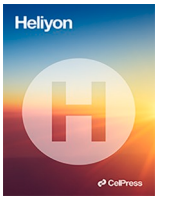
Heliyon 10 (2024) e31067

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Açai (*Euterpe oleracea* Mart.) supplementation promotes histological and ultrastructural changes in rats' alveolar bone

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ARTICLE INFO

Keywords:
Açai supplementation
Alveolar bone
Functional food
Phenolic compounds
Amazon

ABSTRACT

The açai juice contains high concentrations of phenolic compounds, including cyanidin-3-glucoside and others flavonoids. The aim of this study was to evaluate the impact of açai supplementation on healthy mandibular alveolar bone in male albino rats of the Wistar strain. 24 rats were divided into 3 groups, in which one group received a daily dose of saline solution and the other two groups were treated with daily doses of clarified açai juice for 14 or 28 days. After the experiment, hemimandibles were collected and analyzed using Scanning Electron Microscopy (SEM), histological assessments, and micro-CT. Results showed changes in the integrity of the alveolar bone as seen in SEM, increased osteocyte density and higher collagen matrix area in the açai group compared to the control group as seen in histological analysis, and increased bone volume, trabecular thickness and number, and cortical bone as seen in micro-CT analysis. The space between bone trabeculae showed no difference among the groups. These results suggest that açai supplementation may have a structural change effect on alveolar bone, but further research is needed to confirm these findings in humans and to determine the exact mechanisms behind these effects.

1. Introduction

The palm tree *Euterpe oleracea* Mart., commonly known as açai, is found primarily in the eastern part of the Amazon basin and grows in three soil types: várzea (lowlands), terra firme (highlands), and igapó (flooded soil) [1]. The distinct purple hue of the fruit is attributed to its rich anthocyanin content, a category of phenolic compounds that are part of the flavonoid group [2]. This is reflected

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https://doi.org/10.1016/j.heliyon.2024.e31067
Received 17 September 2023; Received in revised form 11 April 2024; Accepted 9 May 2024
Available online 10 May 2024

in the composition of açai pulp, which is notably abundant in these phenolic substances, conferring it a potent antioxidant activity when compared to other fruits, berries, or vegetables [3].

Phenolic compounds, derived from natural sources, are recognized for their health-promoting attributes, encompassing antioxidant, antibacterial, and antitumor capabilities. Moreover, they play a pivotal role on bone turnover through a multitude of action mechanisms. These include the regulation of bone cell populations, inhibition of matrix metalloproteinases, regulation of mesenchymal cell differentiation, and the regulation of gene expression. Such actions contribute to the preservation of tissue density, thereby providing a defense against degenerative conditions like osteoporosis and osteopenia [4].

Bone modeling initiates during the early stages of skeletal growth, influencing both the dimensions and form of bones. Concurrently, the bone remodeling cycle is instrumental in substituting aged or impaired bone tissue and in sustaining mineral equilibrium. This cycle is a meticulously orchestrated sequence, predominantly marked by the resorption of bone by osteoclasts and the subsequent formation of bone by osteoblasts. The interplay between these two cellular activities is crucial for maintaining overall bone density and integrity [5].

A multitude of external determinants, such as the aging process, gender, pathological conditions, and genetic predispositions [6] and reactive oxygen species (ROS) that play a specific role in the formation or bone remodeling. For example, free radicals produced under the control of functional osteoclasts can accelerate the destruction of calcified tissue [7].

Although clarified açai is not the same type of açai consumed by Amazonian populations, long-term and high quantity consumption of açai, as seen in the diets of these populations, may have the potential to modulate bone behavior over time, providing an important strategy for bone modulation in various conditions, such as apical periodontitis, bone defects, and surgical procedures [8].

The alveolar bone is an important structure of the mandible that supports the roots of teeth and creates the bony socket that holds teeth in place, essential for proper tooth function and support [9]. However, although there are several studies [10–12] that have investigated the benefits of açaí in experimental designs and even some clinical studies [13], none have studied the possible effects of systemic administration on healthy bone tissue, without infectious diseases or pre-existing metabolic conditions. Therefore, this study aimed to investigate the micro and macro-structural effects of açai supplementation on mandibular alveolar bone, making a unique contribution to the research on the benefits of açai.

2. Material and methods

2.1. Animals and formation of experimental groups

The research received the endorsement of the Institutional Animal Care and Use Committee at the Federal University of Pará, with the protocol number 6708270820, aligning with the principles laid out in the NIH Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines [14]. The study utilized twenty-four male albino Rattus novergicus of the Wistar strain, each weighing between 150 and 200 g and aged 70–90 days, sourced from the central animal facility of the Federal University of Pará. These subjects were then habituated to the conditions of the Institute of Biological Sciences' vivarium.

Animals were housed under a consistent 12-h light/dark cycle at a stable temperature setting of 25 ± 1 °C, with unrestricted access to water and food. Subsequent to acclimatization, the rats were randomly allocated into three distinct groups: Control [n = 8; receiving distilled water by intragastric gavage (0.01 mL/g), for 28 days]; Açai - 14 Days (n = 8; treated with a daily dose of 0.01 mL/g clarified açai, during 14 days) and Açai - 28 Days (n = 8; treated with a daily dose of 0.01 mL/g clarified açai, during 28 days). Animals in all groups were weighed weekly during the experiment for dose adjustments. The dosage was 0.01 mL/g, based on previous works demonstrating antioxidant properties and acting on pro-inflammatory cytokines [11,15].

2.2. Clarified açai juice for human consumption

The fruit juice used in this work was produced using a patented process (INPI - PI 1003060-3, August 04, 2010). Briefly, clarified açai was made from fresh fruits. After washing the fruit, 0.5 L of water per kilogram of fruits were added for pulping. The juice was then microfiltered using diatomaceous and stored at −22 °C until the experiments. After the process, açai was obtained as a thin, translucent, wine-colored liquid containing no lipids, proteins, or fibers, but rich in phenolic compounds.

The clarified açai was characterized by the composition of total phenolics and anthocyanins. Determination of total phenolics was performed using the Folin-Ciocalteu method [16]. Quantification of major flavonoids was performed using two validated UHPLC-DA methods [17]. Orientin, homoorientin, taxifolin, vitexin, isovitexin, cyanidin-3-glucoside, cyanidin-3-rutinoside (Extrasynthèse, Genay, France) were used as analytical standards.

The major phenolic compounds of açai were identified and quantified as cyanidin-3-glucoside (112.20 mg/L), cyanidin-3-rutinoside (543.30 mg/L), homoorientin (184.15 mg/L), orientin (144.81 mg/L), taxifolin deoxyhexose (13.06 mg/L), vitexin (10.57 mg/L) and isovitexin (10.18 mg/L).

2.3. Hemimandible collection

The animals were deeply anesthetized with ketamine hydrochloride (90 mg/kg) and xylazine hydrochloride (9 mg/kg) and after total loss of paw and corneal reflexes, they were perfused through the left ventricle of the heart with heparinized 0.9 % saline solution, followed by 4 % formaldehyde. Subsequently, the hemimandibles were removed from both sides, post-fixed in 4 % formaldehyde with a liquid volume at least 30 times larger than the specimen. The samples were properly prepared to assess bone characteristics with

ultrastructural analysis by Scanning Electron Microscopy (SEM), histological assessments, and micro-computed tomography (micro-CT).

2.4. Ultrastructural analyses

For the SEM (Scanning Electron Microscopy) based ultrastructural examination, hemimandibles from both the control and experimental groups were utilized. Non-alveolar bone sections were excised with the aid of an alveolotome and dental forceps. This was followed by the preparation of longitudinal sections of the alveolar bone. The specimens were then cleansed in distilled water using an ultrasonic cleaner for 1 min. To eliminate organic residues, the samples were immersed in a 1 % sodium hypochlorite solution for 5 min, followed by another round of ultrasonic cleaning in distilled water for 30 s. Each specimen was immersed for 5 min in an ascending battery of alcoholic solutions (70 %, 90 %, and absolute ethanol alcohol), subsequently drying at room temperature [18]. The prepared specimens were subsequently metalized and examined under a TESCAN Mira3 XMU (Czech Republic) Electron Microscope. Electromicrographs were captured at varying magnifications of 1200x and 2500x to scrutinize different areas within the alveolar bone.

The experimental model and the analyses performed are summarized in Fig. 1.

2.5. Histological evaluation

The other hemimandibles of each animal were collected and post-fixed for 24 h in 4 % formaldehyde. Subsequently, these samples underwent a demineralization process in a 10 % EDTA solution, sustained over a span of 90 days. The pieces were then dehydrated in alcohol, diaphanized in xylene, and embedded in paraplast. Following paraplast embedding, 5 μm thickness sections were obtained by a Leica RM 2045 microtome (Leica Microsystems, Nussloch, Germany) in the mesiodistal plane and placed on individual slides. Three histological evaluations were performed. For the first evaluation, Hematoxylin and Eosin (HE) staining was used, where he sections were first deparaffinized using xylene, hydrated in a gradient of ethanol, and then treated with hematoxylin for 3 min to stain the cell nucleus. After washing for 5 min with running water, the sections were reacted with eosin for 30 s [19]. For the second evaluation, PicroSirius Red Staining was used. The sections were deparaffinized in xylene, dehydrated in a gradient of ethanol, and then stained with 0.1 % picrosirius red for 60 min before being rinsed with hydrochloric acid for 2 min [20]. For the third evaluation, Masson's trichrome staining was performed following the protocol provided in the Polysciences Inc kit (Cat. no. 25088, Warrington, PA, USA) [21]. All sections were observed using light microscopy, and the PicroSirius Red stained samples were analyzed by the aid of a polarizer attached to the microscope. Images for the three analyses were taken with a digital color camera (Cyber Shot DSC W-230,

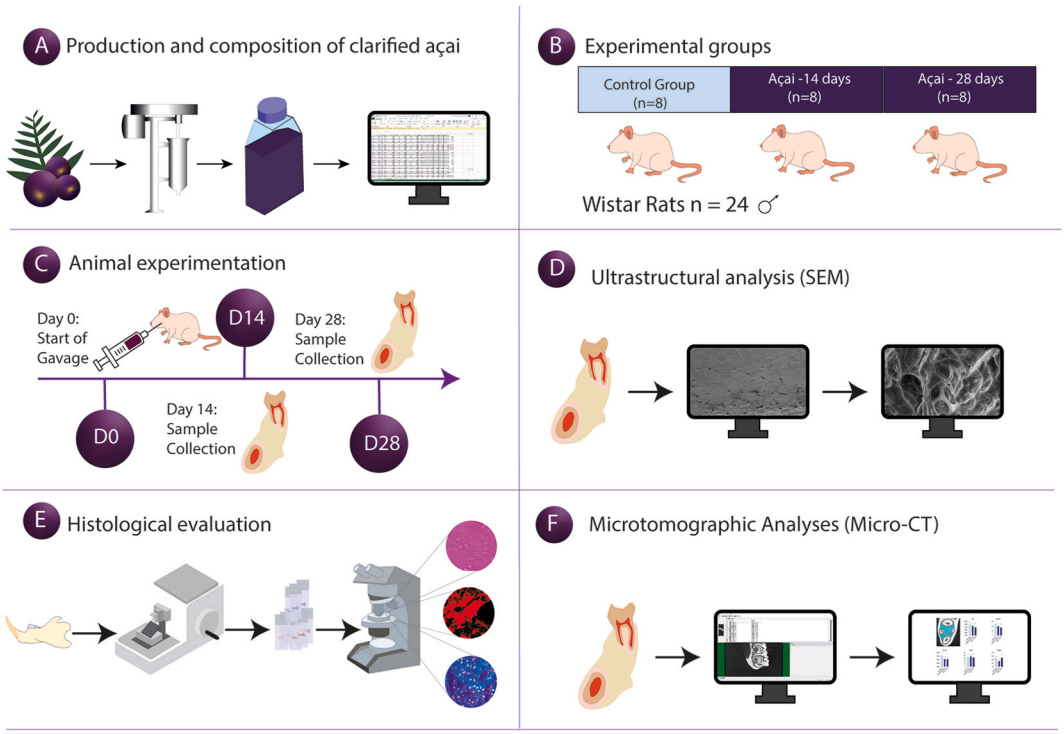

Fig. 1. Flowchart of the experiment: (A) production stage and measurement of the composition of clarified açai; (B) allocation to experimental groups; (C) experimental stages of the pre-collection of the samples on 14 and 28 days; (D) ultrastructural analysis; (E) histopathological evaluation; (F) microtomographic analysis.

optical zoom 4X, Sony, Tokyo, Japan) coupled to a microscope (1.5x, Eclipse E200, Nikon, Tokyo, Japan) at magnifications of 10X and 40X.

The histological assessments were performed according to Chemelo et al. [22]. From each section, three photomicrographs were obtained from different fields of all animals. For osteocyte density assessment, the number of osteocytes was counted under 40x magnification, and dived by the area of alveolar bone in the photomicrograph with the aid of ImageJ software, resulting in number of osteocytes/mm^2. In the case of PicroSirius Red staining, the photomicrographs were taken under polarized light, and three random fields per section were captured in 40x magnification. In order to determinate the collagen content, the collagen area was calculated using the ImageJ software after establishing the arithmetic mean of threshold percentages of the red staining from the sections. The results were expressed as area fraction in percentage.

2.6. Computed microtomography analyses

The entire imaging protocol was based on the work of Kalatzis et al., [23]. Non-demineralized hemimandibles were scanned by a cone beam micro-CT system (Skyscan 1172, Bruker, Kontich, Belgium). Each specimen was mounted on a rotating platform inside a device in a 360° rotation with an intensity of 70 kV and 100 mA. The images were reconstructed in the software InspeXio SMX-90CT (Shimadzu, Kyoto, Japan) with voxel size 10 μm in images at a resolution of 1024x1024 and 14 μm thick.

Initially, image orientation adjustment was performed using the first lower molar as a reference to obtain a standard orientation employing appropriate software (Data Viewer1, version 1.5.0, Bruker, Kontich, Belgium). In the sagittal plane, the alignment of the distal canal's cervical opening and its apical foramen's exit was performed with the X axis. For the coronal plane, the long axis of the distal root was aligned parallel to the Z axis. In the axial plane, the mesial and distal canal were aligned with the Z axis, keeping both canals parallel to it. The Y axis was not used for orientation. Regardless of orientation, the coronal, sagittal and axial planes were kept perpendicular to each other. The images from the sagittal and coronal planes were exported and analyzed in ctan software (Skyscan, Kontich, Belgium). Since in order to distinguish bone from the entire specimen, a suitable threshold is required [24], the threshold was set as the lower limit between 0 and 255 (in gray values) and the upper limit at the top end of the brightness spectrum representing the highest bone density value. The region of interest (ROI) was delimited using the custom function by selecting only the region respective to the interradicular region (coronal sections) in all images. The parameters were Bone Volume (BV), Percent Bone Volume (BV/TV), Trabecular Thickness (Tb.Th), Trabecular Number (Tb.N), Trabecular Separation (Tb.Sp), and cortical bone.

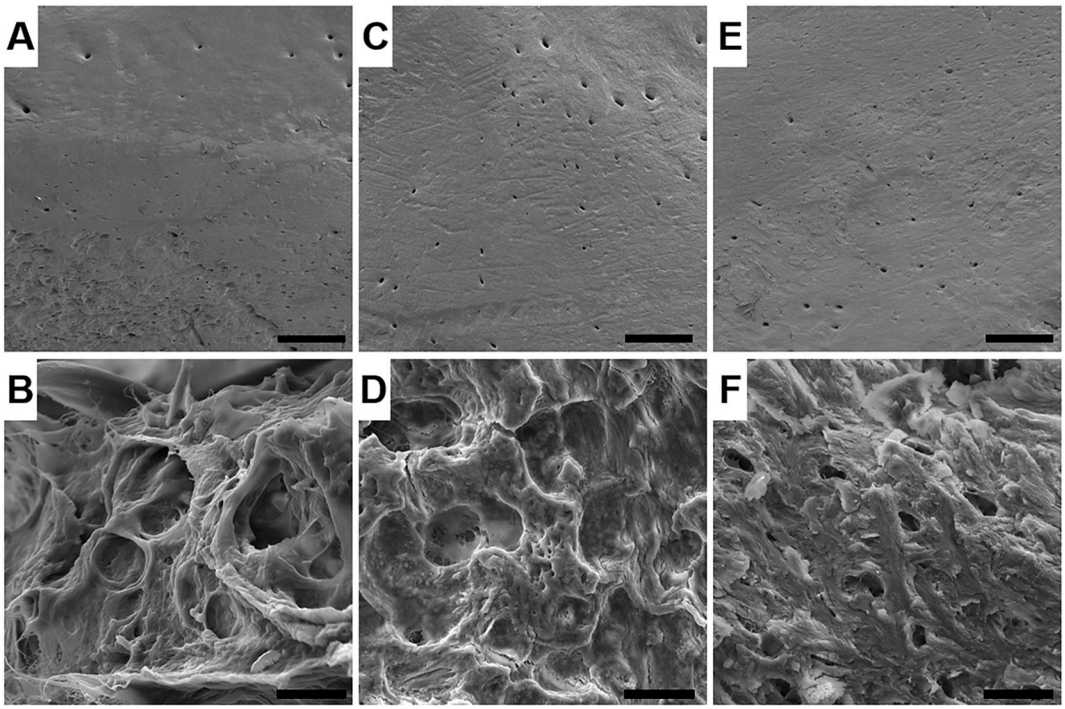

Fig. 2. Ultrastructural analysis of mandible bone: In A, C, and E, electromicrographs of ultrastructural aspects of the cortical bone of the mandibles. In B, D, and F, ultrastructural aspects of the trabecular bone of the mandibles. A and B are the control group; C and D, the Açai – 14 days group; E and F, the Açai – 28 days group. Scale Bar: A, C, and E 200 μm; Scale Bar: B, D, and F 20 μm.

2.7. Statistical analyses

The determination of the requisite sample size was conducted utilizing the GPower 3.1.9.4 [25]. Animals' numbers per group were obtained based on Pinto et al. [26] study. The normalcy of data distribution was scrutinized using the Shapiro-Wilk test. Subsequent statistical evaluations were executed employing a one-way Analysis of Variance (ANOVA), complemented by post-hoc analysis via the Tukey test. For assessments involving picrosirius red staining, the non-parametric Kruskal–Wallis test followed by Dunn's post hoc test was utilized. The results were articulated as the mean ± the standard error of the mean. A threshold for statistical significance was set at $p < 0.05$. The GraphPad Prism version 9.0 software (GraphPad, San Diego, CA, USA) was employed for statistical analyses Additionally, a descriptive analysis was undertaken to elucidate the histopathological findings.

3. Results

3.1. Ultrastructural changes in alveolar bone induced by açai supplementation

Upon SEM examination of the alveolar bone's ultrastructure, notable changes in bone integrity and an augmentation in the intertrabecular spacing were observed. Specifically, in the electron micrographs related to the alveolar bone of the açai groups (Fig. 2), we observed alterations in the integrity of the trabeculae compared to the control group. These findings suggest that the consumption of açai may have an effect on the microstructure of the alveolar bone.

3.2. Histological changes in alveolar bone morphology following açai supplementation

The histochemical assessment with Masson's trichrome (Fig. 3A–C) evidenced a similar pattern of mineralized tissue as that found in SEM, by showing a different pattern of trabecular organization of animals supplemented with açaí in comparison to the control

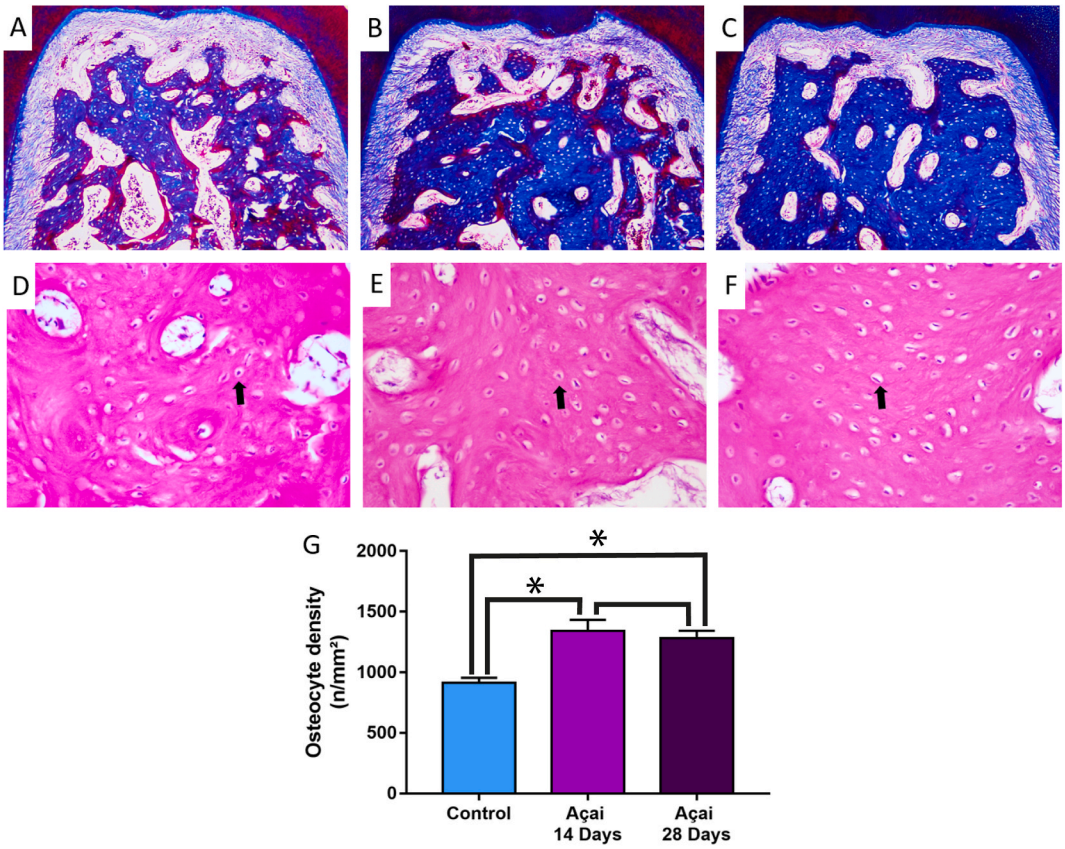

Fig. 3. Histological analysis of mandibular alveolar bone: Effects of açai (0.01 mL/g/day for 14 or 28 days) on the histological organization of rats' mandibular alveolar bone. In A, B, and C, photomicrographs representing the gross histology analysis of bone tissue by Masson's Trichrome staining from Control, Açai – 14 Days, and Açai – 28 Days groups, respectively. In D, E, and F, photomicrographs representing the osteocyte density in the alveolar bone (hematoxylin-eosin staining) from Control, Açai – 14 Days, and Açai – 28 Days groups, respectively. The arrows present in these images point to osteocytes. In G, the results of osteocyte density per mm^2 - The presence of * indicate statistical difference, One-way ANOVA followed by the Tukey test, $p < 0.05$. Scale bar: 200 μm - A, B, and C; 50 μm - D, E, and F.

group also verified in microtomographic assessment. The açaí group (Açai - 14 days and Açai - 28 days) had higher osteocyte density (1004.89 ± 83.56 and 1260.67 ± 67.38, respectively) compared to the control group (948.53 ± 26.46; Fig. 3G). Fig. 3D–F represent photomicrographs of hematoxylin-eosin staining showing osteocyte density in the alveolar bone from Control, Açai - 14 Days, and Açai - 28 Days groups, respectively. Similarly, Fig. 4A–F represent photomicrographs of PicroSirius Red staining showing the collagen fibers content in the alveolar bone from Control, Açai - 14 Days and Açai - 28 Days groups, respectively. Following the same pattern, when analyzing the pattern of collagen fibers, the açai group animals (Açai - 14 days and Açai - 28 days) had higher values for the percentage area (Control: 40.22 ± 0.35; Açai - 14 days: 43.09 ± 0.98; Açai - 28 days: 42.90 ± 0.22; $p < 0.05$, Fig. 4G).

3.3. *Effects of açai supplementation on alveolar and cortical bone quality/volume*

The micro-CT analyses revealed that açai supplementation causes changes in the microstructure of the alveolar bone. The alveolar bone showed a tissue response to açai, with an increased Bone Volume (BV) (Control: 1.67 ± 0.14; Açai - 14 days: 2.53 ± 0.05; Açai - 28 days: 2.80 ± 0.09; $p < 0.05$, Fig. 5G) and an increased bone volume/bone surface ratio (Bv/Tv) (Control: 31.08 ± 3.28; Açai - 14 days: 50.38 ± 0.58; Açai - 28 days: 50.39 ± 1.34; $p < 0.05$, Fig. 5H).

Moreover, açai was able to increase trabecular thickness (Tb.Th) (Fig. 5A–C) (Control: 0.14 ± 0.01; Açai - 14 days: 0.21 ± 0.01; Açai - 28 days: 0.22 ± 0.01; $p < 0.05$ Fig. 5I), also an increase in the trabecular number (Tb.N) when compared to the control group was observed (Control: 2.12 ± 0.07; Açai - 14 days: 2.36 ± 0.05; Açai - 28 days: 2.37 ± 0.06; $p = 0.0240$, Fig. 5J). Açai was also able to increase cortical bone (Fig. 5D–F), (Control: 5.36 ± 0.08; Açai - 14 days: 5.54 ± 0.26; Açai - 28 days: 6.36 ± 0.13; $p < 0.05$, Fig. 5L). Regarding the space between the bone trabeculae (Tb.Sp) there was no difference among the groups (Control: 0.294 ± 0.01; Açai - 14 days: 0.289 ± 0.01; Açai - 28 days: 0.305 ± 0.02; $p = 0.7300$, Fig. 5K).

In the 3D reconstruction (Fig. 6, panels A, C and E), no significant alterations were observed in the cortical bone surface. However, the reconstruction of the deeper bone exhibited similarity with the measurement results (Fig. 6 B, D and F). Animals treated with açai for 28 days presented a greater bone volume compared to those exposed for 14 days (Fig. 6, panels E and F) and also seemed to have a larger bone volume compared to the control group (Fig. 6, panels A and B).

4. Discussion

This unprecedented study investigated the effects of açai supplementation on mandibular alveolar bone. The results indicated a structural change effect after açaí supplementation on the alveolar and cortical bone in rats. Although mechanisms linked to such results need to be understood, histological analyses revealed that the Açai - 14 Days and Açai - 28 Days groups had higher osteocyte density than the control group. Following the same pattern, when analyzing the pattern of collagen fibers, the açai group animals (Açai

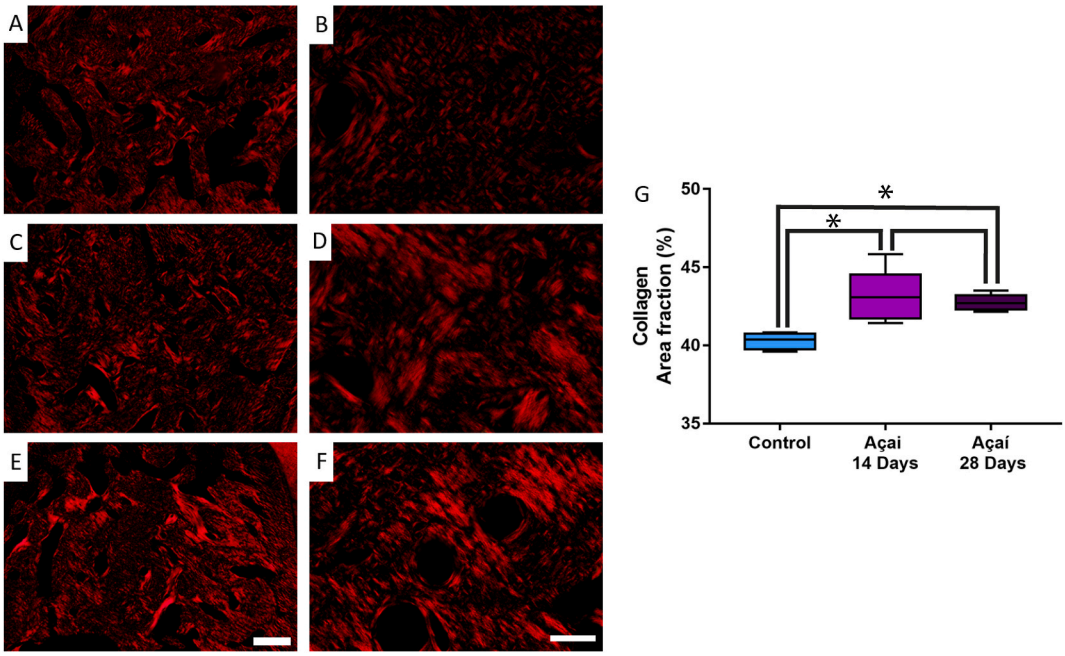

Fig. 4. PicroSirius Red Staining analysis of mandibular alveolar bone: Effects of açai (0.01 mL/g/day for 14 or 28 days) on the histological organization of rats' mandibular alveolar bone. Photomicrographs representing the collagen fibers content in the alveolar bone: in A- B, control; C-D Açai – 14 Days; E-F - Açai – 28 Days. In G, the graph of the percentage of bone collagen area analysis (%) - The presence of * indicate statistical difference, Kruskal–Wallis with Dunn's posthoc test, $p < 0.05$. Scale bar: 10 μm - A, C, and E; 5 μm – B, D, and F. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

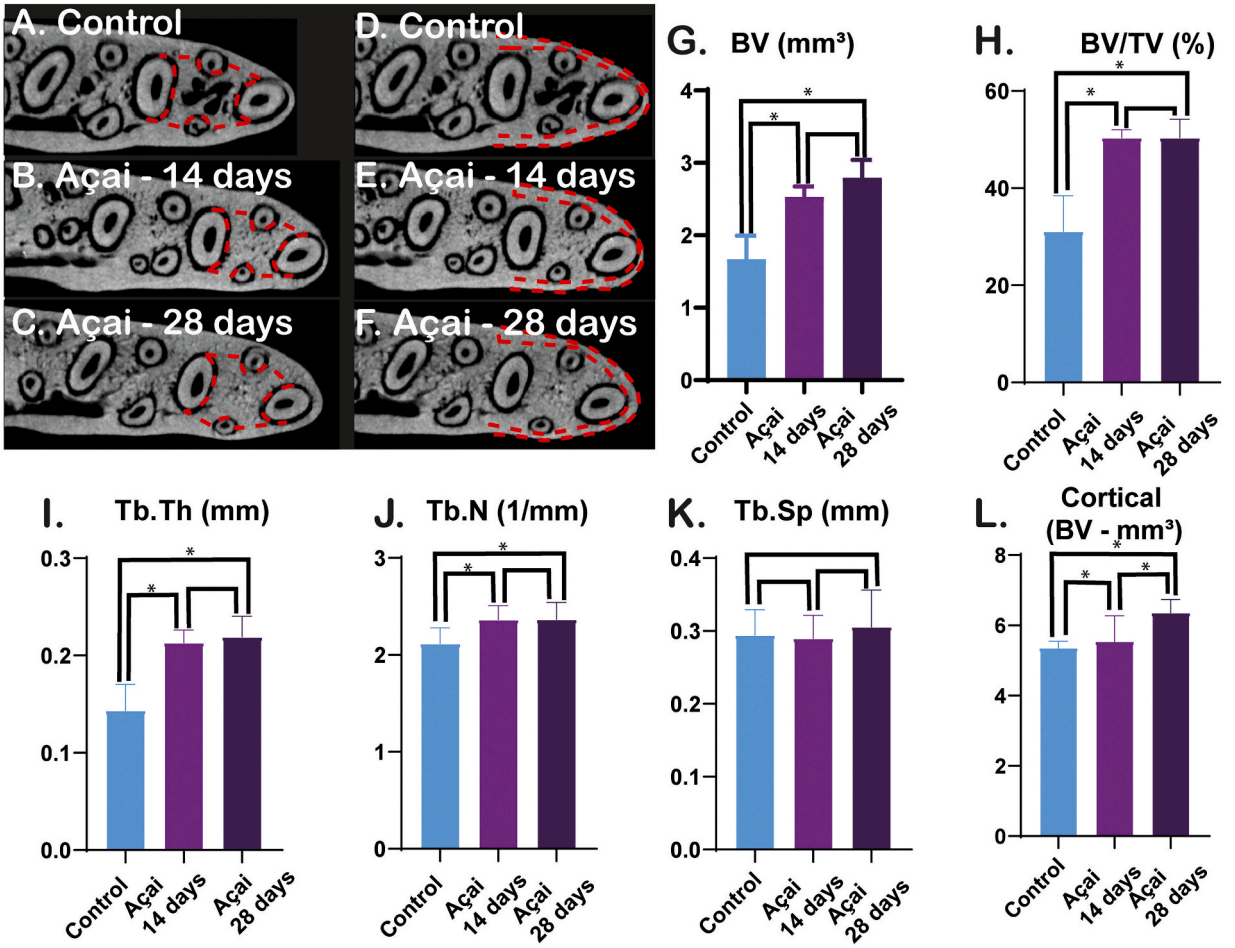

Fig. 5. Analysis of interradicular bone: Effects of açai (0.01 mL/g/day for 14 or 28 days) on interradicular bone of rats mandibles. A, B, and C are coronal slices of the animals hemimandibles with a red dashed line illustrating the region of interest for Bone Volume (BV, mm^3), Percent Bone Volume (%, BV/TV), Trabecular Thickness (mm, Tb.Th), Trabecular Number (1/mm, Tb.N), and Trabecular Separation (mm, Tb.Sp) analysis. D, E, and F are coronal slices of the animals hemimandibles with a red dashed line illustrating the region of interest for cortical bone volume (mm^3). G, H, I, J, K, and L represent the results of the bone parameters investigated of the three experimental groups expressed as mean ± standard deviation. The presence of * indicate a significant statistical difference. One-way ANOVA followed by the Tukey test, $p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

- 14 Days and Açai - 28 Days) had higher values than control group for the total area, and the percentual area. In addition, açai groups demonstrated higher values of all investigated micro-CT parameters (BV, BV/TV, Tb.th, Tb.N, and Cortical) than the control group, except by Tb.Sp that had a equivalent values in all groups. These findings suggest açai potential bone modulation action to the mandibular bone structure.

For this study, the focus was placed on the alveolar bone, identified as one of the most dynamically remodeling tissues within both the periodontal and skeletal systems [9]. The remodeling processes of the alveolar bone are subject to modulation by several factors. Local determinants include phenomena such as tooth eruption, occlusal forces, tooth extractions, and orthodontic manipulations. Concurrently, systemic influences encompass variables such as sex hormone levels and nutritional status, further underscoring the multifaceted nature of alveolar bone dynamics [9]. The interaction between bone and immune cells as modulator factors in physiological bone modeling and remodeling helps preserve mineral homeostasis [27,28]. Due to its distinct features, the alveolar bone differs from other bones and is specifically designed to support tooth activity. Made of compact bone with thick trabeculae and fewer, closer trabeculae than other bones, it indicates high bone mass and mineralization [29]. The alveolar bone also has a unique adaptive response to stimuli, a result of its high bone mass and mineralization, which distinguishes it from other bones [28]. Given the significance and unique traits of the alveolar bone, we chose to examine the impact of açai supplementation on it.

In our study, the chosen dosage of 0.01 mL/g for açaí supplementation was based on previous research that has demonstrated the efficacy of this concentration in exhibiting antioxidant properties and influencing pro-inflammatory cytokines. Notably, the work of Da Silva [30] utilized clarified açaí at the same dosage and found that chronic administration of açaí juice effectively mitigated alterations in spontaneous locomotor activity, bradykinesia, and incoordination induced by alcohol exposure across all behavioral tests. This

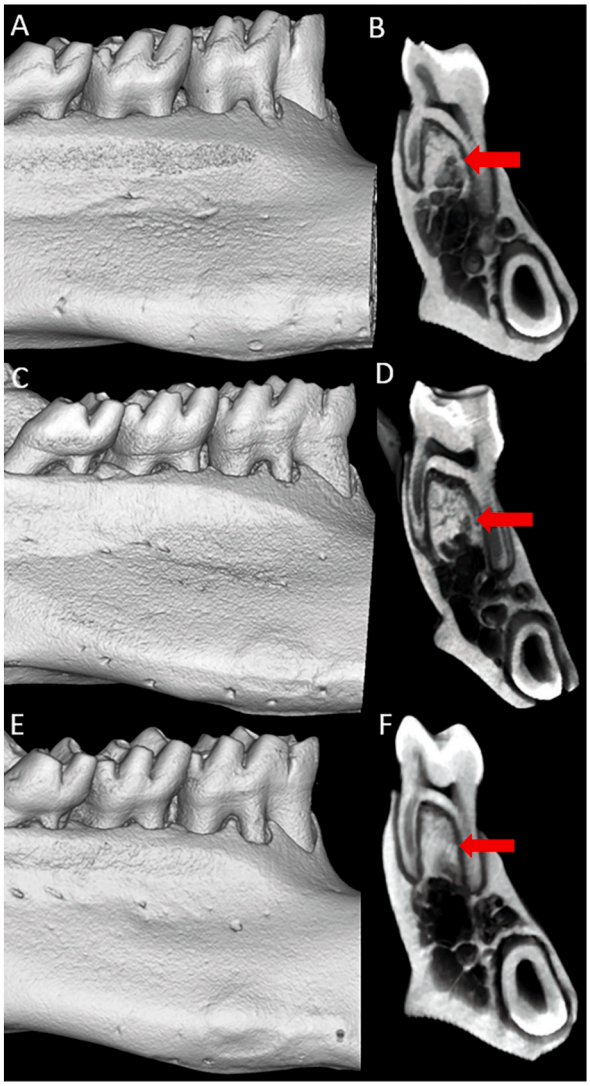

Fig. 6. 3D Reconstruction of Cortical and Deeper Bone Regions. Arrows indicate the deeper bone regions.

suggests a protective role of açaí juice against motor function impairments triggered by binge ethanol exposure in an adolescent model. This finding aligns with Souza-Monteiro's [11] results, which showed that clarified açai significantly safeguarded hippocampal cells from neuronal loss associated with depressive-like states and nitrite level surges—an indirect marker of nitric oxide production. Furthermore, clarified açai administration alone markedly elevated TERT mRNA expression, unveiling a potent anti-aging effect in the brain indicative of neuroprotection against long-term age-related deteriorations. It's worth emphasizing that previous studies have examined the toxicity of açaí, concluding that it is safe for general consumption [31].The micro-CT analyses revealed that in both times studied, açai supplementation caused a change in the microstructure of the alveolar bone. Hence, the alveolar bone showed a tissue response to açai, with an increased BV and an increased Bv/Tv. Moreover, açai was able to increase Tb.Th. Also, an increase in the Tb.N when compared to the control group was observed. Those results corroborate the results of another study of our team, that investigated the açai effects on animals with periodontal disease and that also demonstrated a modulation effect of açai in alveolar bone, even with the installed disease [8].

In addition, regarding the micro-CT results, açai was able to increase the amount of cortical bone in the Açai - 28 Days group when compared to Açai - 14 Days, which in turn was higher than the control group. This pattern was confirmed by MEV images, which demonstrated that the Açai - 28 Days group presented a more compacted cortical bone with fewer flaws. A possible explanation for this result is that açai may play a role in the modulation of alveolar bone maturity [8] which was also observed in the osteocyte density, which showed a higher number of osteocytes in the bone of animals supplemented with açai. Another possible explanation is that açai can reduce osteoclast differentiation [32], leading to lower bone resorption during the remodeling process.

The observed bone modulation effects in the açai group can be attributed to phenolic compounds, known for their antioxidant properties [33]. These compounds are recognized for their significant influence on bone health [34]. Although this study is the first to

investigate the effects of açai supplementation on alveolar bone, prior research has explored the influence of phenolic-rich foods on bones and found that the consumption of green tea enhances the structural and mechanical properties of cortical bones [35], turmeric optimizes bone hemostasis and the expression of key markers for bone metabolism [36], and polyphenol-rich beverages and resveratrol have a beneficial effect on bone modulation, even in the presence of a high-fat diet [37].

The presence and arrangement of collagen in the tissue was assessed using PicroSirius red staining method, which revealed a higher collagen matrix area in the açai groups under polarized light. This data suggests that açai may influence the bone by two ways: first, in the bone formation process, in which the osteoblasts are responsible for the formation of bone through the progressive mineralization of matrix layers primarily composed of type I collagen [38]. And second, in the resorption process, as açai fruit extract has been shown to inhibit osteoclast differentiation and activity potentially through the regulation of various cytokines produced by osteoclast precursor cells [39]. These findings indicate that açai can impact the behavior of cells, the organic matrix, and the mineral matrix of the alveolar bone.

Continuing from the previously discussed impact of açai on bone health and collagen matrix, it's worth noting that Thirupathi et al. [40] aimed to evaluate and compare the effects of treatment with gold nanoparticles reduced with Curcumin (Curcuma longa L.) or Açai (Euterpe oleracea) to a standard commercial treatment of the pharmacological type (Omcilon®) and an electrophysical agent (photobiomodulation) in the palatal wounds of rats. In this context, açai demonstrated a remarkable ability to significantly reduce pro-inflammatory cytokines while increasing anti-inflammatory ones, lower oxidant markers, and diminish inflammatory infiltrate. Moreover, the study observed an increase in the collagen area and the contraction rate of the wound, accompanied by an improved visual quality of the healing process. These results further underscore açai's multifaceted role in tissue repair and regeneration, highlighting its potential to enhance collagen synthesis and organization.

The effect of açai supplementation on bone modulation is still not well understood and requires further investigation. Brito et al. [39] have laid foundational work, suggesting the potential of açai in modulating bone formation and resorption, pivotal processes in maintaining bone integrity. Their research posits that açai's impact extends to the regulation of collagen synthesis, a vital constituent of the bone matrix essential for new bone tissue development. The significance of collagen in bone structure underscores the potential of açai in enhancing bone formation and density.

Further extending this line of inquiry, Brito et al. [32] delve deeper into the molecular interactions, indicating that açai's bioactive compounds might interact with key signaling pathways integral to osteoblast activity, the primary cells responsible for bone formation. This interaction suggests a regulatory effect of açai on osteogenesis, offering a theoretical framework for its role in bone health.

Moreover, the antioxidant and anti-inflammatory properties of açai, as highlighted by Magalhães et al. [41] and by Silveira et al. [42], suggest another dimension to its impact on bone metabolism. The reduction of oxidative stress and inflammation could mitigate the degradation of bone tissue, further supported by açai's influence on cytokine profiles which are crucial in bone remodeling processes.

This emerging evidence collectively underscores the potential of açai supplementation in bone health, advocating for its multifaceted role in promoting osteogenesis, regulating bone matrix components, and modulating inflammatory responses. These findings, while promising, necessitate further research to unravel the complex interplay of açai's bioactive compounds with the cellular and molecular frameworks underlying bone metabolism. Despite the noteworthy findings of this study, there are still some unanswered questions that need to be addressed. For example, the exact mechanism by which açai triggers these changes remains unknown. Additionally, the duration of these changes after the discontinuation of açai consumption is still uncertain. Furthermore, the effects of açai on bones with different histological structures, such as the long bones of the axial skeleton, have yet to be studied. These limitations highlight the need for further research to fully understand the relationship between açai supplementation and bone modulation.

5. Conclusions

Our findings have indicated the osteomodulatory potential of clarified açai extract, such as an increase in osteocyte density, enhancement of collagen fiber deposition, and modulation of the mineralized structure of cortical and medullary bone, which could be further utilized as a functional food in various pathological conditions affecting bone health. Despite such promising results, more studies are needed to deepen the mechanisms involved in the present results, such as biochemical and molecular pathways, however, this study with a diagnostic aim, raises important evidences and new questionings about this Amazon superfruit.

Funding statement

Herve Rogez and Rafael R. Lima researchers from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and received grant under number 313652/2019-8 and 312275/2021-8, respectively. Also, this research was funded by PROCAD Amazônia – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; 23038.005350/2018–78) and PROPESP (UFPA). João Moura and Leonardo Bittencourt received scholarships from Fundação Amazônia de Amparo a Estudos e Pesquisas.

CRediT authorship contribution statement

João Daniel Mendonça de Moura: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Patricia de Almeida Rodrigues:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Vinicius Ruan Neves Dos Santos:** Methodology, Investigation. **Leonardo Oliveira Bittencourt:** Writing – review & editing,

Visualization, Supervision, Methodology, Investigation, Formal analysis. **José Mario Matos-Sousa:** Methodology, Investigation. **Beatriz Rodrigues Risuenho Penaido:** Methodology, Investigation. **José Messias Perdigão:** Methodology, Investigation. **Herve Rogez:** Writing – review & editing, Visualization, Validation, Resources, Formal analysis, Data curation, Conceptualization. **Fabrício Mezzomo Collares:** Software, Resources, Methodology, Investigation. **Rafael Rodrigues Lima:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We are grateful to CNPq and PROCAD Amazônia – CAPES for the fellowship in developing this research.

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