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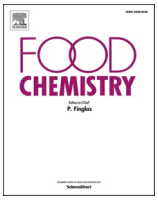

Phenolic composition, antioxidant capacity and digestive enzymes inhibition of Butiá (*Butia catarinensis*, *Butia eriospatha*, and *Butia odorata*) under simulated digestion

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ABSTRACT

Butia is a South American palm genus known for its fruit production. For the first time, the effect of *in vitro* digestion on the phenolic profile, antioxidant activity, and inhibition of digestive enzymes was evaluated in fruits of *Butia catarinensis*, *B. eriospatha* and *B. odorata*. Four phenolic acid derivatives, two catechin derivatives, 3,4-dihydroxybenzoic acid, rutin, and tyrosol were identified by HPLC-DAD in six *Butia* populations. All samples showed antioxidant and enzyme inhibitory activities, with a decrease observed after intestinal digestion. The samples from Encruzilhada do Sul, Torres and Imbituba showed the highest nitric oxide radical scavenging activity, while the Laguna sample stood out against the hydroxyl radical. The Ponte Alta do Norte sample showed the best ratio between α-glucosidase and α-amylase inhibition. Overall, the phenolic compounds exhibited good stability, and in some cases, their concentrations increased, indicating that digestion process efficiently released them from the food matrix.

1. Introduction

In the South of Brazil unique ecosystems can be found, where hundreds to thousands of palms of the *Butia* genus occur. The palms of this genus are known to produce fruit, which are incorporated in local food traditions (Büttow et al., 2009). The gregarious populations of *Butia* are called *Butia* palm groves, or "butiazais", where several animal and vegetal species coexist (Werner-Martins & Freitas, 2023). These aggregations form ecosystems, usually occurring in the grassland vegetation type, with abundant grasses and shrub species, where *Butia* palms predominate in the upper layer of the landscape (Rivas et al., 2023). These ecosystems, however, have not yet been fully recognized by current legislation. As a result, they face the threat of disappearance due to a combination of factors, primarily the reduction and fragmentation of *Butia* palm groves, lack of natural regeneration, and conversion to agricultural and silvicultural monocultures (Sosinski et al., 2019).

Among *Butia* species, *Butia odorata* (Barb. Rodr.) Noblick, *Butia eriospatha* (Mart. ex Drude) Becc and *Butia catarinensis* Noblick & Lorenzi can be found forming extensive populations in Southern Brazil. *Butia catarinensis*, also called "butiá-da-praia" (which means "butiá from the beach"), is an endemic species of the Restinga, which occurs in coastal and sandy areas in the South of Santa Catarina, and North of Rio Grande do Sul states, Brazil (Werner-Martins & Freitas, 2023). The "butiá-da-serra" (or "mountain butiá"), *B. eriospatha*, occurs in high altitude fields

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in the states of Paraná, Santa Catarina and Rio Grande do Sul (Ribeiro et al., 2019). Meanwhile, *B. odorata* is a species that occurs in Southern Brazil and Eastern Uruguay (Eslabão et al., 2016).

Butia fruits are known for their abundance of phenolic compounds (Hoffmann et al., 2018). These compounds have been extensively investigated due to their associations with diverse biological activities, such as antioxidant properties and inhibitory effects on digestive enzymes (Shahidi & Naczk, 2004). Antioxidant capacity is a desirable property to prevent harm caused by oxidative stress and its resulting diseases, such as cancer, neurodegenerative diseases and cardiovascular

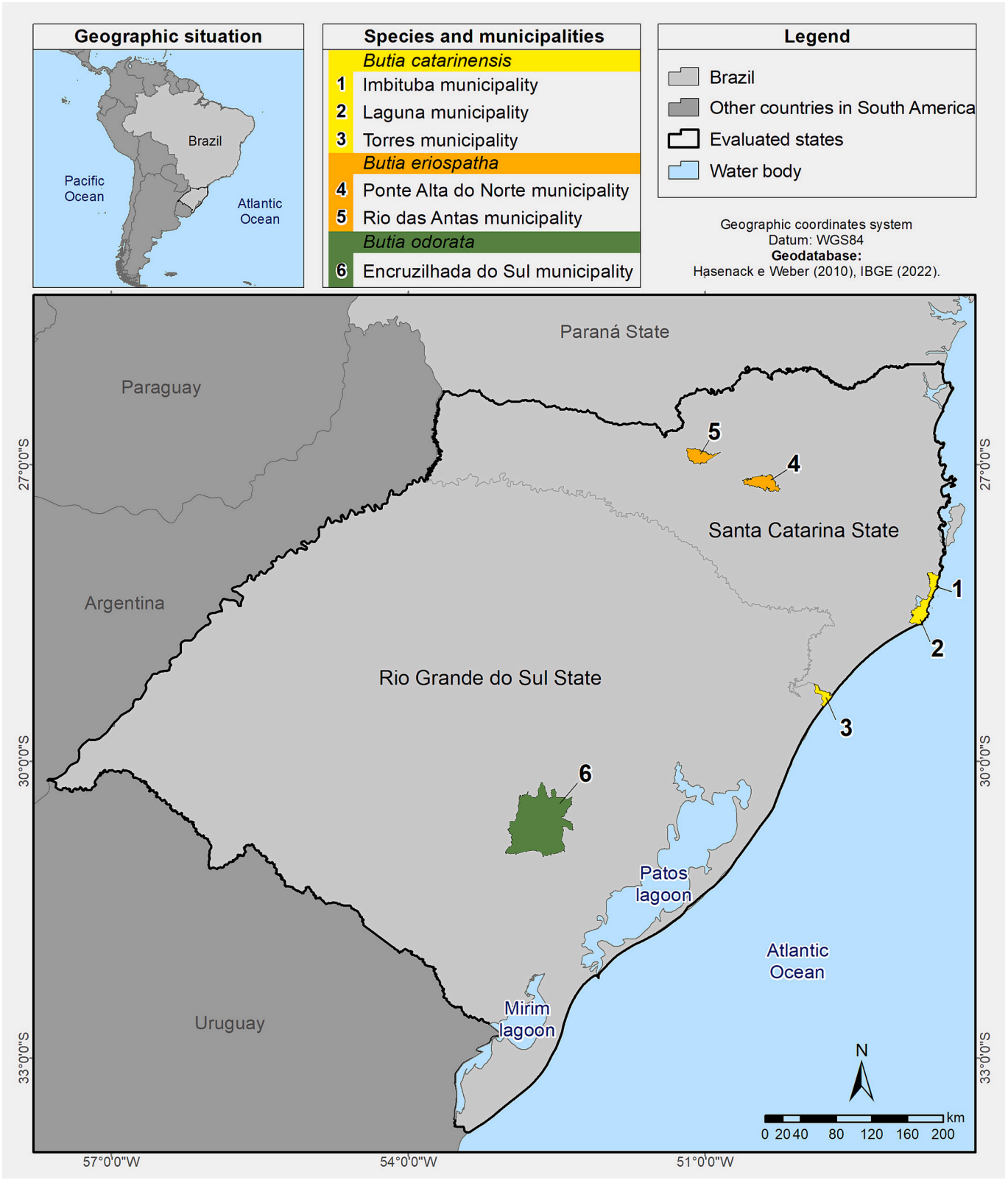

Fig. 1. Collection places for the *Butia* samples (*Butia catarinensis*, *Butia eriospatha* and *Butia odorata*).

disorders (Bhuyan & Basu, 2017). The enzymes α-amylase and α-glucosidase are involved in the digestion and processing of carbohydrates. The inhibition of these enzymes slows carbohydrates absorption, offering a potential strategy for managing type 2 diabetes. In this metabolic disorder, rapid carbohydrate absorption leads to postprandial glycemic spikes (Papoutsis et al., 2021).

Despite the considerable phenolic content observed in *B. catarinensis* (Hoffmann, Carvalho, et al., 2017; Rockett et al., 2020; Rodrigues et al., 2022), *B. odorata* (Vinholes et al., 2017; Hoffmann, Carvalho, et al., 2017; Ma et al., 2019; Boeing et al., 2020), and *B. eriospatha* (Denardin et al., 2015; Rockett et al., 2020), its bioactivity is contingent upon its bioaccesibility. Bioaccessibility is defined as the percentage of the compound that becomes available for absorption after gastrointestinal digestion. To date, no study has been conducted to evaluate the effect of the digestive process on the phenolic profile of *Butia*. The effects of *Butia* extracts on digestive enzyme inhibition have been scarcely investigated (Vinholes et al., 2017, 2018). Likewise, the effect of *in vitro* digestion on enzymatic inhibition and antioxidant capacity has only been assessed in *B. odorata* (Vinholes et al., 2018). Given the dearth of existing information, this study aimed to evaluate the phenolic profile, antioxidant and enzymatic inhibition of fruit extracts from populations of *Butia odorata*, *Butia eriospatha*, and *Butia catarinensis*, under the influence of *in vitro* digestion, hypothesizing that the differences in the phytochemical composition will reflect in contrasting bioaccessibility in the samples, resulting in differences in the nutraceutical potential.

2. Material and methods

2.1. Sample collection and extract preparation

In March 2022 six natural populations of three different *Butia* species (Fig. 1) were accessed in Brazilian states of Rio Grande do Sul and Santa Catarina. From each population, three bunches of *Butia* were collected at the "green-yellow" ripeness stage (25 and 75 % of greenish coloration; Amarante & Meguer, 2008). Bunches were stored at 18 °C until fruit abscission, after which fruits were separated from the rachises and extracts were prepared.

The voucher specimens from *Butia odorata* and *Butia eriospatha* populations were deposited at the Embrapa Clima Temperado Herbarium (ETC), and those of *Butia catarinensis* in the Herbarium of Universidade Federal de Santa Catarina/Curitibanos (CTBS) (Table 1).

2.2. Simulation of biological digestion

2.2.1. Sample preparation

A blend was made with fruits from each population, the bulk was freeze-dried for five days, the endocarps were removed, and then the samples were ground in a domestic mixer (Oster, FPSTHB2610R-057) until a homogeneous powder was obtained. Extracts were prepared using 5 g of a freeze-dried sample using 25 mL of ethanol: water 70:30 v/v as solvent. The mixture was vortexed (Phoenix, AP56), centrifuged (Eppendorf Centrifuge 5810 R) for 10 min at 4000 rpm (3220 x g), and the supernatant collected. The pooled supernatant was rotary evaporated to remove ethanol and resuspended in water to 100 mL immediately before analysis.

An aliquot of 600 μL containing 30 mg of *Butia* dried extract (dw) was reserved for analyses. The remaining extract underwent *in vitro* digestion as described by Gião et al. (2012). After each phase of digestion (oral, gastric, intestinal), aliquots were collected for HPLC-DAD analyses of phenolic compounds and evaluation of enzyme inhibition and antioxidant capacity.

2.2.2. Oral digestion

To simulate the oral digestion, 2 mL of extract was mixed 600 μL of α-amylase solution (100 U/mL; Sigma Aldrich) in a 15 mL Falcon tube and incubated at 37 °C with shaking for 1 min. A 400 μL aliquot (15.38 mg dw) was then collected for HPLC-DAD analysis of phenolic compounds at this digestion phase.

2.2.3. Gastric digestion

After oral digestion, the pH of the solution was adjusted to 2 with HCl (1 M) to simulate gastric conditions and enable pepsin activity. Then, 750 μL of pepsin solution was added, and the mixture was incubated in a water bath at 37 °C for 60 min under agitation. An aliquot of 500 μL containing 14.34 mg of *Butia* dw was collected for phenolic analysis by HPLC-DAD.

2.2.4. Intestinal digestion

To simulate the intestinal phase of digestion, the solution's pH was adjusted to 6.0 using 1.0 M $NaHCO^3$. Bile salt solution (12 g/L in 1.0 M $NaHCO_3$, Sigma Aldrich) and 375 μL pancreatin solution (2 g/L Sigma Aldrich) were then added, followed by incubation at 37 °C for 60 min under vigorous agitation. An aliquot of 500 μL EtOH: H_2O extract, corresponding to 12.44 mg of lyophilized extract (dw) was collected for HPLC-DAD analyses.

2.3. HPLC-DAD determination analysis

Phenolic compounds (Supplemental Table 1) in digested and undigested samples were analyzed using HPLC equipment (Agilent 1260, Agilent Technologies), equipped with a reverse-phase C18 column (HPLC Eclipse Plus, 4.6 × 100 mm, 3.5 um) and diode-array detector, following the methodology proposed by Somkuwar et al. (2018). Digestion samples were freeze-dried (48 h, −18 °C) and resuspended in the corresponding volumes of ethanolic extract (70:30 ethanol:water, *v*/v) reserved at each phase (Undigested: 600 μL; Oral digestion: 400 μL; Gastric digestion: 500 μL; Intestinal digestion: 500 μL). Samples were filtered (0.45 μm, nylon syringe filters, 15 mm, Agilent technologies, Germany) prior to injection.

The mobile phases were: (A) 0.2 % acetic acid in ultrapure water (Milli-Q Gradient System, Millipore Corporation, Massachusetts, EUA), and (B) 0.2 acetic acid in methanol (99.9 % pure, Merck, Darmstadt,

Table 1
Collection places of the *Butia* samples.

Species	Origin (County/State)	ID[1]	Jurisdiction	GC[2] (GPS)	Elevation (m)	VN[3]
Butia odorata	Encruzilhada do Sul/RS	*B. odorata*	Private property	30°51′48"S; 52°5393"W	395	9825 (ECT)
Butia eriospatha	Ponte Alta do Norte/SC	*B. eriospatha* 1	Private property	27°11′32"S; 50°34′48"W	855	9822 (ECT)
Butia eriospatha	Rio das Antas/SC	*B. eriospatha* 2	Private property	26°58′23"S; 51°01′12"W	977	9819 (ECT)
Butia catarinensis	Torres/RS	*B. catarinensis* 1	Private property	29°18′52"S; 49°45′27"W	13	7532 (CTBS)
Butia catarinensis	Imbituba/SC	*B. catarinensis* 2	Areais da Ribanceira - Environmental protection area	28°12′04"S; 48°40′44"W	87	7539 (CTBS)
Butia catarinensis	Laguna/SC	*B. catarinensis* 3	Gravatá beach	28°30′19"S; 48°45'29′W	36	7536 (CTBS)

[1] ID: Identification; [2] GC: Geographic coordinate; [3] VN: Voucher herbarium number.

Germany). The gradient elution was as follows: B from 1 % (2 min), 1–20 % (4 min), 20–30 % (4 min), held at 30 % (5 min), increased to 50 % (5 min), held (5 min), then 50–100 % (5 min), held (5 min), returned to 1 % (2 min), followed by a 3-min before the next injection, totaling 40 min per run.

Injection volume was 5 uL, flow rate 0.8 mL min^{-1}, at 30 °C. Quantification was based on external calibration curves. Method validation included calibration linearity, limit of detection (LOD) and limit of quantification (LOQ) (Supplemental Table 1), with LOD calculated by signal-to-noise ratio method.

2.4. Antioxidant activity

2.4.1. Hydroxyl radical

The hydroxyl radical scavenging potential was estimated using the methodology of Smirnoff and Cumbes (1989), with adaptations by Radünz et al. (2021) for serial dilution. An aliquot of 25 μL digested and undigested extract was added to a microplate (96-well plate), with 110 μL of $FeSO_4.7H_2O$ (8 mM), 75 μL of salicylic acid solution (3 mM) and 50 μL H_2O_2(7.18 mM). The microplate was incubated in spectrophotometer (SpectraMaxn190, Molecular Devices, USA) for 30 min at 37 °C, and then the reading was performed at 515 nm. For each sample, the highest radical-scavenging value observed within the tested concentration range was reported.

2.4.2. Nitric oxide radical

The scavenging activity of nitric oxide radical was quantified according to Baliga et al. (2003). The array was made using 96-well microplate, where 50 μL of digested and undigested extracts were added to 50 μL sodium nitroprusside (20 mM). The plates were incubated for 60 min at 22 °C under light. Then, 50 μL of 2 % phosphoric acid and 50 μL of Griess reagent were added. The mixture was incubated for more 10 min, now in the dark, at 22 °C (SpectraMax 190, Molecular Devices, USA), before the reading at 562 nm.

2.5. Inhibition of digestive enzymes

2.5.1. α-amylase inhibition

The α-amylase inhibitory activity of *Butia* extract was assessed following Kim et al. (2004) with the modifications proposed by Radünz et al. (2021). In a 96-well plate, 15 μL of extract (digested/ undigested) was mixed with 12.5 μL of α-amylase (241.71 U/mL, Sigma Aldrich) and 50 μL of phosphate buffer (pH 7.0), and incubated for 5 min at 37 °C in a spectrophotometer (SpectraMaxn190, Molecular Devices, USA). Then, 62.5 μL of soluble starch was added, following by a 15 min incubation at 37 °C. The reaction was stopped with 12.5 μL of HCl (1 M), and 25 μL of potassium iodide/iodine solution (0.005 M each) was added to form blue complexes with unhydrolyzed starch. Absorbance was read at a wavelength of 690 nm. Acarbose (50 mg/mL) served as the positive control.

2.6. α-glucosidase inhibition

The inhibition activity of α-glucosidase was evaluated with the method of Matsui et al. (1996), in serial dilution array, using 96-well plates. Each well received 10 μL of extract, 50 μL 3.25 mM *p*-nitrophenyl-α-D-glucopyranoside (in phosphate buffer, pH 7.0), and 50 μL of α-glucosidase enzyme (9.37 U/mL, Sigma Aldrich). After incubation at 37 °C for 10 min, absorbance was read at 405 nm. Acarbose (50 mg/mL) was used as positive control.

2.7. Experimental design

A completely randomized design was used, with two factors: six populations (Encruzilhada do Sul, Ponte Alta do Norte, Rio das Antas, Torres, Imbituba, Laguna) and four digestion phases (undigested, oral, gastric, and intestinal digestion), with three replicates. For variables with a normal distribution (antioxidant and digestive enzymes inhibition), a two-way analysis of variance (ANOVA) was applied, followed by the Tukey HSD Test (5 % significance) using the "ExpDes" package (Ferreira et al., 2021). Phenolic compounds were analyzed using non-parametric Aligned Rank Transform ANOVA (ART ANOVA) (Wobbrock et al., 2011), with Sidak's post-hoc test, *via* "pacman" (Rinker & Kurkiewicz, 2017) and "ARTool" packages (Elkin et al., 2021). Analyses were performed in R-4.0.3 software (R Core Team, 2023).

3. Results and discussion

3.1. Phenolic profile

There were differences in the composition of original extracts of the six samples, and a different phenolic profile was observed in the different phases of digestion, where interaction between the factors was found for all the phenolics. Among the 20 phenolics standards available, nine were identified. The phytochemicals identified in *Butia* populations were phenolic acid derivatives, catechin derivatives, 3,4-dihydroxybenzoic acid, tyrosol, and rutin (Table 2). In the Laguna sample (*B. catarinensis*-3) the highest diversity of phytochemicals was identified (5), followed by Encruzilhada do Sul (*B. odorata*), Imbituba (*B. catarinensis*-2) and Rio das Antas (*B. eriospatha*-2), with 4 phytochemicals each, Ponte Alta do Norte (*B. eriospatha*-1) with 3, and Torres (*B. catarinensis*-1) with 2.

Catechin was the only phytochemical present in all samples. Catechin is a well-known antioxidative agent related to apoptosis regulation, formation of new blood vessels (angiogenesis), and prevention of oxidative stress. However, it is important to note that the effectiveness of this flavan-3-ol for human health depends on its absorption in the small intestine and subsequent transport into the bloodstream (Rockett et al., 2020). This applies to all polyphenols, as bioaccessibility will depend on the stability of these compounds throughout digestion to reach the intestine (small and large), which accounts for about 90 % of phenolic absorption (Wojtunik-Kulesza et al., 2020).

Catechin showed variable behavior during *in vitro* digestion, with overlapping patterns, making the interpretation challenging. Peak concentrations occurred at different stages of digestion: *B. eriospatha* samples (1 and 2) and *B. catarinensis*-2 showed their highest concentrations after gastric digestion, while *B. catarinensis*-3 had its peak concentration after intestinal digestion. In contrast, the highest catechin concentrations in *B. catarinensis*-1 were found both after oral and gastric digestion, whereas in *Butia odorata* they were detected in the undigested extract and after gastric digestion. Concentrations ranged from 0.02 μg/mg of *Butia* dried weight (dw) (undigested *B. eriospatha*-1) to 6.39 μg/mg dw (*B. catarinensis*-2 sample after gastric digestion), though *B. catarinensis*-2 showed low stability with a 95.77 % drop after intestinal digestion. *B. catarinensis*-3 stood out with the highest concentration after complete digestion (1.66 μg/mg dw).

Catechin is among the most abundant phytochemicals in *B. odorata* (Boeing et al., 2020; Hoffmann et al., 2017; Ramos et al., 2020; Ma et al., 2019), with epicatechin also reported as a major compound in *B. odorata* (Boeing et al., 2020; Hoffmann et al., 2017; Ramos et al., 2020) and *B. eriospatha* (Rockett et al., 2020). In this study, epicatechin was not detected, but gallocatechin was present in two populations, with notably high levels after gastric digestion: reaching 3301.75 μg/mg dw in *B. odorata* and 431.54 μg/mg in *B. catarinensis*-3. Ma et al. (2020) also observed increases in flavonoids and phenolic acids after gastric digestion of bamboo leaf soup, attributing this to structural changes at low pH that release phenolics from the food matrix (Ma et al., 2020).

High concentrations of rutin were reported in *B. odorata* (Boeing et al., 2020; Hoffmann, Zandoná, et al., 2017) and *B. capitata* (Lahlou et al., 2022). Also, in the evaluation of four *Butia* species (*B. catarinensis*, *B. odorata*, *B. paraguayensis*, *B. yatay*) by Hoffmann, Carvalho, et al.

Table 2
Phenolic compounds identified in different natural populations of *Butia* along *in vitro* gastrointestinal simulation, determined by HPLC-DAD.

ID	Compound	Sample	Digestion phases (compound concentration μg/mg of *Butia* dried weight			
			UND	OD	GD	ID
		B. odorata	0.02 ± 0.00de	0.05 ± 0.00a	n.d	n.d
		B. eriospatha-2	0.008 ± 0.00 g	0.01 ± 0.00ef	0.02 ± 0.00 cd	0.03 ± 0.00bc
1	Caffeic acid	*B. catarinensis*-1	0.008 ± 0.000 g	0.01 ± 0.00f	n.d	n.d
		B. catarinensis-3	0.007 ± 0.002 g	n.d	n.d	0.04 ± 0.00ab
		B. odorata	0.62 ± 0.10bcdef	0.51 ± 0.02 defg	0.81 ± 0.05abcde	0.46 ± 0.07efgh
2	Catechin	*B. eriospatha*-1	0.02 ± 0.00 m	0.17 ± 0.06 jklm	0.40 ± 0.12fghi	0.14 ± 0.02klm
		B. eriospatha-2	0.17 ± 0.04 klm	0.23 ± 0.03 hijk	0.52 ± 0.08defg	0.14 ± 0.01 lm
		B. catarinensis-1	0.22 ± 0.00 ijkl	1.82 ± 0.08 ab	1.22 ± 0.05abcd	0.28 ± 0.05ghij
		B. catarinensis-2	0.13 ± 0.01 m	0.16 ± 0.01 klm	6.39 ± 0.73a	0.27 ± 0.01ghi
		B. catarinensis-3	0.15 ± 0.00 klm	1.95 ± 0.58 ab	0.56 ± 0.09cdefg	1.66 ± 0.29 abc
3	3,4-dihydroxybenzoic acid	*B. eriospatha*-1	0.002 ± 0.00d	0.018 ± 0.00b	0.01 ± 0.00b	0.006 ± 0.001c
		B. catarinensis-2	0.005 ± 0.00 cd	0.006 ± 0.00c	0.03 ± 0.00a	0.01 ± 0.00b
4	Ellagic acid	*B. catarinensis*-2	0.008 ± 0.00b	0.012 ± 0.00a	n.d	n.d
5	Gallocatechin	*B. odorata*	1087 ± 65.94b	1063 ± 145.16b	3301.75 ± 927.21a	283.36 ± 10.57d
		B. catarinensis-3	23.04 ± 1.84f	156.17 ± 32.03e	431.54 ± 62.34c	485.54 ± 19.69c
		B. eriospatha-1	0.01 ± 0.00j	0.24 ± 0.00 gh	n.d	0.49 ± 0.00e
6	Tyrosol	*B. eriospatha*-2	0.06 ± 0.00i	1.73 ± 0.17ab	0.59 ± 0.03 cd	1.45 ± 0.08bc
		B. catarinensis-2	0.02 ± 0.01j	0.40 ± 0.01 fg	8.94 ± 0.34a	0.43 ± 0.02f
		B. catarinensis-3	0.01 ± 0.001j	0.20 ± 0.01hi	n.d	0.50 ± 0.03de
7	p-Coumaric acid	*B. eriospatha*-2	0.007 ± 0.00d	0.01 ± 0.00c	0.02 ± 0.00b	0.03 ± 0.00a
8	Rutin	*B. odorata*	0.02 ± 0.00d	0.04 ± 0.00c	0.06 ± 0.00b	0.29 ± 0.17a
9	Syringic acid	*B. catarinensis*-3	0.14 ± 0.01c	0.22 ± 0.00b	n.d	0.88 ± 0.02a

B. odorata: Encruzilhada do Sul; *B. eriospatha*-1: Ponte Alta do Norte; *B. eriospatha*-2: Rio das Antas; *B. catarinensis*-1: Torres; *Butia catarinensis*-2: Imbituba; *B. catarinensis*-3: Laguna. Und: Undigested extract, OD: Oral digestion, GD: Gastric digestion, ID: Intestinal digestion.
Data presented in means ± standard variation. n.d.: not detected. Average followed by the same lowercase letters in the row and column within the same phenolic compound do not differ statistically.

(2017) rutin was the biomarker that allowed differentiating *B. odorata* from the other species. In this study, rutin was detected only in *Butia odorata* (Table 2), corroborating Hoffmann, Carvalho, et al. (2017). Notably, the highest concentration was observed after intestinal digestion, indicating good stability of this compound.

Of the nine phytochemicals identified, seven had been previously reported in *Butia* species. In contrast, 3,4-dihydroxybenzoic acid ($C_7H_6O_4$) had not been specifically reported before, although related compounds such as hydroxybenzoic acid ($C_7H_6O_3$) (Boeing et al., 2020; Hoffmann et al., 2018; Hoffmann, Carvalho, et al., 2017; Hoffmann, Zandoná, et al., 2017; Lahlou et al., 2022; Ma et al., 2019; Ramos et al., 2020), and non-specified dihydroxybenzoic acid isomers (Rockett et al., 2020) have been detected in other studies. Dihydroxybenzoic acid was described as one of the main compounds quantified by Rockett et al. (2020) in *B. eriospatha* and *B. catarinensis*, and in the current work the isomeric form, 3,4-dihydroxybenzoic acid, was identified in *B. eriospatha*-1 and *B. catarinensis*-2, but not in *B. odorata*.

Tyrosol ($C_8H_{10}O_2$) was successfully identified in *Butia* for the first time, based on matching retention time and absorption spectra with a commercial standard. This phenolic alcohol, known for its antioxidant and cardioprotective properties (PubChem, 2023), was detected in four out of six samples: *B. eriospatha* 1 and 2, and *B. catarinensis* 2 and 3. Previously, only hydroxytyrosol ($C_8H_{10}O_3$) was identified in *B. odorata* (Ma et al., 2019). Although it belongs to the same chemical class as tyrosol, it differs from this phenolic compound in both structure and bioactivity. Although the detection of tyrosol in *Butia* samples in the present study is noteworthy, we acknowledge the limitations of the analytical method employed. We recommend the use of mass spectrometry (MS) in future analyses to confirm this finding and to distinguish possible isomers based on their fragmentation patterns (Hoffmann & Stroobant, 2007).

In general, the highest concentrations of phenolic acids— such as p-coumaric, syringic and caffeic acid— were found after intestinal digestion, a pattern also seen with rutin. Simulated gastrointestinal conditions (mediated by pH and enzymatic activity) can promote chemical and structural changes, favoring the release of phenolic compounds. This process liberates not only compounds that were not extracted by the hydroalcoholic method, but also those that were originally bound to the food matrix (Gutiérrez-Grijalva et al., 2019). Enzymes like amylase promote the hydrolysis of starch, pepsin breaks down proteins and amino acids in the stomach, and the pancreatic enzyme digests carbohydrates, fats, and proteins, enabling the release of bound phenolic compounds (Wojtunik-Kulesza et al., 2020).

Caffeic acid was identified in early digestion phases in *B. catarinensis*-1 and *B. odorata*, and ellagic acid in *B. catarinensis*-2, but both were undetected in later stages. Digestive enzymes may irreversibly degrade phenolic compounds, as shown for quercetin and resveratrol (Lee et al., 2020). This degradation likely results in dissociation of carbon and hydroxyl groups. In addition, pH changes can also lead to oxidation and racemization of phenolic compounds, altering or reducing compound stability (Gutiérrez-Grijalva et al., 2019). Such structural changes may explain the absence of tyrosol in *B. eriospatha*-1 and syringic acid in *B. catarinensis*-3 after gastric digestion.

Variations in compound behavior across samples —such as the inconsistent patterns of tyrosol— may result from differences in the food matrix. These compounds interact with other food constituents (Bermúdez-Soto et al., 2007; Wojtunik-Kulesza et al., 2020) like proteins and polysaccharides (Ma et al., 2020; Wojtunik-Kulesza et al., 2020). The genetic and phenotypic diversity of *Butia* species likely underlies the distinct phenolic profiles observed in Supplementary Fig. 1.

3.2. Antioxidant capacity

Different mechanisms generate free radicals, just as there are different underlying mechanisms to combat these reactive species in organisms. In this work the antioxidant capacity was measured by the neutralization of hydroxyl and nitric oxide radicals and was influenced by both population (origin sites) and the digestion phase. *B. catarinensis*-3 showed the highest OH scavenging potential in the undigested and gastric-digested extracts. After oral digestion, *B. eriospatha* 1 and *B. catarinensis* 3 stood out, though *B. catarinensis* 1 and 3 and *B. odorata* showed no difference (Table 3).

After intestinal digestion, only *B. catarinensis*-1 presented antioxidant capacity against hydroxyl radical, while no sample was able to

Table 3
In vitro antioxidant potential of digested and undigested ***Butia*** extracts, expressed as the highest % of free radical scavenging and inhibition of α-glucosidase/α-amylase inhibition in serial dilutions (starting from 50 mg of dried *Butia* per mL).

Scavenging % OH*						
Digestion phase	*B. odorata*	*B. eriospatha*-1	*B. eriospatha*-2	*B. catarinensis*-1	*B. catarinensis*-2	*B. catarinensis*-3
Undigested	43.47^{aB}	45.35^{bB}	18.68^{aC}	38.09^{aB}	10.04^{aC}	57.85^{bA}
Oral digestion	40.48^{aB}	56.02^{aA}	16.61^{aC}	41.89^{aB}	11.25^{aC}	46.94^{cAB}
Gastric digestion	35.65^{aB}	30.75^{cB}	17.95^{aC}	34.57^{aB}	6.71^{aC}	70.45^{aA}
Intestinal digestion	n.d	n.d	n.d	23.41^{bA}	n.d	n.d
Scavenging % ON*						
Undigested	51.43^{bAB}	22.50^{bC}	35.48^{bBC}	49.89^{aAB}	57.86^{abA}	35.08^{bBC}
Oral digestion	47.47^{bA}	22.90^{bB}	30.17^{bAB}	47.08^{aA}	46.94^{bA}	46.25^{abA}
Gastric digestion	68.16^{aA}	54.57^{aA}	62.21^{aA}	55.73^{aA}	70.45^{aA}	60.18^{aA}
Intestinal digestion	n.d	n.d	n.d	n.d	n.d	n.d
α-glucosidase inhibition %						
Digestion phase	*B. odorata*	*B. eriospatha*-1	*B. eriospatha*-2	*B. catarinensis*-1	*B. catarinensis*-2	*B. catarinensis*-3
Undigested	21.13^{aD}	72.87^{aA}	66.35^{aA}	14.46^{aD}	31.26^{aC}	52.10^{aB}
Oral digestion	18.29^{aC}	61.30^{bA}	54.90b^{AB}	12.57^{aC}	17.49^{bC}	49.82^{abB}
Gastric digestion	3.59^{bD}	63.21^{bA}	36.35^{cB}	6.09^{bCD}	14.96^{bcC}	41.70^{bB}
Intestinal digestion	4.43^{bC}	57.04^{bA}	37.12^{cB}	n.d	8.94^{bC}	40.05^{cB}
α-amylase inhibition %						
Undigested	84.23^{aA}	16.12^{bB}	67.42^{aAB}	72.40^{aA}	55.43^{aAB}	52.76^{aAB}
Oral digestion	82.67^{aA}	44.35^{abA}	74.66^{aA}	94.79^{aA}	56.85^{aA}	87.46^{aA}
Gastric digestion	83.19^{aA}	62.53^{abAB}	44.78^{aAB}	16.81^{bB}	78.16^{aA}	70.43^{aAB}
Intestinal digestion	75.84^{aA}	71.89^{aA}	43.14^{aA}	50.35^{abA}	67.88^{aA}	90.60^{aA}

B. odorata: Encruzilhada do Sul; *B. eriospatha* 1: Ponte Alta do Norte; *B. eriospatha* 2: Rio das Antas; *B. catarinensis* 1: Torres; *B. catarinensis* 2: Imbituba; *B. catarinensis* 3: Laguna.*OH: hydroxyl radical, ON: nitric oxide radical. Average followed by the same capital letters in the horizontal direction do not differ significantly. Average followed by the same lower cases in the vertical do not differ significantly. n.d = not detected.

neutralize nitric oxide after intestinal digestion. This may occur due to degradation of antioxidants agents caused by the alkaline conditions of the duodenum (Ryan & Prescott, 2010).

The populations of *B. odorata*, *B. eriospatha*-2, *B. catarinensis* 1 and 2 have no significant difference in OH scavenging capacity in the different digestion phases, which means a high stability of the bioactive compounds contained in these samples, to withstand pH changes and enzymatic action (Ryan & Prescott, 2010). In turn, *B. catarinensis*-3 and *B. eriospatha*-1 showed different responses during the digestive process.

In *B. catarinensis*-3 the highest hydroxyl scavenging was observed after gastric digestion, while in *B. eriospatha*-1 it occurred during oral digestion. This may result from enzymatic and chemical modifications—such as α-amylase-mediated-transformations—producing metabolites with enhanced radical-scavenging (Wojtunik-Kulesza et al., 2020). Conjugation of phenolic acids with glucuronic acid, for example, can improve absorption and antioxidant potential (Anson et al., 2009). Similar increases in antioxidant capacity post-digestion were reported by Vinholes et al. (2018), with *B. odorata* genotypes showing 15 and 26 % higher OH scavenging than undigested extract.

Regarding nitric oxide neutralization, *B. catarinesis*-2 showed the highest antioxidant capacity (between 46 and 70 %) among the samples, in all phases where activity was detected (undigested, oral digestion and gastric digestion). After oral digestion only *B. eriospatha*-1 differentiates, with lower values. The populations showed no difference after gastric digestion, and the highest percentual of inhibition was achieved in this stage.

To compare the overall performance in terms of antioxidant capacity, the average digestion phases were pooled for each population to investigate the first factor (population) separately. For the capacity to eliminate the hydroxyl radical four distinct statistical classes were formed. The population from Laguna (*B. catarinensis*-3) stood out with the highest capacity, followed by Encruzilhada do Sul (*B. odorata*), Ponte Alta do Norte (*B. eriospatha*-1) and Torres (*B. catarinensis*-1). The population from Rio das Antas (*B. eriospatha*-2) formed the third class, and Imbituba (*B. catarinensis*-2) showed the lowest capacity to neutralize hydroxyl radicals. The populations from Encruzilhada do Sul (*B. odorata*) and Imbituba (*B. catarinensis*-2) exhibited the strongest nitric-oxide-radical-scavenging activity, outperforming both *B. eriospatha* populations. Populations from Torres and Laguna (*B. catarinensis*-1 and -3) formed an intermediate statistical group: their activity did not differ significantly from that of Encruzilhada do Sul (*B. odorata*), Imbituba (*B. catarinensis*-2) or Rio das Antas (*B. eriospatha*-2), yet it was higher than that of Ponte Alta do Norte (*B. eriospatha*-1) (Fig. 2-A).

Despite belonging to the same species (*B. catarinensis*), the samples from Torres, Imbituba and Laguna showed distinct hydroxyl radical scavenging capacities, whereas their nitric oxide scavenging potential did not differ significantly. A similar pattern was observed in *B. eriospatha* samples from both Rio das Antas and Ponte Alta do Norte

When considering only the second factor (the pooled mean of the populations for each digestion phase), for hydroxyl radical scavenging, there were no differences between the potential in undigested extracts, and after oral and gastric digestion (Fig. 2-B). In the intestine, the capacity for scavenging hydroxyl radicals was lower. Reduction in the antioxidant capacity after intestinal digestion was already reported by Rodríguez-Roque et al. (2013). For nitric oxide scavenging, the highest antioxidant capacity occurred at gastric digestion, while the potential did not differ between the original extract and after oral digestion. In the intestine there was an absence of activity, at any concentration tested for any population.

Oral digestion is the shortest of all the simulated steps (just over one min), so the fact that there is no difference between the capacity of the extract before and after oral digestion may be due to the short interaction with α-amylase, its influence being much less evident than in the subsequent (Wojtunik-Kulesza et al., 2020). High stability of phenolic compounds during oral and gastric digestion in fruit species was reported in the literature review of Wojtunik-Kulesza et al. (2020), as well as reduction after intestinal digestion (Bermúdez-Soto et al., 2007).

3.3. Inhibition of digestive enzymes

There was interaction between the population and digestion stage in the inhibition of digestive enzymes. In undigested extracts the inhibition of α-glucosidase varied in the samples from 14 to 72 %, in oral digestion from 12 to 61 %, in gastric from 3 to 63 %, and intestinal from 4 to 57 %. *B. catarinensis*-2 and *B. eriospatha*-1 presented no differences in the activities during the digestion phases, whereas the others presented differences in the inhibition behavior (Table 3).

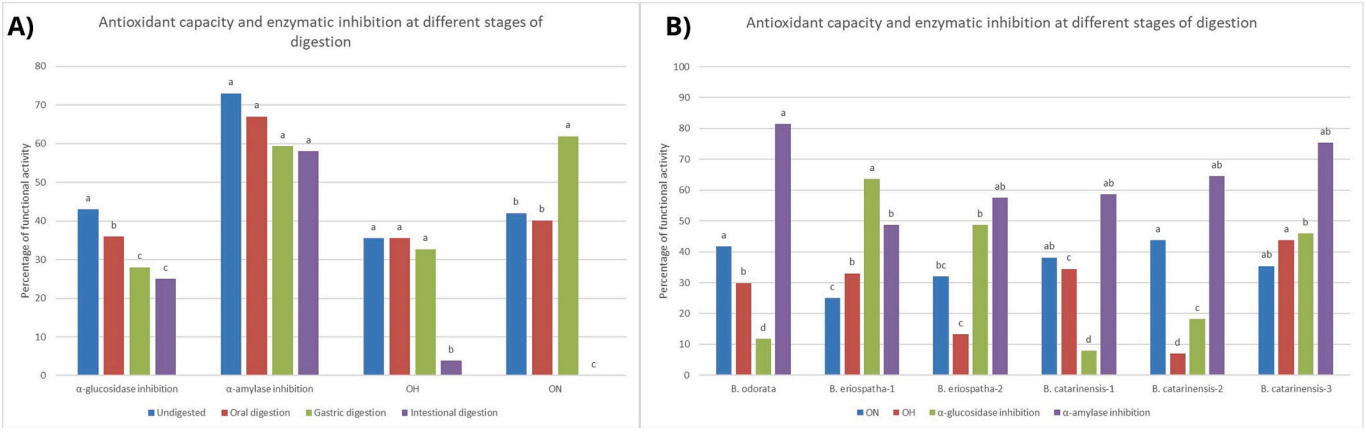

Fig. 2. A) Performance of six *Butia* population in scavenging hydroxyl (OH) and nitric oxide radicals (ON), as well as inhibiting α-glucosidase and α-amylase enzymes during simulated *in vitro* digestion. Results consider the cumulative activity across all digestion phases evaluated: oral digestion, gastric digestion, intestinal digestion, and the original undigested extract. * *B. odorata*: Encruzilhada do Sul; *B. eriospatha*-1:Ponte Alta do Norte; *B. eriospatha*-2: Rio das Antas; *B. catarinensis*-1: Torres; *B. catarinensis*-2: Imbituba; *B. catarinensis*-3: Laguna. B) General capacity of scavenging hydroxyl and nitric oxide radicals and as inhibit α-glucosidase and α-amylase enzymes, observed in different phases of digestion, in extracts of Butia, submitted to *in vitro* digestion. Different letters indicate significant differences supported by HSD Tukey's Test at 5 % of probability.

The lowest reductions in α-glucosidase inhibitory capacity after simulated gastrointestinal digestion were observed in *B. eriospatha*-1 and *B. catarinensis*-3, whose reduction in activity after the whole process was 22 and 23 % respectively, relative to the undigested extract. In turn, *B. eriospatha*-2, *B. odorata* and *B. catarinensis* 1 and 2 showed more severe reductions, of 44 %, 100 %, 71 %, 79 %, respectively.

Unlike reported by Vinholes et al. (2018) for two *B. odorata* genotypes, in this work differences were observed in the capacity of inhibition of α-glucosidase among undigested and orally digested extracts. Nevertheless, in the only sample of *B. odorata* accessed in the present study, the inhibitory capacity of the two fractions (undigested and orally digested) was not different. *B. eriospatha*-1 population excelled, with the highest percentual of α-glucosidase inhibition in the original extract, and after each digestion phase.

Individual factor analysis also provided insight into the overall performance of each population, and the influence of the digestive process on enzyme inhibition. The comparison between the populations revealed differences in both enzyme inhibitions activities (Fig. 2-A). For α-glucosidase inhibition, a larger difference was observed between the samples, where four well-defined statistical groups were formed, whereas for α-amylase, three groups were formed, with an intermediate class (ab) predominating.

The population from Ponte Alta do Norte (*B. eriospatha*-1) showed the strongest α-glucosidase inhibition, averaging 64 %. Rio das Antas (*B. eriospatha*-2) and Laguna (*B. catarinensis*-3) followed 49 % and 48 %, while Imbituba (*B. catarinensis*-2) reached 18 %. The lowest activities were recorded for Encruzilhada do Sul (*B. odorata*) at 12 % and Torres (*B. catarinensis*-1) at 8 %.

Pereira et al. (2020) note that extracts combining strong α-glucosidase with moderate α-amylase inhibition are optimal: excessive suppression of α-amylase allows undigested starch to reach the large intestine, where it is fermented by intestinal bacteria, causing discomfort, flatulence and diarrhea. Given this, the Ponte Alta do Norte population (*B. eriospatha*-1) is the most promising candidate for further *in vitro* validation. Although it showed the lowest nitric-oxide-scavenging activity and only moderated hydroxyl-radical neutralization, it was the only population in which α-glucosidase exceeded α-amylase inhibition overall. These findings underscore how metabolic profiles, shaped by genetic differences and different responses to environmental pressures, translate into distinct nutraceutical potentials.

Vinholes et al. (2018) reported increased enzymatic inhibition of α-glucosidase after gastric digestion, but in this work a decreasing pattern was observed, in which undigested extract showed 43 % inhibition, oral digestion 36 %, and in the latest stages (gastric and intestine) the lowest values of 28 % and 25 % were observed (Fig. 2-B). There are no other investigations on the inhibition of digestive enzymes in *Butia* throughout the digestion process; however, a similar pattern of decreased α-glucosidase inhibitory capacity was observed by Cao et al. (2021) in passion fruit peel. For α-amylase, there was no difference in the enzyme inhibition between digestion phases, with activity between 58 % to 73 %.

The reduction in the inhibition of α-glucosidase after gastric digestion is documented in the literature (Gutiérrez-Grijalva et al., 2019; Radünz et al., 2021; Vinholes et al., 2018). Phenolic content has been associated with α-glucosidase inhibition, where phenols present different inhibitory mechanisms, depending on their structure (Cao et al., 2021). Through digestion, breakdown and absorption processes occur, where polyphenols interact with other food components, resulting in specific patterns of increase or decrease of these compounds, according to the difference between food matrices (Wojtunik-Kulesza et al., 2020).

In this regard, the differences identified in biological activity, phenolic composition, and release patterns of phytochemical compounds among *Butia* samples are expected due to the high variability within the genus. The results of this study highlight *Butia* fruits as a rich source of bioactive compounds while underscoring the invaluable role of *Butia* palm groves in conserving genetic diversity with nutritional and nutraceutical potential.

4. Conclusions

Extracts from different populations of *Butia odorata*, *Butia catarinensis* and *Butia eriospatha* exhibited antioxidant capacity as well as inhibitory effects on the enzymes α-glucosidase and α-amylase. The *in vitro* digestion yielded divergent outcomes in terms of antioxidant capacity, digestive enzymes inhibition and phenolic profile. In general, there was a decrease in the bioactivities at the intestinal digestion phase. The samples from Encruzilhada do Sul, Torres and Imbituba showed the highest nitric oxide radical scavenging capacity, while the one from Laguna showed the highest hydroxyl radical neutralization. The sample from Ponte Alta do Norte stood out with the best ratio between α-glucosidase and α-amylase inhibition. In turn, in the Rio das Antas sample, there were the highest concentrations of tyrosol, which seems to have been reported for the first time in *Butia*. Although the phenolic compounds identified were not the predominant ones, the results provide an initial insight into the chemical variability safeguarded in *Butia*

populations and their behavior under *in vitro* digestion. Further studies are needed to deepen the characterization of the phenolic profile of the species studied and to better its relationship with nutraceutical potential.

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CRediT authorship contribution statement

Julia Goetten Wagner: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Taiane Mota Camargo:** Methodology, Investigation. **Marjana Radünz:** Methodology, Investigation. **Ikram Bashir:** Writing – review & editing, Formal analysis. **Nubia Marilin Lettnin Ferri:** Methodology, Investigation. **Cristian Soldi:** Validation, Resources, Methodology. **Gustavo Heiden:** Writing – review & editing, Supervision. **Marcia Vizzotto:** Writing – review & editing, Supervision, Resources, Conceptualization. **Rosa Lía Barbieri:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

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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.

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Data availability

Data will be made available on request.

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