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Combination of organic acids and heat-moisture treatment on the normal and waxy corn starch: thermal, structural, pasting properties, and digestibility investigation

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Abstract

Resistant starch (RS) has gained interesting because of its health benefits as the control of diseases, such as diabetes. Modifications in starches have been applied in order to increase RS content and consequently the range of industrial food applications. The heat-moisture treatment (HMT) combined with the addition of organic acids was the aim of this study, and also to evaluate the *in vitro* digestibility and other properties of corn starches. In both botanical sources, the RS content increased significantly, for the normal type and the waxy. Among organic acids used, citric, followed by lactic and acetic acid, promoted the most evident alterations and showed promising results in the increasing of RS. The results obtained by this combined method open opportunities for further applications in functional foods as well as starch based encapsulation process.

Keywords: resistant starch; modified starch; amylose content; in vitro digestibility.

Practical Application: Modified starches are excellent ingredients in food applications, contribute to texture and viscosity of the final product. In addition, starch can be an excellent contributor in increasing the content of insoluble fiber in food, with numerous benefits in control diseases.

1 Introduction

Corn is the main botanical source used for starch extraction, which provides over 80% of the global market (Wang et al., 2017). In recent decades, health habits have been growing trends in the life of some people. The constant struggle against diseases such as diabetes, cardiovascular problems and obesity has changed the lifestyle of the consumers, who are looking for foods that in addition to essential caloric needs, bring health benefits and contribute to the proper functioning of the body. Functional foods that stand out can improve general body conditions such as prebiotics and probiotics, lower the risk of some diseases and can even be used to cure them. Prebiotics, for example, are non-digestible food ingredients that selectively stimulate the proliferation or activity of desirable bacterial populations in the gut (colon), benefiting host health. Among currently known prebiotics, resistant starch is an excellent candidate to perform these functions (Zaman & Sarbini, 2016).

According to Englyst, Kingman and Cummings (Englyst et al., 1992) starch can be divided into three categories: rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). For example, RS is the portion that resists digestion and absorption in the intestines of healthy individuals and is available for fermentation in the body intestine.

However, the starch in its native form has limited technological properties. Starch modifications open new application possibilities, improving processing quality, gelatinization and retrogradation properties. In addition, physical and chemical modifications can cause changes in starch digestibility, bringing nutritional benefits, such as its prebiotic function. Research shows an excellent ability of resistant starch to act like fiber in terms of providing consumer satiety (Raigond et al., 2015).

Heat-moisture treatment (HMT), performed at a temperature above the gelatinization temperature with insufficient moisture to gelatinize the biopolymer has been investigated and the data show that the modifications are effective to improve SDS and RS levels compared to starches unmodified (Shaikh et al., 2019). Therefore, the present study investigated the combination of organic acids and heat-moisture treatment on the thermal, structural, pasting properties, and digestibility of normal and waxy corn starch.

2 Materials and methods

For the experiments, the normal (25.53% amylose) and waxy (0.77% amylose) corn starches were kindly donated by Ingredion Brazil LTDA. Porcine pancreatic α-amylase (E.C. 3.2.1.1, 8 x USP specifications, P7545) and *Aspergillus niger* amyloglucosidase

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(E.C. 3.2.1.3, A7095, ≥ 260U/mL) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The remaining reagents were of analytical grade.

2.1 Acid and heat-moisture treatment (HMT)

Following the methodology of Hung et al. (2016) with some modifications, 30g mass (dry basis) of each sample was suspended in volumes of different organic acid solutions (0.2 M lactic acid, 0.2 M acid acetic acid and 0.2 M citric acid) and deionized water. The addition was made to reach the 20% moisture level in pressure-resistant bottles identified with screw caps. Thereafter, the bottles were allowed to equilibrate for 24 h before being treated by HMT in an oven (Tecnal, TE 394/1, Piracicaba, SP, Brazil) at 110 °C for 9 h. The samples were neutralized with a 1.0 M sodium hydroxide (NaOH) solution and then washed with deionized water. All the samples were dried in an oven for 24 h at 40 °C. The samples were identified as follows: HMT H_2O (heat-moisture treatment with distilled water); HMT AA (heat-moisture treatment with acetic acid); HMT LA (heat-moisture treatment with lactic acid) and HMT CA (heat-moisture treatment with citric acid).

2.2 Thermal analysis - Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was performed using the DSC 60 (Shimadzu, Japan) with 99.99% purity standard indium, melting point at 156.6 °C and ΔH_{fusion} = 28.56 J/g. A mass of approximately 2.5 mg of each sample was weighed and homogenized in 10 µL of deionized water (1:4 mass: mass) in hermetically selected aluminum crucibles (Ito et al., 2018) Analysis conditions used were the following: 50 mL/min flow, heating range from 30 to 100 °C at a heating rate of 5 °C/min.

2.3 Powder X-ray diffractometry (PXRD)

The X-ray diffractograms (PXRD) were obtained using an Ultima IV X-ray diffractometer (Rigaku, Japan), and CuKα radiation (λ = 1,541 Å), with a voltage of 40 kV and an electric current of 20 mA. The scattered radiation was detected at an angular range of 3 to 40 ° (2θ) with a scanning speed of 2 °/min, and a step of 0.02 °. Equation 1 was used to calculate the relative crystallinity (Nara & Komiya, 1983).

$$Xc = \frac{A_p}{(A_p + A_b)}(100) \quad (1)$$

Where X_c refers to the relative crystallinity; A_p refers to the crystallinity area of the X-ray diffractogram, and A_b refers to the amorphous area of the diffractogram.

2.4 Pasting properties (Rapid Visco Analyser - RVA)

RVA-4 equipment (Newport Scientific, Australia) was used to evaluate the paste properties of starches. For this, a suspension in water 8% (w/w) of dry starch (28 g of total weight) was prepared and subjected to a heating cycle to 95 °C and controlled cooling under constant circular agitation (do Prado Cordoba et al., 2016).

2.5 In vitro digestibility

The *in vitro* digestibility was determined following the method from Demiate et al. (2016), with modifications. Starch (0.90 g) was suspended in 20 mL of sodium acetate buffer (0.1 M, pH 5.2). The samples were equilibrated at 37 °C and then hydrolyzed using 5 mL of an enzyme solution of porcine pancreatin extract and amyloglucosidase with continuous agitation of 100 strokes per min. For the measurement of the content of rapidly digestible starch (RDS) and slowly digestible starch (SDS), each aliquot of 0.25 mL was placed in 10 mL of 66% ethanol after 20 and 120 min of incubation. The results were determined using a glucose oxidase/peroxidase (GOPOD) assay kit (Megazyme, K-GLUC, USA). The portion that was not hydrolyzed at the end of 120 min was defined as resistant starch (RS).

The final hydrolysate was then denominated as the total glucose concentration (TG). The RDS, SDS and RS portions were calculated using the following equations 2, 3 and 4 (Demiate et al., 2016):

$$RDS = G_{20} x 0.9 \quad (2)$$

$$SDS = (G_{120} - G_{20}) \times 0.9 \quad (3)$$

$$RS = (TG - G_{120}) \times 0.9 \quad (4)$$

Where G_{20} and G_{120} are the percentual starch hydrolyzed after 20 and 120 min, respectively. The 0.9 is the conversion factor (from starch to glucose).

2.6 Statistical analysis

The results were expressed as mean ± standard deviation and were analyzed using Action Stat 3.3 software (Estatcamp, Sao Paulo, Brazil). One-way analysis (ANOVA) of variance was used to study the behavior of the samples under DSC, X-ray diffraction and viscoamylograph analysis. Tukey's tests were conducted to determine the differences between the means at a 95% confidence level ($p < 0.05$) (Ito et al., 2018).

3 Results and discussion

3.1 Thermal analysis - Differential Scanning Calorimetry (DSC)

The DSC curves included in Figure 1, demonstrate the thermal behavior of native (normal and waxy), and modified starches.

The curves obtained by DSC for starches present the endothermic event, related to starch gelatinization, in which a shift of this event to the right was observed in the treatments of HMT H_2O and HMT AA, for both starches, that is, increase in onset (T_o), peak (T_p) and event conclusion (T_c) temperatures (Figure 1). Similar behavior was also observed in normal and waxy corn starches (Jiranuntakul et al., 2011).

The increase in T_o, T_P and T_c (Table 1) can be attributed to structural changes in starch granules involving amylose-amylose and amylose-lipid interactions, suppressing the mobility of starch chains in amorphous lamellae; In other words, due to a new crystalline reorganization and formed bonds,

requiring higher temperature for this process to occur (Lacerda et al., 2014).

Increased conclusion temperature can be attributed to more stable lens formation during HMT (Chen et al., 2017). Another possible explanation for this behavior may be due to a restriction of water penetration in the granules by the new surface layer formed by HMT, delaying granule swelling (Jiranuntakul et al., 2011).

Before HMT treatment, normal and waxy corn starches showed a characteristic endothermic transition occurring between 60 and 80 °C, with waxy corn starch gelatinization enthalpy (Table 1). Gunaratne & Hoover (2002) reported that the value of ΔH_{gel} is representative of the number of double helices that unfold and rupture during gelatinization, so higher ΔH_{gel} values for waxy starch can be attributed to the presence of a larger number of double helices and almost no amylose (Gunaratne & Hoover, 2002; Y. Xie et al., 2019). Similar results were found in studies for corn (Chen et al., 2017), potato (Jiranuntakul et al., 2011) and sorghum (Shaikh et al., 2019) starches.

Modifications by HMT combined with organic acids caused significant changes in the thermal parameters obtained by DSC,

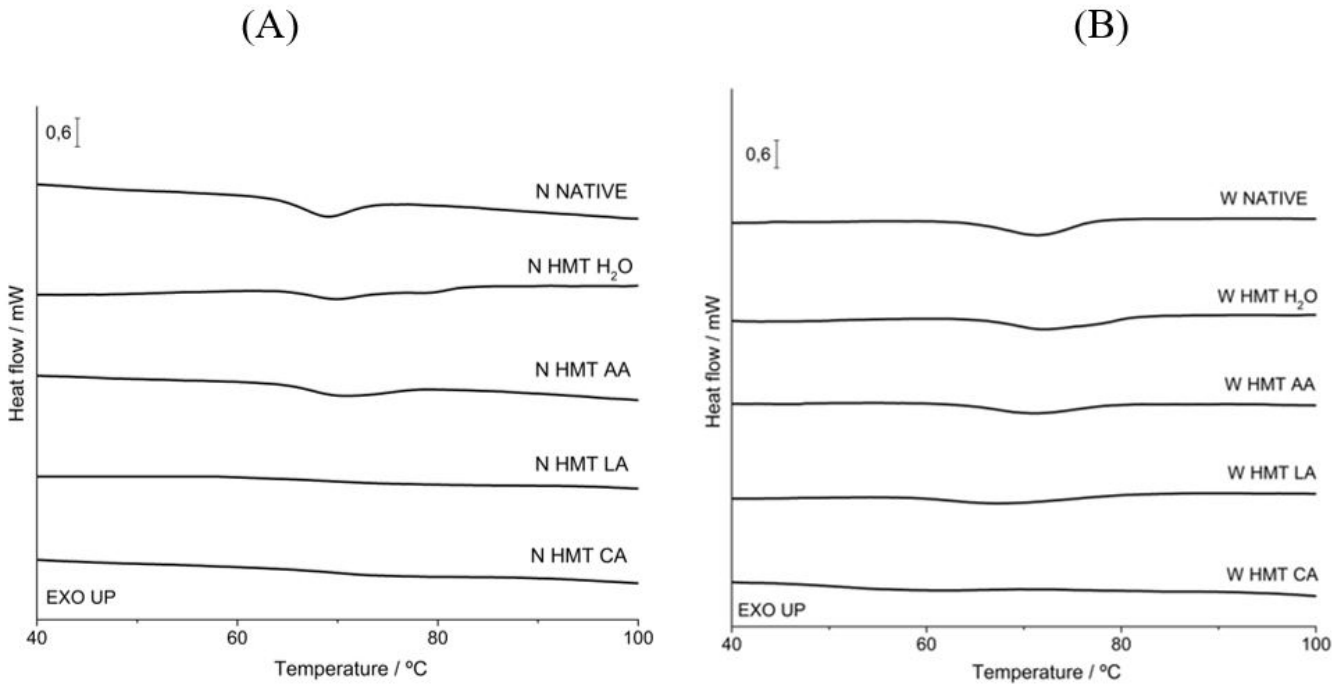

Figure 1. DSC curves of normal (A) and waxy (B) corn starches. N NATIVE - normal native corn starch, HMT H_2O - distilled water heat-moisture treatment, HMT AA - acetic acid heat-moisture treatment, HMT LA - lactic acid heat-moisture treatment and HMT CA - citric acid heat-moisture treatment; W NATIVE - waxy native corn starch, HMT H_2O - distilled water heat-moisture treatment, HMT AA - acetic acid heat-moisture treatment, HMT LA - lactic acid heat-moisture treatment and HMT CA - citric acid heat-moisture treatment.

Table 1. Thermal properties measured by DSC.

Treatments	T_o/ °C	T_p/ °C	T_c/ °C	ΔH_{gel}/ J/g	RC/ %
N Native	$63.6^{b} \pm 0.1$	$69.0^{c} \pm 0.1$	$73.3^{c} \pm 0.3$	$9.4^{a} \pm 0.1$	$29.50^{a} \pm 0.16$
N HMT H_2O	$65.0^{a} \pm 0.2$	$70.0^{b} \pm 0.1$	$82.6^{a} \pm 0.2$	$8.8^{b} \pm 0.1$	$26.04^{c} \pm 0.11$
N HMT AA	$65.3^{a} \pm 0.1$	$70.8^{a} \pm 0.1$	$77.5^{b} \pm 0.3$	$7.9^{c} \pm 0.3$	$27.30^{b} \pm 0.33$
N HMT LA	-	-	-	-	$25.23^{d} \pm 0.15$
N HMT CA	-	-	-	-	$25.30^{cd} \pm 0.20$
W Native	$63.9^{b} \pm 0.3$	$71.6^{c} \pm 0.1$	$77.8^{c} \pm 0.2$	$11.4^{a} \pm 0.5$	$32.60^{a} \pm 0.30$
W HMT H_2O	$66.2^{a} \pm 0.1$	$72.0^{a} \pm 0.1$	$81.4^{a} \pm 0.1$	$11.1^{a} \pm 0.1$	$29.85^{b} \pm 0.18$
W HMT AA	$64.6^{b} \pm 0.3$	$71.5^{b} \pm 0.1$	$79.7^{b} \pm 0.1$	$8.5^{b} \pm 0.2$	$27.90^{c} \pm 0.06$
W HMT LA	-	-	-	-	$28.70^{bc} \pm 0.15$
W HMT CA	-	-	-	-	$23.80^{d} \pm 0.25$

Note: N - normal corn starch; W – waxy corn starch; HMT H_2O - distilled water heat-moisture treatment, HMT AA - acetic acid heat-moisture treatment; HMT LA - lactic acid heat-moisture treatment, HMT CA - citric acid heat-moisture treatment. Values presented as mean values ± standard deviation. The values followed by the same letter in the same column are not significantly different by Tukey's test ($p < 0.05$). Statistics were evaluated between samples from the same botanical source. T_o – onset, T_p - peak temperature, T_c - conclusion temperature, ΔH_{gel} - gelatinization enthalpy, RC - Relative crystallinity.

being the most expressive data for modifications with lactic and citric acid. The endothermic peak gradually decreased or even disappeared with increasing acidic strength for both normal and waxy corn starch. This behavior corroborates with the results for wheat and cassava starch (Li et al., 2019; Mei et al., 2015). This phenomenon may indicate that acid esterification reaction affected starch crystallinity in order to increase the amorphous region of starch. These results confirm the decrease in RC observed by X-ray diffraction.

Li et al. (2019) further report that the absence of the endothermic event in the DSC curves in HMT-modified wheat starch samples with citric acid may have been caused by the formation of starch citrate monoesters during heat treatment. When heated by the DSC, it results in the formation of starch diesters, therefore forming a more resistant, i.e., cross-linking structure.

3.2 Powder X-ray diffractometry (PXRD)

Figure 2 and Table 1 show the X-ray diffraction patterns and relative crystallinity of native and modified starches. Native normal and waxy corn starches and their HMT-modified samples showed characteristic type A diffraction patterns (Figure 2), with principal peaks at approximately 2θ ≈ 15 °, 17 °, 18 °, 23 ° and a gentle peak at 20 °.

The combination of organic acids and HMT on the normal and waxy corn starch did not change the diffraction patterns, This fact was also observed in sweet potato (Hung et al., 2014), cassava (Van Hung et al., 2017), corn and sorghum (Shaikh et al., 2019) starches.

In contrast, in other researchers with potato (Van Hung et al., 2017) and yam (Hung et al., 2014) starch shifted from type B diffraction pattern after type HMT with addition of organic acids. It is possible to relate that the botanical source, amylose content and arrangement of starch structure influence the response to heat treatment. Double helices present in the type B starch chains are more mobile, that is, more prone to rupture than type A (Gunaratne & Hoover, 2002).

After HMT, it is possible to observe significant reductions in relative crystallinity (RC %) - Table 1. The results are consistent with studies using potatoes and cassava (Van Hung et al., 2017), and sweet potatoes (Xia et al., 2016). The combination of HMT with organic acids had a similar influence on crystal structure than in samples treated by HMT alone (HMT H_2O). The reduction in RC of the treated starches is in accordance with the results by the DSC.

The lowest values obtained for RC were in the treatments combining HMT with lactic and citric acid (Table 1). The influence of amylopectin on the crystalline portion of starch confirms the highest RC% values for waxy corn starches. These observations are consistent with the data found in granule type A and B wheat starches (Li et al., 2019), relating this phenomenon to a possible decomposition of starch crystal structure by citric acid.

3.3 Pasting properties

The combination of organic acids and HMT on the normal and waxy corn starch exhibited viscosity profiles shown in Figure 3.

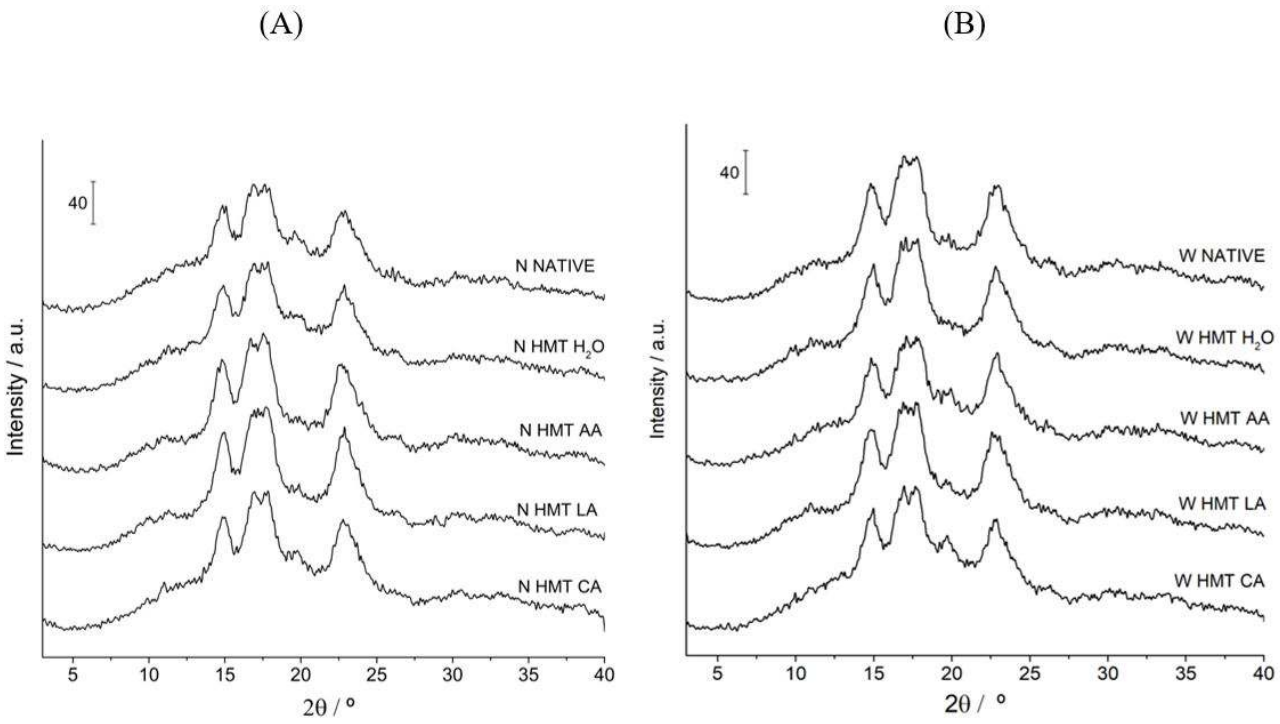

Figure 2. Diffractograms of normal (A) and waxy (B) corn starches. N NATIVE - normal native corn starch, HMT H_2O - distilled water heat-moisture treatment, HMT AA - acetic acid heat-moisture treatment, HMT LA - lactic acid heat-moisture treatment and HMT CA - citric acid heat-moisture treatment; W NATIVE - waxy native corn starch, HMT H_2O - distilled water heat-moisture treatment, HMT AA - acetic acid heat-moisture treatment, HMT LA - lactic acid heat-moisture treatment and HMT CA - citric acid heat-moisture treatment.

Comparing the viscosity profiles of the native samples, non-HMT-treated waxy corn starch obtained high peak viscosity, significant breakdown and low setback value (Figure 3B) when compared to the normal starch sample (Figure 3A). The waxy types of untreated corn, potato and rice starches also exhibited similar paste properties, and these drastic differences between normal and waxy starches attributed to the amylose, lipid and protein content, explain (Jiranuntakul et al., 2011).

Another discrepant parameter is the greater tendency for retrogradation by normal corn starch compared to waxy corn starch, observed by the setback. This fact occurs due to the higher amylose content in the normal starch granule, as it is known that, in the first stage of retrogradation, the main molecule involved is amylose, while amylopectin retrogrades more slowly after days of storage (Weber et al., 2009).

After HMT modification, the pasting temperature increased for water (HMT H_2O) and acetic acid (HMT AA) treated starches for both normal and waxy corn starch, the increase being greater for sample N HMT H_2O. According to previous studies Kaur and Singh (Kaur & Singh, 2019) this increase may be due to the formation of more crosslinking, lower swelling power and improved perfection of crystallites in the HMT, thus requiring more and more heat time for granule disintegration and paste formation.

The other parameters were also influenced by heat treatments. The peak viscosity, final viscosity, breakdown and setback values of all modified samples were lower than the normal and waxy corn starch samples. For samples modified with lactic and citric acid (HMT LA and HMT CA) the RVA equipment did not measure the pasting properties. Decreased pasting viscosity is caused by changes in the amorphous fraction (amylose interacting with amylopectin and branched amylopectin segments) in semi-crystalline and amorphous growth rings (Juansang et al., 2017). These amorphous fractions can be hydrolyzed under acidic conditions, so the starch chains move more freely. This flexible degradation movement can result in close packaging of starch chains, so the combination with HMT can absorb less water (Chatpapamon et al., 2019).

Structural differences such as the number of carboxyl and hydroxyl groups that organic acids have can also affect the hydrolysis of starch molecules to become a linear glucose polymer derived from amylose or amylopectin (Kim & Shin, 2011). The presence of more hydroxyl and carboxyl groups in lactic acid and citric acid may be one of the reasons for the most significant changes in thermal and paste properties in these treatments.

3.4 In vitro digestibility

The RDS, SDS and RS fractions of native and modified starches were tabulated in Table 2. In general, starch digestibility has changed significantly with the combination of HMT and organic acids.

Comparing the RDS, SDS and RS fractions of native samples, the RS content was higher for normal corn starch, about 62.60% compared to waxy corn starch with 49.21%. These differences may be related to the amylose and amylopectin content. (H. Xie et al., 2018) Zaman and Sarbini (Zaman & Sarbini, 2016) reported that higher amylose content of starches correlates with higher resistance to enzymatic digestion due to their compact linear structure. In our study, the RS and amylose showed a positive correlation ($r = 0.82$; $p < 0.05$), suggesting that resistant starch content was influenced by the amylose content. In addition, the presence of hydrogen bonding linking the amylose glycemic chain confers more resistance to enzymatic

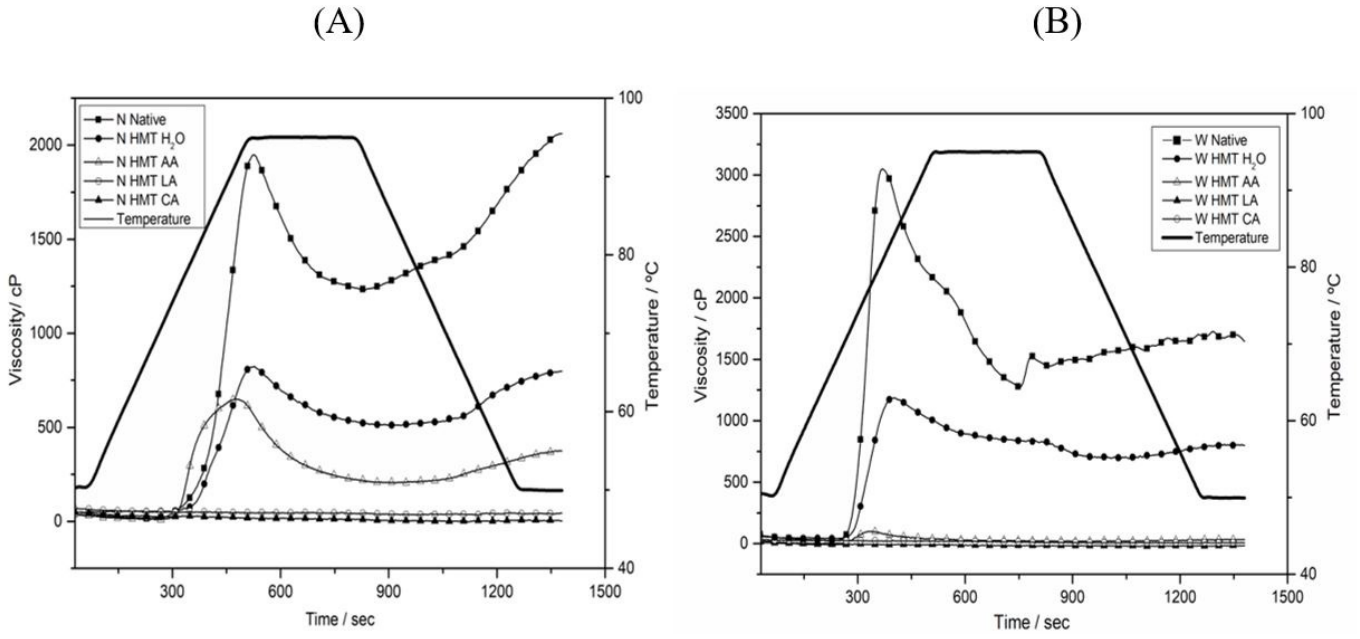

Figure 3. RVA profile of normal (A) and waxy (B) corn starches. N NATIVE - normal native corn starch, HMT H_2O - distilled water heat-moisture treatment, HMT AA - acetic acid heat-moisture treatment, HMT LA - lactic acid heat-moisture treatment and HMT CA - citric acid heat-moisture treatment; W NATIVE - waxy native corn starch, HMT H_2O - distilled water heat-moisture treatment, HMT AA - acetic acid heat-moisture treatment, HMT LA - lactic acid heat-moisture treatment and HMT CA - citric acid heat-moisture treatment.

Table 2. RDS, SDS and RS fractions of native and modified normal corn starches.

Treatments	RDS %	SDS %	RS %
N Native	$15.71^{a} \pm 0.36$	$21.70^{a} \pm 0.72$	$62.60^{d} \pm 0.36$
N HMT H_2O	$15.09^{ab} \pm 0.26$	$17.75^{b} \pm 0.01$	$67.16^{c} \pm 0.26$
N HMT AA	$15.16^{ab} \pm 0.17$	$17.33^{b} \pm 0.45$	$67.51^{c} \pm 0.27$
N HMT LA	$14.33^{b} \pm 0.12$	$14.50^{c} \pm 0.58$	$71.17^{b} \pm 0.46$
N HMT CA	$15.70^{b} \pm 0.17$	$7.60^{d} \pm 0.40$	$76.70^{a} \pm 0.23$
W Native	$14.22^{cd} \pm 0.13$	$36.57^{a} \pm 0.68$	$49.21^{c} \pm 0.81$
W HMT H_2O	$13.39^{d} \pm 0.31$	$34.05^{a} \pm 0.66$	$52.56^{bc} \pm 0.91$
W HMT AA	$26.14^{a} \pm 0.67$	$22.69^{b} \pm 0.57$	$51.18^{bc} \pm 0.10$
W HMT LA	$22.42^{b} \pm 0.40$	$22.81^{b} \pm 0.62$	$54.80^{b} \pm 1.03$
W HMT CA	$15.87^{cd} \pm 0.31$	$23.00^{b} \pm 0.66$	$61.15^{a} \pm 0.36$

Note: The values followed by the same letter in the same column are not significantly different by Tukey's test ($p < 0.05$). Statistics were evaluated between samples from the same botanical source. N - normal corn starch; HMT H_2O - distilled water heat-moisture treatment, HMT AA - acetic acid heat-moisture treatment; HMT LA - lactic acid heat-moisture treatment, HMT CA - citric acid heat-moisture treatment.

activity; However, impact factors on digestibility are not limited to amylose content alone, but it is a plausible explanation for such comparison (Zaman & Sarbini, 2016).

After HMT, the behavior of N HMT H_2O and W HMT H_2O samples were similar. RDS and SDS content decreased and RS increased significantly for both. RS content increased from 62.60% to 67.18% 66.24% and from 49.21% to 52.56% for normal and waxy corn starches, respectively, after HMT. Similar trends were found for potato and cassava starches also treated by HMT (Van Hung et al., 2017). Hung et al. suggests that the increase in RS may be due to interactions formed during HMT, partially restricting the accessibility of starch chains to hydrolysis enzymes (Hung et al., 2016). HMT can be used to increase resistant starch content without disruption of granular structure, reports Cheng et al. (2019).

In addition to organic acids, the changes in the RDS, SDS and RS fractions were more intensified and proportional to the acid intensity, being the most significant values in citric acid treatments, which contains more hydroxyl and carboxyl groups. Citric acid, when heated, dehydrates and produces an anhydride, this citric anhydride may react with this polysaccharide to form citrate starch and alter its properties, especially its digestibility. Mei et al. (2015) reported increasing RS content in cassava starches with increasing concentration of citric acid added to this biopolymer, as more hydroxyls in their molecules were replaced by citric anhydride to form citrate starch, which was composed of the crosslinked structure. This structure could resist enzymatic hydrolysis.

For normal corn starch, SDS content decreased and RS increased on significant values after adding organic acid treatments ($p < 0.05$). Similar performance was observed in waxy corn samples which increased their RS fraction from 49.21% in W Native to 61.15% in W HMT CA. In general, the decrease in SDS in samples modified by HMT and organic acids may have occurred due to the rupture of the double helices that form the starch structure, evidenced by the decrease in gelatinization enthalpy (Chung et al., 2009).

The best impact on citric acid RS content followed by lactic acid in this study is from previous research where the resistant starch fractions in potato and cassava starches (Van Hung et al., 2017) and yam and sweet potato starches (Hung et al., 2014) were superior to their native samples after modifications by HMT and lactic and citric acids.

The authors mentioned, attributed this performance to partial starch hydrolysis, which caused movement and rearrangement of the molecules; therefore a better-observed RS yield (Van Hung et al., 2017). Shaikh et al. (2019) also evaluated the influence of HMT combined with lactic and citric acid on the digestibility of corn and sorghum starches and reported the partial acid hydrolysis as a possible response to this increase. In addition esterification by citric acid may promote the formation of a cross-linked structure more resistant to enzymatic hydrolysis.

Li et al. (2019) evaluated esterification by citric acid in starches. In their study, wheat starch from granules type A and B underwent modification by citric acid followed by HMT and the results corroborate with previous research. High levels of RS were found, attributed to the formation of starch diesters that can resist enzymatic hydrolysis.

In addition to esterification, short and branched starch chain interactions generated by citric acid hydrolysis during HMT can resist digestive enzyme hydrolysis and, consequently, increase RS content.

4 Conclusion

The initial proposal for the evaluation of digestibility becomes of great value, due to the increase in the resistant starch content, especially in the modifications with lactic and citric acid, both normal and waxy corn starch. Foods with high levels of resistant starch can be considered functional foods, acting in the control of blood glucose and the prevention of chronic diseases such as diabetes and obesity.

Applications can be studied for the use of modified starches, bringing nutritional benefits to those who consume them. Modification by HMT, moreover, is a green technology and the combination with organic acids, of food use, keeps the proposal of preservation environmentally and ecologically correct.

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