Ultrasonication improves the structures and physicochemical properties of cassava starch films containing acetic acid

Fuqi Wu[a], Zeguang Zhou[a], Manling Liang[a], Lei Zhong[a,*], Fengwei Xie[b,c,**]

[a] Guangxi Key Laboratory for Polysaccharide Materials and Modifications, School of

Chemistry and Chemical Engineering, Guangxi University for Nationalities, Nanning 530006,

China

[b] International Institute for Nanocomposites Manufacturing (IINM), WMG, University of

Warwick, Coventry CV4 7AL, UK

[c] School of Chemical Engineering, The University of Queensland, Brisbane, Qld 4072,

Australia

[*] **Corresponding author.** Email: leiwin@gmail.com (L. Zhong). Tel. & Fax:

+86-135-5701-1926 (L. Zhong)

[**] **Corresponding author.** Email: D.Xie.2@warwick.ac.uk, fwhsieh@gmail.com (F. Xie)

## Abstract

Cassava starch films were fabricated with acetic acid treatment and ultrasonication. Different ultrasound power levels from 200 W to 750 W were used and the effects of ultrasonication on the morphology, microstructures, and properties of the starch–acetic acid films were investigated. Scanning electron microscopy (SEM) shows a cohesive and compact structure of the films resulting from ultrasonication. X-ray diffraction (XRD) analysis reveals that the crystalline index (CI) was decreased by acid treatment and increased by ultrasonication. The tensile strength and elongation at break of the films first increased and then decreased with increasing ultrasound power level. Ultrasonication also resulted in higher opacity, higher water barrier performance, and lower water adsorption of the films. Thus, our results have shown that ultrasonication can be used as a simple and efficient way to modify the morphology,

microstructure, and performance of starch–acetic acid films to better meet the application needs.

## 1. Introduction

In recent years, with the concerns over the limitation of petroleum resources and pressing environmental issues caused by the use of traditional synthetic plastics, the development of biopolymer materials for food packaging and other applications have attracted great attention.[1-3] Polysaccharides such as starch, cellulose, and chitosan have been considered to be promising alternatives to traditional petroleum-based plastics for developing sustainable and biodegradable packaging materials.[4-6] They are abundant in nature and environmentally friendly. Their application in packaging may effectively reduce costs and wastes, leading to sustainability outcomes.[7]

Starch is an abundant polysaccharide in nature. It has been widely used in the food industry as a variety of food products and additives.[8] Starch is highly potential to be used for food packaging because of its biodegradability and low cost.[9,10] It is well known that native starch consists of two types of biomacromolecule, namely amylose, and amylopectin. Their ratio in starch granules largely determines the properties of starch products.[11] Gelatinized starch exhibits an excellent film-forming capacity and the films fabricated by starch are odorless and transparent and have low oxygen permeability.[12] However, films made of unmodified starch usually have poor mechanical properties. Furthermore, since starch is highly hydrophilic, unmodified starch films are usually very sensitive to water vapor and show poor water barrier properties.[13,14] These drawbacks have greatly limited the applications of starch in the food

packaging. To overcome these limitations, various chemical, physical and enzymatic methods have been applied to improve the properties of starch films.[15-17]

Ultrasonication has been considered as an efficient and eco-friendly physical method for starch modification.[18-20] The energy generated by ultrasound can form acoustic cavitation and the subsequent collapse of cavitation bubbles results in high-speed microjet streaming and strong shock waves, which can break up the aggregations of starch granules.[21] Previous studies suggested that ultrasound-induced forces could degrade amorphous regions in starch granules[22] and disrupt the granule ghosts, which subsequently changed the physicochemical properties of starch, such as increased solubility and decreased viscosity.[23,24] However, it is worth noting that the effects of ultrasound on the starch film properties in previous reports were not always consistent. For example, Garcia-Hernadez et al.[23] found that ultrasonication destroyed the ghost fraction, leading to a decrease in the tensile properties of corn starch films. In contrast, Abral et al.[25] claimed that ultrasonication improved the tensile properties by generating a more compact and homogeneous structure of sago starch films. This inconsistency in results might be due to the difference in starch source and ultrasonication condition for the fabrication of the films.

Organic acids, such as acetic acid, malic acid, and citric acid are commonly used in many food relevant products. They are inexpensive and generally safe, which makes them ideal for starch modifications for the food industry.[26,27] In previous studies, it was found that organic acids had significant effects on the rheological properties of starch. For example, Hirashima et al.[28] investigated the effects of six types of organic acid (acetic, ascorbic, citric, lactic, malic and tartaric acids) on the rheological properties of corn starch. They observed an increase (pH from

5.5 to 3.6) and then a decrease (pH further below 3.5) in paste viscosity caused by addition of these organic acids. They proposed that the increased viscosity was due to the entanglement of leached glucose chains and the decreased viscosity was due to the hydrolysis of starch molecules. More recently, Majzoobi et al.[29] studied the effects of acetic acid on the rheological properties of pregelatinized wheat and corn starches. Their results showed that the apparent viscosity of starch suspensions was decreased and a more cohesive and less turbid starch gel was obtained in the presence of acetic acid. These reports indicated that the addition of acetic acid could improve the film-forming ability of starch suspensions and modify the physicochemical properties of starch-based films.

Although the impact of ultrasonication on the properties of starch has been widely studied, many of these previous reports only focused on the properties of starch suspensions, especially the difference in the swelling behavior of starch granules.[30-35] There have been only limited studies on the effect of ultrasonication on the properties of films based on acid-treated starch.[22,36] To the best of our knowledge, there is still a lack of systematic research on how ultrasonication affects the mechanical, optical and moisture barrier properties of starch films containing organic acids.

Cassava is one of the most significant industry crops in southern parts of China. Over 60% of the domestic production of cassava starch in China is concentrated in Guangxi province.[37,38] Compared with starches from other plant sources such as corn, potato and pea, cassava starch has several advantages such as low gelatinization temperature, high transparency, and good gel stability. [39] Currently, large amounts of cassava starch is wasted due to the difficulties in its processing. Compared with traditional processing methods, ultrasonication can be considered

as a more efficient and economical way to dissolve starch molecules and form homogeneous starch suspensions.[40] Therefore, it is worth developing cassava starch-based films with tailored properties by acid treatment and ultrasonication, which are potential to be applied in packaging applications to alleviate the plastic waste issue.

In this work, cassava starch films were prepared by a solution casting process treated with acetic acid and ultrasound. The effect of the ultrasound power level on the structural characteristics and physicochemical properties of the starch–acetic acid films was systematically investigated. This study aimed to find a facile and efficient way to prepare starch-based films with enhanced properties, potential in food packaging and wider applications.

## 2. Materials and methods

### 2.1 Materials

Food grade cassava starch (19/81 amylose/amylopectin ratio, as determined by the iodine method) was supplied by Guangxi Hongfong Starch Co., Ltd. (Nanning, China). Glacial acetic acid (AR) and glycerol (≥ 99% purity) were purchased from Guangdong Guanghua Co., Ltd. (Guangzhou, China).

### 2.2 Preparation of the starch–acetic acid film

The cassava starch was dispersed in distilled water to obtain a 10% (w/w) starch suspension. Afterwards, glacial acetic acid (20% of starch, w/w) and glycerol (20% of starch, w/w) were added to the suspension. Then, the mixture was heated to 80 °C and stirred at 430 rpm for 60 min, followed by ultrasonication using a 15mm probe-type ultrasonic processor (Model KH-2890J, Kehai Inc., Weihai, China) at 20 kHz for 5 min. A processing temperature of 80 °C should be enough for full gelatinization of cassava starch.[41] Our preliminary experiments showed that longer treatment time could make the films too brittle for tensile tests. Different ultrasonic power levels (200 W, 400 W, 600 W, and 750 W) were used. During ultrasonication, the suspensions were kept in a cold-water bath to limit ultrasonication-induced warming to be below 80 °C.

The resulting suspensions were cooled and kept for at least 60 min at room temperature to eliminate air bubbles. Afterwards, the films were cast by pouring 30 g of the film-forming suspension on a polymethyl methacrylate plate (120 mm × 120 mm), which was dried in an oven at 50 °C for 24 h to obtain starch films. These conditions were established according to our preliminary experiments to avoid the defects in the films.

The control samples were prepared following the same procedure without ultrasonication.

The film thickness measured at six random positions was 0.24±0.02 mm. All the samples were stored at least 2 days at 25±2 °C and 53% relative humidity (RH, achieved using saturated $Mg(NO_3)_2$).

**2.3 Scanning electron microscopy (SEM)**

The cross-sectional surface morphology of the starch films after tensile tests were observed using a scanning electron microscope (Zeiss, Supra 55, Oberkochen, Germany) at 2000× magnification. All the samples were sputter-coated with gold and the tests were carried out with an acceleration voltage of 2 kV.

## 2.4 Fourier Transform Infrared Spectroscopy (FTIR)

Molecular interactions in the starch films were characterized by a Fourier-transform infrared spectrometer (Nicolette Magna 550II, GMI, Ramsey, MN, USA). The samples were ground and mixed with KBr powder. Afterwards, the powder blends were compressed into testing discs. The measurement was conducted over a wavenumber range of 4000–400 $cm^{-1}$ at a resolution of 8 $cm^{-1}$.

## 2.5 X-ray diffraction (XRD)

X-ray diffractograms were recorded by an X-ray diffractometer (MiniFlex 600, Rigaku, Tokyo, Japan) under Kα Cu radiation at 40 kV and 15 mA. The samples were cut into 2 cm × 2 cm squares and placed onto a glass plate. The scanning was conducted over a $2\theta$ range of 3° to 40° with a step size of 0.02°. The crystallinity index (CI) of cassava starch–acetic acid films was calculated by Eq. (1):

$$CI = A_c/(A_c+A_a) \times 100\% \qquad (1)$$

where $A_c$ is the area of the crystalline region, and $A_a$ is the area of the amorphous region.

## 2.6 Thermogravimetric analysis (TGA)

Thermal stability was evaluated by thermogravimetric analysis (TGA) using a simultaneous thermal analyzer STA 449 F3 Jupiter (Netzsch, Selb, Germany) over a temperature range of 35 °C to 600 °C at a heating rate of 10 °C/min under nitrogen. For each measurement, 5 mg of the sample was used. Based on the weight loss data, derivative-weight curves (DTG) were also generated for analysis.

## 2.7 Mechanical properties

Tensile tests were performed according to the method used by Liu et al.[22] with some modifications. The mechanical properties of the starch films were determined by a universal testing machine (JDL-1000N, Tianfa Instruments Co., Ltd., Yangzhou, China). The conditioned samples were cut into rectangular strips (60 mm × 20 mm). The initial grip separation was set as 500 mm, and the testing was conducted at an overhead speed of 10 mm/min. The tensile stress (TS) and elongations at break (EB) were calculated according to the stress–strain curves. For each sample, at least five specimens were tested.

## 2.8 Opacity

Opacity was studied using a UV-spectrophotometer (UV-2600, Shimadzu, Kyoto, Japan) over of wavelength range of 400 to 800 nm. Samples were cut into rectangular stripes (40 mm × 10

mm) before the measurement. According to ASTM D1003-00, the opacity values of films were calculated by the area under the absorbance spectrum and was averaged based on four replicates.

## 2.9 Water vapor permeability (WVP)

Water vapor permeability (WVP) was measured according to ASTM E96-00 with some modifications.[25] The sample film (60 mm diameter) was sealed on the top of a plastic cup (50 mm diameter) containing 30 mL of distilled water (100% RH). Vaseline was used to prevent the leakage of moisture while sealing. After the initial mass of the cup was weighed, it was stored in a closed desiccator filled with 1000 g of silica gel at 25 °C and weighed every 3 h until reaching a stabilized weight. WVP is calculated by Eq. (2):

$$WVP = (\Delta W \times T) / (\Delta t \times \Delta P \times A) \qquad (2)$$

where $\Delta W$ is the weight loss of the cup (g), $T$ is the thickness of the film, $A$ is the area of water permeation, $\Delta t$ is the time change under the partial pressure difference ($\Delta P = 2533$ Pa). The WVP of each sample was obtained based on four replicates.

## 2.10 Moisture absorption (MA)

Moisture absorption (MA) was measured by a two-step gravimetric method used previously[42] with some modifications. The films were cut into square stripes (20 mm × 20 mm) and dried in

a vacuum oven at 50 °C for 24 h. Then, the dried samples were stored in a closed desiccator

with 75% RH for 24 h to reach the equilibrium. MA is calculated by Eq. (3):

MA (%) = $(M_w - M_0) / M_0 \times 100$ (3)

where $M_w$ and $M_0$ are the final weight (g) and initial weight (g) of the sample film, respectively.

Four replicates were conducted for each sample.

## 2.11 Statistical analysis

Data were expressed as mean ± standard deviation. The statistical analysis of the results was

conducted by analysis of variance (ANOVA). The effects of ultrasonication on the properties

of the films at 5% significant difference ($p \leq 0.05$) were confirmed by Tukey's test using

Microsoft Office Excel.

## 3. Results and Discussions

### 3.1 Morphology

The SEM images of cross-sectional surfaces of ultrasonicated films at different power levels

are presented in Figure 1. Ultrasonication clearly affected the surface morphology of the films.

At the same magnification, the surface of the untreated film was rough while the surfaces of

ultrasonicated films became smooth and cohesive. Increasing ultrasonication power level

resulted in smoother and more cohesive surface morphology. A similar observation was

reported by Liu et.al.,[22] who found that sweet potato starch–lauric acid films with more ordered and uniform morphology could be obtained with increasing ultrasonic power density from 240 $W \cdot cm^{-2}$ to 560 $W \cdot cm^{-2}$. In this regard, the ultrasound energy could destroy the insoluble remnants of starch granules, break up the clumps of starch agglomerations, and lead to the formation of starch materials with fewer micro-pores and cracks.

**3.2 FTIR**

The FTIR spectra for native starch and the starch–acetic acid films prepared with ultrasonication at different power levels are illustrated in Figure 2(a). All the characteristics peaks of native starch remain to be evident for the films. The strong and broad band between 3000–3700 $cm^{-1}$ could be ascribed to the O─H stretching vibration of starch, glycerol, or water. The sharp band at 2927 $cm^{-1}$ is attributed to the C─H stretching vibration. The distinctive peak at 1650 $cm^{-1}$ is ascribable to the O─H bending of water molecules absorbed by starch, and the peaks at about 950 $cm^{-1}$ correspond to the vibration of the glycosidic linkage.[19] This FTIR spectrum is similar to the spectrum of sorghum starch treated with acetic acid.[43] Nonetheless, the characteristics peak of acetylated starch at 1750 $cm^{-1}$ could not be observed, indicating ultrasonication could not induce esterification of acetic acid-treated cassava starch.

**3.3 XRD**

Figure 2(b) shows the XRD patterns of native starch and the starch–acetic acid films prepared with different power levels of ultrasonication. The native starch showed four peaks at $2\theta =$ 15.3°, 17.3°, 18.2° and 23.3°, characteristics of the A-type crystalline structure. The addition of acetic acid changed the crystalline structure significantly, the CI decreased from 16% to 7% (see Table 1), and the A-type characteristic peaks disappeared. A similar effect of citric acid on the crystalline structure of cassava starch has been reported.[36] The starch–acetic acid films prepared with ultrasonication showed similar diffraction patterns, with the appearance of two small new peaks at $2\theta = 19.9°$ and 22.1°, corresponding to the B-type crystalline structure. The CI gradually increased from 9% to 16% with increasing ultrasound power level from 200 W to 750 W. It was likely that ultrasound treatment could assist the chain aggregation to form a more ordered structure in the starch–acetic acid films.

To investigate the effect of ultrasound and acid treatments on the crystalline structure further, the same ultrasound power levels were applied to prepare starch films without acetic acid. The results were 15.68%, 13.90%, 14.82% and 15.66%, respectively, confirming that there were no significant changes in CI with different ultrasonication power levels in this study. The results were in agreement with those observed by Abral et al.,[25] who found that no significant changes in the CI of sago starch films with increasing ultrasonication duration. Thus, we suggest that ultrasonication alone might not be able to disrupt and transform the starch structure. The structural changes could be due to the combined effects of ultrasound and acid treatment. The acid treatment hindered the recrystallization of gelatinized starch,[36] while ultrasound might assist the chain alignment of the degraded starch and led to an increase in crystallinity.[44]

## 3.4 Mechanical properties

Figure 3 shows the TS and EB results of the starch–acetic acid films prepared with different ultrasonication power levels. Ultrasonication evidently affected the mechanical properties of the films. The TS of the films treated at 200 W and 400 W (26.38 MPa and 23.38 MPa, respectively) were apparently higher than that of the film without ultrasonication (14.36 MPa), with 83.7% and 62.8% increases, respectively. The improvement might be ascribed to the formation of a more compact and homogeneous structure induced by ultrasound. In a previous study, Liu et al.[22] found a similar result, namely, the TS of sweet potato starch–lauric acid films was increased with ultrasound power densities of 240 W·cm$^{-2}$ and 320 W·cm$^{-2}$, respectively. Moreover, compared with the films treated at 200 W and 400 W, the films treated with higher ultrasound power levels (600 W and 750 W) exhibited lower TS and EB values, which were even lower than those of the control samples. Garcia et al.[23] also observed that when the ultrasonication duration increased to 8 min, compared to the samples without ultrasonication, the TS and EB values of the starch films decreased from 7.3 MPa and 48.8% to 5.9 MPa and 38.7%, respectively. In this regard, high ultrasound energy might degrade starch chains and weaken starch chain interactions, leading to brittle materials

## 3.5 Thermal stability

The thermal stability results, in the form of weight loss and derivative weigh as a function of temperature, of the starch–acetic acid films are shown in Figure 4. Three stages of weight loss

with temperature can be seen. The weight loss at the first stage (30–190 °C) was about 7%, which should be related to moisture evaporation. During the second stage (190–330 °C), the weight loss rate increased dramatically at about 250 °C, and there was over 70% mass loss at this stage. The decomposition of the films completed at the third stage (330–600 °C) but with about 15% material remaining. Zambelli et al.[45] observed similar thermal behaviors for cassava starch modified with different concentrations of acetic acids. Since the TGA and DTG curves of samples with and without ultrasonication were similar and mostly overlapped, we propose that ultrasonication did not have significant effects on the thermal stability of the starch–acetic acid films.

### 3.6 Opacity

Figure 5 shows the relationship between absorbance and wavelength, which can be used to evaluate the opacity of starch–acetic acid films. The results show that the opacity of the samples with ultrasonication was higher than the film without ultrasonication (107.9 AU·nm), and the opacity increased with increasing ultrasound power level. The increase in opacity is in agreement with the increase in CI. These results are in contrast to some previous findings. Garcia-Hernadez et al.[23] claimed that the opacity of ultrasonicated films was reduced because of the rupture of ghosts. Abral et al.[25] reported that there was an increase in film transparency after ultrasonication due to the formation of more compact and homogeneous structures. In our work, the reduced transparency might be due to the formation of new aggregated structures or

crystals from the starch chains partially hydrolyzed by acetic acid and ultrasound, which had a different refractive index.

### 3.7 Moisture adsorption and water vapor permeability

Moisture adsorption and water vapor permeability are crucial parameters to evaluate the capacity of food-wrapping films.[46] Since starch is highly hydrophilic, starch-based films generally show high water adsorption and poor moisture barrier properties.[47]

All the films reached their steady MA states at about 10 h. Table 2 lists the MA values with different power levels of ultrasonication. Ultrasonication led to a gradual reduction in MA. The lowest MA value of 10.48% was shown by the film treated with 750 W ultrasound, representing a 33% decrease compared to that of the sample without ultrasonication. It was proposed that ultrasonication could degrade starch chains and led to a more compact material structure, leading to reduced MA.[22,40]

Figure 6 shows the WVP of the starch films with different power levels of ultrasonication. Ultrasound treatment led to lower WVP. After 21 h, the WVP values of the films prepared with ultrasonication ranged from $1.69\times10^{-10}$ to $1.04\times10^{-10}$ $(g\cdot m^{-1}\cdot s^{-1}\cdot Pa^{-1})$, while that of the film without ultrasonication was $2.22\times10^{-10}$ $(g\cdot m^{-1}\cdot s^{-1}\cdot Pa^{-1})$. Regarding this, ultrasonication could lead to a more compact and homogeneous starch matrix with higher crystallinity, which effectively limited the passage of water molecules.[25,40]

## 4. Conclusion

In this work, we investigated the structure and properties of films based on cassava starch containing acetic acid and treated by different power levels of ultrasound. The results indicate that ultrasonication led to a more compact structure, which might contribute to the improvement of the mechanical and water barrier properties of the starch–acetic acid films. Increasing the ultrasound power level resulted in increased film opacity, which might be due to the growth of new aggregated structure or crystals in acid-hydrolyzed starch assisted by ultrasonication. The ultrasonication with acetic acid did not cause any chemical changes to the starch; the thermal stability of the films was not apparently altered, either. This study has demonstrated the potential of combining ultrasonication with acid treatment as a convenient and effective way to adjust and improve the structure and properties of starch-based films.

**Conflict of interest**

The authors declare no conflict of interest.

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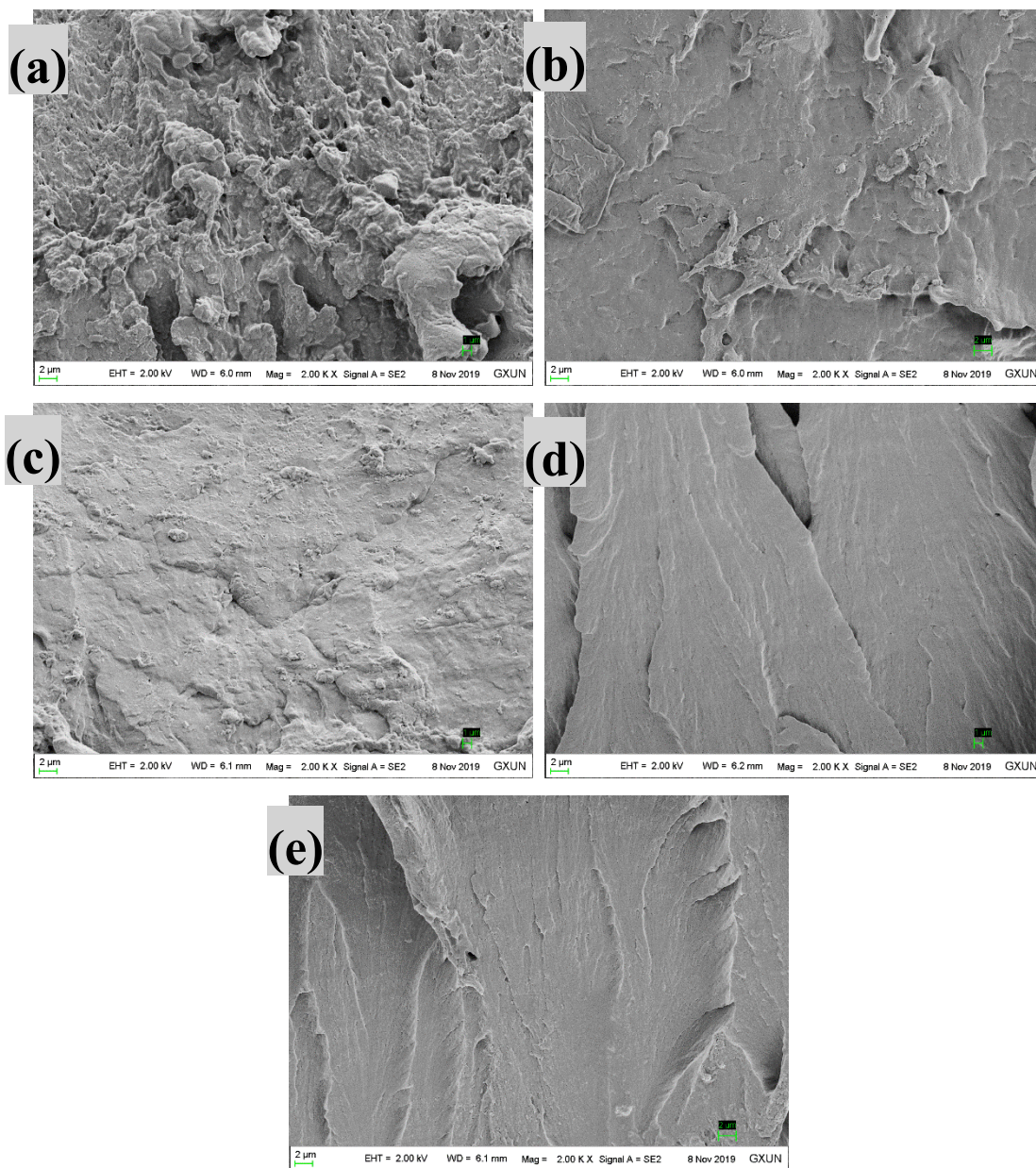[47] M. Petersson, M. Stading, *Food Hydrocoll.* **2005**, *19*, 123.

**Figure legends**



**Figure 1.** SEM images of the starch–acetic acid films prepared with different power levels of ultrasonication (a, without ultrasonication; b–e, with ultrasonication at 200 W, 400 W, 600 W, and 750 W, respectively).
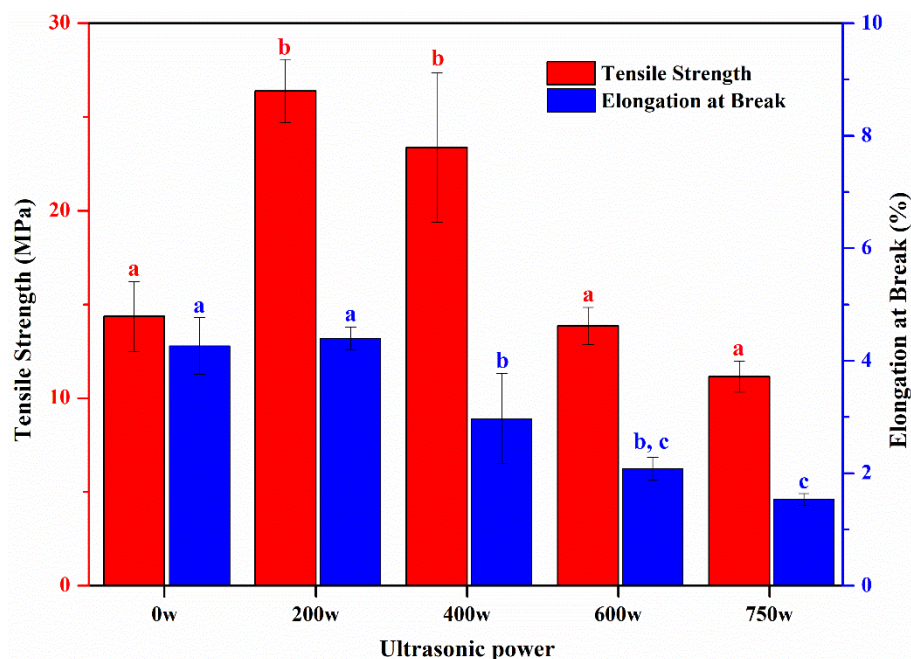
**Figure 2.** a) FTIR spectra and b) XRD patterns for native starch and the starch–acetic acid films prepared with different power levels of ultrasonication.
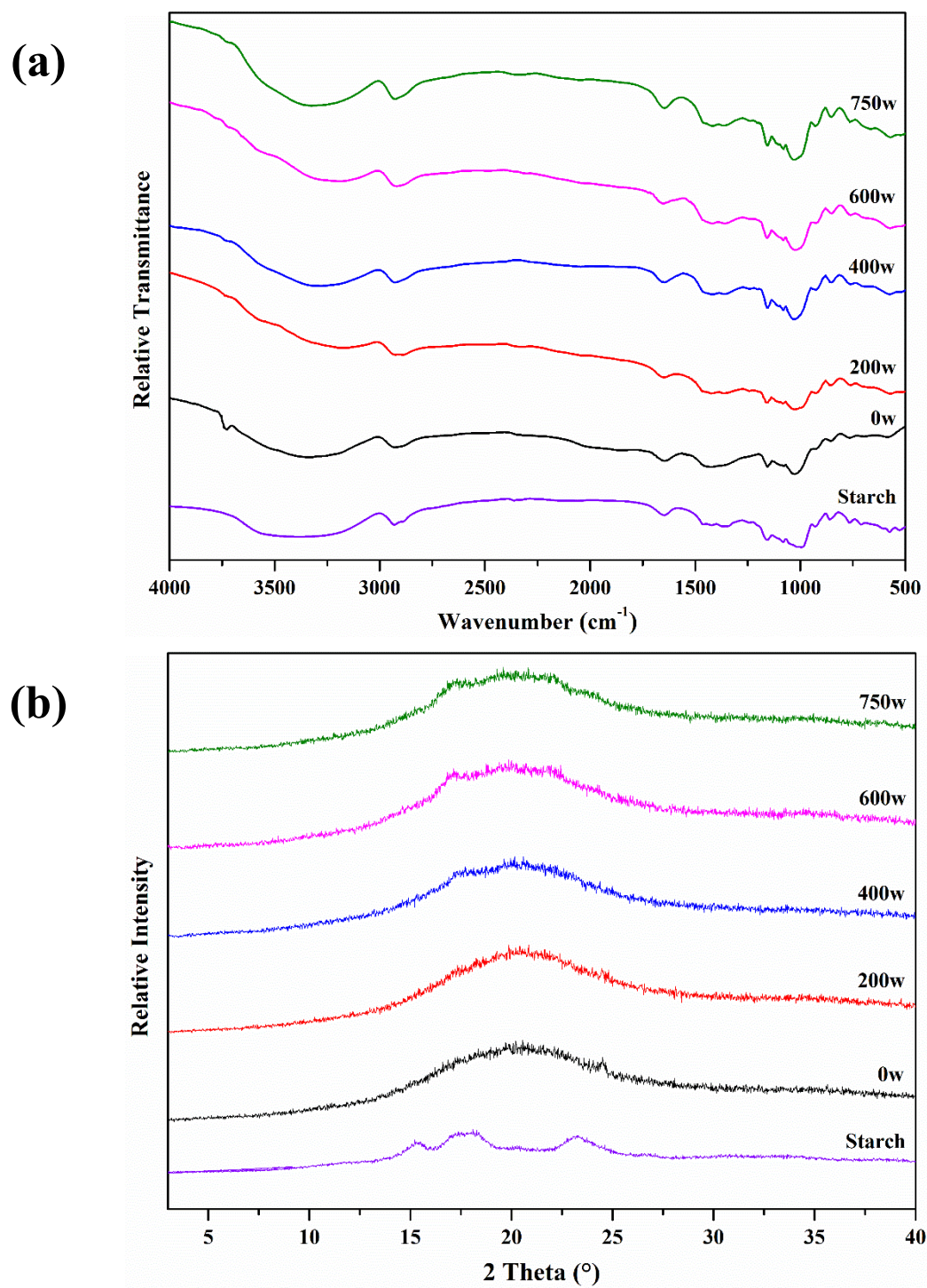
**Figure 3.** Mechanical properties of the starch–acetic acid films prepared under different power levels of ultrasonication. Different letters above the columns show significant differences among samples ($p \leq 0.05$).
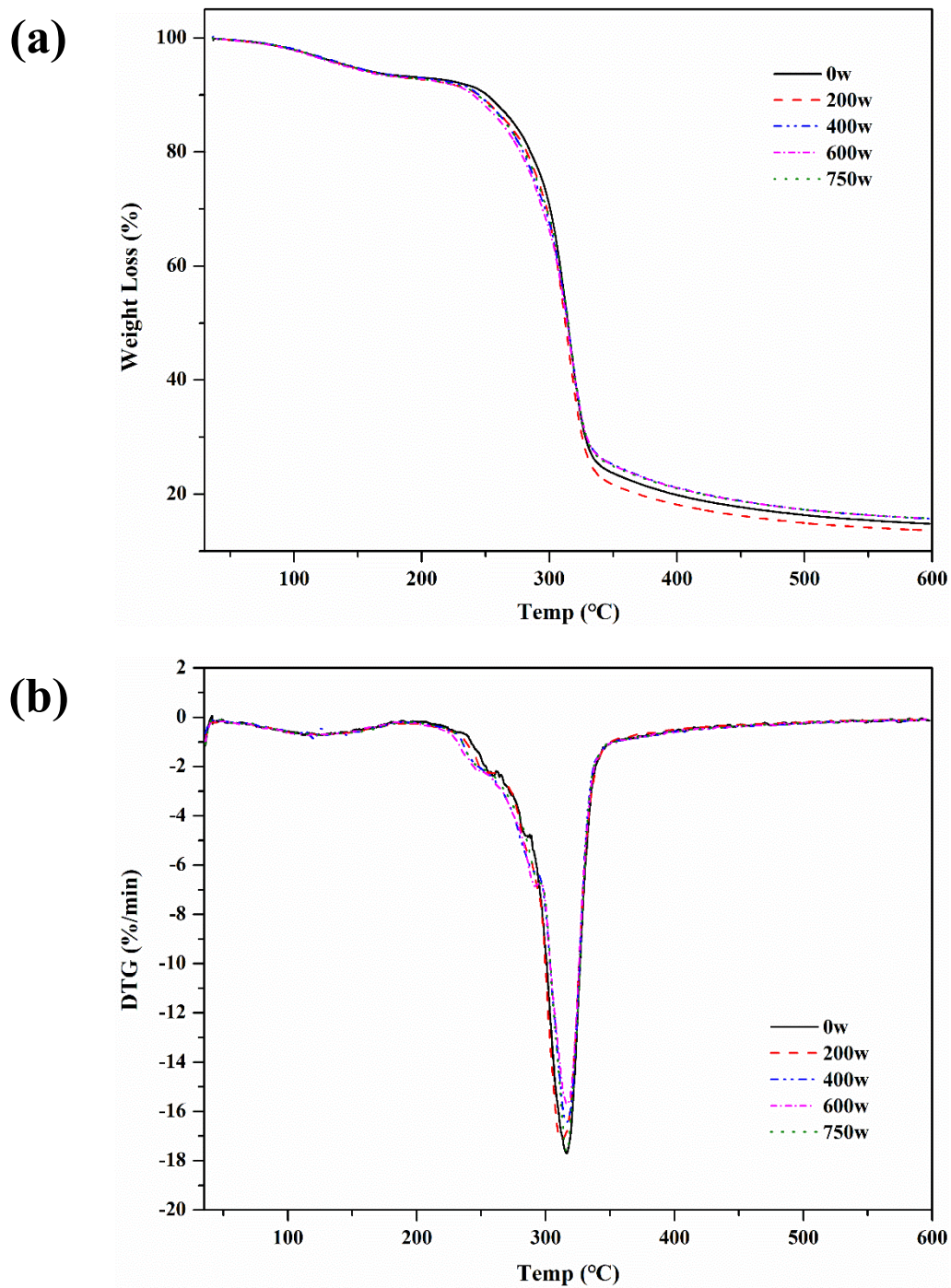
**Figure 4.** Weight loss (a) and derivative weight (DTG) (b) curves for the starch–acetic acid films prepared with different power levels of ultrasonication.
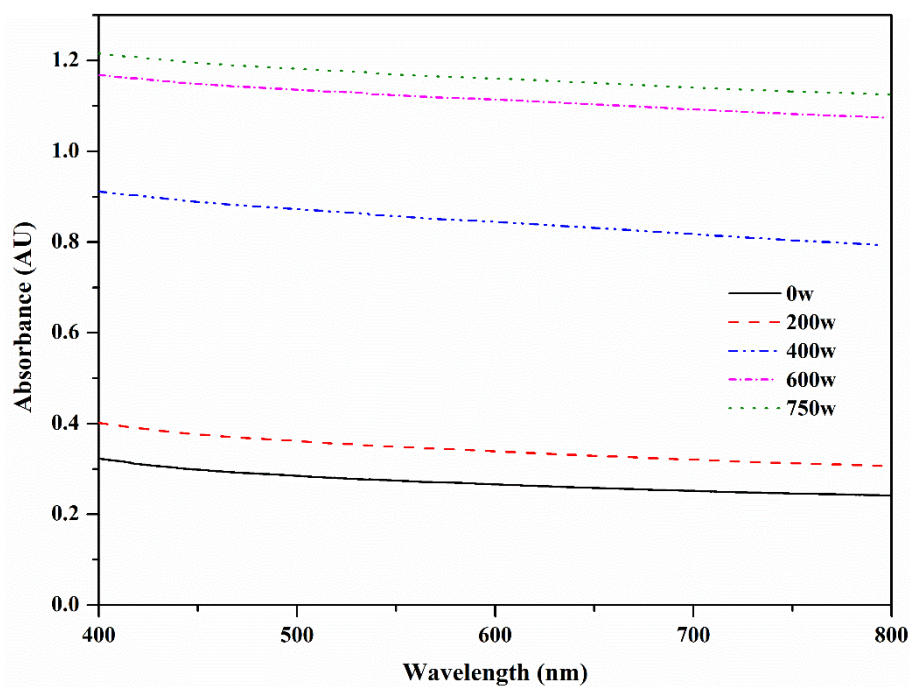
**Figure 5.** Opacity of the starch–acetic acid films prepared with different power levels ultrasonication.
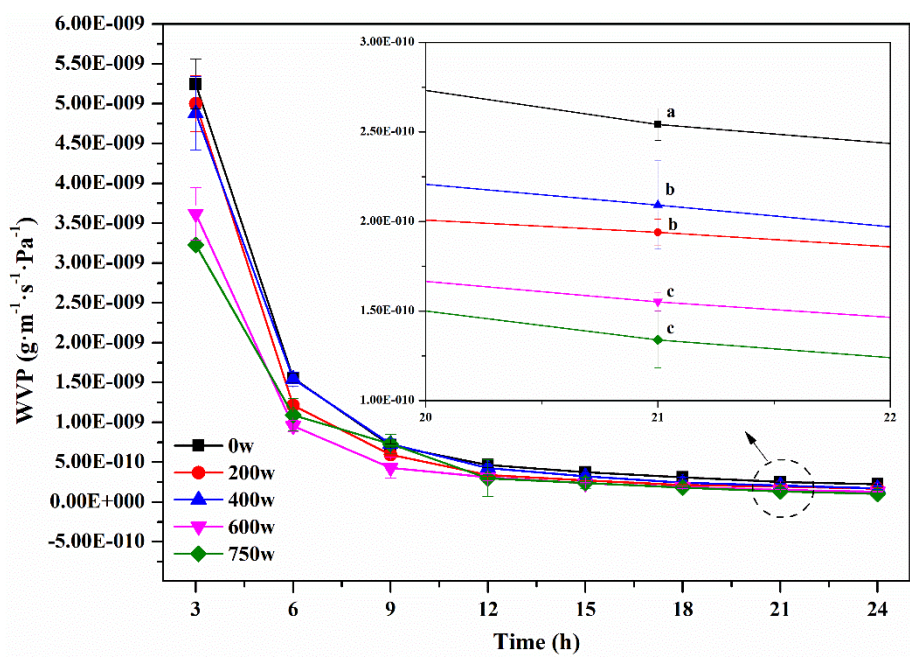
**Figure 6.** WVP of the starch–acetic acid films prepared with different power levels of ultrasonication. Different letters above the curves show significant differences among samples ($p \leq 0.05$).

**Table 1.** Crystallinity index (CI) of the starch–acetic acid films prepared with different power levels of ultrasonication.

| Sample | Ultrasonic power (W) | CI (%) |
|---|---|---|
| Starch–acetic acid films | 0 | $6.69 \pm 0.17^a$ |
| | 200 | $8.96 \pm 0.33^b$ |
| | 400 | $12.07 \pm 0.20^c$ |
| | 600 | $12.72 \pm 0.19^c$ |
| | 750 | $15.64 \pm 0.45^d$ |

a-d: Means with different superscripts in the same column show significant differences among samples ($p \leq 0.05$).

**Table 2.** MA values of the starch–acetic acid films prepared with different power levels of ultrasonication.

| Sample | Ultrasonic power (W) | MA (%) |
|---|---|---|
| Starch–acetic acid films | 0 | $15.65\pm0.41^{a}$ |
| | 200 | $14.94\pm0.69^{a}$ |
| | 400 | $14.73\pm0.12^{a}$ |
| | 600 | $13.16\pm0.82^{b}$ |
| | 750 | $10.48\pm0.53^{c}$ |

a-c: Means with different superscripts in the same column show significant differences among samples ($p \leq 0.05$).

## Layman summary:

As cassava is an important industrial crop, cassava starch is potential for non-food applications such as packaging films. This work demonstrates the fabrication of cassava starch-based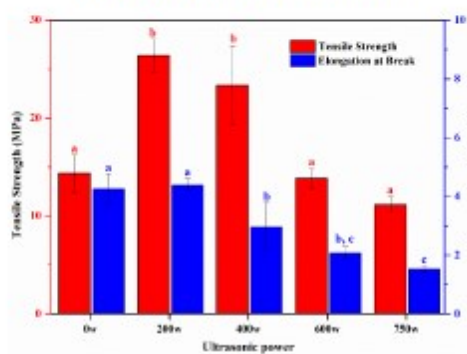 films treated with acetic acid and ultrasonication. Ultrasonication led to improved structural cohesiveness, reduced crystallinity, increased opacity and tensile and water barrier properties of the starch film.
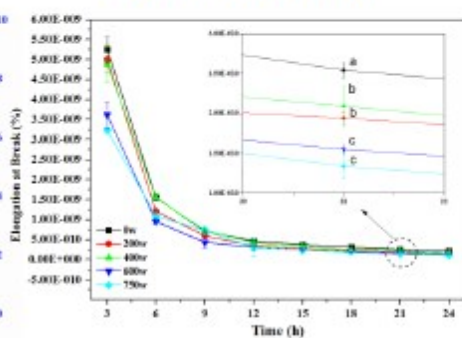
## Starch-acetic acid films
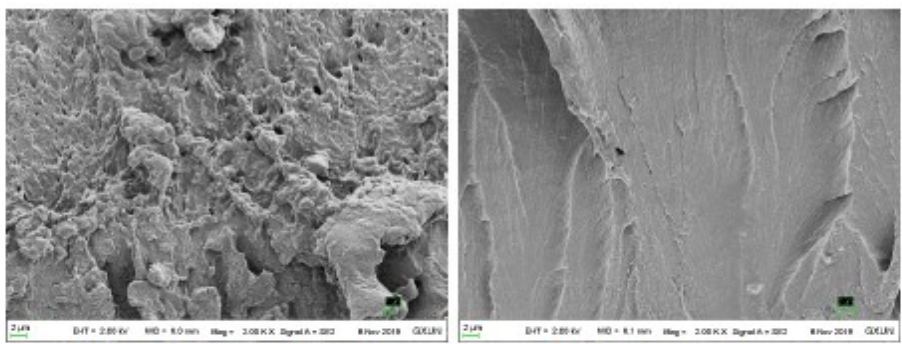
Without ultrasonication

With ultrasonication

Mechanical properties

Water barrier properties