EUROPEAN ACADEMIC RESEARCH
Vol. X, Issue 3/ June 2022

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Green Synthesis of Silver Nanoparticles Mediated by Aqueous Açaí (*Euterpe Oleracea*) Extracts

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Abstract

The production of metallic nanoparticles (MNPs) by green routes stands out for implementing concepts related to Green Chemistry in order to minimize impacts to the environment caused by traditional synthetic routes. The use of plant extracts to mediate the synthesis of MNPs is presented as an

alternative since it contains phytochemicals that can participate in the formation and stabilization of nanoparticles (NPs). In this scenario, açaí, a fruit native to the Amazon region that contains biomolecules with antioxidant properties, exhibits the potential to mediate the synthesis of NPs. The present study investigated the production of silver nanoparticles (AgNPs) mediated by aqueous extracts of açaí, assessing different reaction parameters (açaí extract concentration, pH and temperature). The reaction media exhibited broad bands between 350-430 nm, which is associated with the surface plasmon resonance (SPR) of AgNPs, and were more visible when using diluted fruit extracts and in neutral/alkaline medium at the temperature of 25° C. Furthermore, AgNPs exhibited spheroidal shapes and hydrodynamic radius of 21.0 ± 0.8 nm (25°C); 9.0 ± 0.5 nm (40 °C) and 129.3 ± 5.6 nm (50 °C) and polydispersity indices (PdIs) of 0.283 ± 0.004 (25 °C), 0.452 ± 0.023 (40 °C) C) and 0.191 ± 0.017 (50 °C), with Zeta potential values close to -30 mV. The AgNPs showed lower cytotoxicity to fibroblasts than the $AgNO_3$ solution, and did not exhibit microbiological activity, indicating a strong complexing capability of the phytochemicals present in the açaí extract.

Keywords: alternative synthetic routes; green chemistry; plant extract; Amazon fruits; microbicide; cytotoxicity.

INTRODUCTION

Nanoparticles (NPs) are structures with dimensions between 1 and 100 nm, whose most important characteristics are their high proportion of surface area in relation to the occupied volume, allowing greater interaction with other structures, resulting in enhanced chemical and physical properties in relation to the micro and macrostructures. Thus, NPs have interesting advantages when employed in areas such as catalysis and biomedical sciences (Mat Yusuf *et al.,* 2020; Yadi *et al.,* 2018).

In the nanosynthesis area there is a growing number of studies aimed at investigating the properties of metallic nanoparticles (MNPs) as antimicrobial agents to act against several pathogenic microorganisms (González-Vargas *et al.,* 2017), especially considering their use as surface coatings. Among them, silver nanoparticles (AgNPs) have been the focus of many studies because they are able to kill Gram-positive and Gram-negative bacteria, present less toxicity to mammalian cells and are extensively used in environmental applications, in healthcare industry, in wound dressing development and as disinfectants (Manosalva *et al.*, 2019).

The main methods for the production of MNPs are based on physical and chemical techniques that could potentially release harmful byproducts to the environment in their process, thus, requesting the development of viable and friendlier alternative methodologies from an ecological point of view (Manosalva *et al.*, 2019). In order to obtain more sustainable routes, concepts related to Green Chemistry are adopted for the production of AgNPs, such as the use of plant extracts to mediate the synthesis of these structures (Dada *et al.,* 2019). The synthesis of MNPs mediated by plant extracts possesses lower environmental impact and can be economically competitive, providing applications in a range of areas that would not be allowed when using precursors that present toxicity to humans or other beings (Beyene *et al.,* 2017).

The Amazon region has a rich plant biodiversity and its abundant raw material exhibits favorable characteristics for mediation in the synthesis of metallic nanoparticles, such as the açaí fruit (*Euterpe oleracea*), widely used in the food sector. Açaí has in its phytochemical composition species that have antioxidant and anti-inflammatory properties, especially phenolic compounds, such as the flavonoid group, which has as its main representative in açaí the anthocyanins responsible for the dark color of the fruit. (Yamaguchi *et al.,* 2015). These substances exhibit potential to act as reducing agents of silver ions, allowing the production of silver nanoparticles by a greener approach. Thus, the present study proposes to investigate the production of AgNPs mediated by aqueous extract of açaí by assessing different synthetic parameters, such as concentration, pH and temperature of the reaction medium and their correlation to the microbiological activity of these dispersions.

EXPERIMENTAL

Production of aqueous açaí extracts: Açaí extract was obtained by manual pulping of the fruits obtained from the local market in the city of Itacoatiara-AM. The fruits were previously cleaned in running water and then put in contact with a 1.0 % (v/v) sodium hypochlorite solution for 15 min. Later, the açaí was subjected to homogenization with distilled water using a blender at 25 °C for 30 min using 1 kg of açaí and 1 L of distilled water, yielding the **raw açaí extract**. Subsequently, the raw extract was centrifuged at 4000 RPM for 30 min. For the synthesis of NPs, the raw açaí extract was diluted in the proportion of 10 mL of centrifuged extract: 40 mL of distilled water, yielding the **purified açaí extract**.

Synthesis of silver nanoparticles (AgNPs): To produce silver nanoparticles dispersions, 50 mL of silver nitrate ($AgNO_3$ (P.A), Laderquímica) solution at $0.1x10^{-3}$ mol.L^{-1} was added to a beaker containing 5 mL of purified açaí extracts at different dilutions, yielding a total volume of 55 mL for each reaction media. All vessels were covered with aluminum foil. **Evaluation of the concentration of açaí extract:** from the purified açaí extract, different dilutions were made, which resulted in **condition 1** (1:1 dilution) **condition 2** (1:2 dilution) and **condition 3** (1:4 dilution). **Evaluation of the pH**: The AgNPs were synthesized under **conditions 2** and **3** of the açaí extract dilution by adjusting the pH of the reaction medium to 6.0, 7.0 and 8.0. For this adjustment, a 0.01 mol L^{-1} nitric acid (65 % (wt/wt) HNO_3 (P.A), Vetec) solution or a 0.01 mol L^{-1} sodium hydroxide (NaOH (P.A), Biotec) solution was used. **Evaluation of the temperature**: To investigate the effect of temperature on the production of AgNPs, the synthesis of AgNPs was performed using the **condition 2** of açaí extract dilution at pH 7.0 and the temperatures of 25, 40 and 50 °C during the first 4 h of reaction.

Reaction media monitoring: The evolution of the AgNPs formation process was followed by UV-Vis spectroscopy using a BEL UV-M51 equipment by monitoring the band associated with the surface plasmon resonance of silver located between 380-450 nm. The samples were taken hourly for 4 h, at 8, 24, 36 and 48 h, and daily from 3 to 21 days.

Transmission electron microscopy: Transmission electron microscopy (TEM) images were recorded on a JEOL microscope model JEM 1400 operating at 120 kV. The samples were previously diluted in deionized water and dropped onto carbon-covered Cu grids and allowed to dry at 25 °C for 1 h.

Dynamic light scattering (DLS) and Zeta potential analysis: The hydrodynamic diameter and Zeta potential analysis of AgNPs dispersions were performed by the Malvern Zetasizer NanoZS equipment using a helium-neon laser (4 mW) at 633 nm with a beam angle of 173°. The measurements were recorded in triplicate in automatic mode at 25 °C. The Zeta potential was measured manually in triplicate with a DTS-1070 capillary electrophoresis cell.

Microbicide evaluation: *Streptococcus mutans* ATCC 35688, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 90028 were used in microbiological evaluation of AgNPs by agar diffusion test. Bacteria were suspended in Mueller–Hinton broth and the

yeast was suspended in Sabouraud broth. After that, inoculums were prepared by adjusting the optical density reading at 630 nm to 0.08 - 0.10 (~$1x10^8$ cells mL^{-1}), which matched to 0.5 McFarland standard. Afterward, microorganisms were inoculated on nutrient agar Petri dishes and 8 mm diameter wells were cut from the agar using a sterile cork-borer. Subsequently each well was filled with 50 µL of the extracts and then were incubated for 2 h at 25 ºC and thereafter the plates were incubated under aerobic conditions at 36 °C for 18 h (Brighenti *et al*., 2014). A 0.12% chlorhexidine digluconate solution (Colgate Palmolive Ltd) was used as positive control and sterile distilled water was used as negative control. Inhibition zone was measured in millimeters (mm). The samples were considered bioactive if the inhibition halos were greater than 11 mm (Brighenti *et al.*, 2014).

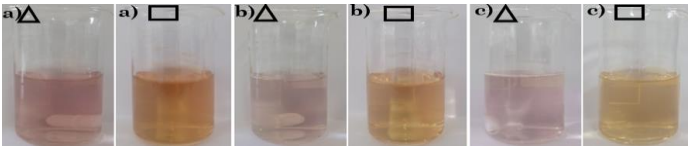
Cell viability evaluation: L929 murine fibroblasts obtained from American Type Culture Collection (ATCC) were cultivated in culture flask containing Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) with 10% fetal bovine serum and 1% of antibiotics containing 10,000 UI penicillin, 10 mg mL^{-1} streptomycin and 25 µg mL^{-1} amphotericin B and kept at 37 ºC in a humidified atmosphere of 5% CO_2. After semiconfluence has been reached, the cells were trypsinized using Trypsin-EDTA 0.25% (Sigma-Aldrich) and then they were counted in a Neubauer chamber. Following, L929 fibroblasts were seeded in a 96-well plate at a density of $1x10^4$ cells $well^{-1}$ in DMEM + 10% FBS for 24 h at 37 ºC and 5% CO_2. Subsequently, the cells were exposed to **A** (reaction media using condition 2 of açaí extract dilution at pH 7 and 25 ºC), **B** (reaction media using condition 3 of açaí extract dilution at pH 7 and 25 ºC), **C** (reaction media using condition 2 of açaí extract dilution at pH 7 and 40 ºC), **D** (reaction media using condition 2 of açaí extract dilution at pH 8 and 25 ºC), **E** (reaction media using condition 2 of açaí extract dilution at pH 8 and 40 ºC) and **F** (0.1×10^{-3} $mol.L^{-1}$ $AgNO_3$ solution) samples by replacing 100 µL of the medium in each well with 100 µL of samples extracts and then incubated for 24 h. The extracts were prepared in two different conditions: 1:1 (v/v) and 1:2 (v/v) using sample:DMEM media. Then, the extracts samples were removed and the wells were washed twice with pH 7.4 phosphate buffered saline (PBS). Following, 0.1 mL of a freshly prepared 1.0 mg mL^{-1} 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich) solution was added to each well for 3 h in the CO_2 incubator. The purple formazan product was completely solubilized using 50 µL of isopropanol. The absorbance values were measured at 570 nm using a microplate reader (SoftMax Pro 5). Cells maintained in DMEM media were

used as negative control and cells treated with DMSO at final concentration of 40% (v/v) were used as positive control. All samples were assayed in three replicates. The cell viability was estimated according to Riss *et al.* (2004).

RESULTS AND DISCUSSION

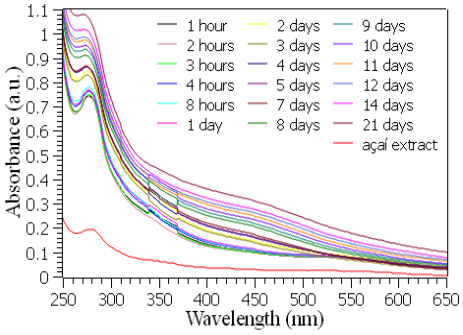
The production of AgNPs was initially carried out at pH 6.0 and color changes were identified with the progress of the reaction, which became light brown (Figure 1). This change in color is associated with the formation of NPs. AgNPs dispersions tend to exhibit yellow to light brown coloration due the surface plasmon resonance (SPR) of silver (Mohaghegh *et al.,* 2020).

Figure 1 - Images of the reaction media for the production of AgNPs at pH 6.0 using the different dilutions of açaí extract after 1 hour (Δ) and 1 day (□) of reaction: a) condition 1; b) condition 2; c) condition 3.



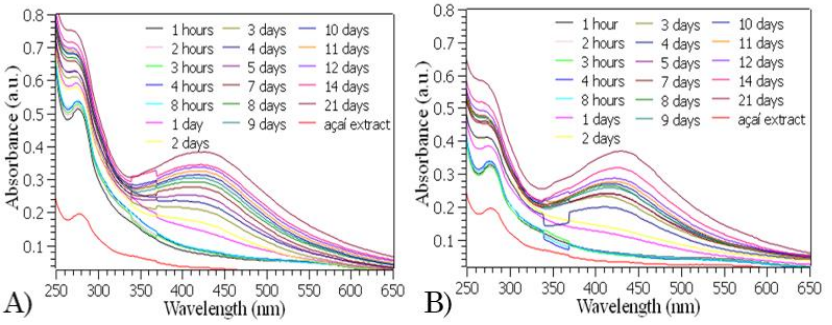
The UV-vis spectra of the reaction medium using the açaí extract dilution of condition 1 at pH 6 exhibit the SPR band with low definition near the range of 380-450 nm (Figure 2). It was noted that an increase of the SPR band was also associated to an increase in the band close to 280 nm, possibly owing to the increase of oxidation of the phenolic compounds present in the açaí extract to carbonyl groups (C=O), which exhibit n→π* electronic transitions (Pavia *et al.,* 2010) around this wavelength value.

Figure 2 - UV-Vis spectra of the reaction medium at pH 6.0 using the açaí extract dilution of condition 1 over the progress of the reaction.



The UV-vis spectra of the reaction media for the conditions 2 and 3 were exhibited in Figure 3. It confirmed the appearance of the SPR band of AgNPs within the 380-450 nm range after 1 day of reaction, with the maximum absorbance recorded around 430 nm. The SPR signal was also narrower for condition 3, indicating the formation of more uniform AgNPs (Mosaviniya *et al.,* 2019).

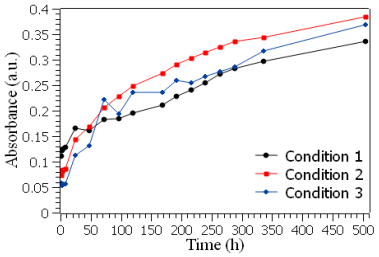
Figure 3 - UV-Vis spectra of the reaction media at pH 6.0 over the progress of the reaction using açaí extract dilution of A) condition 2; B) condition 3.



Analyzing the maximum absorbance of the SPR band versus the progress of the reaction of each reaction media for the three different açaí extract dilutions (Figure 4), it was observed an increase in these values, indicating the formation of more NPs (Guimarães *et al.,* 2020). Since it was observed that for the three conditions there were increments in the absorbance values along the reaction, even in longer reaction times, it indicates that the rate of formation of AgNPs under these experimental conditions was lower.

At higher concentration of açaí extract (condition 1), absorbance values higher than the diluted extracts (conditions 2 and 3) were observed during the first 24 h of monitoring, indicating that the higher concentration of phytochemicals favors initially the rate of formation of the AgNPs. After 48 h, the rate decreases independently of the concentration of the açai extract employed, but it occurs more abruptly at higher concentration. Such effect might be related to the chemical composition of the açaí extracts.

Figure 4 - Variation of the maximum absorbance of SPR bands of the reaction media at pH 6.0 using different açaí extract dilutions over the progress of the reaction.



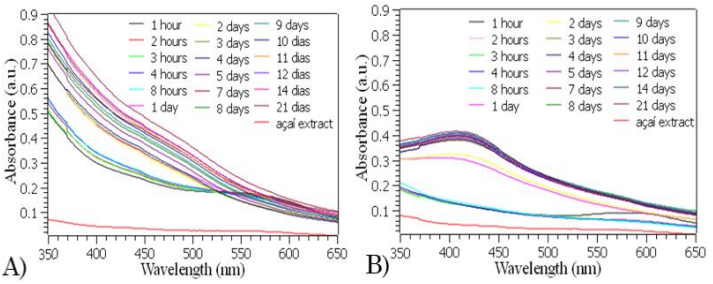
The main phytochemicals are the anthocyanins, especially the cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside biomolecules that are found in larger quantities, which possess -OH groups that can complex metallic ions (De Souza; De Giovani, 2004), such as silver ones. Hence, it is plausible the existence of a competition between the chelating and the reduction effect of silver ions when the concentration of phytochemicals in the açaí extract is high, disturbing the process of AgNPs formation. Considering the more diluted conditions of açaí extract, it was noticed that the production of AgNPs is smaller only at the beginning of the synthesis, and as the reaction progresses, more NPs are formed than the condition 1. However, if the concentration of açaí is further diluted, such as in condition 3, there were fewer phytochemicals that can reduce silver ions, decreasing not only the rate of formation of AgNPs but the number of NPs formed.

The pH is an important physicochemical parameter in the production of NPs, once that the acidity or basicity of the medium can affect the size and shape of the particles (Dada *et al.*, 2019). The açaí extract dilution condition 2 was used at pH 7.0 and 8.0 to produce AgNPs. It was observed a light brown color of each medium in condition 1 of açaí extract dilution at pH 6.0, indicating the formation of AgNPs as suggested earlier (Mohaghegh *et al.*, 2020).

The UV-Vis spectra of the reaction media at these pHs are shown in Figure 5. After one day of the beginning of the synthesis the SPR band is visible around 380-450 nm but less defined at pH 7.0 than pH 8.0. Among the different parameters investigated so far and considering the maximum absorbance of the SPR band recorded as the reaction progresses (Figure 6), the reaction at pH 7.0 yielded the largest amount of AgNPs. The results obtained are in agreement with other studies, where an optimized production of NPs in neutral and alkaline reaction media is reported. Among them, the study by Guimarães *et al.* (2020) regarding the synthesis of AgNPs mediated

by juazeiro leaf extract (*Ziziphus joazeir*o) reports higher yields of NPs within the pH range of 7-11.

Figure 5 - UV-Vis spectra of the reaction media using the açaí extract dilution of condition 2 over the progress of the reaction at: A) pH 7.0; B) pH 8.0.



At pH 7.0, two distinct broad shoulders assigned to the SPR band can be identified, one with lower intensity around 500 nm and another more intense near 430 nm. Thus, the lack of definition of these signals for the selected parameters might be associated with the existence of AgNPs with different sizes and/or shapes, since NPs with different shapes have the potential to form two or more SPR signals in the UV-Vis technique (Mohaghegh *et al*., 2020). The SPR signals at shorter wavelengths are associated with NPs of smaller dimensions (Mat Yusuf *et al*., 2020), indicating that although pH 7.0 favors the presence of species with higher efficiency to reduce Ag^+ ions, the biomolecules of açaí extract in neutral medium exhibit less ability to control the shape and/or size of the synthesized AgNPs.

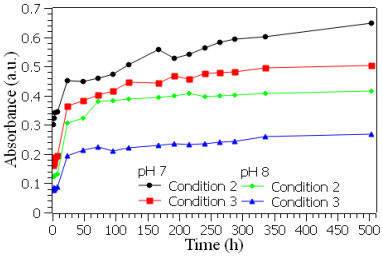
Regarding the maximum absorbance of the reaction media at pH 7.0 and 8.0 of the SPR bands over the time (Figure 6), it is noticed that in all cases, independently of pH and concentration of the açaí extract investigated, there is a higher rate of AgNPs formation up to 24 h of synthesis. As the reaction continues, the rate of formation of NPs is decreased.

After the first day of synthesis, there was a greater production of AgNPs at pH 7.0 when compared to the reaction media at pH 8.0 which may indicate a greater reactivity of the phytochemicals present in açaí extract in neutral media than in alkaline media. The results obtained by the UV-Vis spectroscopy technique indicate that the pH directly influences the reactivity of the biomolecules found in the açaí extracts since the anthocyanins in aqueous media have different chemical stabilities (Jiang *et al.,* 2019).

The anthocyanins exhibit complex chemical structures, and the degree of complexity increases in aqueous media as they are in different chemical equilibriums depending on the pH. In acidic media there is a

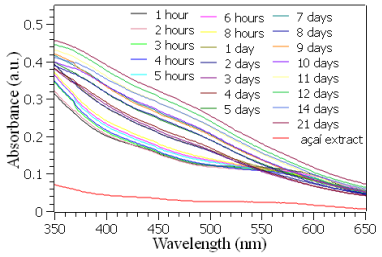
predominance of the flavylium cation form, and in neutral media the hemiacetal structure is predominant. However, in alkaline media, the quinoid bases are the main structures found for the anthocyanins, which have lower antioxidant capacity than the flavylium cations (Freitas, 2019). Thus, the different structures found as a function of pH can lead to the formation of NPs with different morphologies.

Figure 6 - Variation of the maximum absorbance of SPR bands of the reaction media at pH 7.0 and 8.0 using different açaí extract dilutions over the progress of the reaction.



The temperature is an important physicochemical parameter for AgNPs production owing to its ability to change the kinetics of the reactions, influencing the rate of formation and size of AgNPs (Dada *et al.,* 2019). At higher temperatures investigated (40 and 50 °C), it was observed changes in color of the reaction media as the reaction progresses, becoming light brown after several hours, indicating the formation of the AgNPs. However, the color changes were less intense in comparison to the reactions carried out at 25 °C. Figure 7 shows the UV-Vis spectra of the reaction medium using the condition 2 of açaí extract dilution at pH 7 and 40 °C over the progress of the reaction.

Figure 7 - UV-Vis spectra of the reaction medium at pH 7.0 and 40 °C using the açaí extract dilution of condition 2 over the progress of the reaction.

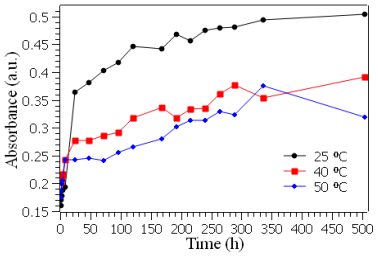


It was observed the presence of the SPR band in the range of 380-450 nm, associated with the formation of AgNPs, although with low resolution after 1 day of synthesis. Comparing the data obtained for the different temperatures employed, it was noticed that the conditions at 40 and 50 °C exhibit lower absorbance values of the SPR band in comparison to those obtained at 25 °C, indicating that lower quantities of AgNPs are formed at higher temperatures. By increasing the temperature of the reaction medium, an increase in the reaction rate was expected, producing more AgNPs, however, a probable degradation of the phytochemicals present in the açaí extract (anthocyanins) occurred when the temperature was increased, hindering the transformation process of silver cations into AgNPs.

When the maximum absorbance of the SPR bands is analyzed over the progress of the reaction using different temperatures (Figure 8), a larger production of AgNPs at higher temperatures was observed in the first hours of reaction, possibly associated with the increase in the kinetic content of the reaction media. However, after 24 h of reaction, the reaction conducted at 25 °C exhibited larger quantities of AgNPs formed. After 48 h from the beginning of the reaction, the absorbance values of the SPR bands for reaction media at 40 and 50 °C are even lower than those obtained at 25 °C, and the reaction media begin to produce gradually less AgNPs after this period of time, possibly related to the decrease in the concentration of the reagents.

With the increase in the temperature, an increase in the rate of formation of AgNPs was expected by increasing the kinetic energy of the reactants to reduce Ag^+ ions into Ag^0, obtaining higher yields and smaller NPs (Perotti and Da Costa, 2021). However, the increase in temperature caused the degradation of different açaí phytochemicals, such as the anthocyanins, possibly involved in the reduction process of silver ions. Costa, Silva and Vieira (2018) evidenced the temperature sensitivity of anthocyanins, which undergo degradation when exposed to temperatures above 40 °C. Therefore, an increase in the temperature of the reaction media can negatively influence the synthesis of AgNPs.

Figure 8 - Variation of the maximum absorbance of SPR bands of the reaction media at pH 7.0 using açaí extract dilution of condition 1 and at different temperature values over the progress of the reaction.



The TEM images of the AgNPs produced at pH 7.0 and açaí extract dilution of condition 2 at 25 °C are shown in Figure 9. It is observed the predominance of populations of spheroidal nanoparticles of different sizes, especially in the 10-50 nm diameter range. The absence of well-defined shapes and sizes indicate that the phytochemicals present in the açaí extract are not able to perform a strict control of these parameters, as is observed for usual chemical reactions derived from the Turkevich method (Perotti and Da Costa, 2021). Additionally, a thin layer of organic material was observed in the recorded images forming more densely spherical regions of AgNPs. This effect may be related to the presence of different carbohydrates in the reaction media from the açaí extract, such as pectin and starch, in addition to other biomolecules, which are present in the aqueous system, once water is removed from the system.

Figure 9 - TEM images of reaction of AgNPs obtained at pH 7.0 and açaí extract dilution of condition 2 at 25 °C.

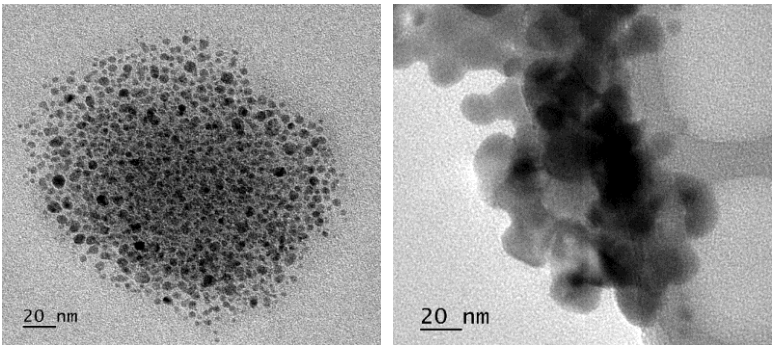
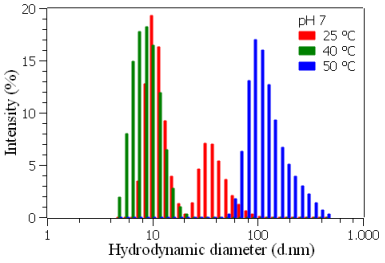


Figure 10 shows the particle size distribution data obtained by the DLS technique for condition 2 of açaí extract dilution at pH 7.0, and temperatures

of 25, 40 and 50 °C. The average hydrodynamic diameters of 21.0 ± 0.8 nm (25 °C), 9.0 ± 0.5 nm (40 °C) and 129.3 ± 5.6 nm (50 °C) were obtained. A bimodal distribution is observed for the condition at 25 °C, indicating the existence of AgNPs with different sizes. For the sample produced at 40 °C, a distribution of AgNPs with smaller average diameter was identified, indicating that with a moderate increase of temperature AgNPs of smaller sizes were formed. As for the reaction medium at 50 °C, NPs were obtained with the highest average hydrodynamic diameter.

The DLS data support the hypothesis that, by increasing the temperature, it is possible to obtain AgNPs with smaller sizes owing to the increase in the kinetic energy of the reactants in the medium, accelerating the reduction of the Ag^+ ion. Studies such as Mat Yusuf *et al.* (2020) obtained smaller AgNPs when employing higher temperatures. However, at 50 °C, the AgNPs obtained in our study showed that the increase in temperature led to a lesser control of the size of the NPs. This result might be related to the agglomeration of the NPs produced, owing to a poorer surface cover of the AgNPs by the degraded biomolecules of the açaí extract.

Figure 10 - Hydrodynamic diameter histogram of the reaction media using açaí extract dilution of condition 2 at pH 7.0 and different temperature values.



For the conditions investigated, polydispersity indices (PdI) of 0.283 ± 0.004 (25 °C), 0.452 ± 0.023 (40 °C) and 0.191 ± 0.017 (50 °C) were obtained. These results indicated the samples obtained at 25 °C and 40 °C exhibited high dispersivity of the AgNPs formed, while at 50 °C the NPs exhibited an average dispersivity (Brito *et al.*, (2020)). Therefore, the phytochemicals present in açaí extract are not able to form monodisperse AgNPs, possibly related to the slow rate of reduction of Ag^+ ions to form the NPs.

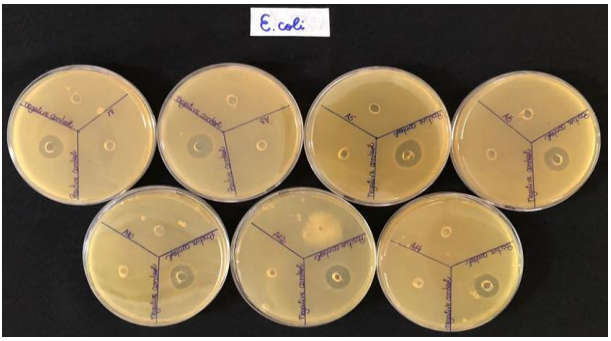
The stability of the AgNPs dispersions can be investigated by analyzing Zeta potential values, since values greater than +30 mV and lower than -30 mV are associated with more stable colloidal dispersions (Manosalva *et al.*, 2019). The following average values of Zeta potentials were obtained for

the different temperatures investigated: -25.7 ± 5.98 mV (25 °C), -29.0 ± 7.3 mV (40 °C) and -37.2 ± 5 .92 mV (50°C).

These negative Zeta potential values may refer to the adsorption of bioactive compounds covering the AgNPs (Mat Yusuf *et al.*, 2020), such as anthocyanins and phenolic compounds present in açaí extract that possess negatively charged groups (carboxylates), creating repellent forces among adjacent particles, hindering the collapse of the colloidal system (Khoshnamvand, Huo and Liu, 2019). These results indicate that an increase in the temperature might produce further oxidized biomolecules acting as capping agents of AgNPs.

It was not possible to identify inhibitory activity against any of the different microorganisms investigated for all samples of AgNPs tested. Moreover, A12 sample favored the proliferation of the microorganisms. Figure 11 shows an example of the results for *E. coli*.

Figure 11 - Evaluation of antimicrobial activity of different samples by well diffusion method against *E.coli*.

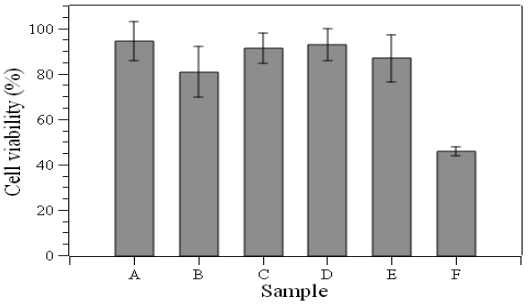


A possible explanation for the absence of bioactivity of the produced AgNPs is related to the composition of the açaí extract, mainly owing to the presence of anthocyanins (cyanidin 3-O-glycoside and cyanidin 3-O-rutinoside). As reported, these substances have a tendency to complex metals (De Souza; De Giovani, 2004) and are found in different chemical balances depending on pH in aqueous media, a competition of the chelating effect and reduction of the cationic silver might be present. Thus, the effect of complexation of silver ions can occur even with more dilute concentrations of açaí extract, reducing the ability of AgNPs to provide cellular damage through the release of Ag^+ ions to interact with the cellular environment. However, it is also important to point out that agar well diffusion test is influenced by several factors, such as the number, size and shape of the particles, electrical charge and polarity of the

substance (Girondi *et al.*, 2017). Larger particles and in higher numbers, and the use of apolar solvents for the preparation of the substances are decisive in the ability to diffuse through the agar and, therefore, can mask its antimicrobial activity (Girondi *et al.*, 2017).

Samples A, B, C, D, E, and F were submitted to the cell viability test (Figure 12), being cytotoxic only for sample F ($AgNO_3$ solution) at 1:1 (v/v) dilution for fibroblasts L929 according to the MTT assay.

Figure 12 - Cell viability of L929 fibroblasts assessed by the MTT assay at 1:1 dilution of the extract using different AgNPs dispersions and $AgNO_3$ solution samples.



Materials that promote a decrease in cell viability below 70% are defined as cytotoxic (ISO 10993-5, 2009). The other samples analyzed did not show toxicity for 1:1 or 1:2 (v/v) dilutions, indicating that the dispersions of AgNPs produced using the açaí extract are less cytotoxic than the $AgNO_3$ solution. This fact may be related to the ability of some phytochemicals present in the açaí plant extract to complex with Ag^+ ions released from the AgNPs, thus reducing the ability of this ion to cause cell damage, as corroborated by the microbiological assays carried out in this study.

CONCLUSION

It was observed that depending on the concentration of the açaí extract in the reaction medium, the rate of formation of AgNPs is different, whereas the more diluted extract conditions led to a better visualization of the SPR signal and produced more stable NPs. Regarding the pH parameter, at 7.0 there was the highest rate of NP formation compared to other media, and at this value, the SPR signals were identified at shorter wavelengths and exhibiting spheroidal shape. An increase in temperature of the reaction media negatively impacts the formation of AgNPs, leading to the degradation of

phytochemicals in the açaí extract and resulting in a smaller yield of nanostructures with larger hydrodynamic diameter. The absence of microbiological activity of the AgNPs obtained may be associated with the presence of phytochemicals present in the açaí extract that prevent the microbicidal action of silver ions through the formation of complexes. This fact was also evidenced by the higher cellular viability of AgNPs dispersions in comparison to the $AgNO_3$ solution.

ACKNOWLEDGEMENTS

The authors are grateful to CAPES (Higher Education Personnel Improvement Coordination) for the financial support to PPGCTRA/UFAM (Postgraduate Program in Science and Technology for Amazonian Resources), to CNPq (National Council for Scientific Development; grant processes no. 404810/2021-7, 313473/2019-6, 407822/2018-6, and INCT-INFO (H.S.B.)), to FAPEAM (Amazonas State Research Support Foundation; grant processes no. 062.00875/2020 and 01.02.016301.03332/2021-12 (G.F.P.), and no. 062.00876/2020 (L.P.C.)), and to FAPESP (São Paulo State Research Support Foundation; grants no. 2011/50318 (V.R.L.C.), 2019/08375-0 (F.L.B.), 2020/07110-0 (A.B.O), 2018/25512-8, 2013/07276-1, and 2017/50334-3 (H.S.B.)) for the financial support to this research. G. S. Ribeiro is grateful for the Master's scholarship granted by FAPEAM (POSGRAD program 2020/2021). H.S.B also acknowledges TA Instruments Brazil for the financial support.

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