Juçara (*Euterpe edulis* Martius) improves time-to-exhaustion cycling performance and increased reduced glutathione: A randomized, placebo-controlled, crossover, and triple-blind study

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Abstract

Purpose: To examine the effects of 7-days juçara powder intake on oxidative stress biomarkers and endurance and sprint cycling performances. **Methods:** In a randomized, placebo-controlled, crossover, and triple-blind study, 20 male trained cyclists were assigned to intake 10 g of juçara powder (JP) (240 mg anthocyanins) or placebo (PLA) for 7-days and performed a cycling time-to-exhaustion (TTE) A 5 s cycling sprint was performed before and after the cycling TTE. Blood oxidative stress biomarkers and lactate concentration where evaluated 1 hour before (T-1), immediately after (T0), and 1 hour after (T1) the cycling TTE. **Results:** The mean duration time for the cycling TTE was 8.4 ± 6.0% (63 ± 17 s) longer in the JP condition (JP: 751 ± 283 s) compared to PLA (688 ± 266 s) ($P < 0.019$). Two-way repeated measures ANOVA showed an increase in the JP condition for reduced glutathione (GSH) ($P = 0.049$) at T0 ($P = 0.039$) and T1 ($P = 0.029$) compared to PLA with a moderate effect size at T0 ($d = 0.61$) and T1 ($d = 0.57$). Blood lactate levels increased over time in both conditions ($P \leq 0.001$). No differences were observed for the post-TTE sprint fatigue index, total phenols, protein carbonyls, and glutathione peroxidase activity. **Conclusions:** Seven-day intake of JP improved cycling endurance performance and increased GSH levels but had no effect on lactate and cycling sprint-induced fatigue.

Keywords: anthocyanins; supplementation; exercise; cycling; sports nutrition.

Clinical Trial Registration: RBR-5d7tcjs - Effect of juçara fruit (*Euterpe edulis* Martius) intake in damage indicators caused by free radicals, fatigue, and performance. Available at: https://ensaiosclinicos.gov.br/rg/RBR-5d7tcjs

1 Introduction

Euterpe edulis Martius (Arecaceae) is a tropical palm tree native to the Brazilian Atlantic Forest that produces juçara fruit (Da Silva et al. 2014; Leitman et al. 2015). The nutritional properties of the juçara fruits have attracted the attention of researchers in recent years due to composition similarities with the açaí fruits (*Euterpe oleracea* Martius and *Euterpe precatoria* Maritus), mainly due to the presence of monounsaturated fatty acids and bioactive compounds, especially anthocyanins (Shulz et al. 2016; Cardoso et al. 2018; Copetti et al. 2020; De Liz et al. 2020).

Anthocyanins comprise a set of plant pigments, soluble in water that are responsible for the red, blue, and purple colors of flowers, fruits, stems, some leaves, and roots (Broullard, 1982). Previous studies (Del Bó et al. 2015; Lila et al. 2016) demonstrated that anthocyanins have a high antioxidant potential, which can alleviate the presence of reactive oxygen species (ROS) during exhaustive exercise (Suzuki et al. 2020). ROS are products of cellular metabolism and are related to physiological processes in the body (Gutteridge and Halliwell 2018) with low exposure of cells and organisms being used for redox signaling addressing specific targets (Sies et al. 2017). However, when in excess and in imbalance with the neutralization of reactive substances by antioxidant defenses, they can lead to oxidative stress, causing the oxidation of biomolecules and, consequently, leading to loss of biological functions and/or homeostatic imbalances (Sies 2018). During exhaustive exercise, ROS production may exert a negative effect on the sodium-potassium pump and calcium release by the sarcoplasmic reticulum, which may cause muscle fatigue (for a review see Powers et al. 2020). Therefore, it is likely that one of the mechanisms related to the process of muscle

fatigue during exercise is related to the production of ROS and may be influenced by anthocyanin intake (Mason et al. 2020).

Anthocyanins act on the vascular endothelium and increase the activity of the endothelial enzyme nitric oxide synthase with the production of nitric oxide, which contributes to skeletal muscle vasodilation (Speciale et al. 2014). Therefore, anthocyanins increase peripheral blood flow during exercise and potentially reduce the effects of peripheral fatigue mechanisms by lowering the presence of fatigue-causing metabolites and lactate. Consequently, a delay in the onset of blood lactate accumulation may have positive implications in prolonged high-intensity exercise and may be predictive of improved performance in endurance exercise (McNeil et al. 2015). Muscle blood flow is paramount for providing oxygen for muscle metabolism and any impediment to blood flow will anticipate peripheral fatigue (McNeil et al. 2015). Furthermore, increased peripheral blood flow may affect oxygen consumption during exercise and is beneficial for exercise performance, especially in sustained exercise over a long period of time (Kalliokoski et al. 2005), such as an exercise time-to-exhaustion (TTE) test. Studies with 7-day intake of anthocyanin-rich berries have provided beneficial effects in different exercise modalities (e.g., haskap berry: 5-km running performance (Howatson et al. 2022); blackcurrant extract: 16.1 km cycling time-trial (Cook et al. 2015). It should also be noted, however, that in addition to anthocyanins, the juçara fruit has a rich variety of active substances and antioxidant compounds (Bicudo, Ribani, and Beta 2014; Inada et al. 2015; Schulz et al. 2015; Schulz et al. 2016), which can naturally act synergistically, modulate the bioavailability of antioxidant compounds, and potentiate its effects on parameters of oxidative stress, fatigue, and exercise performance. Copetti et al. (2020) observed an increase in reduced glutathione and lower fatigue in a high-intensity exercise

session after acute consumption of juçara juice (providing 185 mg day anthocyanins). Morgan et al. (2019) and Cook et al. (2015) also observed improvement in exercise performance with finely powdered freeze-dried Montmorency cherry (providing 256.8 mg day anthocyanins) and New Zealand blackcurrant extract (providing 105 mg day anthocyanins), respectively. Effects of juçara on responses during cycling endurance and cycling sprint exercise have not been examined. We hypothesized that consumption of juçara fruit may improve oxidative stress biomarkers, fatigue, and endurance and sprint cycling performances. Therefore, the aim of the present study was to examine the effects of 7-days intake of juçara powder on oxidative stress biomarkers, cycling sprint-induced fatigue, and TTE cycling performance in male trained cyclists.

2 Methods

2.1 Ethics approval

This study was conducted in line with the principles of the Declaration of Helsinki (WMA, 1997). Procedures involving human participants were approved by the Ethics Committee on Human Research (registration no. 30907020.2.0000.0121). The trial was registered as RBR-5d7tcjs. Participants provided written informed consent.

2.2 Participants

Twenty trained (Pauw et al., 2013) male cyclists (age: 34 ± 7 years, height: 1.75 ± 0.07 m, body mass: 79 ± 13 kg, body fat: 20.1 ± 6.5%, and fat free mass: 62.9 ± 7.6 kg) participated in this study (Table 1) with the following inclusion criteria: male; between 19 and 45 years; and a history of sports practice for more than 6 months with cycling exercises of at least 6 hours per week or 200 km per week. Exclusion criteria were:

smoking, chronic diseases, metabolic disorders, physical disabilities, musculoskeletal injuries; currently supplementing with any of the following: vitamin and mineral supplements, nutritional ergogenic resources (i.e., carnitine, arginine, creatine, caffeine, nitrate, beta-alanine, and sodium bicarbonate), use of steroids in the last six months, and use of medications in the previous week since the use of these substances could influence the variables evaluated.

The calculation of sample size was performed based on the formula by Browner, Newman, and Hulley (2008) for the difference of means. The minimum sample size to detect a statistically significant difference (alpha < 0.05) was calculated based on the power of 80%. To detect a difference of 4.6% (Morgan et al., 2019) in the primary outcome measure TTE cycling performance, the sample size required was estimated to be 17 subjects, assuming a standard deviation of 2.9%. Considering a 20% for dropout, the cohort consisted of 20 participants.

2.3 Experimental Design

Participants were randomized (www.randomizer.org) by the main researcher in blocks to receive juçara powder (JP) or placebo (PLA) for 7-days, including on the day of cycling TTE testing. The JP and PLA were packaged in sachets and coded by a company. The identification codes of the interventions were revealed after the data collection and statistical analysis, characterizing the study as triple-blind (Hochman et al. 2005).

Participants visited the laboratory for three sessions (Figure 1). In the first session, the familiarization and the incremental test on the cycle ergometer were performed. On the same day, the participants received six sachets (10 g each) of JP or PLA for

consumption during 6 days. The participants were instructed to consume the interventions at a single moment of the day and in the way they preferred (i.e. mixed in water or with juices, fruits, smoothies, shakes). Participants were reminded with mobile phone text messages to adhere to the daily intake. On the test day, which was the seventh day of consumption, the JP or PLA sachets were diluted in water and consumed 1 hour before the cycling tests. The cycling tests were performed at the same morning time for each participant. Blood samples from the intermediate vein of the forearm and capillary blood from the fingertips were collected in the week before the first cycling TTE, on the day of the incremental test (before the start of JP or PLA consumption) and the days of the cycling TTE. Blood samples were collected 1 hour before, immediately after, and 1 hour after the cycling TTE to assess oxidative stress biomarkers and blood lactate concentration. After a washout period of 14-days, the treatments were reversed. On the day of delivery of the sachets of JP or PLA for consumption in the second crossover trial (after the washout period), a new collection of venous blood was performed. The flow chart of the study is shown in Figure 2.

The study was conducted at the Federal University of Santa Catarina (Brazil). Data collection took place between November and December 2021, and between June and September 2022.

2.4 Dietary standardization and physical activity

Participants were instructed to maintain their usual diet and training activities for the first 5 days of consumption of JP or PLA but to avoid strenuous exercise 48 hours before and caffeine consumption 24 hours before the tests. Furthermore, they were instructed to record their physical activity for 7 days and their food consumption 48 hours

before the cycling TTE and replicate food consumption of the 48 hours before the first test as much as possible for the crossover test (Cook et al. 2015; Morgan et al. 2019). This measure was taken to ensure that the observations made were due to the intervention alone and not influenced by other diet modifications. In order to control adherence to the interventions, the participants were instructed to bring empty sachets on the days of the experimental tests.

2.5 Characterization of JP and PLA

The product used was JP, which in 10 g contains 20 kcal, 1.54 g of carbohydrates, 0.03 g of proteins, 1.47 g of lipids, 5.61 g of total fibre, being 0.01 g of soluble fiber and 5.59 g of insoluble fiber, 630 mg of total phenols, and 240 mg of anthocyanins. The PLA was composed of maltodextrin as a carbohydrate source, whey protein as a protein source, medium-chain triglyceride as a fat source, and guar gum and microcrystalline cellulose as a source of soluble and insoluble fiber, respectively. The PLA was equivalent to JP except for the absence of total phenols and anthocyanins (Table 2). The references of the methodologies used for the characterization and microbiological analyzes of the JP and their results are in Supplementary Material.

2.6 Characterization of study participants

Weight and height were measured respectively with a digital scale (Welmy® São Paulo, Brazil) with a resolution of 100 g and a stadiometer (Altura Exata® Belo Horizonte, Minas Gerais, Brazil) with a resolution of 0.1 cm. The body mass index (BMI) was adopted as an indicator of nutritional status, according to the classifications of the World Health Organization (WHO 2006) and expressed in kg/m^2. Body composition was

190 measured by dual-energy X-ray absorptiometry (Lunar Prodigy Advance General
191 Electric-GE® Diegem, Belgium). Body fat and fat-free mass were expressed in percentage
192 and kg. Participants completed food records on two non-consecutive weekdays, and one
193 weekend day in the week before the familiarization session to establish everyone's
194 average habitual intake. They also completed two food records for the 48-h preceding
195 each intervention. Participants recorded all foods, preparations, and beverages, as well as
196 the quantities in-home measurements consumed, with the aid of a photo album of home
197 measurements (Zabotto 1996). The dietary information was standardized and transformed
198 into grams and/or milliliters of food and/or beverages with table aid for conversion of
199 home measurements (Pinheiro, Lacerda, and Benzecry 2005). The consumption of
200 calories and macronutrients was calculated according to the Brazilian Table of Food
201 Composition (Nepa 2011). Total anthocyanins intake was calculated using the Phenol
202 Explorer Database (Nevel et al. 2010). For cases of unavailable foods in the databases,
203 data from similar food or food label information were used.
204
205 *2.7 Incremental Test*
206 In the week before the first session, participants underwent an incremental test on
207 a calibrated cycle ergometer (Excalibur Sport, Lode Medical Technology®, Groningen,
208 Netherlands) to allow calculation of the workload for the cycling TTE. The positioning
209 of the athletes on the cycle ergometer was carried out based on the size measurements of
210 the bicycle itself on the first day of evaluations. After that, the cycle ergometer adjustment
211 settings were recorded in the software and kept fixed for the remaining evaluation days.
212 The incremental cycling test started with a load of 100 W for 10 min and subsequent
213 increases of 25 W/min until voluntary exhaustion or until the cadence dropped by 70

rotations per minute (RPM). The preferred cadence was controlled and kept constant, through visual feedback from the cycle ergometer (Lucía et al. 2002; Lanferdini et al. 2020). The participants received verbal encouragement during the final moments of the test to ensure that maximum effort was made. Immediately before and after the incremental test, the participant had 10 s to prepare before starting the sprints and then a 5 s cycling sprint was performed. The fatigue index was measured based on the peak power percentage reductions during the 5 s cycling sprint immediately after the incremental test in relation to the 5 s cycling sprint before the incremental test.

2.8 Time-to-Exhaustion (TTE) and cycling sprint tests

The procedures for the cycling TTE (JP or PLA) were as follows: (1) a 10-min warm-up at 100 W; (2) a 5-min interval for adjustment of the equipment; (3) the cycling TTE at 80% of maximum power obtained in the incremental test with preferred cadence controlled by visual feedback until exhaustion or inability to maintain a cadence of at least 70 RPM (Lucía et al. 2002; Coakley and Passfield 2018). The participants received verbal encouragement during the final moments of the test to ensure that maximum effort was made. Immediately before and after the cycling TTE, the participant had 10 s to prepare before starting the sprints and then a 5 s cycling sprint was performed. The fatigue index was measured based on the peak power percentage reductions during the 5 s sprint immediately after the cycling TTE in relation to the 5 s sprint before the cycling TTE.

2.9 Blood samples and biochemical analysis

Blood samples (8 mL) were collected (see Fig. 1 for time points) by puncturing the intermediate vein of the forearm with a vacuum system (Vacuntainer-BD®, São Paulo,

238 Brazil) in tubes with ethylenediamine- tetra-acetic acid (EDTA). For reduced glutathione
239 (GSH), whole blood was aliquoted into a microtube containing 100 μL of 310 mM N-
240 Ethylmaleimide per milliliter of blood. Plasma samples were obtained through
241 centrifugation (1000 g for 10 min, at 4°C). For the antioxidant enzyme glutathione
242 peroxidase (GPx), a hemolyzed blood sample was used, starting at 100 μL of cells (red
243 blood cells) with 1 mL of hemolyzed solution (4 nM $MgSO_4$ and 1 nM acetic acid). GPx
244 activity was measured by monitoring the oxidation of β-nicotinamide adenine
245 dinucleotide 2′-phosphate reduced tetra sodium salt (NADPH) in the presence of
246 hydrogen peroxide (Wendel 1981) and the results were expressed as mU/mg hemoglobin
247 (Hb). GSH was determined in the whole blood by high-performance liquid
248 chromatography (HPLC), according to the procedures described by Giustarini et al.
249 (2013), and the results were expressed as μmol/g Hb. Total phenols in plasma were
250 measured by the Folin–Ciocalteu colorimetric method (see Serafini et al. 1998), and the
251 results were expressed as mg equivalent gallic acid/liter. The plasma concentration of
252 protein carbonyls (PC) was determined by the colorimetric method, as described by
253 Levine et al. (1990), and the results were expressed as nmol/mg protein. Malondialdehyde
254 (MDA) was measured in plasma by HPLC, according to methodology by Domijan et al.
255 (2014) and Grotto et al. (2007), with modifications, and the resultsexpressed as nmol/L.
256 Hemoglobin was used to express the GSH and GPx results and was evaluated through the
257 colorimetric technique using a (UV-1800 – Shimadzu® Tokyo, Japan), automatic
258 spectrophotometer with Labtest® kit (Lagoa Santa, Minas Gerais, Brazil, Ref: 43).
259 Capillary blood samples (25 μL) for blood lactate were collected through fingertip
260 puncture using a portable lactate analyzer (Accutrend Plus® - Roche) (Basel, Canton,
261 Switzerland) and expressed in mmol/L.

2.10 Statistical analysis

Data were tested for normal distribution and homogeneity by the Shapiro–Wilk and Levene tests Mauchley's test of sphericity and data violations were present and Greenhouse-Geisser adjustments were made. Paired t-tests were conducted for analysis of TTE cycling performance, cycling sprint-induced fatigue index, and dietary variables. Oxidative stress biomarkers, lactate, and cycling sprint-induced fatigue index observations for the treatments (JP and PLA) and the time points were analyzed using two-way repeated measures analysis of variance (RM-ANOVA) with post hoc *t* and LSD tests. Post-hoc LSD pairwise comparisons were used to analyze any significance between groups. To determine the effect size (ES) of responses, Cohen's d was calculated (Cohen 1998). Cohen (1998) described an ES of <0.2 as a trivial, 0.2–0.39 as a small, 0.4–0.69 as a moderate, and >0.7 as a large magnitude of change. Statistical analyses were completed using SPSS 26.0 (SPSS, Chicago, USA) with a significant level ($P < 0.05$).

3 Results

The mean duration time for the cycling TTE was 8.4 ± 6.0% (63 ± 17 s) longer in JP group (751 ± 283 s) compared to PLA group (688 ± 266 s) ($P < 0.019$) (Figure 3). Two-way RM-ANOVA showed a significant effect in time for mean power output during sprint in both interventions ($P = 0.015$), being $P = 0.033$ for the JP group and $P = 0.013$ for PLA group. The rate of fatigue also showed a significant effect in time ($P = 0.001$), being $P = 0.002$ for the JP group and $P = 0.009$ for the PLA group. A significant effect in time for RPM ($P = 0.012$) was observed only in the JP group ($P = 0.042$). No significant differences were observed in the fatigue index at treatment ($P = 0.172$), time ($P = 0.270$), or interaction between treatment and time ($P = 0.486$) in both interventions (Table 2).

Two-way RM-ANOVA showed a significant increase in GSH (P = 0.049) at T0 (P = 0.039) and T1 (P = 0.029) for the JP group. A moderate ES was also observed in GSH at T0 (d = 0.61) and T1 (d = 0.57) in this group. For the PLA group, a significant decrease in MDA (P = 0.003) at T-1 (P = 0.038), T0 (P = 0.013), and T1 (P = 0.001) was observed compared to the JP group, with a moderate ES (d = 0.62) at T1. No significant effects of treatment or interaction between treatment and time in total phenols, PC, and GPx were observed in both groups (Table 3). However, a moderate ES (d = 0.53) was observed in GPx at T-2. Comparisons for the other variables showed trivial or small effect sizes.

Two-way RM-ANOVA showed a significant increase over time in lactate for both interventions $P < 0.001$. The differences observed were between T-1 and T1 ($P < 0.001$) and T0 and T1 ($P < 0.001$) (Table 3).

No significant differences were observed between the consumption of total energy, carbohydrates, proteins, lipids, fibers, and anthocyanins in the food records of the 48 hours prior to each intervention and between each intervention with habitual intake food records (Table 4).

4 Discussion

The main novel finding of this study was that cycling TTE performance was improved following 7-days JP supplementation (240 mg anthocyanins) in a group of trained male cyclists. Cycling TTE performance was improved by 8.4%, however, it needs to be noted that the intra-individual variability in cycling TTE trials has been shown to be high (Faude et al. 2016). Nevertheless, to minimize this variability, a familiarization test was carried out in the week before the cycling TTE, minimizing the learning effect.

Our finding of an 8.4 % increase in TTE in JP group is considerably greater comparable to other studies (Cook et al. 2015; Morgan et al. 2019). For example, Cook et al. (2015) reported enhanced 16.1-km cycling time-trial (TT) performance by 2.4% only following 7-days intake of anthocyanin-rich New Zealand blackcurrant extract (providing 105 mg day anthocyanins). In addition, according to Morgan et al. (2019) the intake of a finely powdered freeze-dried Montmorency cherry (providing 256.8 mg day anthocyanins) during 7 days improved a 15-km TT performance by 4.6%. In general, our findings also support recent observations on the potential of anthocyanin supplementation to improve exercise performance (Murphy et al. 2017; Toscano et al. 2019; Potter et al. 2020; Howatson et al. 2022). An 8.4% increase in TTE cycling performance may represent a significant practical advantage for cyclists because the performance enhancement occurred with no change in training or diet (energy, macronutrients, and anthocyanins) before the cycling TTE. However, future work needs to address the jucara performance effects in TT protocols due to the differences in reproducibility of endurance perfomance tests (Jeukendrup et al. 1996).

We also observed an increase in the levels of GSH in the hour after the cycling TTE cycling test in the JP condition. An increase in GSH was also observed in the group that consumed juçara 1 h after a HIIT session (Copetti et al. 2020). JP compounds might increase the GSH synthesis. It is known that transcription of a gene critical for GSH synthesis in cells can be stimulated by relatively low concentrations of flavonoids (Myhrstad et al., 2002) and that GSH also plays an essential role in oxidative/nitrosative stress induced by intense physical exercise. It was reported that skeletal muscle increases the uptake of GSH from plasma to counteract the excessive production of ROS that occurs after intense and prolonged physical exercise (Lew et al. 1985). Furthermore, the increase

in the activity of the enzyme γ-glutamylcysteine synthetase (γ-GCS) may be related to the exercise-induced increase in GSH in muscle fibers (Ji, 1995). Due to its water-soluble antioxidant properties, GSH metabolism plays a role in the cellular redox status in skeletal muscle (Le Moal et al. 2017), reacting with ROS through its ability to donate hydrogen, and neutralizing the potential effects of ROS by serving as a substrate for the action of the enzyme GPx (Ferreira and Reid 2008; Powers and Jackon 2008), which, in our study, was elevated after 7-days intake of JP with a moderate ES. It was also observed that in the JP condition MDA was maintained, with a significant but irrelevant decrease observed in the PLA condition. Due to the small difference in absolute values observed, such values may not be significant in impacting changes that may be associated with cyclists' performance. No differences were observed for the JP and PLA conditions in total phenols and PC, which might be related to a present high antioxidant status of cyclists due to their training status. The baseline antioxidant profile of an individual is an important determinant of the ergogenic effectiveness of an antioxidant intervention (Paschalis et al. 2018).

As expected, there was an increase in the lactate levels immediately after the cycling TTE and 5-s sprint, which returned to baseline values 1h post with no differences between the JP and PLA conditions. Our findings are in line with the studies with cherry (Clifford, Scott, and Mitchell 2013) and New Zealand blackcurrant extract (Murphy, Cook, and Willems 2017; Perkins, Vine, and Blacker 2015; Willems, Cousins, and Williams, 2016) which also did not observe lactate differences between the conditions. Although anthocyanin ingestion may favor some ergogenic effects on lactate production and/or clearance (Cook et al. 2015; Morgan et al. 2019), there is still no consensus on the most effective dose of anthocyanins to be used.

In the present study, 7-days intake of JP had no effect on fatigue during cycling TTE measured by the cycling sprint-induced fatigue index. This observation contrasts with previous studies on the effects of anthocyanin-rich fruits on fatigue (Torregrosa-García et al. 2019; Copetti et al. 2020). The fatigue index was evaluated during sprints in our study and it is known that during the sprint there is an accumulation of ROS and reactive nitrogen species (RNS), which could be involved in the development of fatigue (Morales-Alamo and Calbet 2013). However, the non-difference in the fatigue index observed herein between the interventions may be related to the absence of difference observed for most of the assessed oxidative stress biomarkers, since these are associated with the onset of fatigue (Finsterer 2012). As already mentioned, we cannot exclude the possibility that the antioxidant status is higher in trained cyclists due to the higher training status, influencing this result. Although no difference was observed in fatigue measured by the cycling sprint-induced fatigue index, it is noteworthy that other markers that were not measured in this study may have affected fatigue. Therefore, the longer TTE time in the JP condition indicates the postponement of the contribution of central and peripheral fatigue mechanisms during the TTE.

In the present study, the performance improvement, even if not accompanied by improvements of certain oxidative stress biomarkers, lactate, and cycling-sprint induced fatigue may be related to the synergistic effects of other substances and antioxidant compounds that are present in JP, such as phenolic acids, carotenoids, ascorbic acid, vitamin E, zinc, and selenium (Inada et al. 2015; Schulz et al. 2015; Schulz et al. 2016; Cardoso et al. 2018). These compounds can naturally act synergistically, modulate the bioavailability of antioxidant compounds, and potentiate their effects on cycling TTE performance.

Food records showed that the usual consumption of anthocyanins by cyclists was 225 ± 392 mg/day, a value higher than studies that also quantified the consumption of anthocyanins by the same database, in which the mean consumption ranged from 46 ± 13 (Montanari et al. 2020; Montanari et al. 2021) to 67 ± 14 mg/day (Strauss et al. 2018) and the dose of anthocyanins administered in the JP group was 240 mg. It is noted that the cyclists already had a good intake of anthocyanins habitually, with the foods that most contributed to consumption: açaí, red fruit pulp, red fruit jelly, strawberry, strawberry pulp, strawberry jelly, grape, juice grape jam, grape jam, wine, plum, blackberry, nectarine, black bean, red lettuce, red onion, and black olive.

The strengths of this study are the study design (randomized, triple-blind, and crossover), which allowed each participant to receive both treatments, minimizing possible confounding factors. In addition, this is the first study to evaluate the effects of a juçara intake in cycling TTE. The findings of this study contribute to strengthening the few published scientific evidence about the effects of juçara fruit on exercise (Copetti et al. 2020; Mendes et al. 2021). On the other hand, some factors may have interfered in the evaluation of the results of this study, such as eventual oblivion to the consumption of JP group and PLA group. However, participants were instructed to bring the empty sachets on the days of the experimental tests as a way of proving adherence to consumption and reminded via text message on their cell phones to consume the interventions daily.

5 Conclusions

Seven-day intake of JP powder improved cycling TTE performance and increased the blood levels of GSH, but had no effect on lactate and cycling sprint-induced fatigue after cycling TTE. Unlike many fruits rich in anthocyanins, the juçara fruit is a complex

matrix, comparable to açaí, due to the presence of monounsaturated fatty acids and bioactive compounds, which can enhance its effects on different outcomes in the context of exercise. It is also considered important to popularize the juçara fruit among the population, stimulating its production, promotion, and appreciation, contributing to environmental sustainability and also to the economic sector of the regions where it is cultivated, since the *Euterpe edulis* Martius palm tree, which originates the juçara fruit is in risk of extinction. Further studies are suggested with different juçara dosing strategies, focusing on clarifying the ideal dose, frequency, and duration of ingestion to verify possible effects on variables that were not affected by JP consumption in this study and that provide optimum benefits in other exercise protocols, enhancing the findings in the field of sport and exercise nutrition.

Author statements

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

CLKC: conception and design of the study; collection, analysis, and interpretation of the data; and writing the manuscript; FD: data collection; analysis, interpretation of the data; and critical revision of the manuscript; FJL: data collection; analysis, interpretation of the data; and critical revision of the manuscript; BFS and BSM: biochemical analysis; and critical revision of the manuscript; ELS; supervision of the biochemical analysis; analysis and interpretation of the data; and critical revision of the manuscript; FGKV and METW: critical revision of the manuscript; and PFDP: conception and design of the study; analysis and interpretation of the data; and critical revision of the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Data availability

Available upon request.

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704 **Table 1 –** Participants characteristics (*n*=20).

Variables	Mean ± SD
Maximum power in the Incremental Test (W)	348 ± 51
Power 80% in the Incremental Test (W)	276 ± 40
Initial Sprint/PPO in the Incremental Test (W)	2182 ± 305
Final Sprint/PPO in the Incremental Test (W)	1981 ± 377
Maximum Heart Rate in the Incremental Test (beats/min)	187 ± 8
Incremental pre-test lactate (mmol/L)	1.7 ± 0.9
Incremental post-test lactate (mmol/L)	10.4 ± 4.9
Habitual intake (per day)	
Total energy (kcal)	2216 ± 712
Carbohydrates (g)	258 ± 91
Proteins (g)	110 ± 35.05
Lipids (g)	80 ± 34
Fibers (g)	22 ± 8
Anthocyanins (mg)	225 ± 392

705 PPO: Power Peak Output; SD: standard deviation.

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717 **Table 2** - Data during time-to-exhaustion (TTE) cycling at 80% of maximal power and 5
718 s cycling sprints.

Variables	Juçara Powder	Placebo
Sprint pre TTE - PPO (W)	2450 ± 281	2368 ± 352
Sprint pre TTE - Mean Power (W)	1372 ± 191	1362 ± 211
Initial Rate of Fatigue (%)	71 ± 6	70 ± 8
Sprint post TTE - PPO (W)	2181 ± 332	2339 ± 314
Sprint post TTE - Mean (W)	1266 ± 251	1271 ± 218
Final Rate of Fatigue (%)	79 ± 10	77 ± 11
RPM (TTE)	88 ± 5	89 ± 5
Mean Heart Rate (TTE) (beats/min)	170 ± 8	170 ± 7

719 Data reported as mean ± SD. PPO: Power Peak Output; RPM: rotations per minute; SD:
720 standard deviation.

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722 **Table 3 –** Concentration of oxidative stress biomarkers and lactate before the incremental test or after washout period (-2); 1 h before cycling
723 TTE (-1); immediately after cycling TTE (0); and 1 h after cycling TTE (1) with the interventions (JP and PLA) in 20 trained cyclists (continue).

Parameters	**Time**	**JP**	**PLA**	***P* value[a]**	***P* value[b]**	***P* value[c]**
Glutathione Peroxidase (mU/mg Hb)	-2	16.3 ± 9.4	12.0 ± 6.8	0.448	0.376	0.683
	-1	16.5 ± 17.7	14.1 ± 7.9			
	0	15.5 ± 9.7	15.2 ± 9.3			
	1	12.3 ± 6.5	13.1 ± 7.3			
Reduced Glutathione (µmol/g Hb)	-2	5.4 ± 1.2	5.5 ± 1.4	0.049*	0.058	0.190
	-1	5.2 ± 1.2	4.8 ± 1.3			
	0	5.9 ± 1.3	5.0 ± 1.5			
	1	5.9 ± 1.5	5.2 ± 0.8			
Total phenols (mg equivalent gallic acid/liter)	-2	101.4 ± 10.8	104.5 ± 27.9	0.809	0.435	0.679
	-1	104.5 ± 16.7	99.8 ± 21.7			
	0	98.9 ± 10.3	97.8 ± 21.4			
	1	102.9 ± 15.9	101.4 ± 21.0			
Protein Carbonyls (nmol/mg protein)	-2	2.1 ± 0.6	1.9 ± 0.5	0.948	0.307	0.347
	-1	2.0 ± 0.4	1.9 ± 0.7			
	0	2.0 ± 0.6	2.2 ± 0.8			
	1	1.9 ± 0.6	2.0 ± 0.6			

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Table 3 – Cont. Concentration of oxidative stress biomarkers and lactate before the incremental test or after washout period (-2); 1 h before cycling TTE (-1); immediately after cycling TTE (0); and 1 h after cycling TTE (1) with the interventions (JP and PLA) in 20 trained cyclists.

Parameters	**Time**	**JP**	**PLA**	***P* value[a]**	***P* value[b]**	***P* value[c]**
Malondialdehyde (nmol/L)	-2	0.36 ± 0.14	0.34 ± 0.16	0.003*	0.177	0.134
	-1	0.38 ± 0.13	0.34 ± 0.16			
	0	0.38 ± 0.16	0.31 ± 0.12			
	1	0.37 ± 0.16	0.28 ± 0.13			
Lactate (mmol/L)	-1	2.0 ± 0.9	1.9 ± 0.8	0.661	< 0.001*	0.048*
	0	8.8 ± 3.9	9.7 ± 3.7			
	1	2.6 ± 1.7	2.1 ± 0.8			

Values are reported as mean ± SD.

Time -2: Before incremental test or after washout period; Time -1: 1 h before cycling TTE; Time 0: immediately after cycling TTE; Time 1: 1 h after cycling TTE.

JP: juçara powder; PLA: placebo; GPx: Glutathione Peroxidase; GSH: Reduced Glutathione; PC: Protein Carbonyls; MDA: malondialdehyde.

RM-ANOVA: [a]Treatment; [b]Time; [c]Treatment and time interaction. *Significant values.

734 **Table 4 –** Dietary intake in the 48h preceding trials.

	Juçara Powder	**Placebo**	***P value***
Total energy (kcal)	2301 ± 789	2106 ± 759.26	0.30
Carbohydrates (g)	268 ± 107	265 ± 124	0.91
Proteins (g)	112 ± 39	99 ± 40	0.91
Lipids (g)	88 ± 35	74 ± 24	0.05
Total fiber (g)	24 ± 10	21 ± 10	0.20
Anthocyanins (mg)	125 ± 327	29 ± 46	0.16

735 Values are reported as mean ± SD.

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Figure captions

Figure 1. Experimental design. The study was divided into three sessions. **Session 1:** In the first session (week before the tests) samples of venous and capillary blood were collected for evaluation of oxidative stress biomarkers and blood lactate, respectively. Then, the familiarization and the incremental test on the cycle ergometer were performed. Soon after, capillary blood samples were collected again to assess blood lactate. On the same day, the participants were randomized to receive the sachets with the juçara powder or placebo for consumption during six days. **Session 2:** On the test day, which was the seventh day of consumption, the juçara powder or placebo sachet was offered by the researcher 1 hour before (T-1) the cycling time-trial to exhaustion (TTE). Venous and capillary blood samples were collected 1 hour before (T-1) (before ingestion of interventions), immediately after (T0) and 1 hour after (T1) the cycling TTE to assess oxidative stress biomarkers and blood lactate concentration, respectively. The 5-s sprints ocurred before and after the cycling TTE. After an interval of 14 days (washout period), the blood samples were collected again and the treatments were reversed **(Session 3).**

 Venous blood

 capillary blood

 sprint 5-s

 incremental test/ cycling time-to-exhaustion

 juçara powder or placebo

Figure 2. Flowchart of the study.

786 **Figure 3.** Cycling time-to-exhaustion (TTE) at 80% of maximal power. Columns show
787 group mean and individual values. Cycling TTE improved after JP ($P < 0.019$). JP =
788 juçara powder; PLA = placebo. *Significant intergroup difference.