

Ultrasound-assisted extraction and characterization of the functional properties of starch from soursop fruits (*Annona muricata* L.)

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ABSTRACT. Starch is one of the most used polysaccharides in the food industry because is an excellent raw material to modify the texture and consistency of food. Also, is the main source for energy storage in horticultural products. The starch from soursop fruits can be used as an alternative source with functional properties and a wide field of application in the food industry. The use of emerging technologies such as ultrasound-assisted extraction has improved sensory attributes and functional properties. To the best of our knowledge, extraction of starch by ultrasound from soursop fruits has not to date been documented. Therefore, the objective of this study was to characterize the functional properties of soursop fruit starches by ultrasound-assisted method and determine the presence of acetogenins. Three times (10, 15 and 20 minutes) and three ultrasonic amplitudes (UA) of 20, 30 and 40% for the ultrasound-assisted extraction were used. Total yield, gelatinization temperature, water absorption and solubility index, swelling index, amylose and amylopectin content, presence of acetogenins and starch granule morphology were recorded. The highest extraction yield (ultrasound) was reached using 10 min and 40% UA (6.34%). For the gelatinization temperature, 10 min and 30% UA (82.33°C) were the best conditions. Water Solubility Index (WSI) values of $6.13 \pm 0.00\%$ using 10 min and 20% UA were recorded. Water Absorption Index (WAI) as well as Swelling index (SI) values of $3.34 \pm 0.12\%$ and $3.26 \pm 0.00\%$ were obtained by conventional extraction, respectively. Starch granules morphology showed different size with circular shapes and truncated geometries. Finally, positive qualitative results for the presence of acetogenins by all the extraction methods were obtained.

Keywords: acetogenins; *Annona*; morphology; polysaccharides; rheology.

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Introduction

Polysaccharides are used in the food industry as gelling agents, stabilizing thickeners, among other applications. Starch is one of the most important polysaccharides and is considered an excellent raw material to modify the texture and consistency of food. In addition, due to its functional properties, the starch has a wide range of applications in the food industry, in the manufacture of paper, adhesives and biodegradable packaging. Also, is a low-cost product for the benefit of the human being (Torres, Durán, & Montero, 2013). Starches are polysaccharides of a predominant food reserve in plants, they are present in the form of compact intracellular granules with structure and characteristic size according to the plant from which they come. The starch is a semicrystalline polysaccharide composed of D-glucopyranoses linked by glucosidic bonds. In the same way, the starch is formed by two polymers of different structures (Tovar-Benítez, 2008). Amylose is a linear polymer of glucose units linked by α (1-4) bonds, in which some α (1-6) bonds may be present. On the other hand, amylopectin is a branched polymer of glucose units linked in 94-96% by α (1-4) bonds and in 4-6% with α (1-6) bonds. These branches are located approximately every 15-25 glucose units. Starch can be extracted by conventional or technified methods. In this regard, emerging technologies can be used as an

alternative to increase the efficiency of extraction through food processes (Chemat, Humma, & Khan, 2011). The advantages of new processing technologies over conventional processes are the retention of sensory attributes, the desired texture and the improved functional properties. Ultrasound is the most investigated and developed emerging technology in the last years for food preservation. One of the main characteristics of this technology is the decrease in the microorganisms concentration and inhibition of enzymatic activity without altering the physical, chemical and nutritional properties of food (Delgado, 2011). The quality of starch is related to the variations between the proportion of amylose and amylopectin which is involved to the changes in gelatinization temperature and time in the extraction process, this is reflected in the behavior with respect to its solubility, volume and water absorption power (Wheatley, 1991; Hernández-Medina, Torruco-Uco, Chel-Guerrero, & Betancur-Ancona, 2008). The quality of starch and the current interest for a healthy nutrition to reduce the risk of disease has led to studies that focus on the search of natural bioactive ingredients for the development of products or functional foods that are beneficial to humans, either in health or in the production of their food (Rojas & Gerschenson, 2001). An example of these bioactives compounds are the acetogenins extracted from anonaceae (AA). The AA are secondary metabolites that have been used as fungicides, bactericides, antivirals, among other contributions. The mode of action of AA is related with the specific inhibition of Complex 1 in mitochondrial respiration (Orru et al., 2003).

The objective of this study was to characterize the functional properties of starches from soursop fruits and determine the presence of acetogenins.

Material and methods

The research was carried out in the laboratories of the Food Technology Unit at the University Autonomous of Nayarit. Soursop fruits at physiological maturity were used as raw material.

Conventional method (control)

Soursop fruit (pulp, peel and seed) was pulverized based on the technique of Flores-Gorosquera et al. (2004) with a wet grinding process using an industrial mill. Subsequently, the paste was filtered and rinsed with distilled water to remove residues until the water with the presence of starch was obtained. Next, it was recovered and allowed to stand for 24h until precipitation. After that time, most of the supernatant was removed and the white residue was centrifuged at 3500 revolutions per minute (rpm) for five minutes. The solid phase was dehydrated in a drying oven (TERLAB® TE-H70DM, Mexico) at 50°C for 24h, the dried starch was weighed on an analytical balance (A&D Company Limited HR-250A, Japan).

Ultrasound-assisted extraction (UAE)

UAE was performed with a ultrasonic processor (Cole-Parmer Instruments, CPX 750, United States) at a frequency of 20 kHz with maximum input power of 750 W. The UA tested were 20, 30 and 40% using operation time conditions of 5, 10, and 15 min under a controlled temperature at 25°C. UAE using three ultrasonic times (10, 15 and 20 min) and three ultrasonic amplitudes (20, 30 and 40%) at 750 W of power was performed. Ten treatments were generated as follows: T1: 10 min-20% UA, T2: 10 min-30% UA, T3: 10 min-40% UA, T4: 15 min-20% UA, T5: 15 min-30% UA, T6: 15 min-40% UA T7: 20 min-20% UA, T8: 20 min-30% UA, T9: 20 min-40% UA and T10: Conventional extraction (control).

Temperature was monitored by a thermocouple and a magnetic stirring grill to keep the soursop pulp suspended during extraction (distilled water was used as a extraction agent). Once the UAE was carried out, the pulp was filtered and washed with distilled water until no residue was observed. Next, the liquid part allowed to stand until the precipitation of starch was recorded and then centrifuged at 3500 rpm for five minutes. The starch was dried in a drying oven (TERLAB® TE-H70DM, Mexico) at 50°C for 24h, the total yield was quantified in percentage.

Starch gelatinization

Starch gelatinization was carried out according to the technique of Grace (1977). 1.0 g of dry base starch (bs) was mixed with 10 mL of distilled water preheated to 60°C. The solution was placed in a water bath (Thermo Fisher Scientific, TSGP10, United States) at 85°C and the temperature was taken gradually with a thermometer until the starch consistency was of gel.

Amylose and Amylopectin content

The technique (ISO, 1987) was used to determine the amylose and amylopectin content. This method is based in the characteristic blue color of the reaction with the 2% iodine stock solution. For this analysis, 0.05 g of starch (dry base), 0.5 mL of ethanol and 4.5 mL of 0.9 N sodiumhydroxide were mixed and then allowed to stand for 24h. After that period, the solution was diluted in 45 mL of distilled water. 2.5 mL of sample were taken and diluted in 25 mL of distilled water. Next, 0.5 mL of 1.0 N acetic acid, 1.0 mL of 2% iodine stock solution were added and mixed to a final volumen of 50 mL. A standard curve was performed from mixtures of pure potato amylose and amylopectin (Sigma Aldrich), with an amylose concentration of 0, 10, 15, 25 and 30%. The readings were performed on a spectrophotometer (BIOTEK, Sinergy-HT, United States) at 620 nm. The amylopectin content was determined by 100% difference from the amylose content by colorimetry.

Determination of the water absorption index (WAI), water solubility index (WSI) and swelling index (SI)

WAI, WSI and SI was analyzed using the protocol reported by Anderson, Conway, Pfeifser, and Griffin (1969). It consisted of heating an aqueous suspension of starch, the granules were swelling due to a progressive and irreversible absorption of water increasing its size. The determination of these indices was measured through the water absorption capacity of the starch granule and the exudation of starch fractions as the temperature of the starch suspensions increased. 1.25 g of starch (bs), 30 mL of distilled water preheated at 60 °C were added to falcon tubes and immediately placed in water bath at 60°C for 30 min. The suspension was stirred every 10 min until the end of heating. Next, the solution was centrifuged at room temperature at 4,900 rpm for 30 min. The volume of the supernatant and the weight of the tubes with the gel were recorded. 10 mL of the supernatant were taken and then placed in a drying oven (TERLAB® TE-H70DM, Mexico) at 70°C for 24h. Soluble weight was recorded.

The following equations were used for the calculation and interpretation of results:

- Water absorption index (WAI) = Gel weight (g) / Sample weight (g) bs
- Water solubility index (WSI) = Soluble weight (g) × V × 10 / Sample weight (g) bs
- Swelling index (SI) = Gel weight (g) / Sample weight (g) bs - Soluble weight (g)

Scanning electron microscopy

The morphology of starch was analyzed in a scanning electron microscope (SEC CO., LTD, SNE-3200M, Korea) according with the methodology described by Casarrubias-Castillo, Méndez-Montealvo, Rodríguez-Ambriz, Sánchez-Rivera and Bello-Pérez (2012). Samples were mounted on a metal slide using a double-sided adhesive tape and coated with a 60 nm gold layer. An accelerating voltage of 15 kV and 20 kV were used during scanning.

Acetogenins

The acetogenins were assessed by the protocol reported by León-Fernández, Obledo-Vázquez, Vivar-Vera, Sayágo Ayerdi and Montalvo-González (2017). This method consisted of a thin layer chromatography test using a *Silica gel 60 F254 TLC plate* with a solution based on methyl chloride, methanol, ethyl acetate and acetone.

Statistical analysis

Data were analyzed by an analysis of variance (ANOVA) with a mean difference (Tukey) with a significance level of 0.05 using the Minitab18 statistical program.

Results and discussion

Total yield

The total percentage obtained by the conventional method was $1.156 \pm 0.319\%$. Otherwise, UAE exhibited yields from 1.15 to 6.34% (Table 1). Among all treatments, T3 (10 min and 40% UA) showed the best yield (6.34 %), these yields were lower compared to other tropical fruits. Quinto, Córdor, Solano, and Silva (2015) obtained 30.62% in quinoa (the white variety julin), Bello-Lara et al. (2016) reported a

yield of 56.53% in banana 'Pera' and Medina et al. (2010) found a yield of 50.80% in mango cotyledons 'hilacha'.

Table 1. Yields and gelatinization temperature of soursop starch.

Time (min)	Ultrasonic Amplitude (%)	Variables	
		Yield (%)	Gelatinization (°C)
10	20	1.28±0.2 ^c	76.33±0.5 ^e
	30	2.81±1.3 ^{bc}	82.33±0.5 ^a
	40	6.34±0.0 ^a	81.33±0.5 ^{bc}
15	20	3.00±0.8 ^{bc}	80.33±0.5 ^{ab}
	30	3.19±0.7 ^{bc}	77.33±0.5 ^{bc}
	40	3.85±1.4 ^b	80.33±1.1 ^{de}
20	20	3.33±0.5 ^{bc}	78.66±0.5 ^{bc}
	30	3.31±0.4 ^{bc}	78.00±0.0 ^{cd}
	40	2.47±0.2 ^{bc}	80.33±0.5 ^{bc}
	Control	1.15±0.3 ^c	70.33±0.5 ^f

Means with different letters are significantly different at $p < 0.05$.

The yields depend directly on the size and the cultivar or variety that is used; it also influences the weather conditions in which they develop and therefore, the size and shape of the granules present in the morphology (Espín et al., 2001). Ultrasound is an effective method to obtain superior performance. The process occurs through the collapse of the cavitation bubbles and the degradation of the polymers through the rupture of the cell as a result of the chemical reaction between the polymer and high-energy molecules produced from the cavitation phenomenon (Chemat et al., 2011).

Gelatinization

We observed a gelatinization temperature of 70.33°C by conventional extraction. Nevertheless, UAE showed temperatures between 75 and 82°C with no differences in treatments (Table 1). The temperatures were lower than those reported by Shittu, Lasekan, Karim and Sulaiman (2016) in banana starch (84.25°C). Li, Wang and Shu (2016) recorded 59.2°C in quinoa starch. Moreover, temperatures of 72.2, 65 and 68°C were reported in cassava and potato starches, respectively (Trinh, Choi, & Moont, 2013; Ascencio Galván, Andrade, & Salcedo, 2016; Paternina, Salcedo, & Romero, 2016). The gelatinization temperature of the soursop starches determines the granule sizes, at a higher temperature, the starches displayed large diameters and at a lower temperature the diameters are small (Li et al., 2016). Gelatinization temperatures vary between starches from different sources, which can be attributed to differences in the degree of crystallinity (Singh Singh, Kaur, Sodhi, & Gill, 2003).

Amylose and Amylopectin content

We obtained by conventional method an amylose and amylopectin content of 35.10% and 64.90%, respectively. On the other hand, amylose contents ranged from 24 to 30% and amylopectin contents from 69 to 76% using UAE (Table 2). Previous studies have shown a 12.45% of amylose and 87.55% of amylopectin content in 'Hilacha' mango fruits (Medina et al., 2010). Solís-Espinoza et al. (2008) reported 36% of amylose and 64% of amylopectin in banana. Further, an amylose content of 29.7% and 21% as well as amylopectin content of 70.63% and 79% has been recorded in corn and potato starch, respectively (Betancur-Ancona, Gallegos-Tintoré, & Chel-Guerrero, 2004). The results found in this investigation are in the range of conventional products for starch extraction, hence, the soursop fruit can be an alternative within unconventional sources.

Table 2. Amylose and amylopectin content.

Time (min)	Ultrasonic Amplitude (%)	Variables	
		Amylose	Amylopectin
10	20	30.90 ^b	69.10 ^b
	30	25.27 ^c	74.73 ^a
	40	24.20 ^c	75.80 ^a
15	20	24.00 ^c	76.00 ^a
	30	29.50 ^b	70.50 ^b
	40	26.27 ^c	73.73 ^a
20	20	24.47 ^c	75.53 ^a
	30	24.90 ^c	75.10 ^a
	40	25.63 ^c	74.37 ^a
Control		35.10 ^a	64.90 ^c

Means with different letters are significantly different at $p < 0.05$.

Water absorption index (WAI), water solubility index (WSI) and Swelling Index (SI)

The WSI of starches is the ability to react with water and dissolve in it, it also indicates the degree of association (intragranular bond) between the ratio of polymers of amylose/amylopectin starch (Araujo de Vizcarrondo, Rincón, & Padilla, 2004). The WSI values of soursop starches was $3.32 \pm 0.06\%$ (Table 3) by conventional extraction. On the otherhand, UAE starches ranged from 2.27% to 6.13%, which leads to no influence on the technology used. These results are smaller than those reported by Torres et al. (2013) who presented 12.8 ± 0.3 and 23.07 ± 0.21 values of white and purple malanga starches, respectively. However, the values obtained in this investigation are higher than those recorded for yam starch (1.25 to 2.79%), cassava (2.60 to 3.70%) and potato (2.97%) (Alvis, Vélez, Villada, & Rada-Mendonza, 2008). We can suggest that these differences in WSI from other starches are by the high amylopectin content in soursop starches and probably, due to the great number of lateral branches of amylopectin. Similarly, a small granule size facilitates the entry of water into the intermolecular spaces, resulting in an increase in the solubility of the polymers, in which the amylopectin have the highest proportion of dissolution (Hwang & Kokini, 1992).

Table 3. Water absorption and solubility index and Swelling Index.

Time (min)	Ultrasonic Amplitude (%)	Variables		
		WSI	WAI	SI
10	20	6.13 \pm 0.00 ^b	1.93 \pm 0.05 ^a	6.12 \pm 0.00 ^a
	30	4.85 \pm 0.03 ^{bc}	1.57 \pm 0.26 ^b	4.84 \pm 0.03 ^b
	40	4.83 \pm 0.00 ^c	1.25 \pm 0.02 ^b	4.82 \pm 0.00 ^b
15	20	4.23 \pm 0.01 ^c	1.23 \pm 0.10 ^c	4.23 \pm 0.01 ^c
	30	4.33 \pm 0.00 ^{bc}	1.34 \pm 0.30 ^c	4.24 \pm 0.12 ^c
	40	2.27 \pm 0.10 ^d	0.38 \pm 0.04 ^f	2.27 \pm 0.10 ^f
20	20	2.37 \pm 0.05 ^d	0.41 \pm 0.09 ^f	2.37 \pm 0.05 ^f
	30	2.36 \pm 0.04 ^d	0.52 \pm 0.20 ^f	2.35 \pm 0.04 ^f
	40	2.78 \pm 0.10 ^d	0.32 \pm 0.09 ^e	2.78 \pm 0.10 ^e
Control		3.28 \pm 0.00 ^a	3.34 \pm 0.12 ^d	3.26 \pm 0.00 ^d

Means with different letters are significantly different at $p < 0.05$.

Moreover, lower WAI values (from 0.32 to 1.93%) were obtained by UAE in comparison with conventional method ($3.34 \pm 0.12\%$) as shown in Table 3. Torres et al. (2013) reported a WAI value in malanga starches of $1.79 \pm 0.1\%$ and Alvis et al. (2008) reported a 5.85% of WAI in potato starches. WAI values of cassava starch ranged from 4.63 to 4.80%, respectively; these results were higher than those reported in soursop starch. The differences in the WAI of starches are directly related to the biological source of the fruit and the size and shape of the starch granule morphology (Lindeboom, Chang, & Tyler, 2004).

The swelling index is related to the water absorption capacity of each starch. SI of the starches is a property of its amylopectin content, in which the amylose is a diluent and swelling inhibitor (Chen, Zhou, Yang, & Cui, 2015). The SI of soursop starch showed $3.30 \pm 0.06\%$. These results were lower than those reported by Gutiérrez, Rivera, Gómez, Bastidas and Izaguirre (2015) for starches from mango almonds and for mango fruits cv 'Alphonso' ($19.53 \pm 0.02\%$). Likewise, the ultrasound-assisted starches showed a SI between 2.27 and 6.12%, presenting average percentages with conventional extraction.

Scanning electron microscopy

Soursop starch granules revealed a circular and elliptical morphology with irregular sizes. Further, some of its geometries were truncated (broken) with cuts at their ends (Figure 1). The ultrasound-assisted starch granules showed similar morphology to control treatment, presenting irregular sizes of circular and elliptical shapes and truncated geometries.

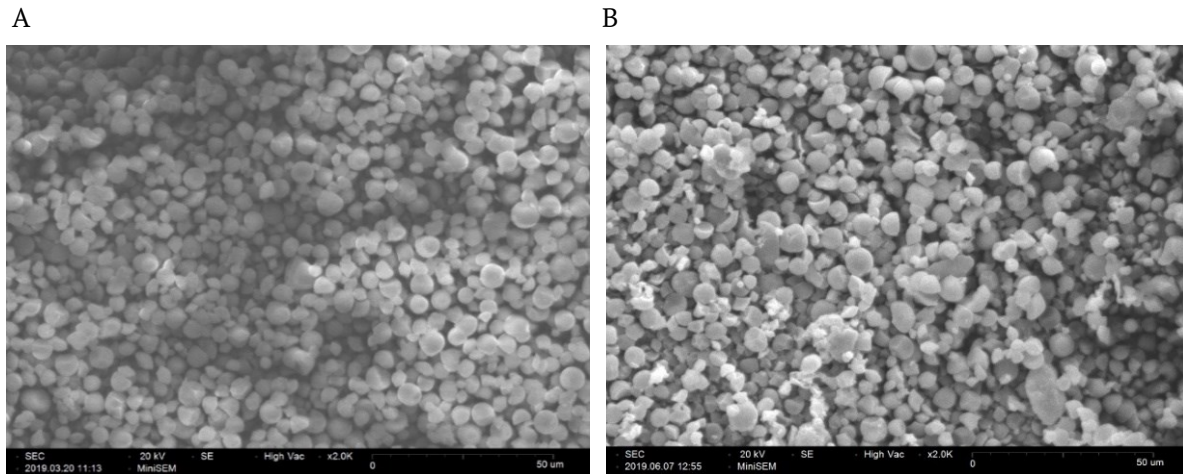


Figure 1. Scanning Electron Microscopy of soursop starch. A) Starch granules extracted by conventional method. B) Starch granules assisted with ultrasound 10x40% AU (conditions: 20 KV - x 2.0 K at 50 μm).

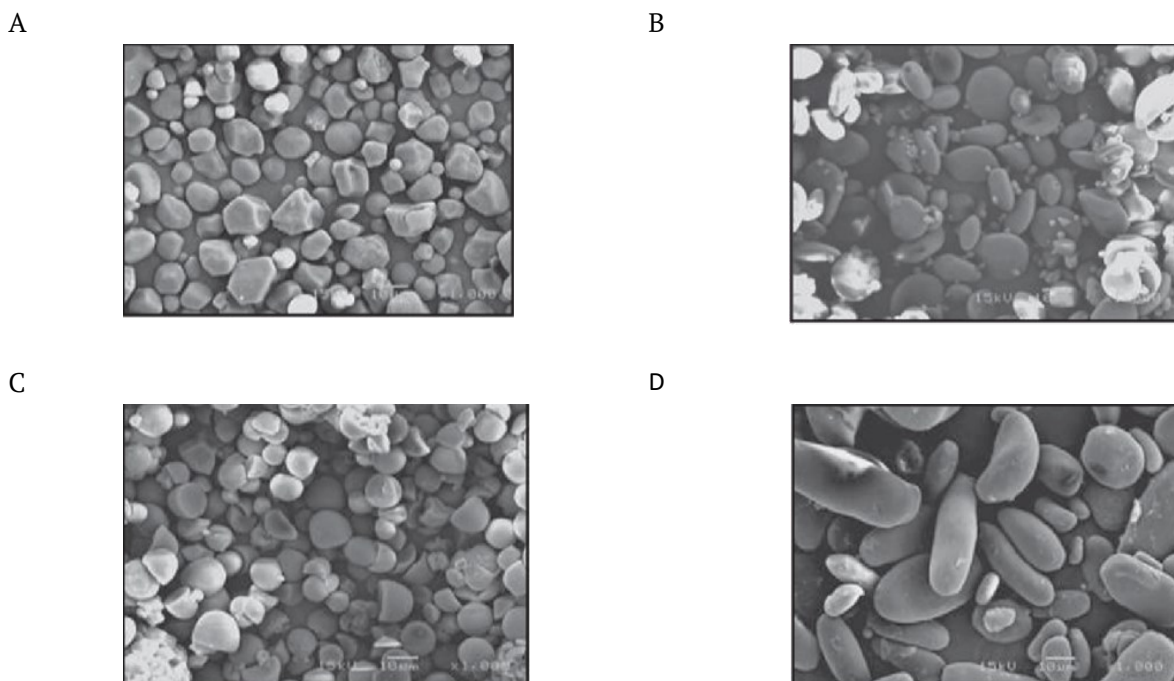


Figure 2. Scanning Electron Microscopy of corn starches (A) barley (B) mango (C) and banana (D); reported by Casarrubias-Castillo et al. (2012).

Soursop starch morphology was compared with barley, mango, banana and corn granules. Corn starch shows spherical, oval and polygonal predominance shapes with small and large sizes, barley starch has small spherical and large lenticular forms, while mango starch granules show a spherical or dome shape and tips, presenting indentations. Finally, banana starch shows larger and longer granules (Casarrubias-Castillo et al., 2012). The size and shape of the starch granules depend directly on the structure of the amylopectin. Additionally, elongated granules have amylopectin with few long chain branches, and small, spherical granules have a superior number of branches, but short chains (Jane et al., 1999). Ultrasound-assisted treatments may affect the structure of starch granules in different dimensions (Zinoviadou et al., 2015).

Indeed, it has been reported that the use of ultrasound can affect the structure of starch granules in different dimensions, presenting fractured or broken granules morphology (Zhu, Li, Chen, & Li, 2012).

Acetogenins

We detected a positive (+) presence of acetogenins (qualitative test) in all extractions. These results indicate that acetogenin molecules adhere to starch due to the presence of this bioactive compound in the final product. In this regard, we proved that the aqueous starch extraction favors the obtention of acetogenins because the polarity of the solvent (Agu et al. (2018)). These results are similar to those reported by León-Fernández et al. (2017) in soursop pulp, in which the presence of total acetogenins was identified using UAE, microwave with chloroform and ethyl acetate. The presence of this bioactive compound in soursop starch gives an added value to the final product, attributing the main benefits that this compound naturally offers.

Conclusion

The highest starch yield of soursop fruits was obtained by UAE in a condition of 10 min and 40% ultrasonic amplitude (6.34%). Gelatinization of the starch at 82.33°C was found using an UA of 30% and 10 min. The WSI was $6.13 \pm 0.00\%$ under conditions of 10 min and 20% UA.

The absorption rate and SI by conventional extraction was $3.34 \pm 0.12\%$ and $3.26 \pm 0.00\%$, respectively. The morphological characteristics of the starch granules showed circular and elliptical shapes with irregular sizes in all conditions tested. Truncated (broken) geometries were observed mainly with assisted extraction. Positive presence of acetogenins (qualitative test) was recorded.

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Appendix 1 – Presence of acetogenins in soursop starch.

Time (min)	Ultrasonic Amplitude (%)	Acetogenins
10	20	+
	30	+
	40	+
15	20	+
	30	+
	40	+
20	20	+
	30	+
	40	+
Control		+