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Phytochemical compounds in soursop fruit starches (*Annona muricata* L.)

Compuestos fitoquímicos en almidones de frutos de guanábana (*Annona muricata* L.)

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ABSTRACT

Researches on starches containing phytochemical compounds are new opportunities in the production of functional foods and dietary supplements. Starches containing these compounds may have beneficial health effects, such as antioxidant properties. Starches are found in a variety of unconventional sources, including soursop fruits. This study aimed to determine the content of phytochemical compounds in starches extracted from soursop fruits by conventional method and ultrasound. Starch was extracted from soursop fruits by conventional method and ultrasound, obtaining two phases for each method. The amylose and lipid content were determined, as well as the content of phytochemical compounds in the starches (phenols, tannins, flavonoids, phytosterols, alkaloids, and acetogenins). The extractions were: ACF1 (conventional starch phase 1), AUF1 (ultrasonic starch phase 1), AUF2 (Ultrasonic starch phase 2), and ACF2 (conventional starch phase 2). The AUF1 starches presented high content of total soluble phenols (2.26 mg GAE/g of d.b.s), total soluble tannins (0.99 mg GAE/g of d.b.s), total soluble flavonoids (1.55 mg QE/g of d.b.s), phytosterols (3.55 mg CE/g of d.b.s), alkaloids (8.42 mg AE/g of d.b.s) and acetogenins (1.81 mg ANE/g of d.b.s). Ultrasound-assisted starch extraction increases the starch content of soursop fruits and phytochemical compounds. The phytochemical compounds present in the starches of soursop fruits are phenols, flavonoids, tannins, alkaloids, phytosterols, and acetogenins.

KEY WORDS: Acetogenins, alkaloids, anonacea, polysaccharides.

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Revista Bio ciencias
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RESUMEN

Las investigaciones en almidones que contengan compuestos fitoquímicos son nuevas oportunidades en la producción de alimentos funcionales y suplementos dietéticos. Los almidones que contienen estos compuestos pueden tener efectos benéficos para la salud, como propiedades antioxidantes. Los almidones se encuentran en una variedad de fuentes no convencionales, dentro de los cuales se encuentran los frutos de guanábana. El objetivo de este estudio fue determinar el contenido de compuestos fitoquímicos en almidones extraídos de frutos de guanábana mediante método convencional y ultrasonido. Se extrajo almidón de frutos de guanábana mediante método convencional y ultrasonido obteniéndose dos fases por cada método; se determinó el contenido de amilosa y lípidos, así como el contenido de compuestos fitoquímicos en los almidones (fenoles solubles totales, taninos solubles totales, flavonoides solubles totales, fitoesteroles, alcaloides y acetogeninas). Las extracciones fueron: ACF1 (Almidón convencional fase 1), AUF1 (almidón ultrasonido fase 1), AUF2 (Almidón ultrasonido fase 2), ACF2 (almidón convencional fase 2). Los almidones de AUF1 presentaron alto contenido de fenoles solubles totales (2.26 mg GAE/g de d.b.s), taninos solubles (0.99 mg GAE/g de d.b.s), flavonoides solubles totales (1.55 mg QE/g de d.b.s), fitoesteroles (3.55 mg CE/g de d.b.s), alcaloides (8.42 mg AE/g de d.b.s) y acetogeninas (1.81 mg ANE/g de d.b.s). La extracción de almidones asistida por ultrasonido incrementa el contenido de almidones de frutos de guanábana y compuestos fitoquímicos. Los compuestos fitoquímicos presentes en los almidones de frutos de guanábana son fenoles, flavonoides, taninos, alcaloides, fitoesteroles y acetogeninas.

PALABRAS CLAVE: Acetogeninas, alcaloides, anonácea, polisacárido.

Introduction

Starch is a polysaccharide widely used in industry due to its high availability, low cost, and great versatility in its applications, highlighting its use as a thickening, stabilizing, and emulsifying agent in the food and paper industry (Schmiele *et al*., 2019; Koev *et al*., 2020). Industrially, starches are extracted from potato (*Solanum tuberosum* L.*),* corn (*Zea mays* L.), rice (*Oryza sativa* L.), and wheat *(Triticum aestivum* L.), cataloged as conventional sources (Carvalho, 2013). Given the growing demand for alternative and innovative food products with nutraceutical characteristics beneficial to health and the increase in the consumption of ultra-processed foods, research has been conducted to evaluate the use of banana starches (*Musa paradisiaca* L.), mango (*Mangifera indica* L.), cherimoya (*Annona cherimola* L.) and soursop (*Annona muricata* L.) because these starches have resistance to enzymatic hydrolysis and a low tendency to syneresis, as well as content of phytochemical compounds (Martínez-Ortiz *et al*., 2022; Espinosa-Solís *et al*., 2009).

Revista Bio ciencias
ISSN 2007-3380

Phytochemical compounds are secondary metabolites produced by plants, these have antioxidant activities mainly (Zubaidi *et al*., 2023). Soursop fruits have phenols, flavonoids, tannins, alkaloids, phytosterols, and acetogenins (Correa-Gordillo *et al*. 2012; Terán-Erazo *et al*., 2019; Aguilar-Hernández *et al*., 2020a), the latter has been identified qualitatively in soursop fruit starches (Ramírez-Balboa *et al*., 2021) and have been studied by determining the content of this compound in seeds and pulp, as well as its biological activity (Grba *et al*., 2022; Prasad *et al*., 2021; Mutakin *et al*., 2022). Phytochemical compounds are present in soursop fruit starches forming binary (amylose-lipid), tertiary (amylose-lipid-protein), and quaternary (amylose-lipid-protein-phenol) interactions (Wang *et al*., 2019; Krishnan *et al*., 2021), in this regard, the phytochemical compound content of starches is directly proportional to the amylose percentage of starches and lipids (Krishnan *et al*., 2021). These compounds present in starches can exert beneficial health effects directly, mainly in the digestive tract, or indirectly through their prebiotic activities on the intestinal microbiota and the formation of bioactive bacterial metabolites that act both locally and systemically (Gasaly *et al*., 2020). In this sense, this study aimed to determine the content of phytochemical compounds in starches extracted from soursop fruits by conventional method and ultrasound.

Materials and Methods

Biological material

Soursop fruits were harvested, considering the harvest index (160 days after anthesis) reported by Balois-Morales *et al*. (2019), the harvest was in the ejido of Venustiano Carranza, Tepic, Nayarit (21° 32’ 2.77’’ N, 104° 58’37.73’’ W, 893 masl). The fruits were transported to the Food Technology Unit, selecting those that did not present mechanical and microbiological visual damage, and washed with a solution of sodium hypochlorite 1% (v / v).

Starch extraction

The research consisted of extracting starches from whole soursop fruits without separating their structures (epicarp, mesocarp, and endocarp) using two extraction methods (conventional and ultrasound):

Conventional starch extraction

It was performed according to the technique of Flores-Gorosquera *et al*. (2004). The fruits were ground in an industrial blender (International LI-5a, Mexico) for 3 min with a solution of citric acid (1%) in a ratio of 1:4 (w / v), the resulting paste was filtered with a sieve No. 100 (150 mics) then in a sieve of No. 200 (75 mics) and 270 (53 mics), respectively. Subsequently, a second grinding with distilled water was carried out in a ratio of 1:2 (w / v) for 3 min and a second filtering.

Revista Bio ciencias
ISSN 2007-3380

Ultrasound-assisted starch extraction

The technique described by Flores-Gorosquera *et al.* (2004) and Ramírez-Balboa *et al*. (2021) were used. Fruits were ground for 3 min with a solution of citric acid (1%) in a ratio of 1:4 (w / v). Subsequently, the resulting paste was sonicated at 300 W power in a 750 W ultrasonic processor (Model CPX750 Cole-Parmer Instruments Vernon Hills, U.S.A.) with 20 kHz probe for 10 min at 25 °C monitored by a manual mercury thermometer (Ramírez-Balboa *et al*., 2021). The sonicated paste was filtered using sieves of the numbers 100, 200, and 270. Subsequently, a second grinding was carried out using distilled water in a ratio of 1:2 (w / v) and then the precipitate (starch) was recovered.

To remove excess water in starches, a centrifuge was used (Hermle Z326K Wehingen, Germany). During this process, it was observed that the starches when centrifuged were separated into two phases (phase 1 and phase 2), so it was decided to evaluate both phases to determine their physicochemical and phytochemical characteristics.

The starch extracted by conventional method and ultrasound was centrifuged at 1537 *g* for 5 min at 25 °C to precipitate and separate the phases that were identified as phase 1 (brown color) and phase 2 (white color). Phase 1 was separated with a spatula, leaving phase 2 at the bottom of the tube. Subsequently, 10 mL of water was added to the tube and stirred (first wash), then centrifuged at 1537 *g* for 5 min at 25 °C repeating the phase separation process. This process was done in triplicate. The starches (phase 1 and phase 2) were dried in a recirculating furnace (LSIS-B2V/VC 55, Germany) for 24 h at 35 °C, pulverized, and sieved (No.100). Finally, they were weighed for quantification and stored. The following nomenclature was used to better interpret the results: ACF1, ACF2, AUF1, and AUF2. The letter A (starch), C (conventional extraction), U (ultrasound-assisted extraction), F (phases); e.g., starch extraction by conventional method phase 1 (ACF1).

Variables evaluated

Lipid and amylose evaluation

Lipid content (%)

It was determined using the AOAC technique (2005). 4 g of starch was added to cellulose cartridges and placed in a grease extractor. 130 mL of petroleum ether in a flask was added at a constant weight. It was left to boil for 4 h. The calculation was determined by the weight difference in the flask.

Revista Bio ciencias
ISSN 2007-3380

Amylose content (%)

It was performed using the Hoover & Ratnayake technique (2001), 100 mg of dry and defatted starch was scattered in 10 mL of dimethyl sulfoxide (DMSO) (90% w/v) and heated in a water bath (Thermo Scientific, TSGP10, Canada) at 85 ° C/15 min in stirring (solution 1). Subsequently, 100 mL was measured with distilled water (solution 2) and 20 mL of the sample was taken at 25 mL (solution 3). Then 1 mL of solution 3 was taken and 5 mL of iodine were added, adjusting the solution to 50 mL. It was allowed to stand 15 min in darkness and the absorbance was read at 600 nm. A standard potato amylose and amylopectin curve was used.

Analysis of phytochemical compounds

Obtaining methanolic starch extract

In 100 mL of methanol, 20 g of starch (ACF1, AUF1, ACF2, and AUF2) were added. The resulting solution was sonicated in an ultrasonic bath (Luzerner, CD-4820, China) at 35 kHz and 160 W for eight cycles of 8 min each, it was stirred at the end of each cycle, then centrifuged at 6147 *g* / 10 min and the liquid phase was recovered, the extracts were filtered with a vacuum pump (EVAR model EV-40, Mexico) in a porcelain funnel, using closed-pore filter paper and subsequently concentrating on a rotary vapor (IKA© model RV-10 Digital V, North Carolina, USA) at 40 °C at reduced pressure (65 cmHg), the concentrated extracts were subjected to 35 ± 2 °C in an oven (LSIS-B2V/VC 55, Germany) for 48 h to evaporate the solvent residues (León-Fernández *et al*., 2021).

Qualitative analysis of phytochemical compounds of the starch extract

The extracts were diluted to a concentration of 4 mg / mL in methanol. Subsequently, the procedures of Sofowara (1993), Harborne (1998) & Evans (2009) were used to determine the presence of the following compounds:

Phytosterols

100 µL of extract, 1 mL of chloroform, and 100 µL concentrated sulfuric acid were added to a test tube. The reddish-brown precipitate at the bottom of the tube indicated the presence (Trease & Evans 1989; Evans, 2002).

Phenols and tannins

In a test tube, 300 µL of extract and 300 µL of ferric chloride ($FeCl_3$) (10%) were placed. Blue or green precipitate indicated presence (Trease & Evans 1989; Evans, 2002).

Revista Bio ciencias
ISSN 2007-3380

Alkaloids

In a test tube were placed 300 µL of extract and 300 µL of hydrochloric acid (HCl) at 2 N and 300 µL of Mayer's reagent, the presence of a pale precipitate at the bottom of the tube, indicates the presence of alkaloids (Trease & Evans 1989; Evans, 2002).

Flavonoids

In a test tube, 300 µL of extract were placed, 333 µL of 10% ammonia (NH_4OH), and 333 µL of H_2SO_4 were added. When the yellow color disappears in the sample, the presence is indicated (Trease & Evans 1989; Evans, 2002).

Acetogenins

1 mL of extract was added to a tube and Kedde's reagent was added (Gu *et al*., 1995) which was prepared with the mixture of solution A (3-5 dinitrobenzoic acid) and solution B (potassium hydroxide KOH 5.7%), 2 mL each. The pink coloration determined the presence of acetogenins (León-Fernández *et al*., 2021).

The results were expressed with the presence (+) or absence (-) of the phytochemical compound evaluated.

Quantitative analysis of phytochemical compounds

Total soluble phenols

They were determined according to the methodology of Stintzing *et al*. (2005). 50 µL of the sample was added in Eppendorf vials, then 250 µL of Folin-Ciocalteu solution (v/v 1:10 in deionized water) and 200 µL of sodium carbonate solution (7.5%) were added, the solution was stirred in a vortex (Genie® G650, U.S.A) and incubated at 23 °C for 30 min in the absence of light. Absorbance was measured in a microplate reader (Power Wave XS, Biotek, U.S.A) at a wavelength of 765 nm. The results obtained were expressed in mg gallic acid equivalents per gram of dry base starch (mg GAE/g of d.b.s).

Total soluble tannins

It was determined by the Folin-Ciocalteu + PVPP technique (polyvinylpolypidorrilone) according to the method of Makkar (2003), 100 mg of PVPP was diluted in 1 mL of distilled water and left to stand for 2 h. 200 µL of sample was added and stored at 4 °C for 15 min, then centrifuged at 6147 *g* for 10 min and the supernatant containing non-tannin phenols was recovered. 50 µL of supernatant was taken and the total phenol content was evaluated using the method of Stintzing *et al*. (2005). Absorbance was measured in a microplate reader (Power Wave XS, Biotek, U.S.A.)

Revista Bio ciencias
ISSN 2007-3380

at a wavelength of 765 nm. The result obtained was subtracted from the content of total soluble phenols found in the sample without PVPP. The results obtained were expressed in mg gallic acid equivalents per gram of dry base starch (mg GAE/g of d.b.s).

The calculation of total soluble tannins was performed using the following equation (equation 1):

Equation 1:

$$T = FT - Fnt$$

Where:

TT= total soluble tannins.

Fn= total soluble phenols.

Fnt= soluble phenols, not tannins.

Total soluble flavonoids

It was performed using the methodology developed by Zhishen *et al*. (1999), 50 µL of the sample was added in Eppendorf tubes, and 100 µL of deionized water. Subsequently, 10 µL of NaOH (15%) was added, stirred in a vortex (Genie G650, U.S.A.), and rested for 6 min in darkness at 23 °C. After a time, 15 µL of $AlCl_3$ (10%) was added and stirred in a vortex (Genie G650, U.S.A.) tag. The solution was rested for 6 min in darkness at 23 °C. Finally, 200 µL of NaOH (4%) was added and stirred. Absorbance was measured at 510 nm (Power Wave XS, Biotek, U.S.A.). The results obtained were expressed in mg of quercetin equivalent per gram of dry base starch (mg QE/g of d.b.s).

Total phytosterols

The total phytosterol content was determined using the Lieberman-Buchard method (Lobato *et al*., 2008). In a glass reaction tube, 50 µL of sample and 50 µL of chloroform were added, the mixture was stirred and an aliquot of 50 µL was taken by adding 50 µL of chloroform and 1 mL of Lieberman-Buchard reagent prepared with acetic anhydride and sulfuric acid (20:1 v/v) cooled to 4 °C. The mixture was subjected to a water bath (Thermo Scientific, TSGP10, U.S.A.) at 35 ± 2 °C for 25 min. After a time, 200 µL was taken and the absorbance was read at 550 (Power Wave XS, Biotek, U.S.A.). The results obtained were expressed in mg cholesterol equivalents per gram of dry base starch (mg CE/g d.b.s).

Revista Bio ciencias
ISSN 2007-3380

Total alkaloids

It was performed using the technique of Fazel *et al*. (2010). 1 mL of extract was dissolved in 1 mL of HCl 2 N (1:1 v/v) and 3 mL of chloroform were added. The precipitate was recovered, and the pH was adjusted to 7 with NaOH 0.1 N. The solution was deposited inside a separation funnel and 5 mL of bromocresol green solution and 5 mL of phosphate buffer at pH 4.7 were added; the mixture was stirred and the alkaloid-green complex of bromocresol was extracted by adding 1 mL of chloroform, subsequently, 7 mL were added recovering the complex for each mL added. The yellow complex was adjusted to 10 mL with chloroform. 200 µL was taken and absorbance was measured at 416 nm in the microplate reader (Metash, UV-5100 UV/VIS, China). Results were expressed in mg atropine equivalents per gram of dry base starch (mg AE / g d.b.s).

Total acetogenins

It was performed using the methodology of Aguilar-Hernández *et al*. (2022). 250 µL of sample and 4 mL of Kedde reagent (Aguilar-Hernández *et al*., 2020a) were added in quartz cells. Absorbance kinetics were performed at 505 nm for 30 s with readings every 5 s and the highest absorbance peak was recorded on a spectrophotometer (Metash, UV-5100 UV/VIS, China). Results were expressed as mg annonacin equivalents per gram of dry base starch (mg ANE/g d.b.s).

Statistical analysis

A complete randomized design was used with a 2 x 2 factorial arrangement (extraction methods and starch phases). Four treatments were established where starches (phase 1 and phase 2) obtained from conventional and ultrasound-assisted extraction were evaluated. Results were analyzed with an ANOVA and a comparison of means per Tukey test ($p \leq 0.05$) using the Statistical Analysis System software (SAS® V. 9.2).

Results and Discussion

Physicochemical analysis

Lipid content (%)

The lipid content (%) in starch extractions was ACF2 (0.016), AUF2 (0.018), ACF1 (2.4), and AUF1 (4.03). The results obtained showed statistical differences in the extractions ($p \leq 0.05$) (Table 1). Krishnan *et al*. (2021) mention that starches can interact with some compounds of a lipid nature through amylose-lipid complexes. Concerning this characteristic, starches can form binary interactions with some lipid compounds of soursop fruits such as phytosterols and acetogenins, such compounds have been reported by Haykal *et al*. (2019) and Aguilar-Hernández *et al*. (2022),

Revista Bio ciencias
ISSN 2007-3380

respectively. Also, starches with high lipid content have been reported to have lower hydrophilic properties, because lipid compounds form complexes with amylose, limiting the availability of hydroxyl groups to interact with water molecules (Henning *et al*., 2021).

Amylose content (%)

The content of amylose (%) in the starch extractions was ACF2 (24.48), AUF2 (23.14), ACF1 (11.95), and AUF1 (11.05), with a difference in the results ($p \leq 0.05$). For the estimation of amylose, the starches were defatted, eliminating the amylose-lipid complex to form an iodine-amylose complex, which is used for the estimation of amylose (Hoover & Ratnayake, 2001); in this research, the high content of amylose in the starches of the ACF1 and AUF1 extractions could be related to the formation of quaternary interactions with other components such as proteins and phenolic compounds (Krishnan *et al*., 2021). As reported by Oliveira-Bernardo *et al*. (2018), this result decreases the water absorption capacity of starch granules. Studies in soursop fruit starches have reported 24.2 and 35.1% amylose in starches extracted by conventional and ultrasound-assisted methods (Ramírez-Balboa *et al*., 2021). Nwokocha & Williams (2009) reported 19% amylose in starches of *Annona squamosa* L. and *Anonna muricata* L. using conventional extraction; they also reported that the ideal range of amylose is 18-30% in native starches. The starches of soursop fruits have a low content of amylose (waxy) and low syneresis (slow retrogradation), so they can be used in the preparation of baked and frozen products (Martínez-Ortiz *et al.,* 2022), in addition to their content of phytochemical compounds they can be considered beneficial for health (Cereceres-Aragón *et al*., 2019; Gasaly *et al*., 2020).

Table 1. Physicochemical analysis of soursop fruit starch.

Extraction	Lipids (%)	Amylose (%)
ACF2	0.016 ± 01^{c}	24.48 ± 0. 56^{a}
AUF2	0.018±0.01^{c}	23.14 ± 1.67^{a}
ACF1	2.4 ± 0.8^{b}	11.95 ± 0.64^{b}
AUF1	4.03 ± 0.4^{a}	11.05 ± 0.3^{b}
LSD	1.24	2.49
CV	29.25	5.39

The same letters per column do not differ statistically (Tukey, $p \geq 0.05$). The values are the means of three repetitions ± standard deviation. LSD: least significant difference. CV: coefficient of variation.

Revista Bio ciencias
ISSN 2007-3380

Qualitative analysis of phytochemical compounds

In the starches extracted by the conventional method and ultrasound, the presence (+) of phytosterols, phenols, tannins, alkaloids, flavonoids, and acetogenins was observed (Table 2). Phytochemical compounds found in soursop fruit starch have been reported by León-Fernández *et al*. (2021) and Aguilar-Hernández *et al*. (2020a), who conducted a study where they evaluated the phytochemical profile of epicarp, mesocarp, and endocarp, however, there is only one indication of acetogenins present in soursop fruit starches (Ramírez-Balboa *et al*. 2021). Lipid compounds such as acetogenins can bind starches by covalent bonds with amylose, forming an amylose-lipid complex (Krishnan *et al*., 2021; León-Fernández *et al*., 2021); in addition, a high amylose content increases the ability to form interactions with other compounds such as polyphenols (Dueñas *et al*., 2018; Wang *et al*., 2020). Likewise, there are reports that starches contain dietary fiber, which has bioactive compounds (Bello-Pérez *et al*., 2006), these are linked to fiber through hydrophobic interactions and covalent bonds (Saura-Calixto, 2011; Agredano-De la Garza *et al*., 2021).

Table 2. Qualitative analysis of starch extracts from soursop fruits.

Phytochemical compound	Extraction			
	ACF2	ACF1	AUF2	AUF1
Steroids	+	+	+	+
Terpenes	-	-	-	-
Phenols and tannins	+	+	+	+
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Acetogenins	+	+	+	+

ACF1: phase 1 conventional starch, ACF2: phase 2 conventional starch, AUF1: phase 1 ultrasound starch, AUF2: phase 2 ultrasound starch, Presence (+), absence (-).

Revista Bio ciencias
ISSN 2007-3380

Quantitative analysis of phytochemical compounds

Total soluble phenols

Phenolic compounds are characterized by having aromatic or benzene rings, with unsaturations and OH groups (Abarca-Vargas & Petricevich, 2021). These compounds interact with starch forming hydrogen bonds, hydrophobic, electrostatic, and ionic interactions (Dueñas *et al*., 2018), binding to amylose by hydrogen bonds between the hydroxyl groups of polyphenols and the oxygen atoms of the glycosidic bonds of polysaccharides (Fernandes *et al*., 2014). In this research, the content of total soluble phenols (mg GAE/g of d.b.s) in the starches of the extractions was ACF2 (0.02), AUF2 (0.06), ACF1 (1.44), and AUF1 (2.26). The results showed statistical difference ($p \leq 0.05$) (Table 3). The starches from AUF1 presented a high content of phenols concerning the rest of the extractions, this result could be because, in ultrasound-assisted extraction, the cavitation effect generates microjets that break the cell walls allowing the release of intracellular compounds (Aguilar-Hernández *et al*., 2022). Zárate-Martínez *et al*. (2021) mention that the shell (epicarp) and seeds (endocarp) of the fruits have a high content of phenolic compounds, in this sense, according to the results obtained in AUF1, compounds of other structures (shell and seeds) may be present.

Total tannins

Tannins are hydrophobic compounds that present hydroxyl groups close to each other in their structure, which strengthens the interaction with carbohydrates such as starch through hydrogen bonds (Amoako & Awika, 2016). Table 3 shows the results of the total tannin content obtained from conventional and ultrasound extractions. The starches obtained in phase 1 by both extraction methods presented statistical differences in the content of total tannins concerning phase 2 ($p \leq 0.05$). In the case of phase 1 (ACF1 and AUF1), the result could be attributed to ultrasonic waves that allow the release and solubilization of compounds in the extraction solvent, allowing the interaction of these with starches (Aguilar-Hernández *et al*., 2019). In the present investigation, it was observed that the starches extracted by ACF1 and AUF1 had lower content of amylose and high content of tannins; in this regard, Dueñas *et al*. (2018) mention that the formation of the amylose-phenol complex is directly proportional to the amount of amylose that composes it. In this sense, Barros *et al*. (2012) mention that this result could be due to the presence of tannins with high molecular weight such as ellagitannins (e.g., catechin-gallic acid) (Olivas-Aguirre *et al*., 2015), which have a greater affinity for amylose.

Total soluble flavonoids

Flavonoids are polyphenols with a hydrophobic character and could bind strongly to starch through covalent interactions with amylose (Barros *et al*. 2012). These compounds are water-soluble and interact with starches during the extraction process (Sarria-Villa *et al*., 2021). In this research, the total flavonoid content (mg QE/g of d.b.s) in the extractions was ACF2 (0.022), AUF2 (0.049), ACF1 (0.99) and AUF1 (1.55). The results obtained showed statistical difference

Revista Bio ciencias
ISSN 2007-3380

($p \leq 0.05$). ACF1 and AUF1 starches were high in flavonoids (Table 3), as reported by Zárate-Martínez *et al*. (2021), these compounds are present in soursop peel, seed, and pulp. So, the high content of flavonoids in these starches (ACF1 and AUF1) may come from husk and seed. While the low concentrations of flavonoids in the starches AUF2 and ACF2, could be related to starches from the pulp, in addition to being polar they are highly soluble in water according to what was reported by Soto-García & Rosales-Castro (2016). Takahama & Hirota (2018), mention that starches with high flavonoid content can be considered resistant because when consumed they reach the intestinal tract intact and serve as a substrate (prebiotic) for the intestinal microbiota.

Total phytosterols

Phytosterols are secondary metabolites produced by plants, they act as a defense against biotic and abiotic stress (Silva *et al*., 2016). Phytosterols due to their lipid structure can interact with amylose and amylopectin (Krishnan *et al*., 2021; Takahama & Hirota, 2018). The content of total phytosterols (mg EC/g d.b.s) was ACF2 (0.86), AUF2 (1.6), ACF1 (2.09), and AUF1 (3.55), with statistical difference ($p \leq 0.05$) (Table 3). In the present study, it was observed that the method of extraction of starches influenced the content of phytosterols, where AUF1 presented high content of phytosterols (3.55 mg EC/g of d.b.s) concerning the rest of the extractions, whose results coincide with the high content of lipids present (Table 1), these characteristics decrease their water absorption capacity (Henning *et al*., 2021). According to these results, starches from AUF1 could present rancidity due to lipid oxidation (Villarroel *et al*., 2018), however, the consumption of starches high in phytosterols reduces the absorption of cholesterol in the intestine by competition, since they have a chemical structure similar to cholesterol (Fernandes & Cabral, 2007). A diet rich in phytosterols can reduce total cholesterol and LDL cholesterol (harmful to health) (Silva *et al*., 2016). The estimated daily intake of phytosterols varies between 160 and 500 mg/day, however, its beneficial action is achieved with consumptions of 1500 mg to 2400 mg daily (Laitinen & Gylling, 2012). In the seeds of fruits of anonaceas, phytosterols (β-sitosterols and β-stigmasterols) with anticancer effects have been reported (Haykal *et al*., 2019; Aguilar-Hernández *et al*., 2022).

Revista Bio ciencias
ISSN 2007-3380

Table 3. Bioactive compounds in soursop fruit starches.

Extraction	TSP	TST	TSF	TPh	TA	TAc
	mg GAE / g of d.b.s		mg QE / g of d.b.s	mg CE / g of d.b.s	mg AE / g of d.b.s	mg ANE / g of d.b.s
ACF1	1.44 ± 0.08^{b}	0.706 ± 0.08^{b}	0.99 ± 0.02^{b}	2.09 ± 0.04^{b}	3.580 ± 0.13^{b}	1.527 ± 0.003^{b}
AUF1	2.26 ± 0.07^{a}	0.993 ± 0.05^{a}	1.55 ± 0.13^{a}	3.55 ± 0.07^{a}	8.429 ± 0.13^{a}	1.818 ± 0.017^{a}
ACF2	0.023 ± 0.001^{c}	0.007 ± 0.0002^{c}	0.022 ± 0.001^{c}	0.86 ± 0.07^{d}	0.012 ± 0.002^{c}	0.034 ± 0.002^{c}
AUF2	0.060 ± 0.007^{c}	0.022 ± 0.002^{c}	0.049 ± 0.003^{c}	1.60 ± 0.04^{c}	0.044 ± 0.005^{c}	0.039 ± 0.0005^{c}
CV	6.04	11.82	10.61	3.03	3.16	0.99
LSD	0.15	0.13	0.18	0.16	0.24	0.02

The same letters per column do not differ statistically (Tukey, $p \leq 0.05$). The values are the means of three repetitions ± standard deviation. TSP: total soluble phenols, TST: total soluble tannins, TSF: total soluble flavonoids, TPh: total phytosterols, TA: total alkaloids, TAc: total acetogenins. CV: coefficient of variation. LSD: least significant difference. ACF1: phase 1 conventional starch, AUF1: phase 1 ultrasonic starch, ACF2: phase 2 conventional starch, AUF2: phase 2 ultrasonic starch.

Total alkaloids

Alkaloids are natural compounds containing basic nitrogen atoms (Coria-Téllez et al., 2018), and have been reported in *Annona muricata* L. mainly isoquinoline, aporfin, and protoberberine type (Riley-Saldaña *et al*., 2017), these compounds may have in their structure a preformed molecule of a phytosterol (Aguilar-Hernández *et al*., 2020b; Xiang *et al*., 2022), allowing the formation of binary amylose-lipid interactions with starches (Krishnan *et al., 2021*). According to De la Cruz-Chacón *et al*. (2011), these compounds are present in seed endosperm, as well as in the shell, and to a lesser extent in the pulp of soursop fruits. In this research, the total alkaloid content (mg AE/g of d.b.s) was ACF2 (0.012), AUF2 (0.044), ACF1 (3.58), and AUF1 (8.42). The results obtained showed statistical differences ($p \leq 0.05$) (Table 3). In this sense, the high content of alkaloids in AUF1 starches can be attributed to ultrasound-assisted extraction, because the cavitation effect breaks cell membranes allowing the release of phytochemical compounds (Aguilar-Hernández *et al*., 2022). Alkaloids from soursop fruits may help reduce blood pressure by blocking calcium ion channels instead of activating endothelium- and nitric oxide-dependent mechanisms (Zubaidi *et al*., 2023). Isoquinoline, coreximina, and anomurin have been shown to have a transient depressive effect on blood pressure (Nwokocha *et al*., 2012). The starches extracted from soursop fruits from this research could have the same effect as reported by these researchers.

Revista Bio ciencias
ISSN 2007-3380

Total acetogenins

Acetogenins are secondary metabolites that contain tetrahydrofuran rings and a lipophilic side chain that allows the formation of binary complexes with amylose (Bachan *et al*., 2013; Krishnan *et al*., 2021). In starches extracted from soursop fruits, the content of total acetogenins (mg ANE/g of d.b.s) was ACF2 (0.034), AUF2 (0.039), ACF1 (1.527) and AUF1 (1.818). The results obtained showed statistical differences ($p \leq 0.05$) (Table 3). In this regard, high content of ACGs was observed in AUF1 starches, this could be due to the presence of seed and husk starches, as well as ultrasound, whose results coincide with what was reported by Aguilar-Hernández *et al*. (2020a)**,** who reported that soursop seeds and shells contain high content of acetogenins and ultrasound-assisted extraction of these compounds increases yield. On the other hand, ACF1 and ACF2 starches presented low content of acetogenins, which could be attributed to the fact that the conventional extraction method has lower extraction efficiency compared to the ultrasound-assisted method (López-Romero *et al*., 2022; Aguilar-Hernández *et al*., 2022). The starches of soursop fruits, due to their acetogenin content, can be used in the development of new innovative products with nutraceutical characteristics, in such a way that they are healthy for the consumer by reducing the problems caused by degenerative diseases. Studies indicate that the consumption of acetogenins has anticancer (Mutakin *et al*., 2022), and antidiabetic (Son *et al*., 2021) effects, in addition, it has been proven that these compounds have antimicrobial activity against *E. faecalis, S. paratyphi, E. coli and L. monocytogenes*, which are the most prevalent harmful bacteria found in food (Siderakou *et al*., 2021).

Conclusions

The ultrasonically assisted starch extraction method increases the content of soursop fruit starches and phytochemical compounds. The phytochemical compounds present in soursop fruit starches are phenols, flavonoids, tannins, alkaloids, phytosterols, and acetogenins.

Authors' contribution

Conceptualization of work (DSMA, BMR, BLJE, LFAE). Development of the methodology (DSMA). Software management (DSMA, BLJE). Experimental validation (DSMA, BLJE, LFAE, RBG). Analysis of results (DSMA, BMR, BLJE, BRPU, JZJO). Data management (DSMA, BMR, BLJE, BRPU, JZJO, RBG). Writing and preparation of the manuscript (DSMA, BMR, BLJE, LFAE). Writing, proofreading, and editing (DSMA, BMR, BLJE, BRPU, JZJO, PPCJ). Project Manager (BMR). Acquisition of funds (BMR).

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Conflict of interest

The authors declare that they have no conflict of interest.

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