease with increasing temperature for HMx, the opposite behavior occurred for HMLAx. The reason for this behavior is not clear at all, but the combined thermal + hydrolytic stress played a different role than the thermal stress alone in the physicochemical changes of starch granules. Differences in the fragmentation sizes of starch chains and the rate of disruption of the chain-chain interactions might be behind the differences exhibited in the viscoelastic response of the gels treated by both methods.[29] Besides, HMT induced a larger reduction in the storage modulus, from about 50 Pa for native starch to about 30 Pa for modified starch, the effect being more pronounced at higher treatment temperatures. The combined treatment did not lead to significant reductions in the storage modulus values. This is indicative that the starch molecular organization was affected to a larger degree by thermal treatment than by the combined treatment, and that lactic acid probably had a buffering effect on the thermal treatment.

3.6. Crystallinity

Figure 5a presents the XRD pattern of native and HMx starches. The native corn starch showed A-type polymorph, with distinctive intensity peaks at 15, 17, 18, 20, and 23 2θ degrees. After HM treatment, the basic crystalline structure of the starch samples remained unchanged (A-type), a result that is consistent with previous reports showing that the temperature level of the heat-moisture treatment did not alter the crystalline pattern of starches with A-type polymorphism.[30] However, the relative intensity of the peaks exhibited visible variations with respect to the treatment temperature. Except the peak at 20°, the intensity of the XRD distinctive bands exhibited important reductions. The estimated relative crystallinity for native and HMx starches is presented in Table 1, where a negative trend with respect to the treatment temperature can be noted. In fact, while the relative crystallinity for native starch was about 27.43%, it was reduced to about 10.27% for HM150. It has been pointed out that the decrease of the relative crystallinity is expected as mobility of water molecules promote the disruption of intra- and intermolecular hydrogen bonding in granules,[31] resulting in this way in decreased regularity of the starch chain packing. On the other hand, the increase of the intensity peak at 20° has been linked to the formation of amylose-lipid inclusion complexes.[32] The HMLA treatment presented a similar trend in the crystalline structure to that exhibited by HM, that is, the treated starches presented A-type crystalline polymorphism (Figure 5b). This suggests that the hydrolysis reactions by lactic acid did not induce important changes in the crystalline structure of corn starches, but rather the most important changes were ascribed to thermal effects. However, the relative crystallinity values for HMLAx did vary from the values exhibited by HMx, with the former exhibiting higher relative crystallinity. This result is not surprising since the hydrolytic action of lactic acid had a higher impact in the amorphous regions of starch granules.[18]

Table 2. Thermal properties of native of the heat-moisture and combined heat-moisture/lactic acid modified starches.

Sample	T_o [°C]	T_p [°C]	T_c [°C]	ΔH [J.g^{-1}]
NS	58.34 ± 0.29^{f}	72.37 ± 0.61^{d}	88.94 ± 0.53^{c}	12.74 ± 0.37^{a}
HM90	71.26 ± 0.12^{e}	80.07 ± 0.57^{c}	91.55 ± 0.58^{b}	7.52 ± 0.22^{b}
HM110	73.72 ± 0.12^{cd}	83.13 ± 0.62^{ab}	92.33 ± 0.61^{ab}	6.41 ± 0.11c
HM130	72.04 ± 0.11^{d}	83.75 ± 0.56^{ab}	92.91 ± 0.42^{ab}	5.14 ± 0.12^{d}
HM150	74.32 ± 0.14^{c}	84.86 ± 0.59^{a}	91.32 ± 0.52^{b}	5.02 ± 0.13^{d}
HMLA90	75.15 ± 0.12^{bc}	82.22 ± 0.55^{b}	93.42 ± 0.43^{a}	6.36 ± 0.13^{c}
HMLA110	76.38 ± 0.12^{b}	83.76 ± 0.56^{ab}	92.38 ± 0.34^{ab}	5.34 ± 0.14^{a}
HMLA130	76.87 ± 0.13^{b}	84.06 ± 0.57^{a}	92.32 ± 0.42^{ab}	5.74 ± 0.18^{d}
HMLA150	78.85 ± 0.09^{a}	84.96 ± 0.53^{a}	91.38 ± 0.39^{b}	5.05 ± 0.15^{d}

Values are means ± standard error, of three replicates; Superscripts with different letters in same column indicate significant differences ($p \leq 0.05$); NS: Native starch, HMX: Heat moisture treatment at "X" temperature, HMLAX: Heat moisture combined with lactic acid treatment at temperature "X;" RDS: Rapidly digestible starch; SDS: Slowly digestible starch; T_o: Onset temperature; T_p: Peak temperature; T_c: Conclusion temperature.

3.7. Thermal Characteristics

The thermal characteristics of the native and treated starches estimated by DSC are shown in **Table 2**. The gelatinization onset, peak, and conclusion temperatures of HMx increased, and

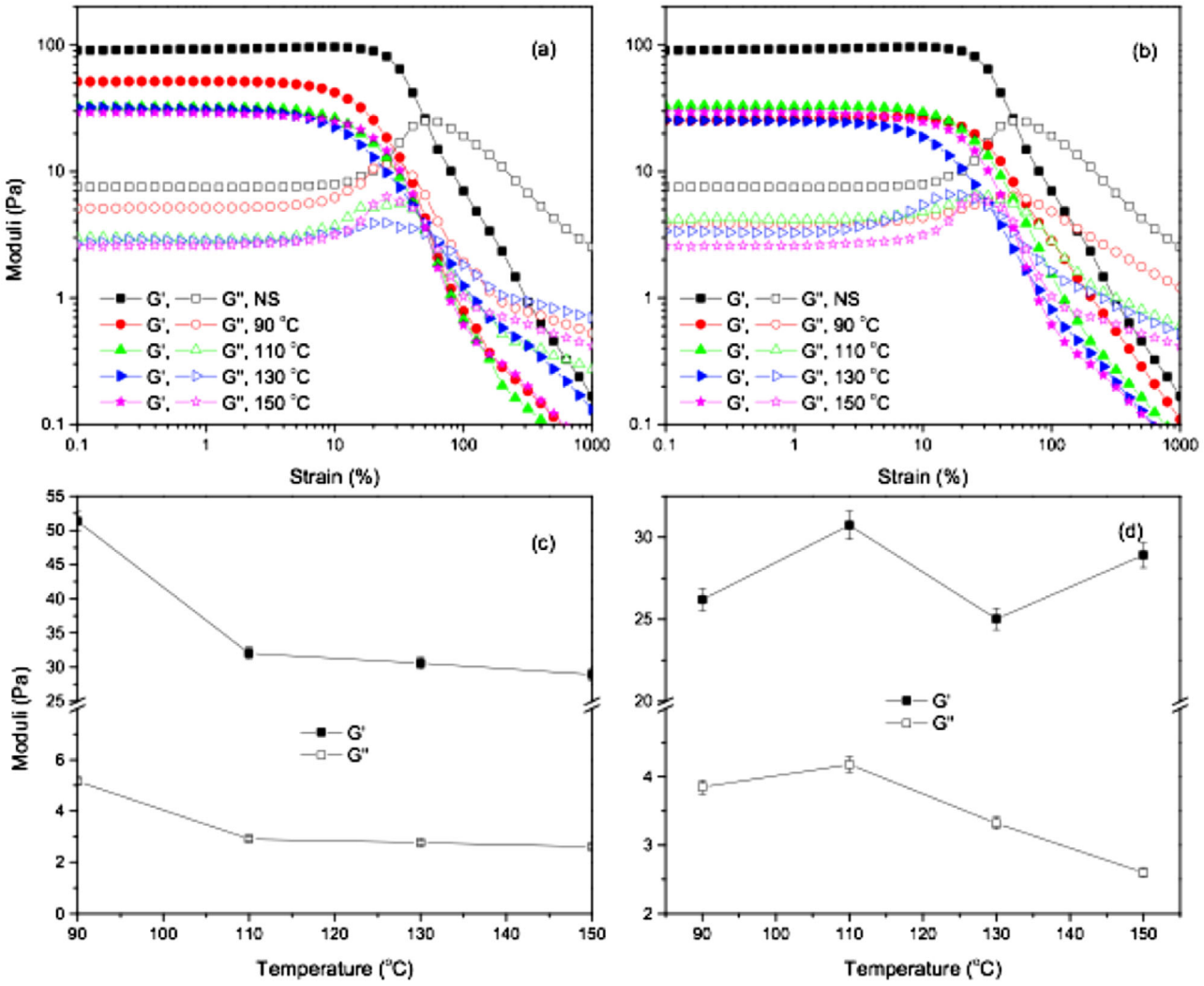

Figure 4. Strain sweep pattern of the native and modified starches obtained by a) heat-moisture and b) combined heat-moisture/lactic acid treatments. Variation of the storage (G′) and loss (G′′) moduli in the linear region for c) heat-moisture and d) combined heat-moisture/lactic acid treatments.

the enthalpy decreased as compared to that native starch. Besides, the gelatinization temperatures (onset, peak, and conclusion) increased with the treatment temperature, suggesting that the thermal treatment had a marked effect in the molecular organization of starch chains in the granule. Although the gelatinization temperatures also increased with the combined treatment, their increase was less pronounced as compared to those produced by HM treatment. Results in this line were reported in previous studies.[12,33] The increase of the gelatinization temperatures and the reduction of the gelatinization enthalpy have been attributed to the reduction of the amorphous regions by thermal stress and the dissociation of double-helix structures to form more complex ordered structures during the heat-moisture treatment.[33] The HMLAx treated starches presented a similar trend as that of the HMx starches. However, the peak temperature was slightly higher for the HMLAx starches, results which are in agreement with studies reporting that acidic hydrolysis attacks predominantly the amorphous regions of the granule, leading to an effective increase of the intra-granular structures.[18]

3.8. Fourier-Transform Infrared Analysis

The FTIR spectra of HMx and HMLAx starches are shown in **Figure 6**a,b, respectively. All the samples presented a broad peak at 3600–3000 cm^{-1} which is attributed to the absorption of hydrogen-bonded OH groups in starch structures.[34] This band is linked to the contribution of water molecules to starch structures. The intensity of the peak decreased after treatments, indicating the reduction of the amount of free water molecules in starch granules. The native starch showed a small peak at about 2930–2870 cm^{-1}, which is commonly ascribed to lipids attached to the granule surface.[35] Both HM and HMLA treatments reduced the intensity of the peak, an effect that could be ascribed to the formation of amylose-lipid inclusion complexes observed in the increased intensity of the 20 degrees XRD peak. The intensity band at 1700–1600 cm^{-1} is linked to Amide I expression of proteins. Interestingly, the intensity of the Amide I exhibited an important reduction after treatment, suggesting that the protein contained on the granule surface was disrupted or even removed

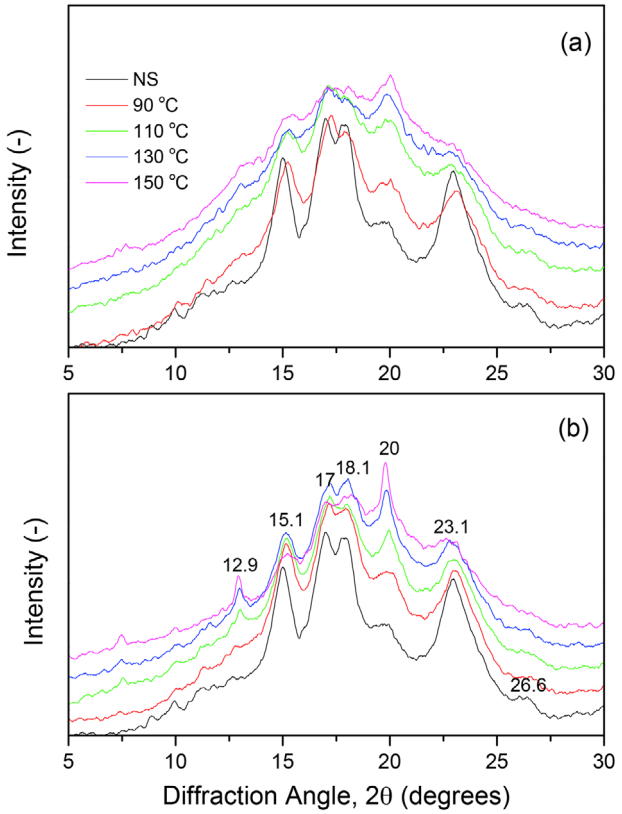

Figure 5. XRD pattern of the native and modified starches obtained by a) heat-moisture and b) combined heat-moisture/lactic acid treatments.

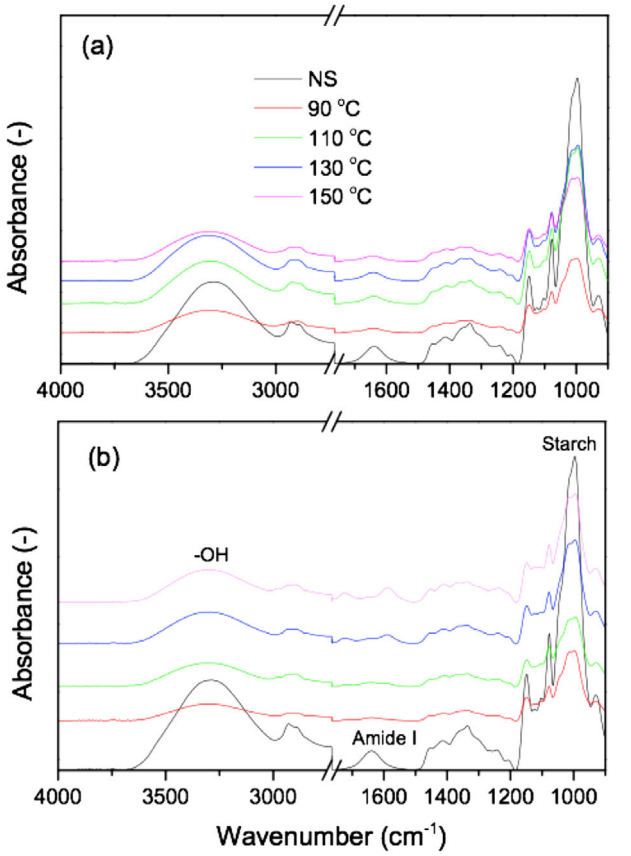

Figure 6. FTIR spectrum of the native starch and the starches obtained by a) heat-moisture treatment and b) combined heat-moisture plus lactic acid treatment.

by the effect of both treatments. The large intensity band at 1050–950 cm^{-1} is linked to starch molecular structures. The band at 1047 cm^{-1} reflects the amount of short-range ordered structures (e.g., double-helix), while the band at 1022 cm^{-1} is characteristic of amorphous domains. Also, the band at 995 cm^{-1} is water sensitive, reflecting intramolecular hydrogen bonding of hydroxyl group at the carbon C6 of the glycolic ring.[36] In this way, the ratio 1047/1022 is an index of the intermolecular short-range order in starch granules, while the ratio 995/1022 quantifies the amount of bound water in starch domains. The results presented in Table 1 show that the HMx showed a maximum in both ratios for HM_{110}, but the combined HMLA treatment induced a marked reduction of both FTIR ratios as the treatment temperature was increased. The XRD results showed that treatments reduced the relative crystallinity of starch granules, an effect that is linked to the disruption of the internal organization (e.g., growth rings) of the granules. In the opposite direction, the treatments increased the short-range ordering, an effect that might be reflecting the stabilization and formation of single- and double-helix structures.[37] It is noted that the combined heat-moisture and lactic acid treatment imprinted important modifications on the molecular organization of starch chains, as reflected by the 1047/1022 and 995/1022 ratios. However, the main contribution can be ascribed to the thermal effects, which led to significant increase of the short-range ordering relative to the native starch. Also, the treatment temperature produced a reduction of the short-range ordering, an effect that points to the partial acidic hydrolysis of the starch granules.

3.9. In Vitro Digestibility

The kinetics parameters of the starch enzymatic hydrolysis according to the model (3) are presented in **Table 3**. The kinetics rate constant k_H was 0.0463 min^{-1}, and the value decreased after the heat-moisture treatment. The smallest value was exhibited by HM_{90} (0.0265 min^{-1}), and increased gradually with the treatment temperature. Similarly, combined HMLA treatment, induced a reduction of the hydrolysis rate constant relative to the value for the native starch. However, the estimated values were smaller for HMLA than for HM, suggesting that the combined heat-moisture and lactic acid treatment had a more pronounced effect in the enzymatic hydrolysis of starch granules. The limiting hydrolysis C_∞ for native starch was 42.32 g per 100 g db. Heat-moisture and lactic acid treatments led to important reductions in the limiting hydrolysis extent. For both HMx and HMLAx, the smallest value of C_∞ was for treatment temperature of 90 °C. The estimated limiting hydrolysis was 23.07 g per 100 g db for HM_{90}, and 19.22 g per 100 g for $HMLA_{90}$, showing the marked impact of the combined treatment on the enzymatic fractionation of starch chains. The relative distribution of the digestible

Table 3. Hydrolysis kinetics parameters and in vitro digestible fractions of the native and heat-moisture and combined heat-moisture/lactic acid modified starches.

Sample	$k_H \times 10^2$ [min^{-1}]	C_∞ [g per 100 g db]	RDS [g per 100 g db]	SDS [g per 100 g db]	RS [g per 100 g db]
NS	4.63 ± 0.09^{a}	42.32 ± 0.61^{a}	34.58 ± 0.63^{a}	7.74 ± 0.17^{g}	57.68 ± 0.75
HM90	2.65 ± 0.12^{c}	23.07 ± 0.57^{g}	8.55 ± 0.58^{f}	12.52 ± 0.17^{f}	86.93 ± 0.87^{a}
HM110	2.72 ± 0.12^{c}	33.73 ± 0.62^{d}	15.33 ± 0.61^{c}	17.41 ± 0.21^{b}	67.26 ± 0.75e
HM130	3.04 ± 0.11^{bc}	36.75 ± 0.56^{c}	15.91 ± 0.42^{c}	18.14 ± 0.22^{a}	65.95 ± 0.68^{ef}
HM150	3.32 ± 0.14^{b}	39.86 ± 0.59^{b}	19.32 ± 0.52^{b}	19.02 ± 0.33^{c}	61.66 ± 0.86^{g}
HMLA90	3.15 ± 0.12^{a}	19.22 ± 0.55^{h}	8.42 ± 0.43^{f}	9.36 ± 0.23^{g}	82.22 ± 0.93^{b}
HMLA110	2.38 ± 0.12^{d}	27.76 ± 0.56^{b}	9.38 ± 0.34^{c}	18.34 ± 0.24^{a}	72.28 ± 0.83^{c}
HMLA130	2.26 ± 0.13^{de}	31.06 ± 0.57^{f}	12.32 ± 0.42^{e}	16.74 ± 0.28^{c}	70.94 ± 0.86^{cd}
HMLA150	2.05 ± 0.09^{e}	32.46 ± 0.53^{ef}	14.38 ± 0.39^{cd}	15.05 ± 0.25^{d}	70.57 ± 0.76^{cd}

Values are means ± standard error, of three replicates; Superscripts with different letters in same column indicate significant differences ($p \leq 0.05$); NS: Native starch, HMX: Heat moisture treatment at "X" temperature, HMLAX: Heat moisture combined with lactic acid treatment at temperature "X;" RDS: Rapidly digestible starch; SDS: Slowly digestible starch; RS: Resistant starch.

(rapidly and slowly) and resistant starch fractions is also shown in Table 3. In general, it was found that the resistant starch fraction was negatively correlated (0.96) with the limiting hydrolysis advance C_∞ estimated by Equation (3); namely, RS $\approx 100 - C_\infty$, all units in %. This means that the model (1) provided an accurate description of the hydrolysis kinetics. The native starch showed values of 34.58 and 7.74 g per 100 g db for RDS and SDS fractions. Heat-moisture treatment led to an important reduction of the SDS content, with the minimum value exhibited by the starch HM_{90} (8.55 g per 100 g db). The increase of the treatment temperature was accompanied by an increase of the RDS content, although the maximum value (19.32 g per 100 g db) was still smaller than the value estimated for the native starch. Regarding the SDS, the heat-moisture treatment increased the content of this fraction, with the highest value (19.02 g per 100 g db) presented by the starch HM_{150}. The combined HMLA treatment reduced to a greater extent the RDS fraction than the HM treatment for all treatment temperatures, but the highest gap in the RDS values between both treatments was at 90 °C, and decreased as temperature increased to 150 °C. The SDS fraction was increased, exhibiting values ranging from about 9.36 g per 100 g db for $HMLA_{90}$ to 15.05 g per 100 g db for $HMLA_{150}$. The SDS values exhibited by HMLAx tended to be lower than those for HMx the same temperature. The results described in Table 3 are in agreement with recent reports showing that the incorporation of organic acids in heat-moisture treatments has a significant negative impact on the in vitro digestibility of modified starches.[12,38]

3.10. Principal Component Analysis

PCA was carried out to assess the correlation between the different attributes of the modified starches. Eight treatments (HM_X and $HMLA_X$) and seventeen variables were considered, which were coded as follows: solubility (SOL), storage and loss modulus in the linear region (GS and GL), total sugars (TS), yield (Y), amylose content (AC), relative crystallinity (RC), 995/1047 FTIR ratio (R995), 1047/1022 FTIR ratio (R1047), onset, peak and conclusion temperatures (T_O, T_P, and T_C), enthalpy (DH), hydrolysis rate constant (KH), limiting hydrolysis (CINF), rapidly and slowly digestible starch fractions (RDS and SDS). The PC1 and PC2 accounted respectively for 36.30% and 22.33% of the total variance. PC1 and PC2 together accounted for 58.53%. The relatively low variance fraction of PC1+PC2 suggests that the combination of heat moisture treatment and lactic acid offers some degrees of freedom to produce modified starch with diverse physicochemical, thermal and digestibility properties. The loading plot is presented in **Figure 7**a, and shows the correlations among the properties and the starch digestion fractions. Two main clusters of variables can be identified. Interestingly, the SDS variable and the gelatinization temperatures (T_O, T_P, and T_C) are contained in the same cluster CL1. On the other hand, the RDS fraction is close to the variable KH (kinetics rate constant), an expected result since increased rates of enzymatic hydrolysis are linked to high content of RDS. It is interesting to note that relative crystallinity (RC) and solubility (SOL) are not aligned with the variables in clusters CL1 and CL2, which indicates that the modulation of these variables together with the FTIR ratio 1047/1022 can be taken as variables offering some degree of freedom to accommodate the characteristics of the modified starches via heat-moisture and lactic acid treatment. Figure 7b shows the distribution of the different starches on the PC1 and PC2 plane. The native starch (NS) is located far from all modified starches, showing that the combined treatment has a great impact on the starch properties. On the other hand, the starches modified by HM treatment are located along an arc close to the y-axis, indicating that these starches have similar PC1 component. That is, the differentiation of the effect of the HM_X starches is ascribed to secondary (i.e., refined) effects reflected in PC2. The distribution of the $HMLA_X$ starches is more concentrated in the second quadrant, and its distance from the native starch (NS) is higher than the distance for HM_X starches. In turn, this indicates that the incorporation of lactic acid to the heat-moisture treatment led to more profound changes on the physicochemical, thermal and digestibility properties of the corn starch.

The in vitro digestibility results presented in Table 3 showed a marked increase of the SDS and RS fractions by effect of the combined treatment. The PCA results in Figure 7 indicated that the SDS fraction was positively correlated with several

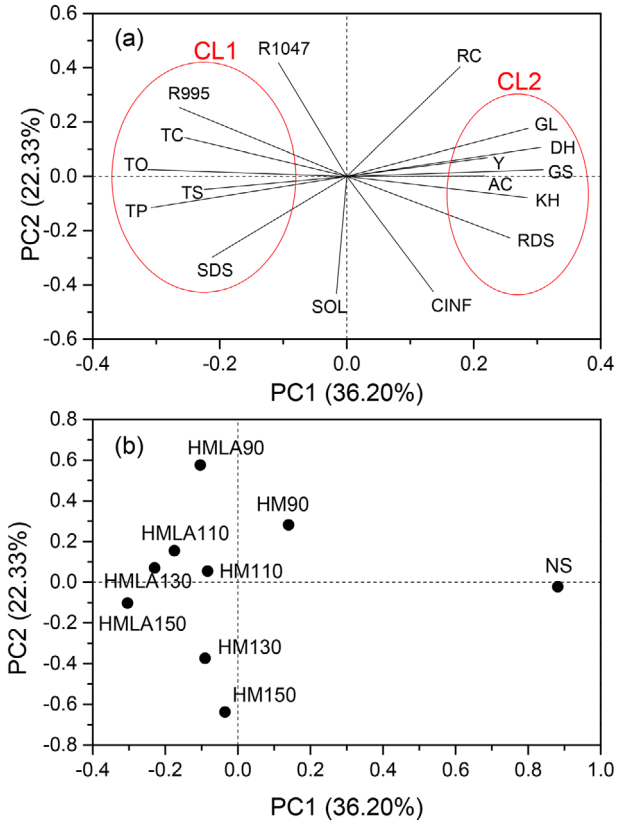

Figure 7. Loading plots obtained from principal component analysis of starches modified by heat-moisture and combined heat-moisture/lactic acid treatments. a) Properties and b) formulations.

physicochemical variables, including the FTIR 995/1022 ratio (R995) and the gelatinization temperatures (onset, peak, and conclusion temperatures—T_O, T_P, and T_C). The FTIR 995/1022 ratio designates the relative content of starch hydrated structures relative to starch amorphous structures. In this regard, the reduction of amorphous structures by effect of starch molecular hydration increases the resistance of starch chains to be degraded by amylolytic reactions. The reduction of amorphous starch structures is in turn reflected as increased gelatinization temperatures. In fact, temperatures T_O, T_P, and T_C are within the cluster of the SDS fraction, which in turn is an indicative of more ordered starch structures.[39]

The case of the maximum hydrolysis, represented by C_∞, is interesting since this variable is positively correlated with solubility. This is not surprising result given that solubilized starch chains are more accessible for amylolytic reactions than aggregated chains. Since RS $\approx 100 - C_\infty$, starch solubility is negatively correlated with the RS fraction, a result already analyzed in previous reports.[12,13] The relative crystallinity (RC) as quantified from XRD analysis is negatively correlated with SDS, meaning that ordered starch structures are less prompted to attack by amylolytic enzymes. However, the relative crystallinity (RC) and the maximum hydrolysis (CINF) are nearly orthogonal, which suggests that these variables are not correlated. That is, the relative crystallinity of the starch granules hardly affected the amount of starch that cannot be degraded by amylolytic enzymes. Interestingly, the FTIR ratio 1047/1022 (R1047) is negatively aligned with CINF, meaning that short-range ordered starch structures determine the extent of the amylolytic degradation, and hence the resistant starch content, a result that to the best of our knowledge has not been explored previously.

4. Conclusions

The temperature used for heat-moisture treatment had a profound impact on the physicochemical, thermal and in vitro digestibility properties of corn starch. The internal organization of starch granules was disrupted, which is reflected in marked reductions of relative crystallinity and gelatinization enthalpy. In contrast, the short-range ordering (e.g., single- and double-helix structures) of starch chains was increased as the temperature was raised. In general, the amylolytic susceptibility of the modified starch was reduced relative to the native starch. Overall, the results showed that temperature treatment offers some flexibility to obtain modified starches with different properties. The incorporation of lactic acid as an additional modifier agent widened the flexibility of the heat-moisture treatment, as demonstrated by a PCA of the properties of native and modified starches. That is, the incorporation of lactic acid (in general, organic acids) offers additional degrees of freedom as those introduced by the heat-moisture treatment alone.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

corn starches, heat-moisture treatment, lactic acid modification, principal component analysis, treatment temperature

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