USE OF SUPERCRITICAL TECHNOLOGY TO INCREASE THE CONCENTRATION OF ANTHOCYANINS FROM AÇAÍ (*Euterpe oleracea*) AND THEIR USE AS pH INDICATORS

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ABSTRACT

The objective of this study is to investigate the use of supercritical CO_2 extraction to increase the concentration of anthocyanins from açaí (*Euterpe oleracea*), as well as their use as pH indicators, aiming at the development of biotechnological products for food quality monitoring. Supercritical fluid extraction of lyophilized açaí pulp was performed using CO_2 as solvent, resulting in a sample of deffated açaí pulp in the extraction bed. Total anthocyanins quantification was performed in both lyophilized and deffated pulp. An anthocyanin extract was prepared using the deffated pulp, added to buffered solutions with pH values from 1.0 to 14.0. The results showed an 84 % increase in total anthocyanins in açaí pulp after supercritical fluid extraction. In addition, the anthocyanin extract of defatted açaí pulp presented a reddish color in more acidic media, orange in media close to pH 4.0, brown in values close to neutral, blue-green in media close to pH 10.0 and yellow in more basic media. The results showed that supercritical extraction using CO_2 is able to increase the anthocyanin content of açaí, and the distinguishable coloration observed throughout the pH range by the anthocyanin extract justifies its use on pH indicators for the formulation of food products.

Keywords: Açaí. Anthocyanins. Bioactive compounds. pH indicators. Supercritical technology.

1 INTRODUCTION

The Amazon basin has one of the greatest biodiversity in the world and encompasses a wide supply of fruit genetic resources, which economic exploitation is of great importance for the region[1]. Many Amazonian species have recognized concentrations of bioactive compounds, whose interest in their exploitation has intensified, aiming at the sustainable development of biotechnological products and processes. Among the Amazonian species rich in bioactives, açaí (*Euterpe oleracea*) stands out, whose fruit has pulp with a high concentration of anthocyanins, such as cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside[2].

For the recovery of bioactives from Amazonian plant species, the use of environmentally friendly and efficient technologies has been sought. In this context, supercritical technology has been gaining prominence. Supercritical fluid extraction is an innovative green separation technique, since it does not use organic solvents that can be harmful to the environment and human health. The technique also allows the obtaining of high extraction yields at relatively low temperatures, enabling the preservation of thermolabile bioactive compounds, such as anthocyanins[3,4].

Supercritical fluid extraction generally uses CO_2 in the supercritical state as a solvent for extraction, due to advantages such as low critical values of temperature (31.3º C) and pressure (73.9 bar), low cost, as it is non-toxic and spontaneously released from the plant extract, dispensing subsequent steps of solvent elimination after extraction. CO_2 is a non-polar solvent, which means that it extracts the lipophilic portion of the plant matrix, leaving a defatted pulp with a higher concentration of polar substances in the extraction cell[4]. Some studies have found that the extraction bed of supercritical extraction concentrates polar bioactive compounds, including anthocyanins, which allows defatted pulps to be better used in industry and minimizes the waste of high-value industrial waste in the environment[5].

Anthocyanins present different colors depending on the pH of the medium in which they are inserted. Generally, in an acidic condition, some of the anthocyanins appear red, while they may have a purple hue at a neutral pH and blue in increasing pH condition. Anthocyanins can present themselves as different chemical species under different pH conditions, such as flavylium cation, anhydrous quinoidal base and chalcone[6]. For this reason, anthocyanins may find application in the development of pH indicators for food products, aiming to monitoring their quality. The pH levels of food products can vary depending on factors such as flavor, consistency, and the formation of chemical reactions, which can affect shelf life, making monitoring interesting[7].

In view of the above, the objective of this study is to investigate the use of supercritical CO_2 extraction to increase the concentration of anthocyanins from açaí (*Euterpe oleracea*), as well as their use as pH indicators, aiming at the development of biotechnological products for food quality monitoring.

2 MATERIAL & METHODS

Açaí (*Euterpe oleracea*) pulp was collected in the municipality of Muaná/PA. The pulp was ground, sieved and lyophilized to remove the water. Supercritical fluid extraction was performed using the Spe-ed™ EFS extractor equipment (Applied Separations, Inc., Allentown, PA, USA) coupled to a recirculator, a compressor with and internal volume of 19.7 L, a CO_2 cylinder (99.9% purity, White Martins, Brazil) and a flow meter connected to the system outlet. The operational parameters were: 50.0 ºC, 350.0 bar, supercritical CO_2 density of approximately 900.0 Kg/m³ and CO_2 flow rate of 4.0 g/min. For the extraction, 15 min of static period and 30 min of dynamic period were used. At the end of the dynamic period, the defatted pulp was collected from the extraction bed for further analysis.

To analyze the influence of extraction on the anthocyanin content of açaí, the quantification of total anthocyanins was carried out in both deffated and lyophilized pulp, according to the differential pH method[8]. The extractions were performed with a solution of acetone:distilled water:acetic acid (70:29,5:0,5 %). The reactions were performed with 75 µL of sample and 2,925 µL of pH 1.0 buffer (0.025 M potassium chloride) and pH 4.5 buffer (0.4 M sodium acetate). The readings were performed in a UV-Vis spectrophotometer at wavelengths of 510 and 700 nm, after 15 and 60 minutes after the beginning of the reaction. A blank was prepared by replacing the sample with distilled water. The total anthocyanin content was expressed as mg of cyanidin-3-glucoside equivalent/100 g.

An anthocyanin extract was prepared from the defatted pulp, using distilled water as solvent, at a concentration of 50,000 mg/L. For this purpose, 1.25 g of defatted pulp was weighed and the volume was completed with water up to a total volume of 25 mL. The set was submitted to sonication for 20 minutes, followed by filtration. To evaluate the functionality of the extracts as pH indicators, 14 buffered solutions from acetic acid and sodium hydroxide, with pH from 1 to 14, were prepared and adjusted with the aid of a potentiometer. Then, 5 mL of each solution was transferred to test tubes and 200 µL of anthocyanin extract was added. The tubes were then vortexed and the colors were observed.

3 RESULTS & DISCUSSION

The lyophilized açaí pulp showed total anthocyanin content of 126.35 mg/100 g, while the defatted pulp obtained after supercritical extraction showed the value of 232.77 mg/100 g. The total anthocyanin content for both samples demonstrate that açaí is a rich source of this class of bioactive compounds, as previously described in the literature, with values up to 224.7 mg/100 g[9]. Supercritical extraction, in turn, proved to be efficient to increase the concentration of anthocyanins from vegetable matrices, a fact observed by the higher content of these compounds in the defatted pulp, about 84 % higher than the value found in the lyophilized pulp.

Vegetal samples with high levels of anthocyanins may encounter various biotechnological applications, making the defatted açaí pulp obtained through supercritical extraction an input of interest. Anthocyanins are compounds recognized for a series of biological properties, such as anti-inflammatory and antioxidant, and the consumption of anthocyanins alone has been encouraged, as they are closely related to the prevention or reduction of the risk of a series of diseases[10]. The development of products from anthocyanins, in turn, is also encouraged. Supercritical technology has proven to be efficient in obtaining anthocyanins and other bioactive compounds at low temperatures without the presence of toxic organic solvents, allowing the preservation of these compounds and expanding their range of applications for the food, cosmetics, and pharmaceutical industries[5,3].

Figure 1 shows the anthocyanin extract obtained from the defatted pulp of açaí in solutions with pH values from 1.0 to 14.0, in ascending order, from left to right.

Figure 1 Anthocyanin extract from defatted açaí pulp subjected to 14 different pH solutions.

Based on the total anthocyanin content of deffated pulp previously reported, about 23.3 µg of anthocyanins were added in each test tube. It was possible to observe that the anthocyanin extract of the defatted açaí pulp presented a distinguishable color in the different buffered solutions. The extract has a reddish color in more acidic media (pH 1.0), orange in media close to pH 4.0, brown in values close to neutral (pH 7.0), blue-green in media close to pH 10.0 and yellow in more basic media (pH 14.0). The change in color observed in the açaí extract under different pH media can be attributed to changes in the structure of the anthocyanins. This is due to the molecular structure of anthocyanins having an ionic nature. At pH 1.0, the flavylium cation is the predominant species and contributes to the red color. At pH values between 2.0 and 4.0, quinoidal-based species are predominant. At pH values between 4.0 and 6.0, four structural forms of anthocyanins can coexist: flavylium cation, anhydrous quinoidal base, colorless carbinol base, and pale yellow chalcone. At pH values higher than 7.0, anthocyanins are degraded depending on their substituent groups. When the pH is increased, the amount of anhydrous base also increases, which can result in blue hues. The increase in the amount of chalcones can result in more yellowish hues, as seen in pH conditions close to 14.0[6,11].

The results show that the anthocyanin content and profile of the defatted açaí pulp allows its use in pH indicators for the development of biotechnological products. Examples of biotechnological applications for anthocyanins as pH indicators include the formulation of films for visual verification of the pH of packaged foods, in the form of smart packaging[12] or their incorporation in conjunction with metal ions, such as Fe^{2+} in zein in the formulation of colorimetric indicators to monitor milk spoilage[13].

4 CONCLUSION

Supercritical extraction using CO_2 allowed an increase in the anthocyanin content in the defatted açaí pulp. In addition, the anthocyanin extract prepared from the defatted pulp showed distinguishable coloration throughout the pH range tested, justifying its use in pH indicators. The use of a clean and efficient technology in the extraction stage, combined with the positive characteristics of the extracts and defatted pulps obtained, represent a promising step towards the sustainable development of biotechnological products from the bioactive compounds of Amazonian plant matrices.

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