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Chemical composition, characterization of anthocyanins and antioxidant potential of *Euterpe edulis* fruits: applicability on genetic dyslipidemia and hepatic steatosis in mice

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Abstract

The significance of polyphenol intake for the prevention of chronic diseases is controversial.

***Objective:* this study investigated the chemical composition and antioxidant potential of an anthocyanin-rich extract from *Euterpe edulis* fruits (LPEF) and its effects on liver steatosis in dyslipidemic apoE$^{-/-}$ knockout mice.**

***Materials and methods:* mice were divided into G1 (C57BL/6) standard diet; G2 (apoE$^{-/-}$) standard diet, G3 (apoE$^{-/-}$) 2% LPEF, G4 (apoE$^{-/-}$) 6% LPEF, G5 (apoE$^{-/-}$) 10% LPEF, G6 (apoE$^{-/-}$) 2% α-tocopherol acetate. After 75 days of treatment, the animals were euthanized. The LPEF contained a high level of monomeric anthocyanins (301.4 mg/100g) and marked antioxidant activity.**

***Results:* Catalase activity was reduced in G3, G4, G5 and G6 compared to G2. Superoxide dismutase was reduced only in G4. The animals in G4, G5, and G6 showed low HDL and triglycerides levels compared to G2. The proportion of lipid droplets in liver tissue was reduced in G4 and G5 compared to G2, G3, and G6.**

***Conclusion:* The results indicated that *E. edulis* pulp is rich in anthocyanins and the LPEF dietary consumption can reduce the severity of liver steatosis in apoE$^{-/-}$ mice, an effect that is potentially mediated by the antioxidant activity of this extract and modulation of triglyceride serum levels.**

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Key words: *Anthocyanins*. *Arecaceae*. Euterpe edulis. *Functional food*. *Liver disease*.

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COMPOSICIÓN QUÍMICA Y CARACTERIZACIÓN DE ANTOCIANINAS Y POTENCIAL ANTIOXIDANTE DE LA FRUTA *EUTERPER EDULIS*: APLICABILIDAD EN LA DISLIPIDEMIA GENÉTICA Y LA ESTEATOSIS HEPÁTICA EN RATONES

Resumen

El papel de los polifenoles en la prevención de enfermedades crónicas es controvertido.

***Objetivo:* este estudio investigó la composición química y el potencial antioxidante de un extracto del fruto de *Euterpe edulis* rico en antocianinas (LPEF) y sus efectos en la esteatosis hepática en ratones apoE-/- knockout con dislipidemia.**

***Material y métodos:* los ratones fueron divididos en los siguientes grupos; G1 (C57BL/6) con una dieta estándar; G2 (apoE$^{-/-}$) con dieta estándar; G3 G3 (apoE$^{-/-}$) con 2% de LPEF; G4 (apoE$^{-/-}$) con 6% de LPEF; G5 (apoE$^{-/-}$) con 10% de LPEF y G6 (apoE$^{-/-}$) con 2% acetato α-tocoferol (α-tocopherol acetate). Después de 75 días de tratamiento, los animales fueron eutanizados. El LPEF contenía un alto nivel de antocianinas monoméricas (301,4 mg/100 g) con notable actividad antioxidante.**

***Resultados:* la actividad catalasa fue reducida en los grupos G3, G4, G5 y G6 comparada con G2. La superoxidasa dismutasa solo se redujo en el grupo G4. Los animales de G4, G5 y G6 mostraron bajos niveles de HDL triglicéridos, comparados con G2. La proporción de lípidos en el tejido hepático fue reducida en G4 y G5, comparado con G2, G3 y G6.**

***Conclusión:* los resultados indicaron que la pulpa de *E. edulis* es rica en antocianinas, y que el consumo de LPEF en la dieta puede reducir la severidad de la esteatosis hepática en ratones apoE$^{-/-}$, un efecto que es potencialmente mediado por la actividad antioxidante de este extracto y la modulación en los niveles séricos de triglicéridos.**

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Palabras clave: *Antocianinas*. *Arecaceae*. Euterpe edulis. *Alimentacion funcional*. *Enfermedad hepática*.

Abbreviations

apoE$^{-/-}$: apolipoprotein E knockout mice.
CAT: catalase.
SOD: superoxide dismutase.
DPPH: 2,2-diphenyl-1-picrylhydrazyl.
HPLC-ESI-MS: High Performance Liquid Chromatography – electrospray tandem mass spectrometry.
LDL: low-density lipoproteins.
LPEF: lyophilized pulp of *Euterpe edulis* fruits.
TMA: total monomeric anthocyanin.
VLDL: very low-density lipoproteins.

Introduction

The species *Euterpe edulis* belongs to the Arecaceae family, the same botanical genus as the Amazonian açaí (*Euterpe oleracea*). *E. edulis* is found in the remnant areas of the Atlantic Forest of Brazil. *E. edulis* fruits have an intense purple color, mainly due to the presence of anthocyanins, which were described as the main phenol compound in this species[1]. Although of *E. edulis* fruits are consumed by various humans groups, the phytochemical composition of these fruits is poorly understood, which is a fundamental limitation in determining its pharmacological potential to be used as a functional food.

Anthocyanins belong to the flavonoids group and have antioxidant and anti-inflammatory activity, which can reduce the installation and progression of liver diseases[2,3]. Evidences indicate that anthocyanins attenuate oxidative stress by increasing the resistance of LDL to oxidation and reduce pro-inflammatory mediators such as cytokines, chemokines, cell adhesion molecules, and matrix metalloproteinases[4,5].

Clinically, dyslipidemias are identified by biochemical changes such as increased blood levels of cholesterol and its fractions[6,7]. In the liver tissue, steatosis represents a severe pathological consequence of dyslipidemias and is often associated with irreversible metabolic and structural damage and eventually hepatocytes death[8]. Due to deficient in apoprotein receptor, apoE$^{-/-}$ mice have elevated cholesterol levels and increased susceptibility to develop atherosclerosis and hepatic steatosis[9]. These animals have been used to investigate risk factors for lipid metabolism disorders and therapeutic potential of natural and synthetic chemicals on liver diseases[10].

The significance of polyphenol dietary intake for the prevention and control of chronic diseases is highly controversial, both in humans and animals, since the consumption of these phytochemicals is generally low and their metabolism not completely understood[11].

Objective

To clarify its relevance, efficacy, and possible mechanism of action, this study investigated the chemical composition and anthocyanins profile of the pulp of *E. edulis* fruits, their antioxidant potential, and the effects of the dietary consumption of this pulp on lipid metabolism and steatosis in knockout apoE$^{-/-\ mice}$.

Materials and methods

Preparation of the lyophilized pulp of E. edulis fruits (LPEF) and chemical composition

Fruits of *E. edulis* were collected in the "Zona da Mata" of Minas Gerais state, Brazil. The pulp was removed and passed through a fine-mesh screen and lyophilized, resulting in a dry extract, named LPEF. The moisture content was determined by dehydrating a 10 g sample at 105°C for 24 h and the ash content was determined after incineration in a muffle furnace at 550°C for 8 h. The extraction and quantification of total lipids was performed by a Soxhlet-type extractor after successive washes in diethyl ether. The protein amount in the LPEF was determined from the nitrogen content by the classical Kjeldahl method. Carbohydrates were quantified by subtracting the total sum of moisture, ash, lipids and proteins[12]. After incineration, aliquots of the LPEF were submitted to enzymatic hydrolysis by heat-resistant α-amylase, protease and amyloglucosidase. For the determination of insoluble fibers, samples were filtered with acetone and 95% ethanol. To determine the amount of soluble fibers, the samples were filtered with 78% ethanol and 95% acetone. For the analysis of dietary fiber, the sample was re-extracted with a Soxhlet extractor[12].

Phytochemical screaning and analysis of anthocyanins by HPLC–ESI/MS

The LPEF was submitted to phytochemical screening by thin-layer chromatography (TLC) under ultraviolet light at 254 and/or 365 nm[13]. The results obtained were compared with specific reference patterns for each phytochemical class analyzed. The total monomeric anthocyanin (TMA) content was determined by the pH-differential method[12]. The TMA content (% w/w) was calculated as follows: $TMA = A \times MW \times DF \times 100/\varepsilon$ where A is absorbance $= (A_{515} - A_{700})_{pH\ 1.0} - (A_{515} - A_{700})_{pH\ 4.5}$; where MW is the molecular weight for cyanidin 3-glucoside (449.2 g.mol^{-1}); DF is the dilution factor; and ε is the molar absorptivity of cyanidin 3-glucoside.

The HPLC-ESI-MS analysis was performed using a 1290 Infinity System (Agilent Technologies, Waldbronn, Germany) coupled with a UV/Vis detector and a triple quadrupolo mass spectrometer with electrospray ionization (ESI), G4226A auto sampler, thermostat sampler G1330B, DAD G4212A, column oven G1316C and binary pump G4220A. The separation

was performed using a reversed phase column C18 (2.1 mm × 150 mm, 5 μm) (Acclaim 120®, Dionex, USA) and a pre-column C18 at 25°C.

A concentrated extract of anthocyanin was obtained after purification of the LPEF by solid-phase extraction. 100 mg LFPE was resuspended in 10 mL 15% methanol and loaded onto a preconditioned Oasis HLB cartridge (Waters, Milford, MA, USA). An anthocyanin fraction was eluted using 60% methanol in acidic water and analysed by HPLC-ESI/MS. The mobile phase consisted of 1% aqueous formic acid (v/v) solution (phase A) and 1% formic acid in acetonitrine solution (phase B) at a flow rate of 0.2 mL/min. The major parameters were optimised as follows: 4.0 kV capillary tension; 35 psi; spraying nitrogen gas, 13 L/min; 340°C for source temperature; 135 V tension; and 200 s dwell time. Anthocyanins were monitored at 310 and 520 nm.

DPPH free-radical scavenging activity

The antioxidant activity of LPEF was determined *in vitro* by the stable organic free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) photo-colorimetric method[14]. DPPH reagent (Sigma-Aldrich®, USA) was resuspended with methanol (PA) to obtain a DPPH working solution at 0.06 mM or 60 μM. Solutions were prepared from LPEF in different concentrations (0.1 to 25.0 mg/mL). An aliquot of each solution (0.1 mL) was added to 3.9 mL DPPH solution (0.06 mM). The solutions were read in a spectrometer at 515 nm and DPPH radical scavenging activities were calculated: % DPPH radical scavenging = $((A_{control} - A_{sample})/A_{control}) \times 100$.

Animals and treatments

Sixteen, 14-week-old mice were investigated. The animals were kept in a controlled environment (temperature of 22 ± 2°C and 60–70% humidity) with a daily light/dark cycle of 12/12 h, receiving water and food *ad libitum*. The diets were prepared in accordance with the American Institute of Nutrition (AIN-93M)[15]. The experimental diets were isocaloric considering the chemical composition of the lyophilized pulp of *E. oleracea*[16] (Table I). The animals were randomized into six groups with 10 animals in each: G1 (C57BL/6), standard diet; G2 (apoE$^{-/-}$), standard diet; G3 (apoE$^{-/-}$), diet with 2% LPEF; G4 (apoE$^{-/-}$), diet with 6% LPEF; G5 (apoE$^{-/-}$), diet with 10% LPEF; G6 (apoE$^{-/-}$), diet with 2% α-tocopherol acetate. After 75 days of treatment, the animals were euthanized (xylazine, 10 mg/kg; and ketamine, 80 mg/kg, i.p.). The research protocol was approved by the Institutional Ethics Committee (CEUA/UFV) (protocol 98/2009).

Food intake, biometric and biochemical analyses

The food intake and weight of the animals were monitored weekly. Measurements were performed in serum for total cholesterol, low-density lipoprotein (HDL), non-HDL cholesterol and triglycerides using enzymatic diagnostic kits (Bioclin, Belo Horizonte, MG, Brazil). An aliquot of liver tissue (100 mg) was homogenized in ice-cold sodium phosphate buffer (pH 7.2), and centrifuged at 6,000 *g* (4°C) for 10 min and the supernatant was used for analysis of antioxidant enzymes. Catalase

Table I
Chemical composition of the diets (g/1000g of chow)

Ingredients	*Standart diet*	*LPEF 2%*	*LPEF 6%*	*LPEF 10%*	*α-tocopherol 2%*
Starch	515.7	521.3	537.4	549.2	495.7
Dextrinised starch (15.5%)	155	155	155	155	155
Saccharose* (10%)	100	91.6	73.2	56	100
Protein* (casein) (9%)	90	88.6	85.5	82.5	90
Fiber* (cellulose) (5%)	50	41.2	21.8	4	50
Mineral Mix (3.5%)	35	35	35	35	35
Vitamin mix (1%)	10	10	10	10	10
Choline Bitartrate (0.25%)	2.5	2.5	2.5	2.5	2.5
L-cystine (0.18%)	1.8	1.8	1.8	1.8	1.8
Soybean oil* (4%)	40	31.8	13.8	-	20
PLFE	-	21.2	64	104	-
α-tocopherol acetate	-	-	-	-	20
Energetic value (kcal/1000g)	3802.8	3802.8	3802.8	3802.8	3802.8

Based on Reeves et al.[15]. * Each ingredient was added in the manipulated diets considering the nutritional value of the lyophilized pulp of *E. edulis* fruits (LPEF).

(CAT) activity was evaluated according Aebi[17] by measuring the rate of H_2O_2 decomposition. Superoxide dismutase SOD activity was estimated by a xanthine oxidase method based on the production of H_2O_2 and the reduction of nitroblue tetrazolium[18].

Tissue processing and lipid droplet analysis

Fragments of the liver (median lobe) were collected and immersed in histological fixative (4% formaldehyde (w/v) in 0.1 M phosphate buffer, pH 7.2) for 48 h. The fragments were embedded in paraffin and sectioned at 5μm thickness[19]. Fifty histological fields from each group (objective lens ×40) were randomly sampled and a total of 3.65×10^4 μm^2 liver area was analyzed. Computer-based image analysis was used to determine the histological area occupied by lipid droplets (mm^2) in the liver tissue[20]. Digital images were analyzed using Image Pro-Plus 4.5 software (Media Cybernetics, Silver Springs, MD, USA).

Statistical analysis

The data were expressed as the mean ± standard deviation (mean ± SD). The biochemical data were compared using One-way ANOVA followed by the Student-Newman-Keuls *post-hoc* test. The morphological data were submitted to One Way Kruskal-Wallis ANOVA on Ranks for multiple comparisons. Statistical significance was established at $p < 0.05$.

Results

The centesimal composition of the LPEF indicated 5.71g moisture, 3.74g ash, 6.98 g proteins, 41.40g lipids, and 42.17g carbohydrates (34.90g total fibers [30.32g insoluble, and 4.58g soluble]). The LPEF showed an energy value of 569.20 kcal/100 g. The phytochemical screening revealed the presence of phenolic compounds, flavonoids and saponins. The content of total monomeric anthocyanins was 301.4mg, expressed as cyanidin 3-glucoside equivalents per 100 g dry extract. It was possible to identify six anthocyanins in LPEF. The major anthocyanins were cyanidin 3-glucoside [λ_{max} nm:520, precursor ion $[M + H]^+$ (m/z): 449, Product ions $[M+H]^+$ (m/z): 287] and cyanidin 3-rutinoside [λ_{max} nm:520, precursor ion $[M + H]^+$ (m/z): 595, Product ions $[M+H]^+$ (m/z): 287, 449]. In addition Cyanidin-3-sambubioside [λ_{max} nm:310, precursor ion $[M + H]^+$ (m/z): 581, Product ions $[M+H]^+$ (m/z): 287], Peonidin-3-rutinoside [λ_{max} nm:520, precursor ion $[M + H]^+$ (m/z): 609, Product ions $[M+H]^+$ (m/z): 301,463], Pelargonidin-3- glucoside [λ_{max} nm:520, precursor ion $[M + H]^+$ (m/z): 433, Product ions $[M+H]^+$ (m/z): 271], Delphinidin-3-glucoside [λ_{max} nm:520, precursor ion $[M + H]^+$ (m/z): 465, Product ions $[M+H]^+$ (m/z): 303]. The LPEF presented high antioxidant potential *in vitro*. The LPEF concentration required to scavenge 50% of DPPH radical (EC_{50}) was 81.70 ppm. The EC_{50} was 6.8g lyophilised/g of DPPH from the standard curve of serial dilutions of the DPPH reagent versus absorbance (data not shown).

The mean and weekly dietary intake was similar in all groups, except in G1, which showed a lower feed consumption (Fig. 1). The mean diary anthocyanin intake per animal in G3, G4, and G5 was 0.45 mg; 1.40 mg; and 2.29 mg, respectively. These values represented a diary consumption of 18.5 mg; 59.27 mg, and 107.46 mg/kg body weight, respectively. In G6, the mean diary α-tocopherol intake was 3.77 mg per animal, corresponding to 75.4 mg/kg. The initial body weight was significantly reduced in G5 compared to in G2, G3, G4, and G6. A higher weight gain was obser-

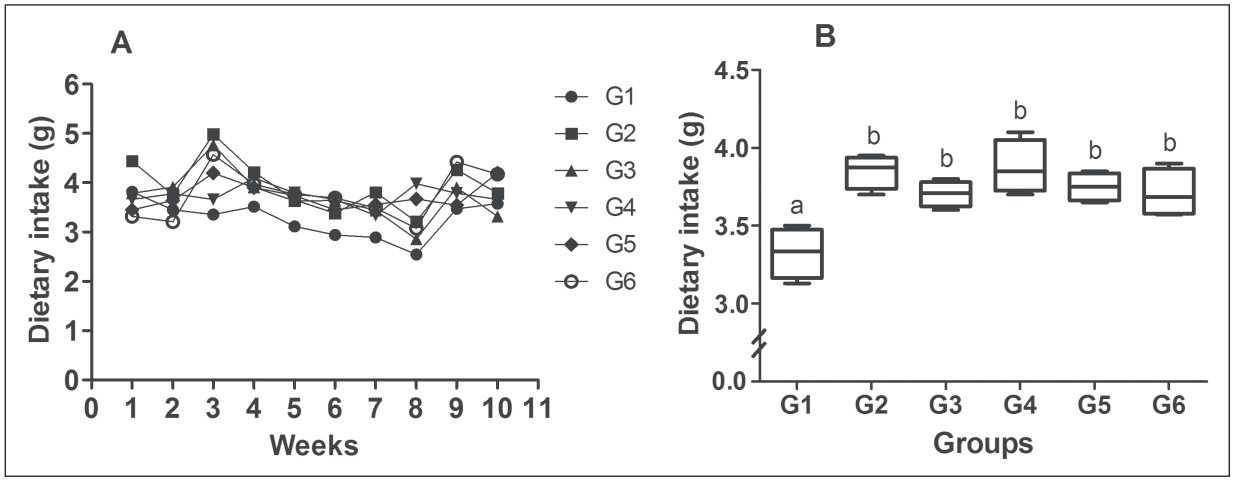

Fig. 1. Evolution of dietary consumption in Apolipoprotein E knockout mices (apoE-/-). (A) Weekly dietary consumption, (B) mean dietary consumption. G1 (C57BL/6): standard diet, G2 (apoE-/-) standard diet, G3 (apoE-/-): diet with 2% LPEF, G4 (apoE-/-): diet with 6% LPEF, G5 (apoE-/-): diet with 10% LPEF, G6 (apoE-/-): diet with 2% α-tocopherol acetate. a,b,c Different letters in columns denotes statistic difference between the groups, p <0.05.

Table II
Effect of E. edulis *lyophilized pulp (LPEF) dietary consumption on serum lipids in Apolipoprotein E knockout mices (apoE$^{-/-}$)*

Groups	*Total cholesterol (mg/dL)*	*HDL (mg/dL)*	*Non-HDL (mg/dL)*	*Triglycerides (mg/dL)*
G1	118.2 ± 13.2^{a}	68.9 ± 6.79^{a}	49.3 ± 5.7^{a}	98.0 ± 28.5^{a}
G2	457.6 ± 84.2^{b}	32.6 ± 5.4^{b}	425.0 ± 49.3^{b}	97.2 ± 20.8^{a,b}
G3	427.5 ± 42.9^{b}	29.3 ± 3.3^{b,c}	398.8 ± 33.1^{b}	55.2 ± 22.3^{b,c}
G4	399.5 ± 66.2^{b}	25.3 ± 4.2^{c,d}	374.2 ± 30.6^{b,c}	52.3 ± 12.4^{c}
G5	364.4 ± 54.3^{b}	16.4 ± 5.1^{e}	348.0 ± 24.9^{c}	41.2 ± 17.1^{c}
G6	406.2 ± 102.8^{b}	19.6 ± 6.8^{d,e}	386.6 ± 61.2^{b}	92.8 ± 35.1^{a,b}

HDL, High-density lipoprotein. G1 (C57BL/6): standard diet, G2 (apoE$^{-/-}$) standard diet, G3 (apoE$^{-/-}$): diet with 2% LPEF, G4 (apoE$^{-/-}$): diet with 6% LPEF, G5 (apoE$^{-/-}$): diet with 10% LPEF, G6 (apoE$^{-/-}$): diet with 2% α-tocopherol acetate. ^{a,b,c,d,e} Different letters in columns denotes statistic difference between the groups, p <0.05.

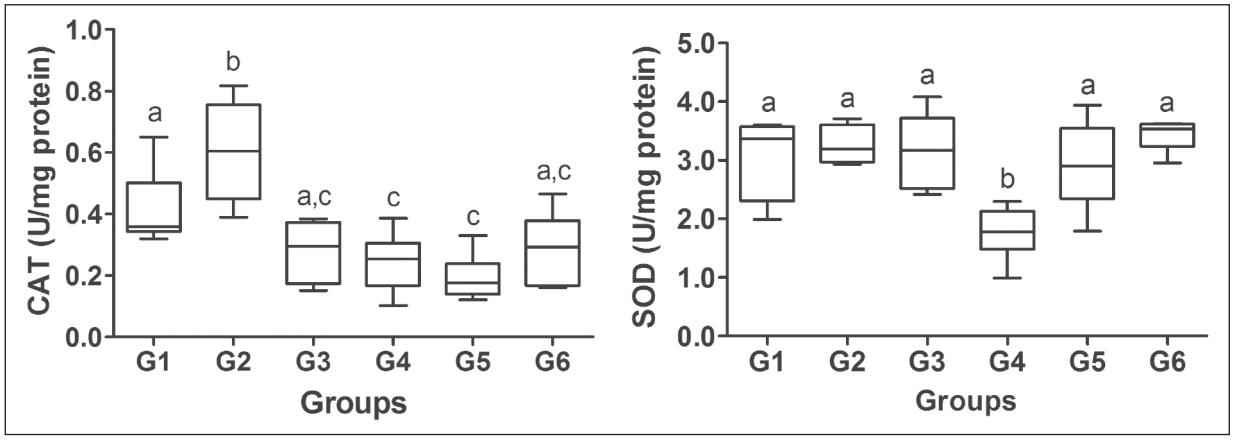

Fig. 2. Effect of E. edulis dried pulp (LPEF) dietary consumption on antioxidant enzymes in liver tissue from Apolipoprotein E knockout mices (apoE-/-). CAT, catalase; SOD, superoxide dismutase. G1 (C57BL/6): standard diet, G2 (apoE-/-) standard diet, G3 (apoE-/-): diet with 2% LPEF, G4 (apoE-/-): diet with 6% LPEF, G5 (apoE-/-): diet with 10% LPEF, G6 (apoE-/-): diet with 2% α-tocopherol acetate. a,b,c Different letters in columns denotes statistic difference between the groups, p <0.05.

ved in G5 and G3. The other groups showed a weight loss during the experimental period.

Total cholesterol and Non-HDL cholesterol levels were reduced and HDL cholesterol was higher in G1 compared to the other groups. Non-HDL cholesterol was reduced in G5 compared to G2, G3 and G6. The animals in G4, G5 and G6 showed a low HDL level compared to G2. This parameter was lower in G5 compared to the other groups, but similar to G6. Triglyceride levels were lower in G3, G4, and G5 compared to G1. The same characteristic was observed in G4 and G5 compared to G2 (Table II). The activity of CAT was higher in G2 compared to the other groups. CAT activity in G4 and G5 was similar to G3 and G6 but was higher in G1. The activity of SOD was reduced in G4 compared to the other groups (Fig. 2).

The microscopic structure of the liver tissue with macrovesicle steatosis and the computational analytical method for lipid droplet quantification. Lipid droplet accumulation was lower in G1 compared to the other groups. This reduction was also observed in G4 and G5 compared to G2, G3, and G6 (Fig. 3).

Discussion

The LPEF showed carbohydrate and protein levels close to those of *E. oleraceae* (*açaí*), which contained 44.20g and 8.13g/100g lyophilized pulp, respectively[16]. The energy value of LPEF and lipid levels were similar to those described for *E. oleraceae*, which were 489.39kcal and 40.75g[21]; or 533.90kcal and 32.50g[16], respectively. The LPEF contained high anthocyanin levels (301.4 mg/100g) and a marked antioxidant potential *in vitro*. The total anthocyanin content in LPEF was comparable to that obtained by Brito et al.[22] and Schauss et al.[16], who identified 290mg/100g and 319mg/100g lyophilized pulp of *E. oleraceae*, respectively. Borges et al.[23] found a wide variation in the anthocyanin content in *E. edulis* fruits collected in different geographic regions, ranging from 14.84 to 409.85mg/100g fresh matter. Cyanidin-3-rutinoside was the major anthocyanin in LPEF, followed by cyanidin 3-glucoside. The mean consumption of anthocyanins was variable in the groups that received LPEF, but was higher compared to the human dietary

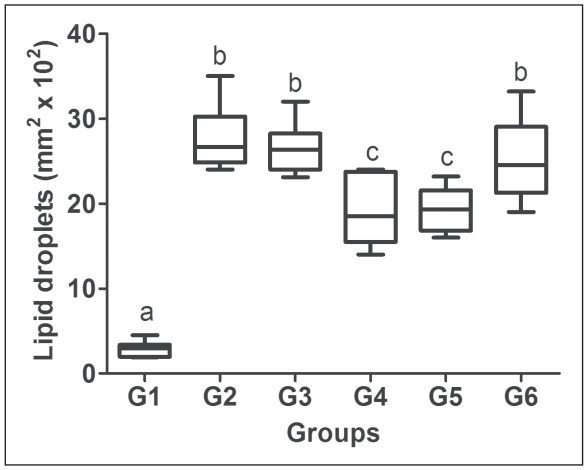

Fig. 3. Effect of E. edulis dried pulp (LPEF) dietary consumption on lipid droplets in liver tissue from Apolipoprotein E knockout mices (apoE-/-). G1 (C57BL/6): standard diet, G2 (apoE-/-) standard diet, G3 (apoE-/-): diet with 2% LPEF, G4 (apoE-/-): diet with 6% LPEF, G5 (apoE-/-): diet with 10% LPEF, G6 (apoE-/-): diet with 2% α-tocopherol acetate. a,b,c Different letters in columns denotes statistic difference between the groups, p <0.05.

intake (12.5 mg/day) based on other anthocyanin sources[24]. Hertog et al.[25] evaluated the mean daily intake of flavonoids, which was 0.33 mg/kg for a subject with 75 kg. Even with this low level of intake, the authors identified an inverse relationship between flavonoid ingestion and mortality from heart disease in humans. Flavonoids were considered initially to be substances without any benefit for humans. Later, it has been reported that they exert multiple biological effects due to their antioxidant, anti-inflamatory and free radical-scavenging abilities[26, 27].

In the present study, apoE$^{-/-}$ mice showed high total and non-HDL cholesterol serum levels, which represent a typical metabolic response of these dislipidemic animals[28]. Although the LPEF dietary consumption showed no beneficial effects on serum cholesterol, there was a remarkable effect on triglycerides, which were reduced by more than half in G5 compared to apoE$^{-/-}$ animals receiving the standard diet. It is possible that the duration of the experimental protocol might not have been sufficient to reduce cholesterol levels in apoE$^{-/-}$ mice. However, studies evaluating the effect of foods and plant extracts rich in polyphenols in these animals produced controversial results on lipid control. Xie et al.[29] showed no anti-hypercholesterolemic effect of 5% freeze-dried açai juice (*E. oleracea*) administered for 20 weeks in apoE$^{-/-}$ mice. This finding was corroborated by Miyazaki et al.[9], who investigated the consumption of purple sweet potato rich in anthocyanins in the apoE$^{-/-}$ model. However, Xia et al.[6] evaluated dietary supplementation with 300 mg/kg/day lyophilized ethanol extract from black rice rich in anthocyanins, which was effective in reducing HDL and triglyceride serum levels in apoE$^{-/-}$ mice. Similar results were observed by Peluzio et al.[28], who reported a reduction in plasma cholesterol in apoE$^{-/-}$ mice treated with *Vitis vinifera* extracts for 11 weeks. These results indicated the polyphenols cause differential biological effects, which are potentially related to the specific profile of these phytochemicals present in different plant extracts.

Similar to the results observed in G6 mice, Peluzio et al.[28] and Koga et al.[30] also found no reduction in cholesterol levels in apoE$^{-/-}$ and LDL$^{-/-}$-deficient mice and rabbits, respectively, treated with different doses of α-tocopherol. Although α-tocopherol acetate possesses recognized antioxidant activity, current evidence indicates a limited effect on cholesterol control, especially in dyslipidemic syndromes with genetic etiology[31]. It has been described that the main applicability of α-tocopherol in dyslipidemias is associated with its ability to minimize LDL oxidation and the subsequent metabolic and cardiovascular pathological events associated with this process, such as induction of oxidative stress, inflammation and atherogenesis[1].

The activity of CAT was higher in apoE$^{-/-}$ mice compared with wild type animals. This response is consistent with the pro-inflammatory and pro-oxidant status typically observed in dyslipidemias[9]. With a regular intake of antioxidants in the diet, a reduction in oxidative stress can occur without the need to increase the activity of antioxidant endogenous enzymes. In fact, animals treated with LPEF or α-tocopherol showed a marked reduction in CAT activity compared to non-treated apoE$^{-/-}$ animals. The SOD content was also lower in G4 mice compared to those in the other groups. These findings indicate that the intrinsic *in vitro* antioxidant activity of LPEF is reproduced *in vivo*. As this result was not influenced by the LPEF concentration, it is possible that even a low intake of this extract might offer benefits to the body as a functional antioxidant food. A similar effect was described by de Souza et al. [32], who observed a reduced concentration of SOD and glutathione peroxidase in serum of hypercholesterolemic mice that were fed with a diet supplemented with açaí pulp (*E. oleracea*) rich in anthocyanins. However, the consumption of phenolic compounds cannot always influence the activity of antioxidant enzymes[10]. Thus, these contradictory results indicate that endogenous antioxidant activity is influenced by multiple factors, such as the etiology of oxidative stress, the animal model used and the type and source of the dietary antioxidant investigated.

There is evidence demonstrating the antioxidant effect of the anthocyanins identified in the present study[33]. Tsuda et al.[33], reported that the administration of cyanidin-3-O-glucoside was effective in suppressing the activity of antioxidant enzymes in rats. The authors found that this anthocyanin has a high intestinal absorption and tissue distribution, where its metabolites react with reactive oxygen species and attenuate hepatic tissue damage. Furthermore, it was demonstrated that cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside, found in eleven commercial

E. oleracea pulps, showed high antioxidant activity against peroxyl, hydroxyl and peroxynitrite radicals[33]. Other anthocyanins (cyanidin-3-sambunoside, delphinidin-3-glucoside, peonidin-3-rutinoside, pelargonidin-3-glicoside and delphinidin-3-glucoside) identified in LPEF, as reported in this study, also play a role as antioxidant agents.

A remarkable effect of the LPEF was the marked reduction in lipid droplets deposition in the liver tissue in dose-dependent response. Hepatic steatosis is characterized by the intense deposition of triglycerides in hepatocytes, a typical condition observed in apoE$^{-/-}$ mice[28]. A direct correlation has been shown between fatty acids and triglyceride serum levels with hepatic steatosis[34,35]. It is possible that the attenuation of hepatic steatosis in apoE$^{-/-}$ animals might be due to a reduction in circulating triglyceride levels induced by LPEF. This disease has aroused increasing scientific interest in view of its frequent association with cardiovascular risk factors[5,34]. In hepatic steatosis, the secretion of pro-inflammatory cytokines by hepatocytes, kupffer and ito cells, induces a state of liver inflammation, in which the release of systemic pro-thrombotic factors involved in the progression of atherosclerosis occurs as well as rupture of the atherosclerotic plaques, which increases the morbidity and mortality of cardiovascular diseases [5,34].

Conclusion

Taken together, the results indicated that dietary consumption of *E. edulis* pulp can reduce liver steatosis in apoE$^{-/-}$ mice, an effect potentially mediated by the antioxidant activity and negative modulation of triglyceride serum levels. However, before the applicability of *E. edulis* pulp as a functional food can be determined, further research is needed to elucidate the mechanism by which LPEF anthocyanins act to selectively modulate lipid metabolism, and to determine the optimal level of consumption of this pulp that promotes satisfactory effects without causing damage to the organism, especially considering its high lipid content.

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Conflict of interest

The authors have declared no conflicts of interest.

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