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Protective Effect of Ultrasound-Processed Amazonian Sapota-do-Solimões (*Quararibea cordata*) Juice on *Artemia salina* Nauplii

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Abstract: Juice processing by non-thermal technology has been extensively studied, aiming at microbial inactivation and quality improvement. However, the knowledge about the possible toxic effects that those technologies can produce in foodstuffs due to the production of reactive oxygen species is still unknown. In this study, sapota-do-Solimões juice processed by ultrasound (2, 6, and 10 min) was evaluated by a toxicity test and protective effect through stress biomarkers (catalase, superoxide dismutase, and lipid peroxidation) using *Artemia salina* nauplii. The non-thermal processed juice was nontoxic to *A. salina.* However, the juice fibers imparted some damage to the animal's body. The ultrasound-processed juice (2 and 6 min) decreased the *A. salina* mortality to 30% compared to the control assay with H_2O_2 where mortality was 80% after 48 h of exposure. However, after 72 h of exposure, the *A. salina* was entirely degraded by H_2O_2-induced toxicity. Furthermore, the catalase and superoxide dismutase presented the highest activity after *A. salina* was exposed to the unprocessed juice. Thus, sapota-do-Solimões juice processed by the ultrasound could promote a protective effect on *A. salina,* revealing this technology's potential to enhance juice features without toxicity.

Keywords: toxicity; non-thermal technology; catalase; superoxide dismutase; lipid peroxidation; fruit juice

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1. Introduction

Non-thermal technologies have evolved, especially to promote food quality avoiding the adverse effects of thermal technologies [1,2]. Among non-thermal technologies, ultrasound uses a short processing time and automated equipment that uses less energy and decreases labor costs, improving food quality [3].

The ultrasound produces sound waves that generate the alternate compression and rarefaction of the particles in the medium and the consequent collapse of the bubbles, causing cavitation [4]. Ultrasound frequencies promote physical and chemical phenomena such as vibration, agitation, cavitation, acoustic flow, pressure, shock waves, shear forces, microjetting, compression, and sparseness, as well as generating free radicals (hydroxyl and hydrogen) [5,6]. Among the effects on the foodstuffs, this process can break cell walls, promotes decomposition of water molecules, and produces free radicals with strong oxidative properties (singlet oxygen, hydrogen, and hydroxyl) [3]. This technology has been applied to modulate different juice features, including sugar concentration, bioactive compounds, color, consistency, and turbidity, as well as enzyme and microbial stability [1,7–10].

Applying ultrasound on the juice of sapota-do-Solimões (*Quararibea cordata* Vischer) could be an excellent opportunity to improve the juice features. Sapota-do-Solimões is an exotic fruit that has been highlighted in its properties to develop different foodstuffs, i.e., jelly, functional juices, wine, and ice cream [11,12]. The main characteristic of this fruit is its intense orange color due to carotenoids and the fibrous pulp that enhances its potentiality as a matrix for different food processes [13]. However, few studies highlight the potentiality of this fruit in respect of its components, such as minerals (Mg, P, and Fe), and fatty acids (oleic acid, linoleic acid, and α-linolenic acid) [12,14]. Moreover, the effective scavenger against RNS (reactive nitrogen species) and ROS (reactive oxygen species) were demonstrated by phenolic compound (epicatechin) and carotenoids (zeaxanthin, lutein, and β-carotene) [15]. Recently, the first study that applied non-thermal technologies to sapota-do-Solimões juice demonstrated that cold plasma could enhance juice quality by increasing its antioxidant power, sugar, and amino acid content [16].

Nevertheless, studying the possible toxicity of non-thermal technologies using in vivo organisms is extremely relevant, since it brings knowledge about the safety of these evolving alternatives for thermal treatments. Thus, biological systems are used as preliminary toxicity studies for acute toxicity tests. *Artemia salina* is a popular model organism for toxicological tests due to its short life cycle, ease of culture, and purchase of its cysts. Moreover, many different parameters can be selected for toxicological evaluation, including hatching, mortality, swimming, morphology, and biomarkers [17]. *A. salina* has been applied in foodstuffs such as pomegranate juice, tangerine (*Citrus reticulata*), and grape pomace (*Vitis vinifera* L.) [18–20]. The toxicity was shown for *A. salina* only at the highest tested concentration (40 $mg \cdot mL^{-1}$) for the extract of grape juice from the cultivars Sagrantino [21]. With respect to non-thermal technologies, coconut (*Cocos nucifera* L.) water treated with ozone presented 6.5% lethality at the maximum tested concentration (1000 $\mu g \cdot mL^{-1}$) after 2 and 6 min of ozone exposure, whereas the cold plasma and ultrasound did not promote toxicity to the *A. salina* [22].

In addition to the toxicity assay, the protective effect is also important to evaluate if the juice components mitigate damage against stressors. Therefore, many studies suggested that H_2O_2 can induce oxidative stress by producing free radicals (hydroxyl, superoxide, peroxyl, hydroperoxide, singlet oxygen) and altering the levels of components in the antioxidant defense system, such as antioxidant defense enzymes [catalase (CAT), superoxide dismutase (SOD)] and oxidative stress biomarker [lipid peroxidation (LPO)] [18,22–24]. SOD and CAT are the main defense enzymes for protecting against free radicals [25]. SOD catalyzes the degradation of highly toxic ROS ($O_2^{\bullet -}$) to less toxic H_2O_2 and O_2, whereas CAT decreases organism damage by using H_2O_2 converting into H_2O and O_2 [26].

Moreover, lipid peroxidation occurs in response to the chain reactions intermediate by free radicals that lead to oxidative degradation of polyunsaturated fats, mainly targeting an organism's cell membranes [27]. The increase in antioxidant enzyme activities and LPO due to the increased oxidative stress to protect the organism from damage was shown in different studies [17,27]. However, the decrease is also possible due to the inhibition and degradation process [28,29].

Therefore, the present study aims to investigate the toxic and protective effects of sapota-do-Solimões juice unprocessed and processed by ultrasound on *A. salina* nauplii.

2. Material and Methods

2.1. Raw Material

Sapota dos Solimões fruits were collected at Federal University of Amazon (Manaus, Brazil). The fruits were washed and manually peeled, the seeds were removed, and the juice was produced using a domestic blender by mixing the fruit pulp and water. The juice soil content was adjusted to 2 °Brix. The juice brix was measured using a Brix refractometer at 25 °C. The juice was stored frozen (−20 °C) [11].

2.2. Ultrasound Processing of Sapota-do-Solimões Juice

The ultrasound processing was carried out using a probe ultrasound (Ultronique QR500, Indaiatuba, SP-Brazil). The maximum potency of 500 W and frequency of 19 kHz was applied. A 13 mm titanium probe was immersed 15 mm below the liquid surface (potency density of 373 W/cm^2). For each experiment, 150 mL of sapota-do-Solimões juice was placed in a glass jacketed beaker (250 mL, D = 7.0 cm, h = 9.5 cm). The juice was subjected to ultrasonic irradiation for 2 min (UL2), 6 min (UL6), and 10 min (UL10) [30]. The processing was thermostated to keep the temperature around 25 °C, but the internal heat generated by the process did not surpass 32 °C.

2.3. Sapota-do-Solimões Juice Lyophilization

The sapota-do-Solimões juice at °Brix 2 (150 mL) was distributed in Petri dishes and lyophilized for 24 h. The lyophilized juice was triturated, and a dried orange powder (2 g) was obtained and kept at −4 °C. The juice brix was measured using a Brix refractometer at 25 °C.

2.4. Protective Effects of Sapota-do-Solimões Juice against Hydrogen Peroxide-Induced Toxicity in Artemia salina Nauplii

The toxicity of sapota-do-Solimões juice (unprocessed and ultrasound processed samples) was evaluated by the *A. salina* lethality assay. Commercial dried cysts of brine shrimp were hatched in seawater (35 g/L, pH 8) with aeration for 48 h. The lyophilized juice was dissolved in seawater and transferred to 24-well plates to obtain concentrations of 10, 100, and 1000 μg·mL^{-1} in 2 mL of seawater with 10 nauplii in each well. In positive control, the wells were filled with 1 mL of seawater, 1 mL of potassium dichromate (0.5 M), and 10 nauplii. The wells were filled with 2 mL of seawater and 10 nauplii in the negative control. After 24 h incubation at 25 °C, the number of viable nauplii was counted. The unprocessed and processed samples did not affect the viability of *A. salina* within the range of 1–1000 μg·mL^{-1}. Thus, the same experiment was performed, but the nauplii were exposed for 1 h to hydrogen peroxide (255 mM). After that, the sapota-do-Solimões juices were added to the wells to observe the protective effect. Negative control (seawater) and two positive controls were evaluated. One was filled with 1 mL of seawater, 1 mL of potassium dichromate (0.5 M), and 10 nauplii, and the other was filled with 1 mL of seawater, 1 mL hydrogen peroxide (255 mM), and 10 nauplii. The 24-well plates were incubated under constant light at 25 °C, and the viability of *A. salina* was studied at 24 h, 48 h, and 72 h [18]. The percentage of survival was calculated Equation (1).

$$\%\text{Artemia } mortality = \frac{deadnaplii}{totalofnauplii} \times 100 \quad (1)$$

2.5. Determination of Antioxidant Enzymes on Artemia salina Nauplii after Protective Effect Test

2.5.1. Extract Preparation of *Artemia salina* Nauplii after Protective Effect Test

The *A. salina*, after the assay of protective effect (item 2.3), was washed with distilled water (3-fold), and the extract (2:1, *w*/*v*) was prepared by adding about 1.5 g of *A. salina* in 0.75 mL of 0.2 M potassium phosphate buffer (pH 6.5). The mixture was stirred with glass beads and then centrifuged (9000× *g*, 4 °C, 20 min) to separate the extract (supernatant).

2.5.2. Protein Determination of *Artemia salina* Nauplii after Protective Effect Test

The protein content was determined using the Coomassie Blue G-250 dye based on the previously described method [31]. The reaction was carried out by adding 20 μL of the sample extract (diluted 50-fold) to 180 μL of Bradford reagent. After 10 min, an ELISA reader measured the absorbance at a wavelength of 595 nm. The standard curve for protein was built using bovine serum albumin (5–50 μg/100 μL).

2.5.3. Catalase Activity (CAT) of *Artemia salina* Nauplii after Protective Effect Test

CAT activity was measured at 240 nm for 4 min. The reaction mixture containing 1 mL of 200 mM/2 mM Tris-HCl–EDTA (pH 8.5) and H_2O_2 (20 mM) reacted with 50 µL of nauplii extract. Change in absorbance per min (ΔA240) was then calculated and used in the estimation of enzyme activity (extinction coefficient 39.4 $L{\cdot}M^{-1}{\cdot}cm^{-1}$) [26,32].

2.5.4. Superoxide Dismutase Activity (SOD) of *Artemia salina* Nauplii after Protective Effect Test

The SOD activity was calculated according to a previous study with modification [29]. The assay was performed in a 96-well plate by the addition of the following reagents: 100 µL of 0.1 M of Na_2CO_3 buffer (pH 10.2), 20 µL of 10 mM EDTA, 20 µL of 240 µM of NBT, 20 µL of 1 mM hydroxylamine hydrochloride, 20 µL of 0.3% of Triton X-100, and 20 µL of the nauplii extract. The blank was kept in the dark. Meanwhile, the sample reaction mixture was exposed to light for 20 min. The plate was read at 560 nm using an ELISA reader. The SOD activity was calculated according to Kono [33].

2.6. Determination of Lipoperoxidation (LPO) of Artemia salina Nauplii after Protective Effect Test

The lipoperoxidation was measured by the modified method using cumene hydroperoxide (CHP) equivalents [34,35]. An extract was prepared by adding *A. salina* to cold methanol (1:9, *w*/*v*). The *A. salina* was homogenized after stirring with glass beads and then centrifuged (9000× *g*, 4 °C, 20 min). Finally, the supernatant was used as an extract. The analysis was carried out by adding 15 µL of the sample extract, 90 µL $FeSO_4$ 1 mM, 35 µL H_2SO_4 0.25 M, 35 µL xylenol orange 1 mM, and 175 µL of water to 96-well microplates. After 1 h, the absorption was measured at 580 nm in an ELISA reader. Then, 10 µL of CHP 175 µM was added to the wells, and after 15 min, the plate was read again. Lipoperoxidation was expressed as CHP equivalents in µmol/g of *A. salina*.

2.7. Morphological Analysis under Light Microscopy of Artemia salina Nauplii

To evaluate the morphological characteristic of the *A. salina* after 48 h of toxicity assay and 72 h of the protective effect assay were washed in seawater and mounted on a glass slide. Thus, the morphological changes were analyzed under a light microscope (Primo Star-Zeiss) equipped with Zen Lite software [22].

2.8. Morphological Analysis under Scanning Electron Microscopy of Artemia salina Nauplii

For SEM morphological analysis, samples of *A. salina* were collected as described in item 2.6 for light microscopy and fixed in a solution containing glutaraldehyde 2.5%, formaldehyde 4.0% in sodium cacodylate buffer 0.1 $mol{\cdot}L^{-1}$ (pH 7.2, 25 °C, 24 h). Then, the material was rinsed in sodium cacodylate buffer 0.1 $mol{\cdot}L^{-1}$, pH 7.2, three times for 45 min each and post-fixed (1 h, 25 °C) with 1.0% osmium tetroxide in sodium cacodylate buffer 0.1 $mol{\cdot}L^{-1}$, pH 7.2. Subsequently, the samples dehydrated with acetone increased series for 45 min each step. Finally, the dehydrated samples were critically point dried (Q150T ES), placed in stubs, and sputtered with 20 nm gold. Observation and documentation were performed in the scanning electron microscope (Quanta FEG 450 FEI) [22].

2.9. Statistic

The results were analyzed and reported as means and standard deviations. When appropriate, the variables were submitted to a one-way ANOVA analysis followed by Tukey's multiple comparisons test with a significance level of 5% ($p \leq 0.05$) using the STATISTICA program (Statsoft v 13.0).

3. Results and Discussion

3.1. Toxicity Assay of Ultrasound Processed Sapota-do-Solimões Juice

After 24 h and 48 h of interaction, no toxic effect of unprocessed or ultrasound-processed sapota-do-Solimões juice was observed in *A. salina*, even at the higher sample

concentration (1000 μg·mL^{-1}). The positive control ($K_2Cr_2O_7$ 0.5 M) was lethal for the organisms presenting a 100% death rate of *A. salina* after 48 h of the assay as shown in Table S1 (Supplementary Material). At the same time, negative control (seawater) did not present lethality against *A. salina.* Food toxicity seems to depend on processing and the food matrix. Ozone-processed coconut water presented low toxicity to *A. salina* nauplii, resulting in the death of 6.5% of the individuals at a coconut water concentration of 1000 μg·mL^{-1}. In contrast, coconut water processed by cold plasma and ultrasound presented lower toxicity than the ozone sample, causing the death of 3.0% of the individuals at 10 and 100 μg·mL^{-1}, respectively [22]. Pomegranate juice did not affect the viability of *A. salina* nauplii within the range of 1–1000 μg·mL^{-1} [18]. Grape pomace flour (*Vitis vinifera* L.) was considered nontoxic since all *A. salina* remained alive after the toxicity assay [19], whereas the extract of grape juice (*Vitis vinifera*) from the cultivar Sagrantino promoted a 100% of lethality effect for *A. salina* at the highest tested concentration (40 mg·mL^{-1}) [21].

3.2. Morphological Analysis of Artemia salina Nauplii after Toxicity Assay

3.2.1. Morphological Analysis of *Artemia salina* Nauplii after Toxicity Assay under an Optical Microscope

The intake and internalization of unprocessed and ultrasound processed (UL2, UL6, and UL10) juices can be observed in *A. salina* gut (Figure 1). A darker gut area is notable at highest concentration (1000 μg·mL^{-1}) for unprocessed sample. On the other hand, the ultrasound processed juice UL10 showed the same darker area for the three different concentrations (10, 100, and 1000 μg·mL^{-1}). This result indicates that ultrasound processing for longer times (10 min) favored the intake of juice particles. This is because the ultrasound decreases the pulp particle size [7], and, as a non-selective filter feeder, *A. salina* can ingest particles of a suitable size (<50 microns).

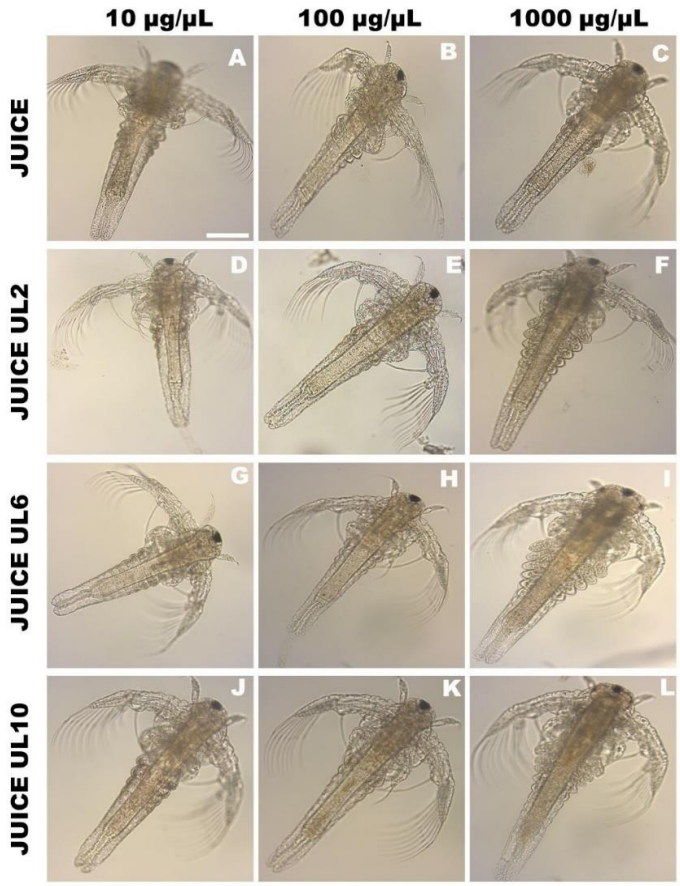

Figure 1. Morphological changes in the *Artemia salina* nauplii after 48 h of exposure under optical microscopy after toxicity test of unprocessed (juice) and ultrasound processed (2/6/10 min) sapota-

do-Solimões using *Artemia salina* nauplii. Unprocessed sapota-do-Solimões 10 μg·mL^{-1} (**A**), 100 μg·mL^{-1} (**B**), and 1000 μg·mL^{-1} (**C**). Ultrasound processed for 2 min sapota-do-Solimões juice (UL2) 10 μg·mL^{-1} (**D**), 100 μg·mL^{-1} (**E**), 1000 μg·mL^{-1} (**F**). Ultrasound processed for 6 min sapota-do-Solimões juice (UL6) 10 μg·mL^{-1} (**G**), 100 μg·mL^{-1} (**H**), 1000 μg·mL^{-1} (**I**). Ultrasound processed for 10 min sapota-do-Solimões juice (UL10) 10 μg·mL^{-1} (**J**), 100 μg·mL^{-1} (**K**), 1000 μg·mL^{-1} (**L**). Bars, 200 μm.

On the other hand, Schiavo et al. 2018 [36] demonstrated the particle size effect on *A. salina* feeding using zinc oxide nanoparticles that quickly aggregated in the *A. salina* digestive system, causing a feed hindrance for the organisms due to insufficient food intake. Moreover, silver nanoparticles at the 12 nM concentration accumulated in the entire gut region of the *A. salina* causing high toxicity and degrading the animal's tissue, whereas the control presented mouth area and the gut region clear [37]. Contrary to nanoparticles, the fruit pulp can be metabolized by *A. salina*, and the size reduction does not impart any toxic effect.

3.2.2. Morphological Analysis of *A. salina* Nauplii after Toxicity Assay under an Electronic Scanning Microscope

As expected, *A. salina* exposed to seawater (negative control) did not present morphological changes such as damage to the appendages and antennae and no particle ingestion (Figure 2A). The antennas, gut, and appendages of the *A. salina* in the seawater are well preserved (Figure 2B–D). Conversely, *A. salina* exposed to $K_2Cr_2O_7$ 0.5 M (positive control) showed particle accumulation in the animal's body characterized by a darker image and a dense digestive area. Morphological changes in *A. salina,* such as surface wrinkling and severe damage to the gut and appendages, are evident in Figure 2F–H.

Optical microscopy (Figure 1) does not allow us to see any morphological changes in *A. salina,* as allowed by scanning electron microscopy (Figure 2). The exposure of *A. salina* to unprocessed sapota-do-Solimões juice (1000 μg/mL) resulted in a wrinkled surface in the abdomen (Figure 2J). Compared to the negative control, the abdomen exhibited slight deformation and loss of body integrity (Figure 2J–L). The sapota-do-Solimões juice is rich in fibers, mostly pectin, which may promote an environment with particles of different sizes [13]. The feed assimilation process is particularly susceptible to stressing action causing harsh damage, including reduced animal body length. For instance, the exposure of *A. salina* to zinc oxide nanoparticles caused malnutrition due to nanoparticle agglomeration in the gut, presenting a wrinkled abdomen area [36]. The increase in the concentration of silver nanoparticles ultimately damages *A. salina* gut due to its high toxicity causing cell apoptosis and destroying the animal tissue [37].

The *A. salina* (Figure 2M,Q,U) exposed to ultrasound-processed juice presented a larger abdomen than the *A. salina* exposed to unprocessed sapota-do-Solimões juice (Figure 2I). This might be due to the decrease in particle size of sapota-do-Solimões juice caused by the ultrasound process favoring the *A. salina* feed [38]. Moreover, *A. salina* exposed to the ultrasound processed juices UL2, UL6, and UL10 (1000 μg/mL) presented a wrinkled surface characterized by folding in the abdomen area, dense body, and total gut area (Figure 2M,Q,U).

After being exposed to the juice sample UL2, the *A. salina* under scanning electron microscopy presented a wrinkled abdomen, and the appendages showed slight damage (Figure 2N–P). Regarding UL6 (Figure 2R–T) and UL10 (Figure 2V–X) juices samples. *A. salina* also presented morphological changes in the anus area with holes and a loss of integrity. *A. salina* is susceptible to environmental stressors causing morphological changes in its body. Damages to the *A. salina* body was described for previous food samples. Coconut water processed by cold plasma and ozone promoted a loss of body integrity and deformations in the animal abdomen. Without morphological changes, the ultrasound process was less harmful to *A. salina* [22]. Irreversible lipid membrane damage was induced

by the attachment of oxidized multi-walled carbon nanotubes on *A. salina*, such as holes in the labrum, abdomen, and notum, leading the surface to wither [39].

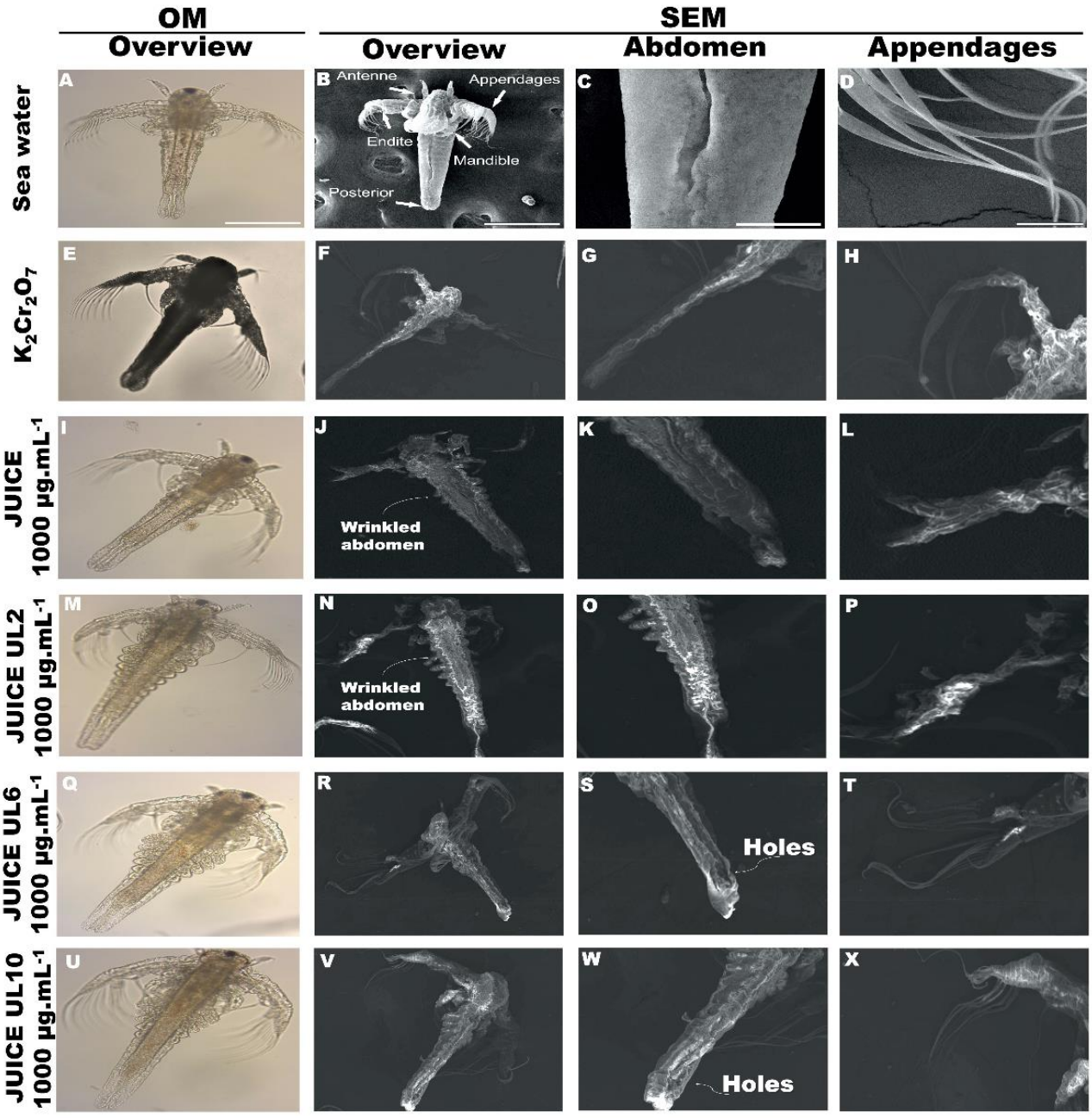

Figure 2. Morphological changes in the *Artemia salina* nauplii after 48 h of exposure under optical and scanning electron microscopy after toxicity test of unprocessed and ultrasound processed (2/6/10 min) sapota-do-Solimões juice using *Artemia salina* nauplii. Bars, 200 µm.

3.3. Protective Effect of Unprocessed and Ultrasound-Processed Sapota-do-Solimões Juice against H_2O_2 Induced Toxicity on Artemia salina

The *A. salina* in seawater (Figure 3A) was not affected since after 72 h of exposure only 10% of the animals died. In contact with $K_2Cr_7O_2$ (0.5 M), the *A. salina* was affected in the first 24 h with 100% mortality. In contrast, *A. salina*, when exposed to the stress inducer, H_2O_2 (127.45 M), increased the mortality rate to 80% after 48 h of exposure. The mortality rate rises to 100% after 72 h. As expected, seawater did not present damage to the animal body (Figure 3B,E), while $K_2Cr_7O_2$ (0.5 M) promoted a particle accumulation in the *A. salina* body (Figure 3C,F). The H_2O_2 (127.45 M) completely degraded the *A. salina*

body (Figure 3D,G). Toxic compounds can cause sub-lethal effects to *A. salina* impairing food uptake, motility, and multiple molting. That was shown after prolonged exposure of *A. salina* to polystyrene, which affects the survival due to aggregates adhering to the external surface and appendages [40].

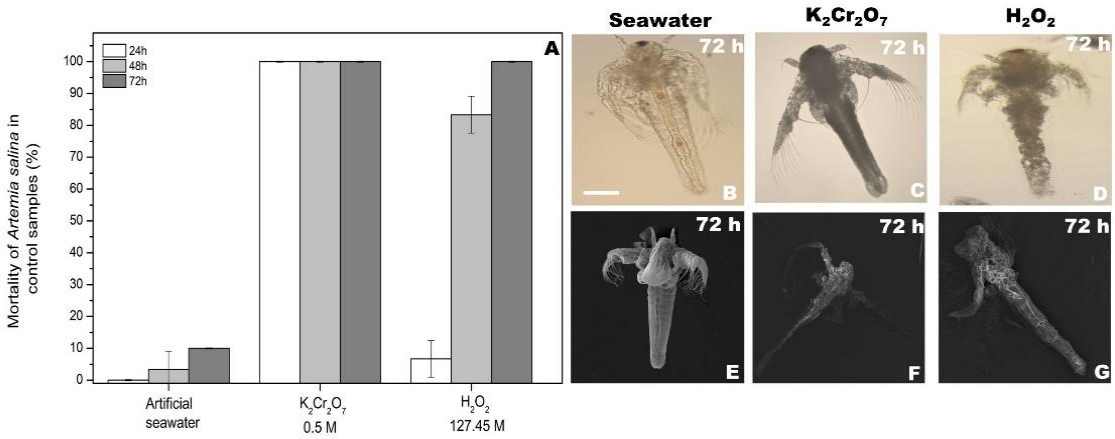

Figure 3. Control samples during protective effect in the 24/48/72 h interval against H_2O_2 in *Artemia salina* nauplii (**A**). Morphological changes in the *Artemia salina* nauplii after 72 h of exposure under optical microscopy seawater (**B**), $K_2Cr_2O_7$ (**C**), and H_2O_2 (**D**). Morphological changes in the *Artemia salina* nauplii by scanning electron microscopy seawater (**E**), $K_2Cr_2O_7$ (**F**), and H_2O_2 (**G**). Bars, 200 μm.

Figure 4A shows that the unprocessed sapota-do-Solimões juice decreased the toxicity induced by H_2O_2 (127.45 M) to *A. salina* mortality by 40% (10 μg·mL^{-1}) and 35% (1000 μg·mL^{-1}) compared with the (Figure 3A) after 48 h of exposure. After 72 h, none of the *A. salina* survived. The improvement of *A. salina* survival can be explained by different compounds present in the juice, including antioxidant compounds that mitigated the action of free radicals [15]. For instance, short-chain fatty acids increased the survival of *A. salina* infected by the pathogenic *Vibrio campbellii* strain [41]. After 72 h of the protective effect assay (Figure 4B–D), the *A. salina* presented damage in the entire body with a deterioration aspect in the three different concentrations (10–1000 μg·mL^{-1}). Figure 4E shows the complete degradation (wrinkled body, and loss of body parts) caused by H_2O_2 (127.45 M) in the *A. salina*. After exposure to a higher concentration (160 mg·L^{-1}) of zinc oxide nanoparticles, *A. salina* suffered deformations of the eyeball, shrinking of the body and intestinal gut, and degradation of the outer shell, antenna, and head. The authors suggested that, besides the concentration and time of exposure, the higher salinity of seawater decreased the electrostatic repulsion between the nanoparticles improving their aggregation in *A. salina* [42].

In Figure 5A, the sapota-do-Solimões juice UL2 decreased the *A. salina* mortality to 30% with the highest concentration (1000 μg·mL^{-1}) compared with the H_2O_2 (127.45 M) (Figure 3A) after 48 h of exposure. However, after 72 h, the mortality increased to 90%. The sapota-do-Solimões pulp has epicatechin as the major polyphenol compound. Cell breakdown can improve this content after the ultrasound process [9,14]. Therefore, polyphenols exerted a protective effect reducing cytotoxicity and against oxidative stress [43].

Furthermore, the protective effect can also be related to the production of important molecules in response to stress conditions. For instance, *A. salina*, when exposed to a non-lethal heat shock, enhanced the synthesis of heat shock proteins, which are known to improve the tolerance to a stress environment [44]. The *A. salina* presented the same deteriorated aspect as the control H_2O_2 (127.45 M) (Figure 3A) with a wrinkled body and completely deformed appendages (Figure 5E). The deformation of the swimming setae was observed in *A. salina* treated with copper oxide, which was related to the reduction of the normal swimming behavior [45].

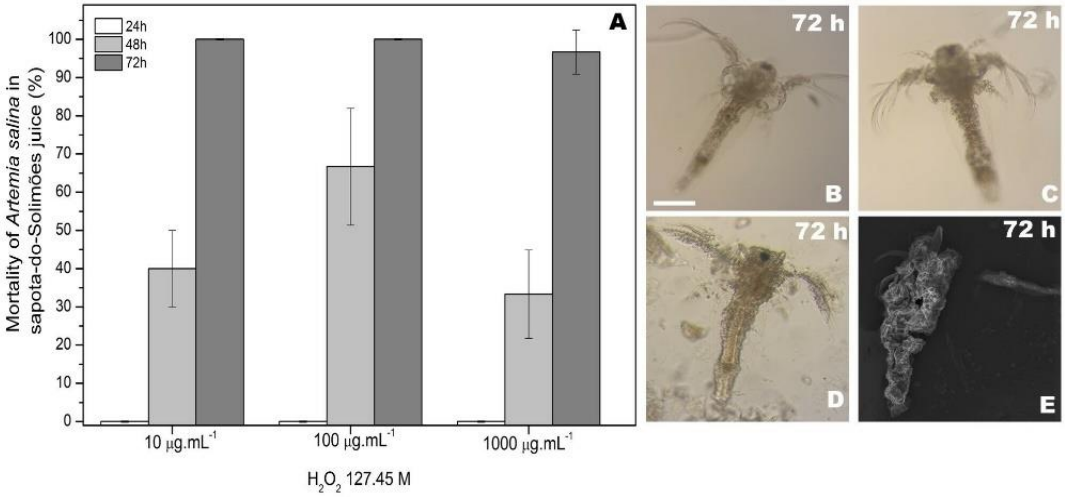

Figure 4. Protective effect of unprocessed sapota-do-Solimões in the 24/48/72 h interval against H_2O_2 using *Artemia salina* nauplii (**A**). Morphological changes in the *Artemia salina* nauplii after 72 h of exposure under optical microscopy unprocessed sapota-do-Solimões 10 µg·mL^{-1} (**B**), unprocessed sapota-do-Solimões 100 µg·mL^{-1} (**C**), unprocessed sapota-do-Solimões 1000 µg·mL^{-1} (**D**). Morphological changes in the *Artemia salina* nauplii by scanning electron microscopy unprocessed sapota-do-Solimões 1000 µg·mL^{-1} (**E**). Bars, 200 µm.

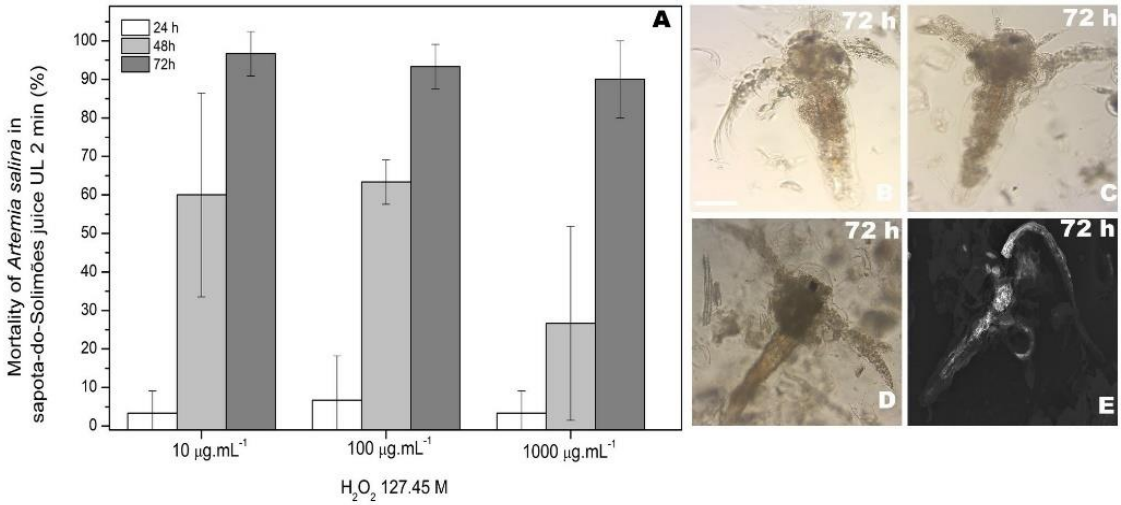

Figure 5. Protective effect of ultrasound-processed sapota-do-Solimões juice (UL2) in the 24/48/72 h interval against H_2O_2 using *Artemia salina* nauplii (**A**). Morphological changes in the *Artemia salina* nauplii after 72 h of exposure under optical microscopy ultrasound-processed sapota-do-Solimões juice 2 min (UL2) 10 µg·mL^{-1} (**B**), ultrasound-processed sapota-do-Solimões juice 2 min (UL2) 100 µg·mL^{-1} (**C**), ultrasound-processed sapota-do-Solimões juice 2 min (UL2) 1000 µg·mL^{-1} (**D**). Morphological changes in the *Artemia salina* nauplii by scanning electron microscopy ultrasound-processed sapota-do-Solimões juice 2 min (UL2) 1000 µg·mL^{-1} (**E**). Bars, 200 µm.

In Figure 6A, the sapota-do-Solimões juice UL6 decreased the *A. salina* mortality to 37% with the highest concentration (1000 µg·mL^{-1}) compared with the H_2O_2 (127.45 M) (Figure 3A) after 48 h of exposure. It increased to 90% after 72 h. The *A. salina* (Figure 6B–D) after 72 h presented a deformed body with a loss of abdomen and eye integrity. Nauplius's eye deformation was also reported in a study with bisphenol, which might affect the pathway of eye development in arthropods by disrupting endocrine-mediated processes inhibiting ecdysteroid biosynthesis [46].

In Figure 7A, the sapota-do-Solimões juice UL10 had the highest mortality rate, 53% at 1000 µg·mL^{-1}, compared with UL2 and UL6 and unprocessed juices after 48 h of

exposure. In contrast, after 72 h the mortality rate was also 90%. The components of the food matrix can enhance the protective effect. For example, ultrasound-processed coconut water presented a remarkable protective effect against H_2O_2 with a mortality rate of 60% after 72 h of exposure [22]. After 72 h of the protective effect assay (Figure 7B–D), the deterioration of the *A. salina* body was observed with a difference in the body density of the highest concentrations (100–1000 μg·mL^{-1}). Figure 7E shows that *A. salina* (1000 μg·mL^{-1}) after 72 h presented a loss in body integrity. The morphological development of *A. salina* decreased when the concentration of tributyltin chloride (antifouling substance) increased. The abnormalities are head width, abdominal width, tail width, and swimming setae, resulting in an imbalanced and irregular swimming pattern [47].

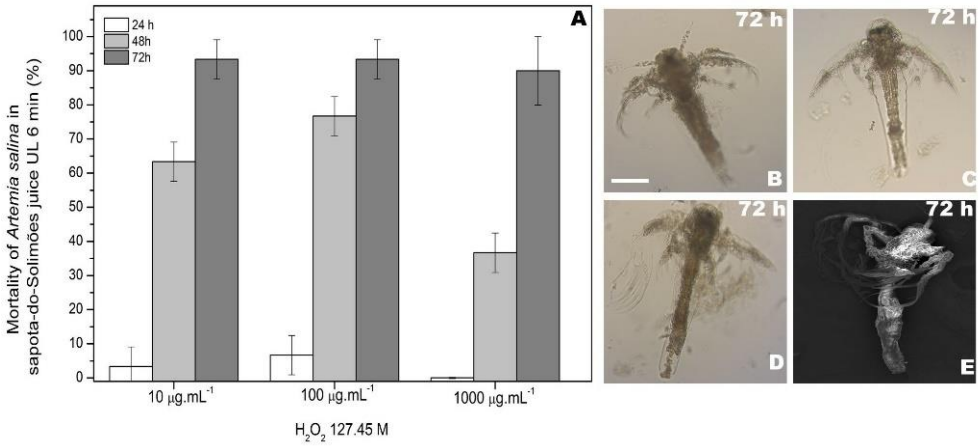

Figure 6. Protective effect of ultrasound-processed sapota-do-Solimões juice (UL6) in the 24/48/72 h interval against H_2O_2 using *Artemia salina* nauplii (**A**). Morphological changes in the *Artemia salina* nauplii after 72 h of exposure under optical microscopy ultrasound-processed sapota-do-Solimões juice 6 min (UL6) 10 μg·mL^{-1} (**B**), ultrasound-processed sapota-do-Solimões juice 6 min (UL6) 100 μg·mL^{-1} (**C**), ultrasound-processed sapota-do-Solimões juice 6 min (UL6) 1000 μg·mL^{-1} (**D**). Morphological changes in the *Artemia salina* nauplii by scanning electron microscopy ultrasound-processed sapota-do-Solimões juice 6 min (UL6) 1000 μg·mL^{-1} (**E**). Bars, 200 μm.

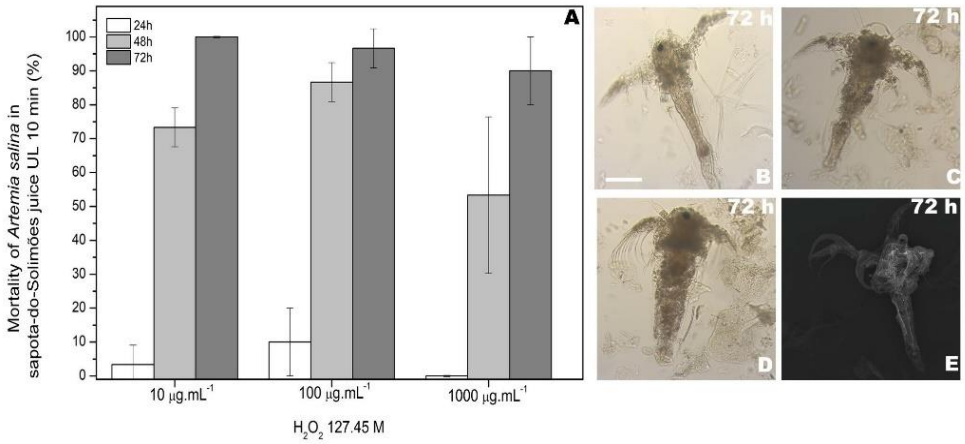

Figure 7. Protective effect of ultrasound-processed sapota-do-Solimões juice (UL10) in the 24/48/72 h interval against H_2O_2 using *Artemia salina* nauplii (**A**). Morphological changes in the *Artemia salina* nauplii after 72 h of exposure under optical microscopy ultrasound-processed sapota-do-Solimões juice 10 min (UL10) 10 μg·mL^{-1} (**B**), ultrasound-processed sapota-do-Solimões juice 10 min (UL2) 100 μg·mL^{-1} (**C**), ultrasound-processed sapota-do-Solimões juice 10 min (UL10) 1000 μg·mL^{-1} (**D**). Morphological changes in the *Artemia salina* nauplii by scanning electron microscopy ultrasound-processed sapota-do-Solimões juice 10 min (UL10) 1000 μg·mL^{-1} (**E**). Bars, 200 μm.

3.4. Evaluation of the Stress Biomarkers on Artemia salina after Protective Effect Assay of Sapota-do-Solimões Juice

3.4.1. Protein Concentration of *Artemia salina* after Protective Effect Assay of Sapota-do-Solimões Juice

The protein content in Figure 8 was higher for the H_2O_2 (positive control). The unprocessed juice had different protein content from processed juices ($p \leq 0.05$). The environmental conditions can induce the synthesis of stress proteins. For instance, in vivo study concluded that PHB (poly-ß-hydroxybutyrate) induced the production of heat shock protein (Hsp) 70 in *V. campbellii* challenged *A. salina* [48]. Moreover, the production of these proteins can enhance the protective effect against adverse environmental conditions as shown after exposure to a non-lethal heat shock at 37 °C improved tolerance to lethal levels of pH, salinity, and ammonia stress [44].

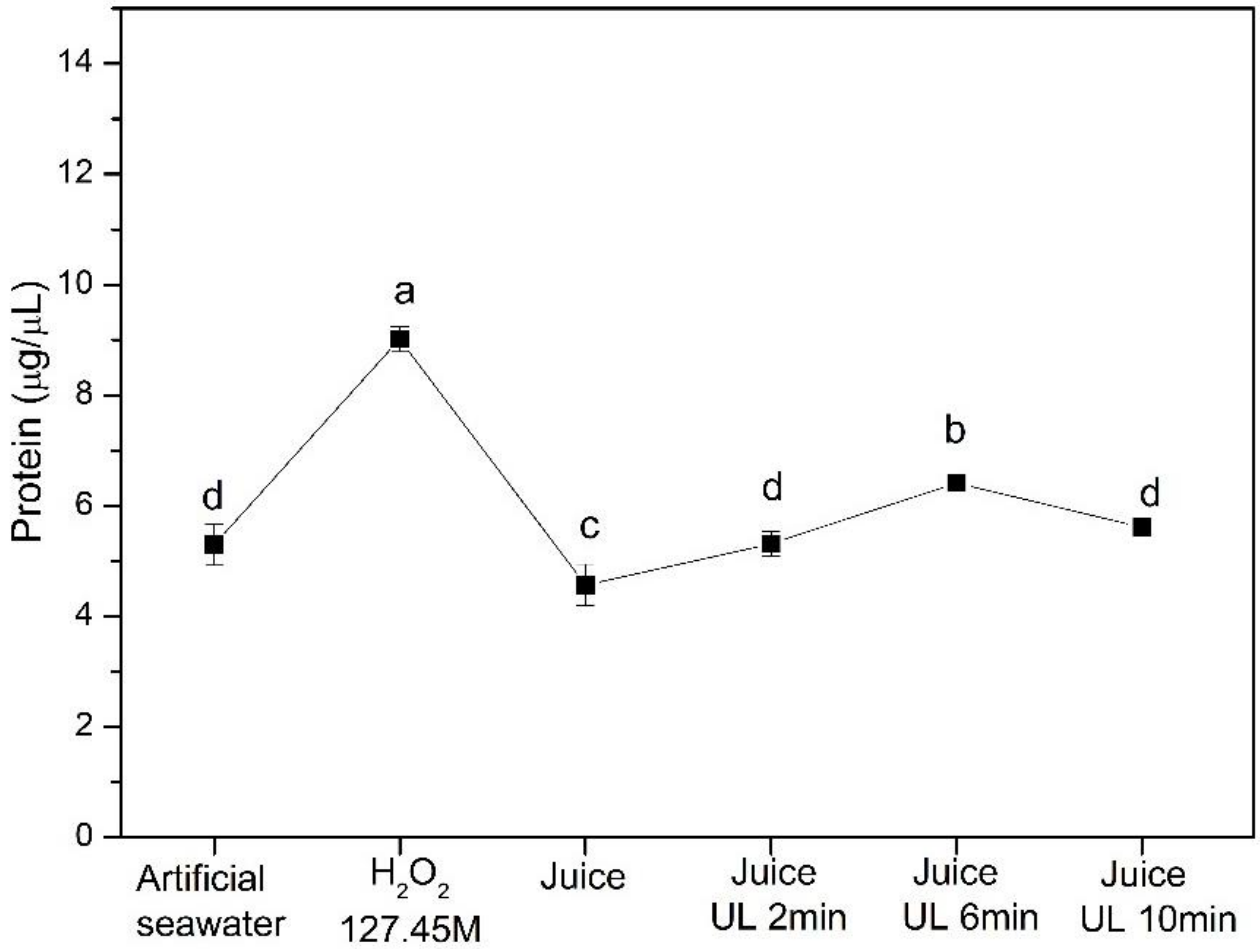

Figure 8. The protein content of unprocessed sapota-do-Solimões juice and processed by ultrasound (1000 µg·mL^{-1}) on *Artemia salina* nauplii after 72 h of H_2O_2 exposure. The different letters indicate statistically significant differences ($p \leq 0.05$).

3.4.2. Catalase Activity on *Artemia salina* Nauplii after Protective Effect Assay of Sapota-do-Solimões Juice

Figure 9 shows that CAT activity was higher in the positive control with only H_2O_2. In contrast, a gradual decay was observed for the increment of ultrasound process time (from 0 to 10 min) being statistically different ($p \leq 0.05$). Meanwhile, the unprocessed juice had similar CAT activity ($p \leq 0.05$) compared to the H_2O_2 (positive control). Thus, in some way, the ultrasound process promotes changes in the juice composition that did not impart any change in CAT activity compared with seawater (negative control). This is probably due to the decrease in particle size during the homogenization process caused by ultrasound,

where the juice fibers are broken down due to mechanical waves [38]. In this regard, the formation of micron and submicron-sized agglomerates zinc oxide nanoparticles could produce toxic effects in *A. salina* caused by a feed hindrance for the organisms [37].

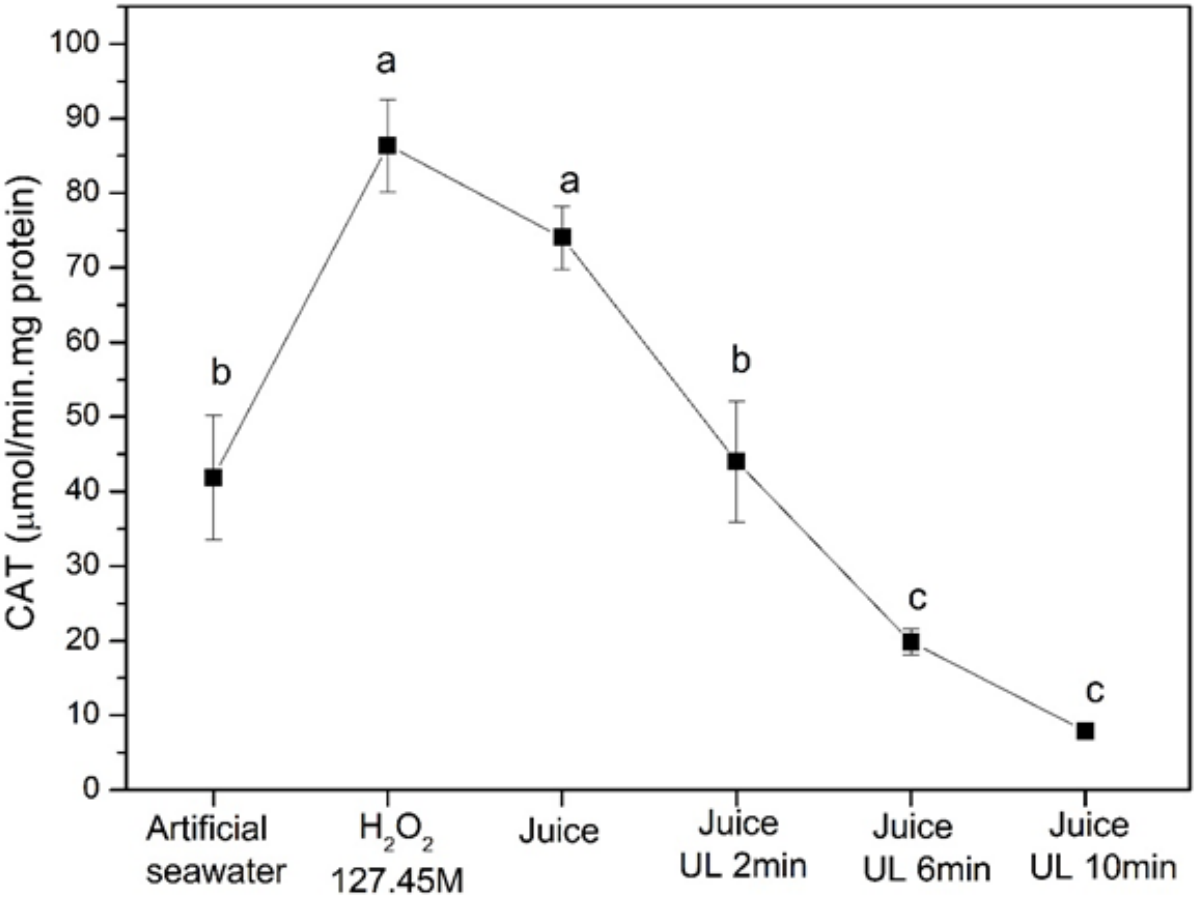

Figure 9. Catalase (CAT) activity of unprocessed sapota-do-Solimões juice and processed by ultrasound (1000 µg·mL^{-1}) on *Artemia salina* nauplii after 72 h of H_2O_2 exposure. The different letters indicate statistically significant differences ($p \leq 0.05$).

Moreover, increasing the concentration of zinc oxide and titanium dioxide nanoparticles enhanced CAT activity in *A. salina* [42]. Under stress, antioxidant defense mechanisms such as SOD and CAT can be activated to protect the cells from oxidative damage. These enzymes work together to form the first defensive steps, thus, H_2O_2 radicals produced by SOD can be used by CAT, decreasing cell damage [29]. Nonetheless, the overproduction of ROS can decrease the CAT activity in marine organisms. *A. salina* treated with copper oxide decreased the CAT activity due to the interaction of dissolved Cu^{2+} ions with H_2O_2, leading to the production of hydroxyl radicals through the Fenton and Haber Weiss pathway, impairing CAT's inactivation [45].

3.4.3. Superoxide Dismutase Activity on *Artemia salina* Nauplii after Protective Effect Assay of Sapota-do-Solimões Juice

In Figure 10, the SOD activity for the unprocessed juice was the highest compared with the controls and ultrasound-processed samples. For magnetic O-carboxymethyl chitosan-loaded silver nanoparticles in *A. salina,* the higher SOD activity was a response against oxidative stress caused by superoxide, where SOD catalyzes the degradation of highly toxic ROS ($O_2^{\bullet -}$) to less toxic H_2O_2 and O_2 [26]. Meanwhile, pristine titanium dioxide nanoparticles decreased SOD activity compared with the control [29]. Lower SOD activity implies the failure of the antioxidant system to scavenge the excessive ROS leading to inhibition or degradation of SOD [24,29]. In this regard, the particles or components in

the unprocessed juice caused oxidative stress for *A. salina*, which is demonstrated by high catalase and SOD activity.

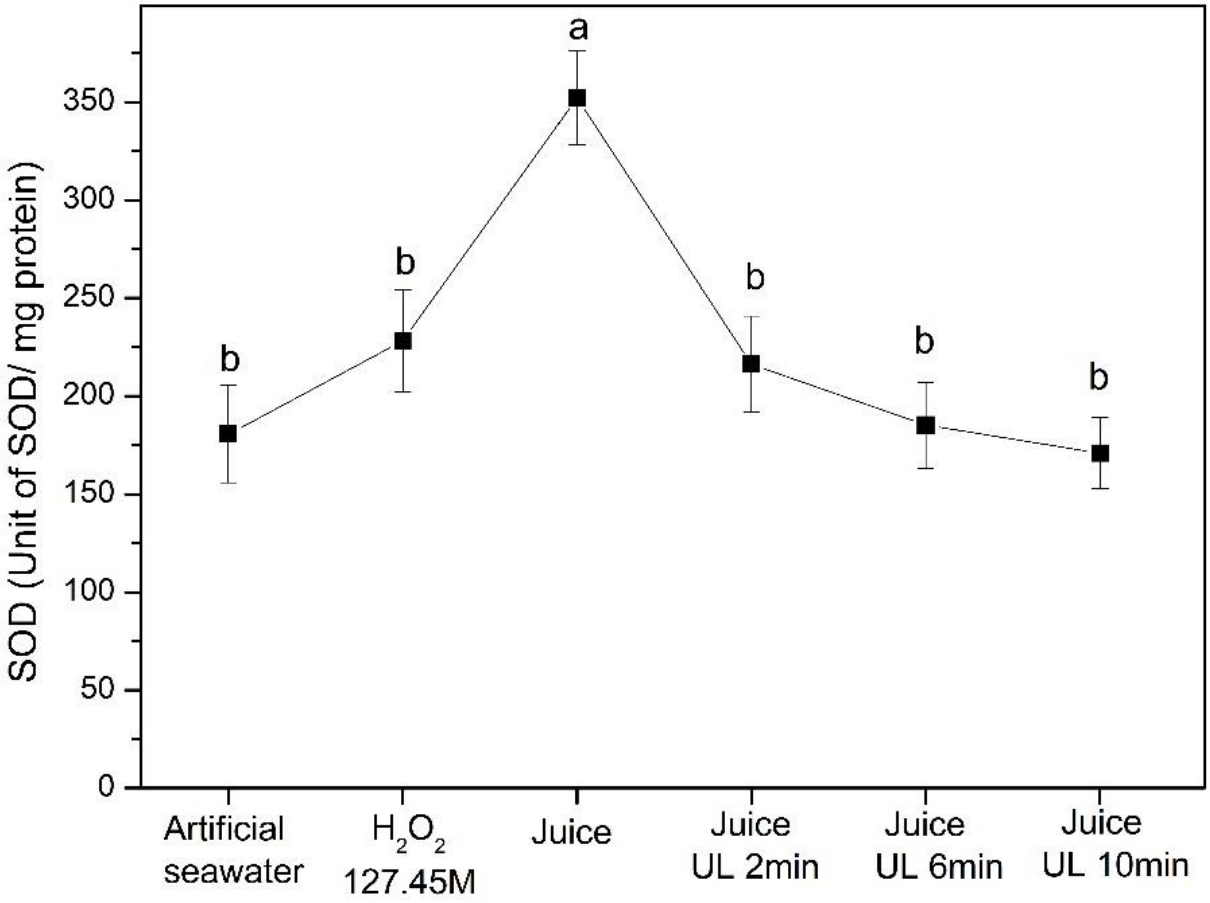

Figure 10. Superoxide dismutase (SOD) activity in response to 72 h of H_2O_2 exposure of *Artemia salina* nauplii on unprocessed sapota-do-Solimões juice and processed by ultrasound (0 to 10 min). The different letters indicate statistically significant differences ($p \leq 0.05$).

3.4.4. Lipid Peroxidation Level on *Artemia salina* Nauplii after Protective Effect Assay of Sapota-do-Solimões Juice

In Figure 11, the LPO shows a lower level for peroxide hydrogen and unprocessed juice. LPO is an important indicator of oxidative stress in aquatic organisms since oxidative stress can cause an increase in the lipoperoxidation process [24]. The exposure of the *A. salina* to acid (pH 6.8) seawater increased the level of lipid peroxidation [27]. LPO increased when exposed to Cd and Hg, while it did not change for Zn. This effect was explained by the metallothionein synthesis, an important antioxidant that contributes to the reduction of reactive oxygen species. Thus, this defense mechanism against lipid peroxidation can be inhibited by Cd and Hg, or induced by Zn [28].

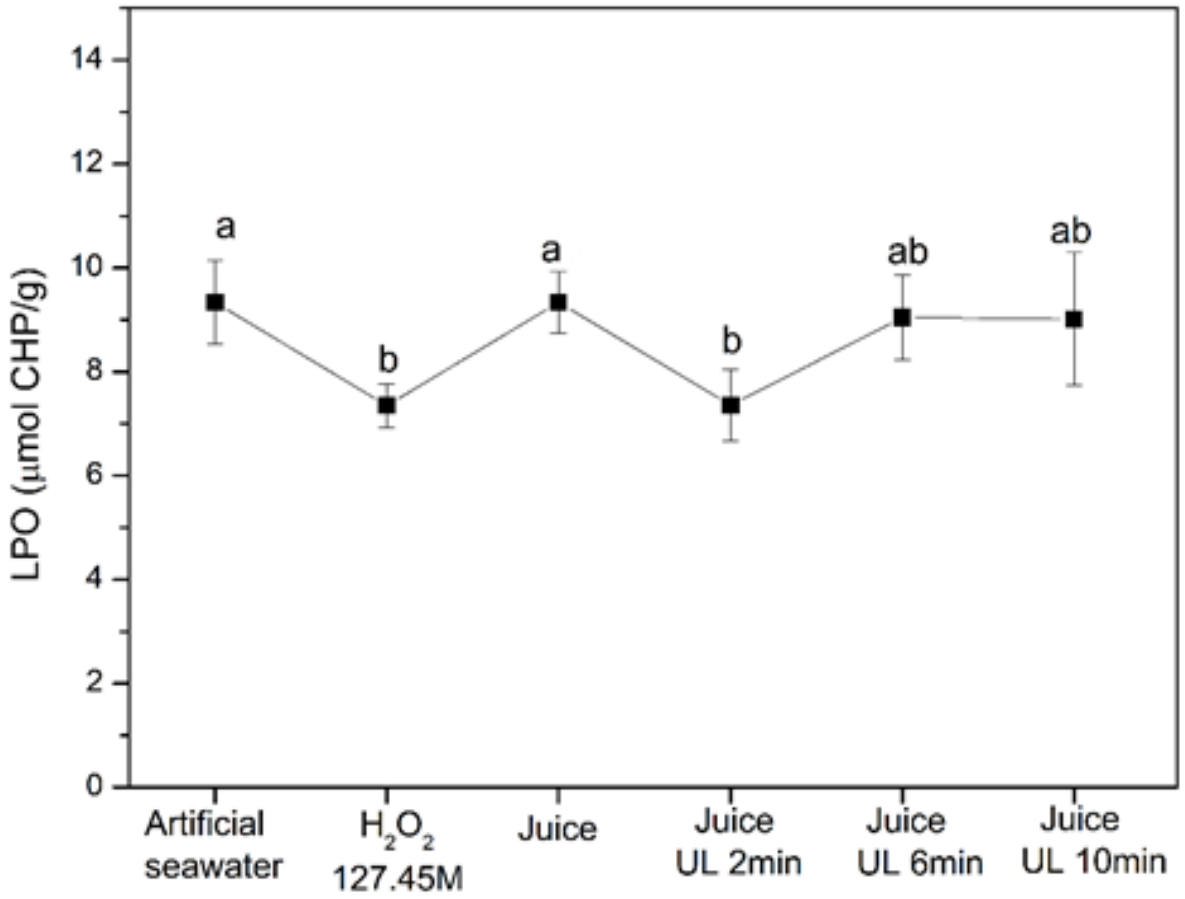

Figure 11. Lipid peroxidation (LPO) of unprocessed sapota-do-Solimões juice and processed by ultrasound (1000 µg·mL^{-1}) on *Artemia salina* nauplii after 72 h of H_2O_2 exposure. The different letters indicate statistically significant differences ($p \leq 0.05$).

4. Conclusions

The sapota-do-Solimões juice processed by ultrasound did not promote a toxicity effect on *A. salina.* Conversely, the juice particles promote morphological changes in the animal's body, including wrinkled surfaces and holes. The ultrasound-processed sapota-do-Solimões juice (UL2 and UL6) had the lowest mortality rate by around 40% after 48 h of protective effect assay showing that juice components were able to decrease the mortality against H_2O_2 toxicity. Regarding the stress biomarkers, the antioxidant enzymes CAT and SOD had the highest activity in the unprocessed juice, whereas LPO had similar results for the different processes. Further studies on different toxicity models might complement this study.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr10091880/s1, Table S1: Acute toxicity of unprocessed and ultrasound processed sapota-do-Solimoes juice.

Author Contributions: Conceptualization: R.M.d.S., T.B.A.R.M., E.d.C.M., P.H.C., F.A.N.F. and S.R.; methodology, R.M.d.S., T.B.A.R.M., E.d.C.M. and S.R., validation, R.M.d.S., T.B.A.R.M. and E.d.C.M.; formal analysis, R.M.d.S., T.B.A.R.M., E.d.C.M. and S.R.; investigation, R.M.d.S., T.B.A.R.M., E.d.C.M., P.H.C., F.A.N.F. and S.R.; resources, P.H.C., F.A.N.F. and S.R.; data curation, R.M.d.S., T.B.A.R.M., E.d.C.M. and S.R.; writing—original draft preparation, R.M.d.S., T.B.A.R.M. and E.d.C.M.; writing—review and editing, R.M.d.S., T.B.A.R.M., E.d.C.M., F.A.N.F. and S.R.; visualization, R.M.d.S., T.B.A.R.M., E.d.C.M., P.H.C., F.A.N.F. and S.R.; supervision, F.A.N.F. and S.R.; project administration, F.A.N.F. and S.R.; funding acquisition, F.A.N.F. and S.R. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data that support the findings of this study are available on request.

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