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EFFECT OF ULTRASOUND ON THE THERMAL, STRUCTURAL, PASTING AND MORPHOLOGICAL PROPERTIES OF *MARANTA ARUNDINACEA* STARCH

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ABSTRACT

Starch extracted from maize, rice and potato is widely used in various sectors of different industries. However, other alternative sources have proved equally interesting for industrial applications, arrowroot being one of them. The starch of this rhizome has been studied, especially when subjected to modifications, such as physical by means of ultrasound, and its technological properties should be investigated. Therefore, the objective of this work was to evaluate the thermal, structural, morphological and paste properties of arrowroot starch modified by ultrasound. It was observed that the modification of arrowroot starch caused a decrease in the initial gelatinization temperature, and gradual reduction of thermal stability at temperatures of 270 ºC. In the analysis of the paste properties, the sonicated samples showed a greater tendency to retrogradation and syneresis, and higher final viscosity. Regarding structural properties, a decrease in relative crystallinity was observed. The morphology of the granules was little affected by the ultrasonic waves. Thus, it is stated that ultrasonic modification was able to alter some properties of arrowroot starch.

1.Introduction

Starch is the main carbohydrate reserve of plants. It is found mainly in seeds, roots, stems, tubers, leaves and fruits. The starch granule is composed of amylose and amylopectin chains, alternating in amorphous and crystalline regions, which influence the crystallinity of the granules (Lorenzo et al., 2022; Ai, Jane, 2015). The granules have different shapes and sizes, which vary according to the species and the developmental stages of the plant (Paes et al., 2019).

In the presence of water and with increasing temperature, starch undergoes the gelatinization process. This process causes changes in the structural organization and loss of crystallinity of the granules. As the granules absorb water, they swell and may deform, causing the amylose to leach out, forming a fluid paste. With decreasing temperature, a partial reorganization of amylose and amylopectin occurs, a step called retrogradation (Shenglin et al., 2023; Fan et al., 2018).

As far as its application is concerned, starch is widely used in the textile, chemical,

pharmaceutical, and paper industries. This is due to its versatility, easy availability, and low cost. In the food industry, starch is used as a gelling, thickener, emulsifier and encapsulant agent. This natural biopolymer is mostly obtained from corn, cassava, wheat, potato and, to a lesser extent, rice (Afolabi et al., 2012; Watershoot, Gomand, Fierens, 2015; Liu, Xu, 2019). Studies point to a search for new sources of starch with potential application in the industry. In this aspect, tropical countries have an advantage in relation to the main world producers of starch, located in temperate regions, due to the variety of tropical starch crops (Maniglia, Tápia-Blácido, 2016; Tarique et al., 2021). In this sense, arrowroot, classified as a rhizome, can be considered an unconventional source of starch, containing 25-30% of this polysaccharide.

Starch is rarely used in its native form due to limitations such as low stability to heating and shear, as well as higher tendency to retrograde. Thus, starch is modified to improve its characteristics, through chemical, enzymatic, or physical methods (Ai, Jane 2015; Hoover, 2010; Kupervaser et al., 2023). The application of ultrasound is a physical method that has been widely researched and applied in the food industry, as well as to modify starches. This technology is considered environmentally friendly, since it does not use chemicals, nor the generation of polluting waste (Sukja, 2017). Ultrasound consists of mechanical acoustic waves with a frequency above the threshold of human hearing (>15 - 20 kHz) (Vela, Villanueva, 2023; Sukja, Janroz, 2013). Ultrasonic treatment can promote changes in the morphology of starch granules through the phenomenon of cavitation, causing cracks, depressions, or pores on the surface. Such structural damage can modify the amorphous and crystalline parts of the starch, making it more permeable to water. Changes can also occur in the thermal stability as well as in the paste properties and crystallinity of the starch granules (Sukja, 2017, Zheng et al., 2013; Zhu, 2015). The effect of ultrasound on granules depends on the type of starch and the conditions used such as: power, frequency, temperature, and treatment time (Zhu, 2015; Obadi et al., 2021). These changes can result in obtaining starches with different technological properties of industrial interest (Bernardo, Ascheri, Carvalho, 2016; Zheng et al., 2013).

Thus, the aim of this study was to evaluate the thermal, structural, morphological and paste properties of a commercial arrowroot starch modified by ultrasound.

2. Materials and methods

2.1. Materials

2.2.1.Samples

Arrowroot starch (HEMA, lot 1903LOJ) was purchased from a local market in Curitiba-PR, Brazil.

2.2.2.Starch Modification

The commercial starch sample was modified by ultrasound according literature (Yu et al., 2018) with some modifications: Solutions containing 12 g of starch and 200 mL of deionized water were subjected to ultrasonic treatment (Vibra-Cell 500W - Sonics & Material Inc, USA) with an ice bath to maintain a temperature of 25 °C in order to avoid gelatinization of the starch during the process. The probe (25 mm) was immersed in the solution and four tests at a constant frequency of 20 kHz were performed, with amplitudes of 50 % and 100 %, at times of 25 and 50 min (25’ and 50’). After, the suspension was centrifuged (8000 rpm, 10 °C) and the starch was oven dried at 35 °C for 24 h. To perform the analyses, the samples were named as follows: commercial without modification (A), 25'50 % (B), 25'100 % (C), 50'50 % (D), 50'100 % (E).

2.2.3.Differential Scanning Calorimetry (DSC)

To obtain the differential scanning calorimetry curves of the native and modified starch fractions, the methodology described by Bet et al. (2018).

2.2.4.Thermogravimetry (TG)

To obtain thermogravimetric curves of native starch fractions and modified modifications, the methodology described by Bet et al. (2018).

2.2.5.X-Ray Powder Diffractometry (DRX)

The diffraction pattern of native and modified starches was determined by adapting the method described in Kuk et al. (2017).

2.2.6.Pasting Properties (RVA)

To obtain the mass profile of the samples, a viscoamylograph, model RVA-4 (Newport, Australia), was used. The methodology proposed by Ito et al. (2018) was used with some adaptations.

2.2.7.Field emission gun-scanning electron microscopy (SEM-FEG)

The diameter and shape of the starch granules were observed by using Scanning Electron Microscope with Field Emission Gun (SEM-FEG) model MIRA 3 (Tescan, Czech Republic). The diameter and shape of the starch granules were observed using a Scanning Electron Microscope with Field Emission Gun (SEM-FEG) model MIRA 3 (Tescan, Czech Republic). Using the methodologies described by Hornung et al., (2017) and Ito et al., (2018).

2.2.8. Statistical Analysis

Instrumental results were performed by analysis of variance (ANOVA) on data obtained in triplicate, considering the analysis of difference between samples with a confidence level of 95% ($p<0.05$). Variations between treatments were evaluated by Tukey's test ($p\leq0.05$). All statistical analyses were performed using Action Stat software version 3.3 (Estatcamp, São Paulo, Brazil).

3.Results and discussions

3.1.Differential Scanning Calorimetry (DSC)

The DSC curves are represented in Fig. 1, in which the gelatinization of the native and modified arrowroot starch samples was evaluated. According to Biliaderis, Maurice (1980), the endothermic process of gelatinization occurs with starch granules in the presence of water when both are subjected to heating.

From Fig. 1, it is possible to observe a similarity between the endothermic curves representing the phenomenon of starch gelatinization. During the gelatinization process, the amorphous regions of the granules undergo hydration as they absorb water, resulting in disorganization of the crystalline structure and the release of hydroxyl groups. Table 1 shows the temperature range values obtained for gelatinization to occur.

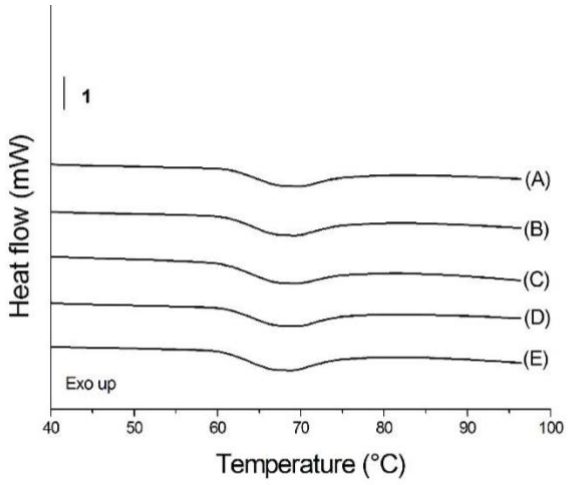

Figure 1. DSC curves of native commercial arrowroot starch (A), commercial modified 25'50 % (B), commercial modified 25'100 % (C), commercial modified 50'50 % (D), commercial modified 50'100 % (E).

Table 1. DSC and Relative Crystallinity (RC) results for samples of native commercial arrowroot starch (A), commercial modified 25'50 % (B), commercial modified 25'100 % (C), commercial modified 50'50 % (D), commercial modified 50'100 % (E).

	To /°C	Tp /°C	Tc /°C	ΔH_{gel} /Jg^{-1}	RC (%)
A	61.8±0.1^{a}	68.0±0.1^{a}	75.2±0.3^{ab}	6.84±0.2^{c}	29.4 ± 0.8^{a}
B	61.1±0.1^{b}	67.7±0.0^{b}	75.4±0.3^{ab}	8.11±0.2^{ab}	26.6 ± 0.7^{b}
C	61.0±0.2^{b}	67.5±0.0^{c}	76.1±0.4^{a}	8.25±0.3^{ab}	27.1 ± 0.7^{b}
D	61.1±0.1^{b}	67.6±0.1^{bc}	75.2±0.6^{ab}	7.73±0.5^{b}	27.0 ± 0.6^{b}
E	60.7±0.2^{c}	67.2±0.0^{d}	75.0±0.2^{b}	8.51±0.3^{a}	27.1 ± 0.6^{b}

*Values with the same letter subscribed in the same column do not show statistical difference between them by the Tukey Test ($p<0.05$). T_o (onset temperature), T_p (peak temperature), T_c (conclusion temperature), ΔHgel (gelatinization enthalpy).

There was a decrease in the onset temperature (T_o) in all samples after modification (Table 1). Sample (E), which was treated with the highest amplitude (100 %) and treatment time (50 min), showed the lowest values of T_o and T_p, starting the gelatinization process at a lower temperature when compared to the native sample. T_o showed a reduction after the treatments, while for Tc there was no significant difference between the treated samples. There was an increase in ΔH_{gel} after the treatments, with a statistical difference between the commercial sample and the samples treated with longer time (50') and vibration amplitudes of 50 and 100%. Wang et al. (2018) obtained a higher gelatinization enthalpy (12.96±0.25 J g^{-1}) for commercially available native arrowroot starch.

Gelatinization temperatures illustrate the stability of starch crystallinity, while enthalpy is related to the energy required for crystal melting (Lopez-Rubio et al., 2008). Ultrasound treatment can cause distortions in the amorphous and crystalline regions of starch granules, which can result in the modification of the granular structure, thus altering its initial characteristics such as crystallinity, enthalpy, and gelatinization temperatures (Zhu, 2015; Ye, et al., 2023; Guo et al., 2022; Jambrak, 2010).

3.2.Thermogravimetry and Derivative Thermogravimetry (TG/DTG

The TG/DTG curves of commercial arrow starch are illustrated in Fig. 2, where it is observed that arrowroot starch shows three main mass losses. The ultrasonically treated samples showed similar curves with only slight shifts in the temperatures of each mass loss as shown in Table 1.

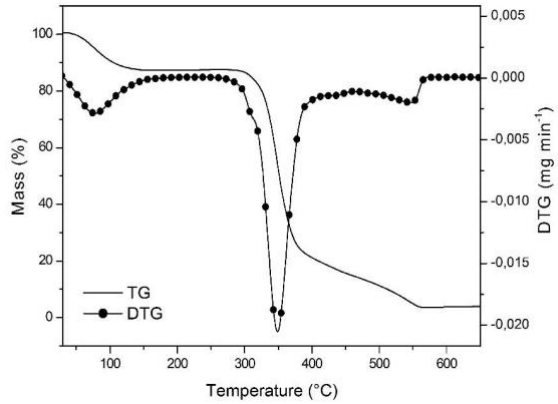

Figure 2. TG/DTG curves of untreated arrowroot starch.

The first mass loss, commonly observed in starches, is associated with the dehydration of the sample (Beninca et al., 2019). According to Table 2, the native sample showed the highest mass loss (13.2 %), and sample (C) exhibited the lowest water loss (8.6 %). It is suggested that ultrasound application may result in starches that are more resistant to dehydration, i.e. with the water fraction more strongly bound to the starch structure (Valencia et al., 2012).

Table 2. TG/DTG results for samples of: native commercial arrowroot starch (A), commercial modified 25'50 % (B), commercial modified 25'100 %(C), commercial modified 50'50 % (D), commercial modified 50'100 % (E).

Thermal event	**1st**			**Stability**	**2nd**			**3rd**		
Sample	Δm, %	ΔT, °C	Tp, °C	ΔT, °C	Δm, %	ΔT, °C	Tp, °C	Δm, %	ΔT, °C	Tp, °C
A	13.2	30-150	81	150-280	69.1	280-422	348	15.0	422-572	502
B	10.5	30-160	81	160-277	72.5	277-421	345	14.2	421-590	509
C	8.6	30-150	83	150-274	74.4	274-409	341	13.3	409-590	494
D	9.7	30-158	84	158-270	74.7	270-417	338	14.2	417-582	497
E	9.1	30-159	86	159-267	73.5	267-416	341	15.2	416-585	501

Δm (mass loss %), ΔT (Difference between the Initial and Final Temperatures °C) of each step (thermal event), T_p (peak temperature °C).

After the first event, a period of stability was identified before the onset of thermal decomposition. Native arrowroot starch showed higher thermal stability, withstanding a temperature up to 280 °C. According to Minakawa et al., (2019), the higher thermal stability of native starches is related to the more compact semi crystalline structure and the high degree of polymerization of native starches. Thus, more ordered structures require more energy to be thermally degraded.

In the second mass loss, it was observed that at temperatures above 267 °C there was a high thermal degradation of the starches, verified by the highest mass decrease in Fig. 2. Sample (A) presented the lowest mass loss in this thermal event (69.1 %), and sample (D) is related to the highest of them (74.7 %). Similar results were reported by Sandoval Gordillo et al., (2014) for native arrowroot starch. According to Valencia et al., (2012), in this step, the thermal degradation of organic compounds of starch, such as fibers and lipids, as well as amylose and amylopectin chains occurs

The native sample presented the highest initial degradation temperature (280 °C), and the starch sonicated with 100 % amplitude for 50 minutes (sample E), exhibited the lowest temperature (267 °C). It is observed that, inversely to the time and amplitude of the treatments, the sonication of the samples caused a decrease in the resistance of the starches to thermal degradation.

The third loss is related to the oxidation of the matter which results in the formation of ash Costa et al., (2013). At this stage, the samples presented mass losses between 13.3 and 15.2 %. Such values may be related to the high content of inorganic residues in arrowroot, such as phosphorus, sodium, and potassium, and to a lesser extent, iron, magnesium, zinc, and calcium Pérez et al., (2005).

3.3. X-Ray Powder Diffractometry (XRD)

At the molecular level it is possible to verify, through X-ray diffraction analysis, the structural characterization of starch, determining the degree of crystallinity through the ratio between amorphous and crystalline regions (Lacerda et al, 2014; Colman, Demiate Schnitzler, 2014). The crystalline material of the granules has typical X-ray diffraction pattern shapes, based on the packing configuration of amylopectin and variations in the water content of the molecule (Chrungoo, Devi, 2015; Pérez, Bertoft, 2010). According to (Pérez, Bertoft, 2010), most cereal

starches have an A-type pattern, some tuber, rhizome and amylose-rich cereals a B-type pattern, and most legume starches a C-type pattern.

The diffraction patterns of native and sonicated arrowroot starches (Fig. 3) are characteristic of type A. A similar result was reported by Wang et al., 2018.

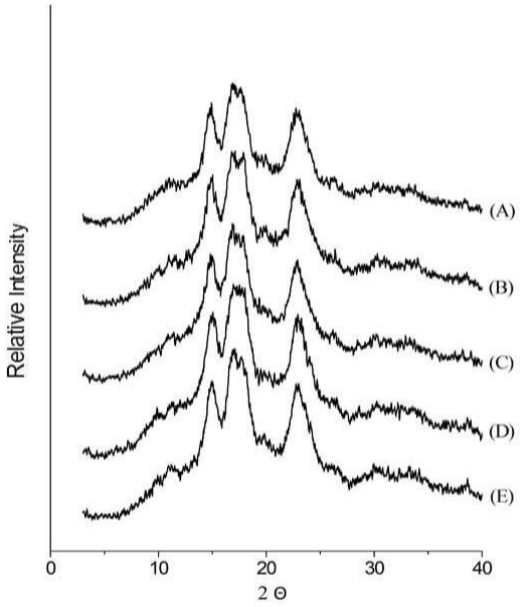

Figure 3. Diffractograms of native commercial arrowroot starch samples (A), commercial modified 25'50 % (B), commercial modified 25'100 %(C), commercial modified 50'50 % (D), commercial modified 50'100 % (E), obtained by X-ray diffraction.

After ultrasonic modification, slight changes were observed in the peaks at 17° and 18° (2θ). This means that the ultrasonic treatment did not severely affect the starch granule structure. However, the modification promoted a decrease in the relative crystallinity (RC) of the samples (Table 1). These results indicate that the application of ultrasonic waves may have caused changes in the ordered structure (amylopectin) and disorder of the double helices of the starch granule, thus decreasing the relative crystallinity (Dar et al., 2018).

3.4. Pasting Properties (RVA)

Starch in the presence of water and increased temperature undergoes a structural change in the granule, with the gelatinization process occurring in a certain temperature range (Mesquita et al., 2016). Viscoamylographic analysis analyzes the thermal behavior of starches during heating and cooling cycles (Wang et al., 2018). Figure. 4 shows the viscoamylographic curves of native and sonicated commercial arrowroot starch samples.

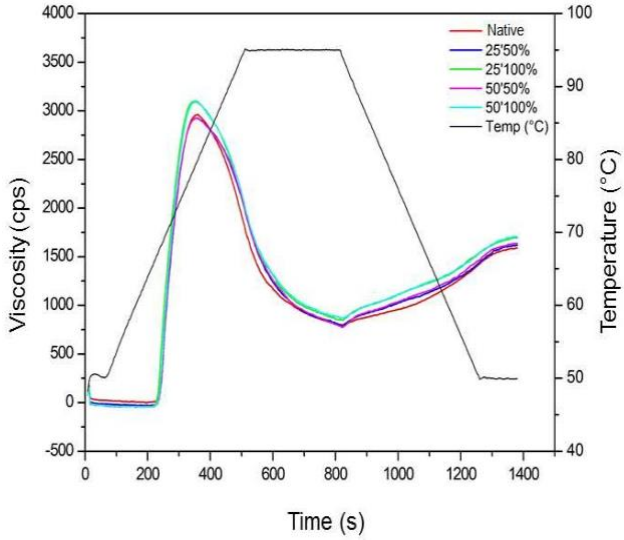

Figure 4. RVA curves of native commercial arrowroot starch samples, commercial modified 25'50 %, commercial modified 25'100 %, commercial modified 50'50 %, commercial modified 50'100 %.

The temperatures of the arrowroot starch samples ranged between 65.8 and 67.2 °C (Tab. 3). A similar result was found by Maniglia, Tapia-Blácido (2016) for native arrowroot starch (67.1 °C). It was observed that the ultrasound treatment with amplitude of 100 % after 25 minutes (sample C), provided the reduction in the temperature (65.8 °C) of starch paste formation, possibly due to a decrease in the resistance to swelling of the granules. This data corroborates with the Tp observed by DSC (Table 1).

Table 3. RVA results for samples native commercial arrowroot starch (A), commercial modified 25'50 % (B), commercial modified 25'100 % (C), commercial modified 50'50 % (D), commercial modified 50'100 % (E).

Sample	Peak viscosity (mPa^{-1})	Breakdown (mPa^{-1})	Final Viscosity (mPa^{-1})	Setback (mPa^{-1})	Peak Time (min)	Pasting Temperature (°C)
A	2960±1.0^{c}	2161±1.0^{c}	1590±1.0^{e}	791±1.0^{e}	6.1±0.1^{a}	66.7±0.0^{b}
B	2922.7±0.6^{e}	2132±1.0^{e}	1616±1.0^{d}	826±1.0^{d}	5.9±0.0^{b}	67.2±0.1^{a}
C	3101±0,0^{a}	2251±1.0^{a}	1694.6±0.6^{b}	845±1.0^{b}	5.9±0.0^{b}	65.8±0.1^{c}
D	2925.3±0.6^{d}	2148.3±0.6^{d}	1636±1.0^{c}	860±1.0^{a}	5.9±0.0^{b}	66.7±0.1^{b}
E	3093±1.0^{b}	2224±1.0^{b}	1706±1.0^{a}	837.3±0.6^{c}	5.9±0.0^{b}	66.7±0.1^{b}

*Values with the same letter subscribed in the same columns do not show statistical difference between them by the Tukey Test ($p<0.05$).

The use of ultrasound resulted in an increase in setback and final viscosity values in all samples. The setback is the ratio of the final viscosity to the minimum viscosity and is directly proportionally related to the tendency for retrogradation and syneresis (Ai, Jane, 2015; Sheglin et al., 2023; Zórtea-Guidolin, 2017). Thus, sonication of the samples resulted in pastes with a higher tendency to retrogradation and syneresis compared to the native sample (A).

In the treatments with 100 % amplitude, (C) and (E), an increase in the peak viscosities and a breakdown were recorded. In this case, despite the increase in peak viscosity, there was a decrease in the ability of the gel to resist both shear stress and heating. On the other hand, in the treatments with 50 % amplitude, (B) and (D), a decrease in these two parameters was observed.

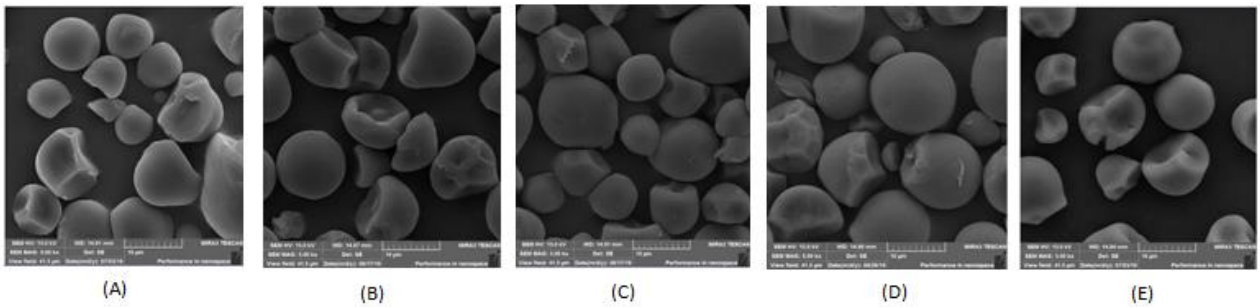

Figure 5. Microimages (5000 X) of unmodified commercial arrowroot starch samples (A), commercial modified 25'50 % (B), commercial modified 25'100 % (C), commercial modified 50'50 % (D), commercial modified 50'100 % (E).

3.5. Field emission gun-scanning electron microscopy (FEG - SEM)

Scanning electron microscopy is an important technique to verify the structure, morphological characteristics, and size determination of starch granules (Paes et al., 2019).

Native arrowroot starch presents a mixture of circular and oval granules with a diameter ranging from 9 to 42 µm (Maniglia, Tapia-Blácido, 2016). Suastegui-Baylón et al., (2021) point out the small number of circular granules and the predominance of the bean shape. In this study, commercial arrowroot starch exhibited a predominantly circular shape (Fig. 5) and a granule diameter between 6 and 13 µm. Similar values were recorded by Astuti et al., (2016) in native arrowroot
starch.

The morphological characteristics of arrowroot starch granules were little affected by the treatments. A similar result was found by Kang et al., 2016, in which the application of ultrasound to maize and rice starches caused some small cracks and depressions that were not very evident on the surface. Obadi, Xu, 2021, suggests that the effects of an ultrasound on starch granules depend on the intensity of the treatment, in addition to factors such as power, frequency, temperature, treatment time and the properties of the starches.

4. Conclusions

The application of ultrasound to modify arrowroot starch caused some changes in the properties of the starches. Regarding thermal properties, the sonicated samples showed lower initial gelatinization temperature compared to the native sample. The use of ultrasound also resulted in starches with a lower thermal stability compared to the native sample, withstanding temperatures up to 270 °C. Regarding paste properties, the modified samples showed a higher tendency for retrogradation and syneresis, although higher viscosity was achieved, especially when higher amplitude and sonication time were used. The analysis of the morphological properties revealed that the structures of the starch granules were little altered by the ultrasonic waves, however, there was a decrease in the crystallinity of the sonicated samples.

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