

Techno-functional properties and biological activity of starches of soursop fruits (*Annona muricata* L.)

Propiedades tecno-funcionales y actividad biológica de almidones de frutos de guanábana (*Annona muricata* L.)

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ABSTRACT

Soursop fruit starches contain phytochemicals, making them a potential alternative for the food and pharmaceutical industries. The objective was to determine the techno-functional properties and biological activity of soursop fruit starches extracted by conventional and ultrasound-assisted methods. Gelatinization temperature, absorption index, solubility, swelling power, acute toxicity, and antioxidant capacity were evaluated. The extractions were: CSP1 (Conventional Starch Phase 1), USP1 (Ultrasound Starch Phase 1), USP2 (Ultrasonic Starch Phase 2), CSP2 (Conventional Starch Phase 2). CSP1 and USP1 starches gelatinized at 72.3 and 72.8 °C, CSP2 and USP2 starches gelatinized at 71.1 and 71.4 °C. CSP2 and USP2 starches had a high water absorption index (9.3 and 7.6 g gel/g sample), water solubility (4.5 and 3.2 %), and swelling power (9.5 and 7.7 g gel/g sample). The toxicity (LD₅₀) of the extracts from CSP1 and USP1 starches was 12.88 and 6.83 µg/mL, respectively. In CSP2 and USP2, the toxicity was 23.58 and 21.18 µg/mL, respectively. CSP1 and USP1 starches showed high antioxidant capacity (mg AAE/g of d.s.) ABTS (10.4 and 11.4), DPPH (59.1 and 69.6), and FRAP (22.2 and 23).

KEY WORDS : Annonaceae, antioxidant, polysaccharide, toxicity..

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RESUMEN

Los almidones de frutos de guanábana contienen fitoquímicos, siendo una alternativa para la industria alimentaria y farmacéutica. El objetivo fue determinar las propiedades tecno-funcionales y actividad biológica de almidones de frutos de guanábana extraídos por método convencional y asistido por ultrasonido. Se evaluaron temperatura de gelatinización, índice de absorción, solubilidad, poder de hinchamiento, toxicidad aguda y capacidad antioxidante. Las extracciones fueron: CSP1 (Almidón convencional fase 1), USP1 (almidón ultrasonido fase 1), USP2 (Almidón ultrasonido fase 2), CSP2 (almidón convencional fase 2). Los almidones CSP1 y USP1 gelatinizaron a 72.3 y 72.8 °C, los almidones CSP2 y USP2 gelatinizaron a 71.1 y 71.4 °C. Los almidones de CSP2 y USP2 presentaron alto índice de absorción de agua (9.3 y 7.6 g de gel/g de muestra), solubilidad en agua (4.5 y 3.2 %) y poder de hinchamiento (9.5 y 7.7 g de gel/g de muestra). La toxicidad (DL_{50}) de los extractos de los almidones CSP1 y USP1 fue de 12.88 y 6.83 en USP1 $\mu\text{g/mL}$, en CSP2 y USP2 fue de 23.58 y 21.18 $\mu\text{g/mL}$. Los almidones CSP1 y USP1 mostraron alta capacidad antioxidante (mg AAE/g of d.s.) ABTS (10.4 y 11.4), DPPH (59.1 y 69.6) y FRAP (22.2 y 23).

PALABRAS CLAVE: Anonáceas, antioxidante, polisacárido, toxicidad.

Introduction

Soursop (*Annona muricata* L.) is cultivated in Mexico, with an annual yield of 30,000 t (SIAP, 2024), its fruits are consumed fresh, as well as its derived by-products (leaves, roots, bark, and branches) which are used in ethnomedicinal applications as antiparasitic, antipyretic, anti-inflammatory, antihypertensive, antioxidant, antidiabetic, anticancer, analgesic, and antiviral activity (Aguilar-Hernández *et al.*, 2024). The pulp of soursop fruits has been used to make functional products, due to its content of bioactive compounds and phytochemicals (Nolasco-González *et al.*, 2023). The extraction of polysaccharides from these fruits, mainly starch, has also been reported; it should be considered that the soursop also contains other polymers such as cellulose and pectins (Martínez-Ortiz *et al.*, 2022). Ramírez-Balboa *et al.* (2021) and Martínez-Ortiz *et al.* (2022) have reported yields of up to 7 % starch isolated from soursop fruits at physiological maturity. In turn, it has been reported that to evaluate the starches of soursop fruits, wet extraction and separation by centrifugation are carried out (Flores-Gorosquera *et al.*, 2004; Ramírez-Balboa *et al.*, 2021); however, during this process, a starch is obtained that is separated into two phases, which are differentiated by color (white and brown). These starches have different physicochemical properties, including amylose and lipid content, as well as phytochemical compound content (De

los Santos-Santos *et al.*, 2023). These starches, like those extracted from custard apples, have a higher amylopectin content, considering them within the waxy classification, with a techno-functional properties such as water absorption capacity, solubility index, and swelling power similar to corn starches (Martínez-Ortiz *et al.*, 2022). These characteristics allow its use in the production of food products as thickening agents, stabilizers, and emulsifiers, pharmaceuticals as a drug carrier, in addition to its thermoplastic properties and gluing agent (polishing, gloss, and firmness) is possible to use it in the textile industry (Nwokocha & Williams, 2009; Martínez-Ortiz *et al.*, 2022; Tesfaye *et al.*, 2018; Schmieles, 2019; Yongfeng & Jay-lin, 2015; Solarte-Montúfar *et al.*, 2019).

On the other hand, the phytochemical compounds reported to be present in these starches are phenols, tannins, flavonoids, phytosterols, alkaloids, and acetogenins (De los Santos-Santos *et al.*, 2023). Likewise, it has been investigated that these compounds have antioxidant and cytotoxic capacity, due to their ability to inhibit free radicals and inhibit the growth of tumor cells (Koev *et al.*, 2020; Md-Roduan *et al.*, 2019). Antioxidant capacity is evaluated by inhibition or equivalence (mg/of ascorbic acid) of synthetic free radicals as in the DPPH method, which uses the free radical 2,2-diphenyl-1-picrylhydrazil that changes color from purple to yellow when reacting with antioxidants and the ABTS method [2,2'-azino-bis-(3-ethylbenzthiazolin-6-sulfonic acid)] in which the radical changes color from green to colorless when reacting with antioxidants (Donoso-Bustamante *et al.*, 2025), another effective method to determine the antioxidant capacity of compounds is the Ferric Reducing Antioxidant Capacity Assay "FRAP", which measures the reduction of the ferric ion complex (Fe^{3+})-ligand to a ferrous complex (Fe^{2+}) by the action of antioxidants in an acidic medium (Da Silva *et al.*, 2024). On the other hand, toxicity can be evaluated by different assays, the most used is with aquatic organisms (*Artemia salina*), because it is a quick and simple technique (Okumu *et al.*, 2021), this assay allows determining the degree of toxicity of the compounds on a scale of very toxic, toxic, harmful, and non-toxic (Villamil, 2015). In this sense, starches from soursop fruits have been studied in the formulation of bioactive materials such as coatings and edible packaging (De los Santos-Santos *et al.*, 2020); however, there is little information about their techno-functional and biological properties. Hence, this research aimed to evaluate the techno-functional properties and biological activity of starches from soursop fruits.

Material and Methods

Plant material

Starches were extracted from soursop fruits (physiological maturity), with a harvest index of 160 d after anthesis (Balois-Morales *et al.*, 2019). The fruits were harvested in the ejido of Venustiano Carranza, Tepic, Nayarit (21° 32' 2.77" N, 104° 58'37.73" W, 893 masl). The starches were extracted using two methods: conventional and ultrasound-assisted. Then they were centrifuged to obtain starches and separate the surplus water.

Extraction by the conventional method

A citric acid solution (1 %) in a 1:4 ratio (w/v) was used for grinding in an industrial blender (International LI-5a, Mexico) for 3 min. A filtration of the grind obtained with sieves with pores of 150, 75, and 53 micrometers was carried out, separating the fiber from the liquid phase. This fiber was milled with distilled water in a ratio of 1:2 (w/v) for 3 min and filtered (150, 75, and 53 μm). The resulting liquid phase was left to stand for 24 h at 7 °C, then the precipitate containing starch was recovered, and the supernatant liquid was removed by decantation (Martínez-Ortiz, 2024).

Ultrasound-assisted extraction

Soursop fruits were ground for 3 min with a citric acid solution (1 %) in a ratio of 1:4 (w/v). The ground material was stored for 10 min at 15 ± 2 °C, then sonicated in an ultrasonic processor (Model CPX750 Cole-Parmer Instruments Vernon Hills, Illinois, U.S.A.) of 750 W at 40 % power, equipped with a 20 kHz probe for 10 min at 25 °C. The resulting material was then filtered with sieves (150, 75, and 53 μm), separating the liquid phase from the fiber. The fiber was then ground with distilled water in a 1:2 ratio (w/v) for 3 min and filtered (150, 75, and 53 μm). The liquid phase was allowed to stand for 24 h, and then the starch-containing precipitate was recovered, and the supernatant was discarded by decantation (Martínez-Ortiz, 2024; Ramírez-Balboa *et al.*, 2021).

Starch centrifugation

The precipitate (obtained by conventional and ultrasonically assisted methods) with excess liquid was centrifuged at 1537 xg for 5 min/25 °C in a centrifuge (Hermle Z326K, Wehingen, Germany) equipped with an angular rotor number 221.18 with a radius of 11.2 cm. After centrifugation, the precipitate was separated into two phases; the precipitate that presented a green/brown color was called PHASE 1 (P1), and the white precipitate PHASE 2 (P2). The P1 product was removed using a spatula, leaving the P2 product at the bottom of the tube. The resulting starch was supplemented with 10 mL of distilled water and stirred for 30 s in a vortex, then centrifuged at 1537 xg for 5 min at 25 °C (First Wash). Three washes were carried out, separating the resulting phases. The starches obtained were dried in a recirculating oven (LSIS-B2V/VC 55, Germany) for 24 h at 35 °C, then pulverized in a mortar and sieved (150 μm). They were finally weighed for quantification and stored in airtight jars. The interpretation of the polysaccharide results was carried out using the following nomenclature: CSP1, USP1, CSP2, and USP2, where the letter A corresponds to starch, the letters C and U (conventional extraction and ultrasound-assisted extraction, respectively), and F (phases).

Gelatinization temperature

In a beaker containing 50 mL of water, 1 g of starch was added, along with 10 mL of distilled water at 60°C, and the mixture was brought to a double boiler (85 °C). Temperature readings were taken until a paste or gel formed (Sánchez & Aristizabal, 2007).

Absorption rate, water solubility, and swelling power

Eppendorf tubes (50 mL) were used at constant weight, 1.25 g of starch plus 30 mL of distilled water (60 °C) were added to each tube, stirred in a vortex, and placed in a double boiler (70 °C/30 min); the suspension was stirred every 10 min until the end of heating. It was then centrifuged at 3012 xg/30 min/25 °C. The volume of the supernatant (liquid phase) and the weight of the tubes with the gel (solid phase) were recorded. Subsequently, 10 mL of the supernatant was collected and transferred to a 50 mL beaker, previously weighed. Subsequently, they were placed in a recirculating oven for 24 h at 70 °C; afterwards, the beaker containing the insolubles was weighed (Sánchez & Aristizabal, 2007).

Calculations and interpretation of the results:

1. Water absorption index: $WAI = \text{weight of the gel (g)} / \text{weight of the sample (g) of starch on a dry basis (g/1.25 g s.d.b.)}$.

2. Water Solubility Index: $WSI = \text{gel weight (g)} \times \text{volume} \times 10/\text{g of starch d.b.}$ It is expressed as a percentage (%).

3. Swelling power. $SP = [\text{gel weight (g)}/\text{g starch (g)}] - \text{soluble weight (g)}$. It is expressed in g of gel/g of starch.

Obtaining extracts

The starches (CSP1, USP1, CSP2, and USP2) were dispersed in methanol at a concentration of 1:5 (w/v), then the heterogeneous mixture was sonicated in an ultrasonic bath equipment (KYMEN® Digital Ultrasonic Cleaner model JP-4820, Shenzhen, China) at 35 kHz for 64 min, then centrifuged at 1537 xg/5 min/4°C in a refrigerated centrifuge (Hermle Z326K, Wehingen, Germany) and the liquid phase was recovered, which was centrifuged again at 6147 g/10 min/4°C. The liquid phase was filtered using closed-pore filter paper (1 µm) and concentrated at reduced pressure in a rotary evaporator (IKA® model C-MAG HS7, North Carolina, USA) at 40 °C/80 rpm/65 cmHg. The extracts were then dried in a recirculation oven (LSIS-B2V/VC 55, Germany) for 24 h at 35 °C. The dry extracts were reconstituted in dimethyl sulfoxide (DMSO) at a concentration of 20 mg/mL and stored in amber bottles.

Determination of the acute toxicity of starch extracts

Extracts of CSP1, USP1, CSP2, and USP2 reconstituted in DMSO (20 mg/mL) were used to prepare suspensions at different concentrations (5, 25, 50, 100, and 150 µg/mL) of each with distilled water. Subsequently, 9 mL of saline water (25 mg/L), 1 mL of suspension, and 10 organisms with 3 weeks of growth were placed in test tubes. As a negative control, 1 mL of DMSO (1 %), 9 mL of saline water, and 10 organisms were used. Acute toxicity was determined after 24 h of exposure. The number of survivors was quantified, and *Artemia* was considered dead if they did not present internal or external movements during 1 min of observation (León-

Fernández *et al.*, 2019). The trial was conducted in triplicate. The mean lethal dose (LD_{50}) was calculated using a PROBIT regression (LD-Probit V.1.0 Universidad Autonoma Chapingo. Department of Agricultural Parasitology) and the toxicity scale for aquatic organisms was used [Very toxic ($LD_{50} < 1 \mu\text{g/mL}$), toxic ($LD_{50} 1\text{-}10 \mu\text{g/mL}$), harmful ($LD_{50} 10\text{-}100 \mu\text{g/mL}$), and non-toxic ($LD_{50} > 100 \mu\text{g/mL}$)] (Villamil, 2015).

Antioxidant capacity

DPPH Assay

A solution of the DPPH• radical (2,2-Diphenyl-1-picrylhydrazyl, Sigma-Aldrich) was prepared at a concentration of 7.4 mg/100 mL in 80 nm ethanol, the solution was stirred (IKA®, Germany) for 60 min in the absence of light, then diluted with 80 % methanol to an absorbance value of 0.70 ± 0.02 nm at a wavelength of 520 nm. 50 μL of extract was added to 1.5 mL Eppendorf tubes, and 250 μL of the DPPH solution (0.70 ± 0.02 nm) was added, stirred in a vortex, and allowed to stand for 30 min in the absence of light, then the absorbance was read in a spectrophotometer (Biotek, PowerWave XS, USA) at a wavelength of 520 nm (Morales & Jiménez-Pérez, 2001). A standard curve of 80 mg ascorbic acid equivalents (mg EAA) was developed. Antioxidant capacity was expressed in mg ascorbic acid equivalents per g of dry sample (mg AAE/g d.s.).

ABTS Assay

The determination of the inhibitory capacity of the radical cation 2,2'-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid) (ABTS •+) was quantified according to the methodology of Re *et al.* (1999). Solutions of ABTS (7 mM) and potassium persulfate (2.45 mM) were prepared with distilled water. The solutions were mixed in a 1:1 ratio (v/v), then incubated for 16 h in the dark at $23 \pm 1^\circ\text{C}$ and kept in constant agitation to form the ABTS •+ radical. The solution was initially diluted at a concentration of 1:20 (v/v) with ethanol (20 %) until an absorbance of 0.70 ± 0.02 nm at 730 nm was reached. 10 μL of the sample was then mixed with 490 μL of ABTS •+ for seven min. Absorbance was read at 730 nm. A calibration curve (400 mg ascorbic acid equivalents, mg EAA) was prepared. Antioxidant capacity was expressed in mg ascorbic acid equivalents per g of dry sample (mg EAA/g d.s.).

Ferric Reducing Antioxidant Power (FRAP)

The determination was performed following the method of Benzie & Strain (1996). The FRAP reagent was prepared by mixing three solutions, first, an acetate buffer was prepared by dissolving 1.36 g of sodium acetate, 8 mL of acetic acid, in 400 mL of distilled water, then the solution was sonicated in an ultrasonic bath (KYMEN® Digital Ultrasonic Cleaner model JP-4820, Shenzhen, China) for 8 min, then the pH was adjusted to 3.6 and measured to 50 mL (solution 1), ferric chloride (FeCl_3) solution was prepared by dissolving 1.351 g at 250 mL (solution 2). The TPTZ reagent (Tris(2-pyridyl)-s-triazine) was prepared by dissolving 2.3 mg of TPTZ with 33 μL of hydrochloric acid (HCl), and the solution was diluted to 10 mL with distilled water ($37^\circ\text{C}/15$ min) (solution 3). The solutions were mixed in a 10:1:1 ratio and stored without light. 30 μL of

sample and 250 mL of the FRAP reagent were added to test vials, and the sample was shaken and allowed to stand for 30 min. Absorbance was measured at 695 nm. A standard curve of 50 mg ascorbic acid equivalents (mg EAA) was developed. Antioxidant capacity was expressed in mg ascorbic acid equivalents per g of dry sample (mg EAA/g d.s.)

Statistical analysis

A complete randomized design with a 2 x 2 factorial arrangement (extraction methods and phases) was used. Four treatments were established in which starches were evaluated (phases 1 and 2), obtained by conventional and ultrasound-assisted extraction. The results were analyzed with a two-way ANOVA and a Tukey post hoc test ($p \leq 0.05$) using the Statistical Analysis System (SAS® V. 9.2) software.

Results and Discussion

Techno-functional properties of starches

Gelatinization temperature (°C)

The gelatinization temperature in CSP2 starches was 71.1 °C, while in USP2 it was 71.4 °C; likewise, CSP1 starches were 72.3 °C, and USP1 was 72.8 °C (Table 1). Xu *et al.* (2020) reported that these starches gelatinize when heated in aqueous systems, resulting in an irreversible transition by hydration, swelling, and solubilization, which causes a disorder due to the interruption of the multiple levels of organization of the starch granules. In this study, which focused on ultrasound-assisted starch extraction, the gelatinization temperature increased under these conditions. In this regard, Park & Han (2016) reported that ultrasound increases the gelatinization temperature, associated with the melting of the weakest crystals, and the presence of the strongest crystals is increased. These have long amylopectin chains, which require higher temperatures to achieve granular disruption and to absorb water. Another factor influencing gelatinization temperature is the granule size (Oliveira-Bernardo *et al.*, 2018); starches extracted from soursop fruits have been reported to be 6-9 microns in size, leading to rapid gelatinization (Nwokocha & Williams, 2009). On the other hand, it has been reported that gelatinization occurs initially in amorphous regions, because hydrogen bonds are weaker in these areas, in this sense, gelatinization temperatures and enthalpies associated with gelatinization endotherms vary between starches from different sources, which can be attributed to differences in the degree of crystallinity, amylose content, presence of lipids and proteins, in addition to granular size (Amini *et al.*, 2015).

Water absorption index (WAI)

WAI indicates the volume of water that gelled starch can absorb and retain during hydration (Huamani *et al.*, 2020). The WAI (g gel/1.25 g s.d.b.) in this study were CSP2 (9.3), USP2 (7.6), CSP1 (5.08), and USP1 (4.62) (Table 1). P2 starches had high WAI; this may be due to the high availability of hydrophilic groups of starches that interact with water molecules (Dun *et al.*,

2020), as well as differences in the amylose/amylopectin ratio and chain length distribution of starches (Ikegwu *et al.*, 2010). In contrast, starches high in phytochemical compounds such as phenols, alkaloids, and acetogenins have been reported to have a reduced ability to absorb water molecules (Humerez-Flores *et al.*, 2022; De los Santos-Santos *et al.*, 2023). Therefore, these compounds, when interacting with starches, reduce the availability of functional groups that can bind phytochemicals, as reported by Méndez *et al.* (2023), who observed that the addition of bioactive compounds such as vanillin to polysaccharides affects their techno-functional properties.

Water Solubility Index (WSI)

WSI is an indicator of the solubilization of granular components; it measures the amount of soluble components (amylose) released, particularly by starch after heating (Ekielski *et al.*, 2020). The results obtained in this research on WSI of starches yielded values of CSP2 (4.5 %), USP2 (3.2 %), CSP1 (4.6 %), and USP1 (3.5 %) (Table 1), which indicates that WSI is low for these starches. Yousf *et al.* (2017) reported that a high WSI is associated with improved digestibility, suggesting greater gelatinization and dextrinization. Some research reports that the heating temperature influences the solubility index of starches, as reported by Ramírez-Balboa *et al.* (2021) and López-Flores *et al.* (2020), who observed that at 60 °C the solubility index is lower (1.25 and 1.43 %), indicating that this parameter is proportional to the heating temperature of the starch. This behavior is because amylose is released into the aqueous medium as the surface area of the granules increases (due to gelatinization). This leaching initially occurs through the pores and granular channels, with greater diffusion of water molecules within the granule, thereby increasing solubility (Amini *et al.*, 2015).

Swelling power (SP)

Starch is not soluble in water, however, as temperature increases, the granules are hydrated by increasing their mass in non-solubilized starch (before releasing amylose), as a result of the absorption of water by the OH groups of amylose and amylopectin (Nwokocha & Williams, 2009); in this research the SP (g gel/g starch) of starches extracted from the soursop fruit was evaluated, and whose results are CSP2 (9.5), USP2 (7.7), CSP1 (6.1), and USP1 (4.68) (Table 1). P2 starches had high SP and low solubility index in water; this condition could be related to the low amylose content present in these starches (De los Santos-Santos *et al.*, 2023), which restricts the swelling of the granules by reinforcing the internal network. Meaño-Correa *et al.* (2014) reported that, in native yam starch, the heating temperature influences the swelling power of starches, which increases with temperature, reaching a maximum of 49.05 g gel/g of starch at 95 °C. This could be attributed to the breaking of intermolecular bonds, the breakdown of the crystalline molecular structure, and the binding of water molecules to amylose- and amylopectin-free hydroxyl groups and to morphological and permeability changes in starch granules (Amini *et al.*, 2015). However, when the swelling power is low, it can be related to a low amylose content, the presence of lipids, and/or phytochemical compounds. Xu *et al.* (2020) reported that proteins and lipid phytochemical compounds can interact with amylose and restrict the swelling power.

Table 1. Techno-functional analysis of soursop fruit starches

Polysaccharide	Extraction method	Gelatinization Temperature (°C)	WAI (g/1.25 g of s.d.b.)	WSI (%)	SP (g gel/g of starch)
CSP2	Conventional	71.1 ± 0.05 ^c	9.3 ± 1.8 ^a	4.5 ± 0.8 ^a	9.5 ± 1.9 ^a
USP2	Ultrasound	71.4 ± 0.5 ^b	7.6 ± 1.08 ^{ab}	3.2 ± 0.5 ^a	7.7 ± 1.1 ^{ab}
CSP1	Conventional	72.3 ± 0.05 ^{ab}	5.08 ± 0.1 ^{bc}	4.6 ± 0.1 ^a	5.1 ± 0.1 ^{b^c}
USP1	Ultrasound	72.8 ± 0.11 ^a	4.62 ± 0.06 ^c	3.5 ± 0.1 ^a	4.68 ± 0.06 ^c
	MSD	0.71	2.75	1.41	2.9
	CV	0.38	15.79	13.5	16.35

Water Absorption Index (WAI), Water Solubility Index (WSI), and Swelling Power (SP) at 70°C. The values are the measures of three repetitions ± standard deviation, different letters per column indicate significant differences (Tukey, $P \leq 0.05$). MSD: minimum significant difference. CV: coefficient of variation.

Acute toxicity of soursop fruit starch extracts

The LD₅₀ of the starch extracts was 12.88 µg/mL in CSP1, as well as in USP1 (6.83), CSP2 (23.58), and USP2 (21.18) (Table 2). It has been reported that an extract is considered active in *Artemia* trials when LD₅₀ < 1000 µg/mL is present (Rupprecht *et al.*, 1990), coinciding with the results obtained in this research. Villamil (2015) has reported that the toxicity levels of chemicals for aquatic organisms, such as *Artemia salina*, are classified as very toxic (LD₅₀ < 1 µg/mL), toxic (LD₅₀ 1-10 µg/mL), harmful (LD₅₀ 10-100 µg/mL) and non-toxic (LD₅₀ > 100 µg/mL). In this sense, the results obtained with the extracts of starches are classified as harmful (CSP2, USP2 and CSP1) and toxic (USP1), which may be related to the structural part of the fruit (seed, pulp, peel) from which the starches come, in the case of this research the starches of P1 come mainly from peel and seed, which have a high content of cytotoxic compounds such as alkaloids and acetogenins (De los Santos-Santos *et al.*, 2023; Riley-Saldaña *et al.*, 2017). Aguilar-Hernández *et al.* (2020) conducted research on soursop fruit peel and seeds and reported high content of alkaloids and acetogenins; these compounds present synergism (Galvis-García *et al.*, 2012), as LD₅₀ of acetogenic fractions of up to 2.60-3.18 µg/mL has been reported in soursop pulp and 21.97 µg/mL in *Annona cherimolioides* bark (León-Fernández *et al.*, 2019; Galvis-García *et al.*, 2012), in this regard, it has also been documented that isolated acetogenins have greater cytotoxic activity than extracts, however, it has been widely investigated that anonyceous acetogenins that exert cytotoxic activity do not damage healthy cells (Vila-Nova *et al.*, 2013; Jiménez *et al.*, 2014). Soursop fruit starches are an alternative in the production of bioactive materials (Martínez-Ortiz *et al.*, 2022), so these results could serve as a basis for future research to evaluate the antifungal activity of these starches, since *Artemia* they are biocontrol organisms with which the ability of compounds to inhibit the growth of fungi, kill insects and/or exert a wide range of pharmacological effects is predicted.

Table 2. Determination of toxicity of starch extracts from soursop fruits

Polysaccharide	Lethal dose $\mu\text{g/mL}$ (LD_{50})
CSP2	23.58 ± 2.14^a
USP2	21.18 ± 3.48^a
CSP1	12.88 ± 2.49^b
USP1	6.83 ± 0.9^b
MSD	6.37
CV	15.13

Means with equal letters in the columns are not statistically different (Tukey, $P \leq 0.05$). CSP1: conventional starch phase 1, USP1: ultrasonic starch phase 1, CSP2: conventional starch phase 2, USP2: ultrasonic starch phase 2. CV: Coefficient of Variation, MSD: Minimum Significant Difference.

Antioxidant capacity

ABTS assay

The ABTS assay measures the ability of antioxidants to eliminate the $\text{ABTS}^{\bullet+}$ cation radical, which is a blue-green chromophore with maximum absorption at 734 nm that decreases its intensity in the presence of antioxidants such as phytochemical compounds (López-Martínez *et al.*, 2022). The antioxidant capacity (mg EAA/g d.s) per ABTS of starches in this study was CSP2 (0.693), USP2 (0.644), CSP1 (10.44), and USP1 (11.4) (Table 3). In this regard, extracts of CSP1 and USP1 starches have a high capacity to trap the $\text{ABTS}^{\bullet+}$ radical compared to CSP2 and USP2 starches, as reported by Prior *et al.* (2005). These results may be due to the high presence of phenolic compounds. Novaes *et al.* (2019) reported that the presence of phenolic compounds such as flavonoids increases the antioxidant capacity of an extract due to the structure of 3 benzene rings. The results obtained in this research are lower than those reported by Balois-Morales *et al.* (2019) and Jiménez-Zurita *et al.* (2017), who researched soursop fruit pulp. This behavior may be attributed to starch extraction under aqueous conditions, a medium in which compounds such as phenols are solubilized. In this sense, the lower antioxidant capacity by the ABTS method could be because soursop fruit starches, when extracted with water, decrease their concentration of polar antioxidant compounds such as phenols, tannins and flavonoids mainly (Rodrigues *et al.*, 2021), since these are solubilized in the extraction medium (water) and are eliminated when the supernatant is discarded from the starch phases.

DPPH assay

The DPPH assay detects soluble components in organic solvents, especially alcohols (ethanol and methanol), unlike some other methods (ABTS and FRAP), which measure the

inhibitory capacity of hydrophilic molecules (Álvarez-Gómez *et al.*, 2016). The antioxidant capacity per DPPH (mg AAE/g d.s) of starches was CSP2 (1.75), USP2 (1.76), CSP1 (59.1), and USP1 (69.6) (Table 3). Starches extracted from soursop fruits contain compounds with antioxidant capacity, such as acetogenins (De los Santos-Santos *et al.*, 2023), since, when obtained from methanol extractions, acetogenins are solubilized and bioavailable to inhibit the DPPH radical. Studies have reported that acetogenins have high antioxidant capacity against the DPPH radical, similar to ascorbic acid (AA); this is mainly due to the α,β -unsaturated lactone ring that is also present in ascorbic acid (Lima *et al.*, 2010). Soursop fruit starches, as they contain phytochemical compounds with biological activity, could be used to produce biopolymers, such as coatings, packaging, and films, with enhanced protective properties (Martínez-Ortiz *et al.*, 2022).

Ferric reducing antioxidant power (FRAP)

The FRAP assay evaluates the combined effect of non-enzymatic antioxidant defenses present in biological samples as an index of the ability to resist oxidative damage (Benítez-Estrada *et al.*, 2021). The FRAP antioxidant capacity for CSP2 and USP2 starches was 1.26 and 1.33 mg EAA/g d.s, respectively, while CSP1 and USP1 starches presented 22.2 and 23 mg AAE/g d.s, respectively. The results showed a significant difference ($P \leq 0.05$). Soursop fruit starches are high in polyphenols (De los Santos-Santos *et al.*, 2023). These compounds have a high antioxidant capacity due to their ability to donate an electron and reduce some compounds, such as lipids that cause rancidity (Benzie & Devaki, 2017). In the seeds of soursop fruits, the presence of lipids Stigmasterol and β -Sitosterol has been reported (Spain *et al.*, 2023). These lipids could be present in the starches, particularly in P1, since this fraction was obtained by grinding whole fruits, allowing their interaction during extraction. In this sense, these lipid compounds could help prevent oxidation owing to compounds with high antioxidant capacity, such as flavonoids and acetogenins (León-Fernández *et al.*, 2019; De los Santos-Santos *et al.*, 2023).

Table 3. Determination of antioxidant capacity of starch extracts from soursop fruits

Polysaccharide	ABTS mg AAE/g of d.s.	DPPH mg AAE/g of d.s.	FRAP mg AAE/g of d.s.
CSP2	0.693 $\pm$ 0.05 ^a	1.75 $\pm$ 0.19 ^a	1.26 $\pm$ 0.05 ^a
USP2	0.644 $\pm$ 0.01 ^a	1.76 $\pm$ 0.03 ^b	1.33 $\pm$ 0.01 ^a
CSP1	10.446 $\pm$ 0.59 ^b	59.1 $\pm$ 2.9 ^c	22.2 $\pm$ 0.2 ^b
USP1	11.4 $\pm$ 0.79 ^b	69.6 $\pm$ 1.5 ^c	23 $\pm$ 0.5 ^b
MSD	1.3	4.4	0.85
CV	8.5	5.1	2.74

Means with equal letters in the columns are not statistically different (Tukey, $P \leq 0.05$). CSP1: conventional starch phase 1, USP1: ultrasonic starch phase 1, CSP2: conventional starch phase 2, USP2: ultrasonic starch phase 2. CV: Coefficient of Variation, MSD: Minimum Significant Difference.

Conclusions

The pure starches extracted from soursop fruits (phase 2) exhibited techno-functional properties, including a high absorption index, high solubility, high swelling, and an intermediate gelatinization temperature. In this extraction phase, toxicity was observed in *Artemia salina* organisms, indicating that the starches were harmful. Starches (phase 1) had high antioxidant capacity and showed harmful toxicity in high concentrations in *Artemia salina* organisms. The results obtained suggest that soursop fruit starches could be used as functional ingredients in the food and pharmaceutical industry due to their absorption capacity, solubility and antioxidant capacity; however, due to the toxicity presented in *Artemia salina* organisms (high concentrations), it is recommended to perform cytotoxicity studies with cell models, as well as in their stability and behavior in complex matrices such as coatings and biofilms. In addition, further research could focus on the chemical and/or physical modification of starches to reduce their toxicity without compromising their techno-functional properties.

Authors' contribution

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

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Conflict of interest

The authors declare no conflict of interest.

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