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<https://doi.org/10.17113/ftb.64.04.26.9515>

original scientific paper

Ultrasonic Extraction of Bioactive Compounds from *Butia eriospatha* Using NADES: Screening and Process Optimization

Running head: Ultrasonic Extraction of Butiá Bioactives with NADES

Daiana Alves Machado^{1,2*} and Renata Dinnies Santos Salem²

¹Federal Institute of Education, Science and Technology of Santa Catarina, Avenida dos Expedicionários, 2150, 89466-312 Canoinhas, SC, Brazil

²Graduate Program in Food Science and Technology, State University of Ponta Grossa, Avenida Carlos Cavalcanti, 4748, 84030-900 Ponta Grossa, PR, Brazil

Received: 5 December 2025

Accepted: 18 October 2026



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SUMMARY

Research background. *Butia eriospatha* (Mart. ex Drude) Becc. is a palm native to southern Brazil whose fruits contain high levels of antioxidant compounds, including phenolic compounds, carotenoids and ascorbic acid. Driven by the demand for sustainable extraction routes, natural deep eutectic solvents (NADES) combined with ultrasound-assisted extraction (UAE) have emerged as a promising alternative to conventional organic solvents for plant matrices.

Experimental approach. This study aimed to optimize the extraction of bioactive compounds from the pulp–peel mixture of *B. eriospatha* using NADES as extraction media in combination with UAE. Three formulations (ChCl–Gly, ChCl–TA and ChCl–LA) were screened, resulting in the selection of ChCl–Gly (1:2 mol/mol). Subsequent extractions were performed using UAE (25 kHz; 165 W)

*Corresponding author;
Phone: +5547997264896
E-mail: daiana.machado@ifsc.edu.br

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based on Box–Behnken and 3^2 factorial designs, varying temperature, extraction time and liquid-to-solid ratio, with 50 % (by volume) ethanol as the control solvent. Extracts were analyzed for total phenolic content (TPC), total flavonoid content (TFC), total carotenoids (TC), ascorbic acid (AA) and antioxidant activity (DPPH, ABTS and FRAP).

Results and conclusions. Quadratic modeling integrated with a desirability function identified 41 °C and 60 min as the optimal condition, yielding TFC≈8.3 mg QE/g, TPC=166.5 mg GAE/g, ABTS≈139.0 mg TE/g and TC=93.5 mg β -carotene/100 g, values superior or comparable to ethanol. In the 3^2 design, DPPH, FRAP and AA were also quantified outside the desirability model, showing high performance in the optimized extract (DPPH≈112.0 mg TE/g; FRAP≈1000.0 mg TE/g; AA≈5.0 mg/100 g). The optimized extract exhibited high thermal stability (60–80 °C for 120 min) and photostability over 96 h (<2 % variation in TPC), indicating low molecular degradation and preservation of antioxidant activity.

Novelty and scientific contribution. The ChCl–Gly/UAE system showed superior extraction efficiency, strong selectivity toward bioactive compounds and high metabolite stability, establishing it as a high-yield, reproducible and industrially applicable green technology. The findings highlight *B. eriospatha* as a regional source of functional ingredients and position NADES as stable and strategically sustainable solvents for food, pharmaceutical, cosmetic and nutraceutical applications.

Keywords: *Butia eriospatha*; NADES; ultrasound-assisted extraction; bioactive compounds; antioxidant activity; native fruits

INTRODUCTION

The genus *Butia* (Arecaceae) comprises several palm species native to southern South America, among which *Butia eriospatha* (Mart. ex Drude) Becc. holds substantial ecological, nutritional, and sociocultural importance in southern Brazil. Its fruits exhibit a sweet–acidic flavor, high juiciness, and considerable potential for industrial, culinary, and nutraceutical applications [1-3]. Beyond their gastronomic value, *B. eriospatha* fruits serve as key resources for traditional communities, support a wide range of native birds, insects, and mammals, and form characteristic landscapes known as butiazais. Habitat fragmentation and limited natural regeneration have led to the inclusion of this species in Brazil's National List of Threatened Species [5], underscoring the need for applied research that enhances its economic, scientific, and conservation relevance [3-5].

Chemically, *B. eriospatha* fruits are rich in specialized metabolites of functional interest, including phenolic compounds, flavonoids, carotenoids, and ascorbic acid. These biomarkers exhibit

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strong antioxidant activity, with documented roles in mitigating oxidative stress, inflammation, cellular damage, and degenerative processes. The combination of diverse phytochemicals, functional potential, and regional availability positions *B. eriospatha* as a promising native species for developing bioactive extracts [1,2,6] and natural value-added ingredients [1,6].

In this context, demand has increased for extraction processes that are sustainable, selective, and efficient, capable of recovering bioactive compounds without structural degradation or loss of functionality. Ultrasound-assisted extraction (UAE) has emerged as a green technology, as acoustic cavitation disrupts cellular matrices and enhances mass transfer [7-10], shortening processing time and increasing extraction yield while minimizing degradation of labile compounds [11,12]. In parallel, natural deep eutectic solvents (NADES) have gained prominence as environmentally friendly alternatives to organic solvents due to their low toxicity, biodegradability and tunable physicochemical properties [13,14], as well as strong affinity for phenolics, flavonoids and carotenoids [15].

Scientific interest in combining NADES with ultrasound has grown significantly, with positive results reported for fruits, agro-industrial residues, and antioxidant-rich plant matrices. Several studies reported synergistic effects when NADES were combined with UAE, leading to higher extraction yields and reduced processing time [16-18], with superior performance over conventional solvents in specific matrices [19,20]. Additionally, NADES can be incorporated directly into food products without evaporation steps, expanding their industrial applications and adding sensory and nutritional value to final formulations [14].

Despite the rapid technical progress, no consolidated studies have optimized the extraction of bioactive compounds from *Butia eriospatha* using ultrasound combined with NADES, revealing an important scientific gap. Therefore, the objective of this study was to optimize the extraction of bioactive compounds from *B. eriospatha* pulp and peel using integrated NADES–UAE, supported by mathematical modeling and multivariate analysis. Furthermore, the thermal and photochemical stability of the optimized extract was evaluated to support its potential application in technological, nutraceutical, and food systems.

MATERIALS AND METHODS

Reagents and materials

Analytical-grade Folin–Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), TPTZ (2,4,6-tripyridyl-s-triazine), and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The enzymes α -amylase, protease, and amyloglucosidase were obtained from

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Merck/SA (Darmstadt, Germany). Choline chloride was supplied by Êxodo Científica (Sumaré, SP, Brazil). The other reagents were of analytical grade.

Sample collection and preparation

Ripe fruits of *Butia eriospatha* were collected between January and February 2024 from three palm trees located in Canoinhas (SC, Brazil) at the georeferenced points 26°11'18.5"S 50°24'32.6"W; 26°11'22.3"S 50°24'46.4"W; and 26°12'25.1"S 50°24'08.8"W, with four fruit clusters sampled per plant. Taxonomic identification was confirmed by the Herbarium of the State University of Ponta Grossa (PR, Brazil), with voucher specimen 23.220.

Fruits were transported to the Plant Processing Laboratory at the Federal Institute of Santa Catarina (IFSC) – Canoinhas Campus, where they were selected, washed, and sanitized in sodium hypochlorite solution (200 mg/L, 15 min). After manual depulping, the pulp (mesocarp) and peel (epicarp) were combined and divided into two portions: one was stored fresh, and the other was dried in a forced-air oven (Lucadema, 82/480, SP, Brazil) at 60 °C until constant weight (~15 h). The drying step resulted in a mass reduction of 90.8 %, corresponding to a dry matter yield of 9.2 g per 100 g fresh material. The dried material was ground, homogenized, and sieved through a 60-mesh screen. Samples were vacuum-packed and stored at –18 °C until analysis.

Synthesis of NADES and preliminary screening

NADES were synthesized following Bosiljkov *et al.* [21] by heating the mixtures at 80 °C under continuous stirring until complete homogenization. Stability was evaluated after standing for 20–24 h at room temperature, according to Ali *et al.* [22]. Viscosity and polarity adjustments were performed by adding distilled water, as described by Nekkaa *et al.* [23].

Three NADES formulations were evaluated, each containing 20 % water (by mass): (1) choline chloride:glycerol (ChCl–Gly 1:2 mol/mol), (2) choline chloride:tartaric acid (ChCl–TA 2:1 mol/mol), and (3) choline chloride:lactic acid (ChCl–LA 1:3 mol/mol). Screening was carried out under standardized extraction conditions (40 °C; 60 min; liquid-to-solid ratio of 30 mL/g). Selection of the formulation for subsequent steps was based on the highest total flavonoids yield.

Preparation of extracts

Extractions were performed according to Weremfo *et al.* [24], with adaptations. Aliquots of 1 g of dried sample were subjected to UAE in an ultrasonic bath (Eco-sonics Q 5.9/25-A, 25 kHz, 165 W; Indaiatuba, SP, Brazil). The volumes of NADES (with different water contents) or 50 % (by volume)

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aqueous ethanol (control) were determined by the experimental designs. Extracts were cooled at 5 °C for 24 h, centrifuged at 2332×g for 10 min (Novatecnica NT810, Piracicaba, SP, Brazil), filtered, and stored at 5 °C protected from light.

Experimental designs

The first experimental design followed a Box–Behnken model [25] evaluating three independent variables: liquid-to-solid ratio (X_1), temperature (X_2), and water content in the NADES (X_3), with extraction time fixed at 60 min. The design included 15 runs, with three center points to estimate repeatability, plus a 50 % (by volume) aqueous ethanol control.

Variables were evaluated at three coded levels (−1, 0, +1): liquid-to-solid ratios of 20, 30 and 40 mL/g; temperatures of 30, 40, and 50 °C; water contents of 20, 25, and 30 % (by mass). Center point conditions (30 mL/g; 40 °C; 25 %) ensured model robustness.

Data were fitted to the quadratic model (Eq. 1) according to Bruns, Scarmino, and Barros Neto [26]:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad /1/$$

where Y is the predicted response; β_0 is the intercept; β_1 , β_2 , and β_3 are the linear coefficients; β_{11} , β_{22} , and β_{33} are the quadratic coefficients; β_{12} , β_{13} , and β_{23} are the interaction coefficients; and X_1 , X_2 , and X_3 , are the coded independent variables corresponding to liquid-to-solid ratio, temperature, and NADES water content, respectively.

After the initial screening design identified the most relevant factors and non-significant terms were removed, a second reduced experimental design (3^2 factorial) was conducted to refine the optimization of temperature (X_1) and extraction time (X_2). The liquid-to-solid ratio was fixed at 30 mL/g and the NADES water content at 25 % (by mass). Variables were studied at 30, 45, and 60 °C and 30, 45, and 60 min, resulting in nine experimental runs. A 50 % (by volume) aqueous ethanol extract was prepared under the central condition and used for comparison.

The reduced quadratic model (Eq. 2) was applied [26]:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad /2/$$

where Y is the predicted response; β_0 is the intercept; β_1 and β_2 are the linear coefficients; β_{11} and β_{22} are the quadratic coefficients; β_{12} is the interaction coefficient; and X_1 and X_2 are the coded independent variables corresponding to temperature and extraction time, respectively.

Optimal conditions were determined using desirability function and validated experimentally.

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Quantification of total flavonoid content (TFC)

TFC was quantified following Ramos *et al.* [27]. Aliquots of 500 μL extract were mixed with 1.8 mL of 50 % (by volume) aqueous ethanol and 200 μL of 5 % (m/V) AlCl_3 . After 30 min of dark incubation at room temperature, absorbance was measured at 425 nm in a UV–Vis spectrophotometer (BEL Photonics Gold Spectrumlab 53, Piracicaba, SP, Brazil). TFC content was determined using quercetin calibration curves. The calibration equations for NADES and ethanol were obtained as follows (Eqs. 3 and 4, respectively):

$$y=2.1635x+0.0086 \quad /3/$$

$$y=2.5707x+0.0086 \quad /4/$$

where y is the absorbance and x is the quercetin concentration. The coefficients of determination were $R^2=0.9953$ and $R^2=0.9979$ for NADES and ethanol, respectively, and TFC was expressed as mg QE/g.

Determination of total phenolic content (TPC)

TPC was quantified by the Folin–Ciocalteu method [28]. Reaction mixtures contained 100 μL extract, 8.4 mL distilled water, and 500 μL Folin–Ciocalteu reagent, incubated at 25 °C for 3 min. Then 1 mL of 20 % (m/V) Na_2CO_3 was added, and samples were left to react for 1 h at room temperature. Absorbance was read at 725 nm in a UV–Vis spectrophotometer. Quantification used gallic acid calibration curves, with results expressed as mg GAE/g. The calibration equations for NADES and ethanol were obtained as follows (Eqs. 5 and 6, respectively):

$$y=3.2859x+0.0991 \quad /5/$$

$$y=3.3955x+0.091 \quad /6/$$

where y is the absorbance and x is the acid gallic concentration. The coefficients of determination were $R^2=0.9928$ and $R^2=0.9926$ for NADES and ethanol, respectively.

Antioxidant activity

DPPH radical scavenging was conducted according to Brand-Williams *et al.* [29]. Aliquots of 150 μL extract were added to 2.85 mL of 1 mmol/L DPPH solution and kept in the dark for 24 h. Absorbance was measured at 515 nm, and results were expressed as mg Trolox equivalent per gram of dry sample (mg TE/g), using Trolox calibration curves. The calibration equations for NADES and ethanol were obtained as follows (Eqs. 7 and 8, respectively):

$$y=-5.4605x+0.9281 \quad /7/$$

$$y=-6.8312x+1.1293 \quad /8/$$

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where y is the absorbance and x is the Trolox concentration. The coefficients of determination were $R^2=0.9992$ and $R^2=0.9997$ for NADES and ethanol, respectively.

ABTS scavenging activity followed Re *et al.* [30]. The ABTS⁺ radical was generated by reacting 7 mmol/L ABTS with 140 mmol/L K₂S₂O₈ for 16 h in the dark, then diluted to absorbance 0.70 ± 0.05 at 734 nm. Aliquots of 30 μ L diluted extract were added to 3 mL of ABTS⁺ solution, and absorbance was read at 734 nm after 6 min. Results were expressed as mg TE/g. The calibration equations for NADES and ethanol were obtained as follows (Eqs. 9 and 10, respectively):

$$y = -20.324x + 0.6882 \quad /9/$$

$$y = -19.689x + 0.6667 \quad /10/$$

where y is the absorbance and x is the Trolox concentration. The coefficients of determination were $R^2=0.9985$ and $R^2=0.9977$ for NADES and ethanol, respectively.

FRAP activity was determined according to Benzie and Strain [31]. Reactions contained 100 μ L extract, 100 μ L FeCl₃ (3 mmol/L), and 1.8 mL TPTZ solution (1 mmol/L), incubated at 37 °C for 30 min (Solab SL150, Piracicaba, SP, Brazil). After 10 min stabilization in the dark at room temperature, absorbance was measured at 620 nm. Results were expressed as mg TE/g, using Trolox calibration curves. The calibration equations for NADES and ethanol were obtained as follows (Eqs. 11 and 12, respectively):

$$y = 0.5199x + 0.0854 \quad /11/$$

$$y = 0.5811x + 0.0055 \quad /12/$$

where y is the absorbance and x is the Trolox concentration. The coefficients of determination were $R^2=0.9952$ and $R^2=0.9915$ for NADES and ethanol, respectively.

Quantification of total carotenoids (TC)

TC was quantified directly from the extracts according to Rodrigues-Amaya [32], using the Lambert–Beer law (Eq. 13). Absorbance was read at 450 nm, and results were expressed as mg β -carotene/100 g dry sample:

$$TC = \frac{A \cdot V \cdot 10^6}{A_1^{1\% \text{ cm}} \cdot 100 \cdot m} \quad /13/$$

where TC is the total carotenoid content (mg β -carotene/100 g sample), A is absorbance at 450 nm, V is the extract volume (mL), $A_1^{1\% \text{ cm}}=2592$ is the absorption coefficient of β -carotene in petroleum ether, and m is sample mass (g).

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Determination of ascorbic acid (AA)

AA was quantified by titration with 2,6-dichlorophenolindophenol (DCPIP) following AOAC [33] and Benassi and Antunes [34], with modifications allowing direct quantification from extracts. Aliquots of 2 mL extract were combined with 5 mL of 2 % (m/V) oxalic acid and titrated with standardized DCPIP solution. The standardization factor was calculated according to Eq. 14:

$$f = \frac{m}{V} \quad /14/$$

where f is the correction factor, m is the sample mass (g), and V is the DCPIP volume (mL).

AA was calculated according to Eq. 15 and expressed as mg/100 g sample:

$$AA = \frac{V_{DCPIP} \cdot f \cdot 100}{V_{extract}} \quad /15/$$

where V_{DCPIP} is the DCPIP volume (mL), f is the correction factor, and $V_{extract}$ is the extract volume (mL).

Extract stability

Thermal stability of the optimized extract was assessed following Mercali *et al.* [35] and Peron, Fraga and Antelo [36], with modifications. Samples were heated in a thermostatic water bath (Solab SL150) at 60, 70, and 80 °C. Aliquots collected at 0, 15, 30, 60, and 120 min were cooled immediately and analyzed for TPC.

A first-order kinetic model was used (Eq. 16) to determine degradation rate constant (k), half-life ($t_{1/2}$), activation energy (E_a), enthalpy (ΔH), Gibbs free energy (ΔG), and entropy (ΔS), according to Eqs. 17–21.

$$C_A = C_{A0} e^{-kt} \quad /16/$$

where C_A is the TPC at time t (mg/g), C_{A0} is the initial TPC (mg/g), t is time (h), and k is the degradation rate constant (h^{-1}).

$$t_{1/2} = \frac{\ln(0.5)}{k} \quad /17/$$

$$\ln k = \ln A - \frac{E_a}{RT} \quad /18/$$

$$\Delta H = E_a - RT \quad /19/$$

$$\Delta G = -RT \ln\left(\frac{kh}{k_B T}\right) \quad /20/$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} \quad /21/$$

where A is the pre-exponential factor (h^{-1}), R is the universal gas constant (J/(mol·K)), h is Planck's constant ($6.6262 \cdot 10^{-34}$ J·s), and k_B is Boltzmann's constant ($1.3806 \cdot 10^{-23}$ J/K).

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Photostability was evaluated according to Dai *et al.* [37], with extracts exposed to fluorescent light (40 W) in a controlled photoperiod chamber (Deluq DL552, Florianópolis, SC, Brazil) at (24±2) °C for 96 h. TPC was monitored at 0, 3, 19, 24, 48, 72, and 96 h.

Statistical analysis

All experiments were performed in triplicate and results were expressed as mean±standard deviation (SD). Differences among treatments were evaluated by one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test at $p \leq 0.05$, selected due to its sensitivity for detecting pairwise differences among mean values. Kinetic parameters were estimated by nonlinear regression using the Gauss–Newton algorithm. All statistical analyses were performed using Statistica v. 7.1 [38].

RESULTS AND DISCUSSION

Preliminary screening and selection of NADES

The initial screening revealed marked differences among the three formulations tested. The choline chloride:glycerol NADES (ChCl–Gly 1:2 mol/mol) showed the highest total flavonoid content, with (8.9±0.1) mg QE/g, a value statistically higher ($p \leq 0.05$) than those obtained with ChCl–TA 2:1 ((3.9±0.3) mg QE/g) and ChCl–LA 1:3 ((4.3±0.1) mg QE/g). The difference among formulations was significant, highlighting that the yield obtained with ChCl–Gly was approximately twice that observed for the acidic NADES (ChCl–TA and ChCl–LA), confirming the greater affinity of this system for the flavonoids present in the pulp and peel of *Butia eriospatha*.

This performance can be attributed to the combination of glycerol, a polyhydroxylated HBD, and choline chloride, a quaternary ammonium HBA, which together form a eutectic matrix with a high density of hydrogen bonds and intermediate polarity. This microstructure favors interactions with hydroxyl and carboxyl groups of flavonoids, as well as dipole–dipole and van der Waals forces, thereby enhancing solubility and stability during extraction [39].

The good performance of ChCl–Gly is consistent with studies reporting choline chloride and glycerol as a versatile, thermally stable, and tunable combination for the extraction of phenolic compounds and flavonoids from plant matrices [39–41]. In naturally acidic matrices, subtle differences in polarity between the solvent and target metabolites, combined with a high hydrogen bond donor/acceptor capacity, tend to maximize the recovery of bioactive compounds [42]. Thus, ChCl–Gly was selected as the NADES for the subsequent optimization steps.

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Bioactive characterization of the extracts

The Box–Behnken design assessed the effects of liquid-to-solid ratio, temperature, and water content in the NADES on the extraction of TFC and TC. The results are shown in [Table 1](#).

TFC ranged from 5.9 to 8.3 mg QE/g, while TC varied from 46.0 to 146.9 mg β -carotene/100 g, evidencing a strong influence of processing conditions. In general, the best responses were associated with a liquid-to-solid ratio of 30 mL/g, suggesting that a higher solvent volume reduces the concentration gradient between the matrix and the extracting medium and favors mass transfer, in agreement with reports for other pigment- and phenolic-rich matrices [43-45].

A water content of 25 % in the NADES provided the best overall performance, indicating a balance between viscosity and polarity. Small additions of water reduce viscosity, increase molecular mobility, and unblock interaction sites in the NADES, whereas excess water disrupts the supramolecular hydrogen-bond network, decreasing solvation capacity [42,43]. When the water fraction exceeds thresholds around 50 %, the eutectic structure is partially destroyed, impairing extraction efficiency [23,43].

Temperature emerged as the most critical variable, with significant main and interaction effects. Intermediate conditions yielded higher TFC and TC without compromising thermal stability, a result consistent with studies on *Butia* spp. pulps and other tropical fruits rich in phenolic compounds and carotenoids [45,46]. Based on these findings, temperature was kept as a key variable in the subsequent design, whereas liquid-to-solid ratio (30 mL/g) and NADES water content (25 %) were fixed at the most promising conditions.

Based on the Box–Behnken design, the 3^2 factorial design evaluated the influence of temperature and extraction time on TFC, TPC, TC, AA, and antioxidant activity (DPPH, ABTS, FRAP), comparing the NADES (ChCl–Gly) with 50 % ethanol (control). The results are presented in [Table 2](#).

Overall, the NADES/UAE system showed superior or at least comparable performance to 50 % ethanol in all responses. For TPC, values ranged from 123.0 to 166.5 mg GAE/g with NADES, compared with 113.7 mg GAE/g for ethanol. For TFC, NADES extracts presented 5.2–8.3 mg QE/g, whereas ethanol reached 4.7 mg QE/g. This increase is consistent with the proposed mechanism for hydrophilic NADES, in which extensive hydrogen-bond networks involving choline chloride and glycerol strongly interact with hydroxyl and carboxyl groups of polyphenols, enhancing solubility and reducing susceptibility to oxidation [46,49,50].

Ultrasound further enhances this effect: acoustic cavitation generates microjets and pressure gradients capable of disrupting cell walls, reducing particle size, and accelerating solvent diffusion into the matrix. When combined with a highly interactive eutectic medium, this physical disruption

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results in greater release and stabilization of intracellular compounds, allowing for high yields at moderate times and relatively low temperatures. Recent studies report similar behavior for different plant matrices, reinforcing the synergy between NADES and UAE in intensifying extraction processes [47,48].

TC ranged from 32.5 to 93.5 mg β -carotene/100 g in NADES extracts, reaching values up to about threefold higher than ethanol under certain conditions. The intermediate polarity and presence of glycerol confer to the NADES a capacity to solubilize and stabilize lipophilic pigments, forming microenvironments less susceptible to oxidation and isomerization, in line with recent data on carotenoid stability in eutectic systems [16,18,32,45]. Ultrasonic cavitation, in turn, facilitates the disaggregation of lipid structures in the mesocarp, increasing pigment availability [50].

For AA, values ranged from 4.3 to 5.5 mg/100 g, slightly higher than ethanol (4.2 mg/100 g). Despite the highly unstable nature of AA, the NADES appears to act as a protective medium, reducing oxygen diffusion and stabilizing the molecule through intermolecular interactions. There is evidence that ChCl–Gly systems form dense, low-volatility networks capable of mitigating the degradation of thermolabile compounds [13,49,50].

Antioxidant activity corroborated the extraction efficiency of the NADES/UAE system. Values ranged from 108.5 to 114.6 mg TE/g (DPPH), 124.8 to 141.5 mg TE/g (ABTS), and 988.6 to 1013.5 mg TE/g (FRAP), whereas the ethanolic extract showed 103.5, 137.1, and 742.5 mg TE/g, respectively. The higher FRAP response indicates a strong reducing capacity, reflecting the extraction of metabolites with high electron-donating potential, while DPPH and ABTS tests indicate good radical scavenging activity toward both hydrophilic and lipophilic species. Studies with Brazilian native fruits and other plant matrices report a direct association between total phenolic content and hydrophilic antioxidant capacity [6,35]. In addition, superior performance of NADES compared with conventional solvents has been previously reported in extraction systems [13,21], likely due to the physicochemical properties of glycerol-based eutectic media [49].

The combined influence of temperature and time suggests that intermediate conditions (≈ 50 – 60 °C) maximize phenolics and carotenoids, whereas excessively long extraction times do not promote additional gains and may even favor thermal degradation. This pattern is consistent with optimization principles described for multivariate systems [26] and with thermal behavior reported for pigment-rich fruit matrices [35,45].

Taken together, the data demonstrate that the ChCl–Gly NADES/UAE system has a high extraction capacity and overall performance superior to 50 % ethanol, while preserving the “green”

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character of the process and opening possibilities for application in food, nutraceutical, and cosmetic ingredients based on native resources.

Optimization of the extraction process

Based on the results of the 3^2 factorial design, the quadratic models fitted for TFC, TPC, ABTS, and TC are presented in **Table 3**, together with significant coefficients, adjusted R^2 , and lack-of-fit p-values.

The analysis revealed significant linear and quadratic effects of temperature (X_1) and time (X_2) on all responses, with excellent goodness of fit for TPC and TC (adjusted $R^2 \geq 0.94$). From the models, the desirability function was applied, both individually and globally, to simultaneously maximize TFC, TPC, ABTS, and TC.

Keeping the liquid-to-solid ratio (30 mL/g) and water content in the NADES (25 %) constant, the maximum global desirability was obtained at 41 °C and 60 min, which was adopted as the optimal process condition. For the individual responses, the optimal conditions were close, but not identical: TFC was maximized at 41 °C/60 min; ABTS at 48 °C/60 min; TPC at 49 °C/37.5 min; and TC at 30 °C/52.5 min, indicating greater thermal sensitivity of carotenoids compared with phenolics.

Table 4 shows the comparison between predicted and experimental values for each response optimized individually and for the global response.

Differences between predicted and observed values were below 5 % for TFC, TPC, and ABTS, confirming the robustness of the models and the adequacy of the desirability-based approach. For TC, the experimental values exceeded the upper limit of the predicted confidence interval, suggesting additional synergistic effects not fully captured by the model, possibly related to carotenoid stability and matrix interactions previously reported for fruit systems [45], considering analytical principles described by Rodrigues-Amaya [32].

The response surface plots and contour maps shown in **Fig. 1** illustrate the simultaneous influence of temperature and time on TFC, TPC, ABTS, and TC, reinforcing the existence of compromise regions between hydrophilic and lipophilic fractions.

The proximity between the optimal conditions for TPC and ABTS indicates a strong association between TPC and hydrophilic antioxidant potential, a phenomenon widely described in plant matrices [6,8,35]. In contrast, the distinct optimal condition for TC highlights the need for trade-offs when multiple responses are optimized simultaneously, especially when they involve compounds with different solubility and stability profiles [26,45].

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Overall, the results validate the use of response surface methodology associated with a desirability function as an effective tool to design and predict the performance of the NADES/UAE system for extracting bioactive compounds from *B. eriospatha*.

Stability of the optimized extracts

The thermal stability of the optimized extract (TPC-optimized conditions) was evaluated at 60, 70, and 80 °C for up to 120 min. TPC and TPC/TPC₀ values are presented in Table 5.

Variations in TPC were below 2 % relative to the initial value (166.1 mg GAE/g), with no significant differences among times and temperatures ($p > 0.05$). The TPC/TPC₀ parameter remained very close to 1 under all conditions, indicating that the extract is highly stable to heating within the evaluated range. This behavior contrasts with that observed in aqueous or ethanolic systems, where substantial phenolic losses are often reported above 60 °C [35].

The kinetic and thermodynamic parameters fitted to the apparent first-order model are shown in Table 6.

The k values were on the order of 10^{-3} h^{-1} , resulting in high half-life ($t_{1/2}$) values ($\approx 59\text{--}144 \text{ h}$), which confirm extremely slow degradation. The activation energy ($E_a \approx 19.7 \text{ kJ/mol}$) and enthalpy (ΔH) values indicate a mildly endothermic process, while the Gibbs free energy ($\Delta G = 118\text{--}124 \text{ kJ/mol}$) suggests non-spontaneous degradation under the tested conditions [35,36]. The negative entropy ($\Delta S < 0$) indicates formation of a more ordered transition state compared with the reactants, consistent with the structured nature of NADES systems previously described [50], which may limit oxygen diffusion and preserve antioxidants under stress conditions [37,46].

These results support the role of NADES not only as extraction solvents but also as protective matrices for bioactive compounds. The high stability up to 80 °C indicates that the extract can be subjected to moderate thermal operations (e.g. slow pasteurization and high-temperature short-time (HTST)) with minimal phenolic losses, in contrast to reports for aqueous/ethanolic extracts [35,36]. This broadens the technological applicability of NADES-based extract in juices, functional beverages, and other thermally processed foods.

Photostability was evaluated at 24 ± 2 °C for up to 96 h. TPC and TPC/TPC₀ values are shown in Table 7.

TPC remained between 163.4 and 164.6 mg GAE/g, with variations below 1 % relative to the initial concentration and no significant differences among times ($p > 0.05$). The fact that TPC/TPC₀ remained close to 1 indicates the absence of relevant photoinduced degradation under the tested conditions. This behavior may be related to the low volatility and high hydrogen-bond density of the

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ChCl–Gly matrix, which reduces oxygen diffusion and limits the propagation of photo-oxidative reactions [37,49,50].

Studies with plant extracts stabilized in NADES report similar phenomena, with preservation of phenolic content after prolonged light exposure, particularly in viscous and structured systems [37,44,49]. Although the total TPC remains constant, intraclass rearrangements (e.g. interconversion between glycosylated and aglycone forms) may occur without affecting the overall sum, as suggested for phenolic extracts subjected to thermal and light stress [35,36].

Even so, the combined thermal and photochemical stability observed indicates that the ChCl–Gly NADES/UAE system yields extracts with high robustness under typical processing and storage conditions, reinforcing its suitability for the development of functional ingredients and value-added products based on *B. eriospatha*. The protective action of the eutectic solvent, associated with high extraction yields, consolidates this approach as a promising and sustainable alternative for the valorisation of native fruits.

CONCLUSIONS

The application of NADES for the extraction of bioactive compounds from *Butia eriospatha* demonstrated high technological and environmental potential, representing a green alternative to conventional organic solvents. The ChCl–Gly NADES system combined with UAE promoted higher recovery of phenolic compounds, flavonoids, carotenoids, ascorbic acid, and antioxidant activity than 50 % ethanol, confirming the synergy between the eutectic medium and ultrasound. Response surface modeling identified the optimal temperature and extraction time, with close agreement between predicted and experimental values. Stability assays showed high retention of total phenolic content under thermal and photochemical stress, indicating that ChCl–Gly NADES can also act as a stabilizing medium. Overall, the ChCl–Gly/UAE system represents an efficient, safe, and sustainable strategy for the valorization of *B. eriospatha*, with potential applications in food, pharmaceutical, and cosmetic products. Future studies should evaluate detailed bioactive profiles, real food matrices, and industrial processing conditions.

ACKNOWLEDGEMENTS

The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES), the State University of Ponta Grossa (UEPG), the Graduate Program in Food Science and Technology (PPGCTA), and the Federal Institute of Santa Catarina – Canoinhas Campus for institutional and infrastructural support. AI-assisted tools (ChatGPT, OpenAI) were used exclusively

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for English language revision, grammar correction, and textual clarity. All scientific content, data analysis, interpretation, and final decisions were performed solely by the authors.

FUNDING

This research received institutional support from the Federal Institute of Santa Catarina (IFSC – Canoinhas Campus), the State University of Ponta Grossa (UEPG), and the Coordination for the Improvement of Higher Education Personnel (CAPES).

ETHICAL APPROVAL

Ethical approval was not required, as this study involved only plant materials and did not include experimentation with humans or animals.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

D. A. Machado conceived and designed the study, prepared the samples, performed laboratory and statistical analyses, contributed to experimental planning, organized and interpreted the data, prepared tables and figures, drafted the first version of the manuscript, and completed the final revision for submission. R. D. S. Salem supervised the entire study, contributed to experimental design, data interpretation, and statistical analysis, secured and managed the financial and laboratory resources, and critically reviewed the manuscript. Both authors approved the final version of the manuscript.

ORCID ID

D. A. Machado <https://orcid.org/0009-0007-1061-0231>

R. D. S. Salem <https://orcid.org/0000-0001-6211-9697>

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Table 1. Concentration of bioactive compounds in *Butia eriospatha* extracts obtained using a Box-Behnken design

Run	Ratio/(mL/g)	Temperature/°C	w(water in NADES)/%	w(TFC as QE)/(mg/g)	w(TC as β -carotene)/(mg/100 g)
1	20	30	25	(7.3 $\pm$ 0.7) ^{abcde}	(74.7 $\pm$ 0.6) ^g
2	40	30	25	(6.7 $\pm$ 0.5) ^{cdef}	(79.7 $\pm$ 1.5) ^f
3	20	50	25	(6.8 $\pm$ 0.2) ^{bcdef}	(56.2 $\pm$ 1.1) ^k
4	40	50	25	(8.2 $\pm$ 0.5) ^a	(95.0 $\pm$ 2.3) ^d
5	20	40	20	(7.8 $\pm$ 0.2) ^{ab}	(56.8 $\pm$ 1.0) ^k
6	40	40	20	(6.5 $\pm$ 0.4) ^{def}	(46.0 $\pm$ 1.9) ^l
7	20	40	30	(6.4 $\pm$ 0.1) ^{ef}	(82.8 $\pm$ 1.5) ^e
8	40	40	30	(7.8 $\pm$ 0.2) ^{ab}	(59.4 $\pm$ 0.7) ^j
9	30	30	20	(7.4 $\pm$ 0.3) ^{abcd}	(146.9 $\pm$ 3.7) ^a
10	30	50	20	(6.9 $\pm$ 0.2) ^{bcdef}	(70.5 $\pm$ 1.3) ^h
11	30	30	30	(7.1 $\pm$ 0.4) ^{bcde}	(62.5 $\pm$ 3.40) ⁱ
12	30	50	30	(6.0 $\pm$ 0.2) ^f	(76.2 $\pm$ 3.4) ^g
13	30	40	25	(8.3 $\pm$ 0.4) ^a	(110.3 $\pm$ 0.7) ^b
14	30	40	25	(6.6 $\pm$ 0.3) ^{cdef}	(111.1 $\pm$ 2.3) ^b
15	30	40	25	(7.5 $\pm$ 0.1) ^{abc}	(111.9 $\pm$ 0.7) ^b
Ethanol (ϕ =50 %)	30	40	25	(4.4 $\pm$ 0.4) ^g	(97.7 $\pm$ 0.5) ^c

Values are expressed as mean $\pm$ S.D. (dry basis). Extraction time was fixed at 60 min for all runs. TFC=total flavonoid content, TC=total carotenoids, QE=quercetin equivalents. Different letters within the same column indicate significant differences ($p\leq 0.05$)

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Table 2. Bioactive compound contents and antioxidant activities in *Butia eriospatha* extracts obtained with NADES and ethanol

Run	Temperature/ °C	t(extraction)/ min	w(TFC as QE)/(mg/g)	w(TPC as GAE)/(mg/g)	w(DPPH as TE)/(mg/g)	w(ABTS as TE)/(mg/g)	w(FRAP as TE)/(mg/g)	w(TC as β - carotene)/(mg/ 100 g)	w(AA)/(mg/ 100 g)
1	30	30	(6.8±0.2) ^b	(128.4±3.1) ^c	(108.5±1.5) ^{ab}	(127.9±1.0) ^b	(1005.2±10.1) ^{ab}	(45.3±1.0) ^d	(5.5±0.9) ^a
2	30	45	(8.2±0.4) ^a	(131.1±2.5) ^c	(108.5±1.1) ^{ab}	(124.8±3.3) ^b	(993.8±7.8) ^b	(85.6±3.6) ^b	(5.5±0.9) ^a
3	30	60	(8.3±0.6) ^a	(129.8±4.4) ^c	(112.2±2.0) ^a	(127.8±6.9) ^b	(1004.6±4.6) ^{ab}	(93.5±4.7) ^a	(5.0±0.9) ^{ab}
4	45	30	(6.9±0.3) ^{ab}	(155.1±2.3) ^b	(113.3±1.2) ^a	(126.6±3.3) ^b	(1011.8±9.5) ^a	(35.2±2.8) ^{ef}	(5.0±0.9) ^{ab}
5	45	45	(6.9±0.4) ^{ab}	(166.5±5.5) ^a	(112.6±2.0) ^a	(132.4±3.6) ^{ab}	(998.8±13.0) ^{ab}	(33.4±1.2) ^f	(4.4±0.1) ^{ab}
6	45	60	(8.3±0.4) ^a	(124.2±3.0) ^{cd}	(111.3±1.2) ^a	(141.5±1.0) ^a	(1013.5±4.3) ^a	(51.0±4.2) ^c	(5.0±0.9) ^{ab}
7	60	30	(5.2±0.6) ^c	(123.0±4.2) ^{cd}	(114.6±2.9) ^a	(138.5±1.1) ^a	(997.2±10.5) ^b	(32.5±0.6) ^f	(4.3±0.3) ^{ab}
8	60	45	(6.9±0.4) ^{ab}	(123.0±3.2) ^{cd}	(112.9±4.4) ^a	(128.7±2.1) ^b	(1001.6±3.7) ^b	(35.5±3.1) ^{ef}	(5.0±0.9) ^{ab}
9	60	60	(7.6±0.3) ^a	(129.1±3.5) ^c	(111.3±3.6) ^a	(131.5±4.8) ^{ab}	(988.7±9.1) ^b	(39.2±2.0) ^e	(4.3±0.3) ^{ab}
Ethanol (φ =50 %)	45	45	(4.7±0.5) ^c	(113.7±3.0) ^e	(103.5±0.8) ^c	(137.1±2.4) ^a	(742.5±13.3) ^c	(33.0±0.5) ^f	(4.2±0.3) ^b

Values are expressed as mean±S.D. (dry basis). Ratio was fixed at 30 mL/g, and water in NADES at w=25 %. TFC=total flavonoid content, TPC=total phenolic content, TC=total carotenoids, AA=ascorbic acid, QE=quercetin equivalents, GAE=gallic acid equivalents, TE= Trolox equivalents. Different letters within the same column indicate significant differences ($p \leq 0.05$)

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Table 3. Significant regression coefficients of the fitted models for the dependent variables

Dependent variable	Factors	Coefficient	Standard error	t-value	p-value	95 % CI lower	95 % CI upper
w(TFC as QE)/(mg/g)	Constant	6.882	0.252	27.305	<0.001	6.353	7.412
	X ₁ (L)	-0.648	0.178	-3.641	0.002	-1.023	-0.275
	X ₂ (L)	0.693	0.178	3.890	0.001	0.319	1.068
	X ₂ (Q)	0.742	0.309	2.404	0.027	0.094	1.391
	Adjusted R ²	0.818					
	p-value (lack of fit)	0.412					
w(TPC as GAE)/(mg/g)	Constant	134.461	0.700	192.024	<0.001	132.990	135.933
	X ₁ (L)	-2.349	0.858	-2.739	0.014	-4.151	-0.547
	X ₁ (Q)	10.596	0.743	14.267	<0.001	9.036	12.156
	X ₂ (L)	-3.882	0.858	-4.355	<0.001	-5.691	-2.073
	X ₂ (Q)	4.289	0.743	5.775	<0.001	2.729	5.850
	Adjusted R ²	0.943					
w(ABTS as TE)/(mg/g)	Constant	132.418	2.049	64.628	<0.001	128.113	136.722
	X ₁ (Q)	-5.694	2.509	-2.269	0.036	-10.967	-0.422
	X ₂ (L)	7.440	1.449	5.135	<0.001	4.396	10.484
	Adjusted R ²	0.667					
	p-value (lack of fit)	0.119					
w(TC as β -carotene)/(mg/100 g)	Constant	50.124	0.563	89.014	<0.001	48.941	51.307
	X ₁ (L)	-19.531	0.690	-28.320	<0.001	-20.980	-18.082
	X ₁ (Q)	-7.956	0.597	-12.889	<0.001	-8.954	-6.435
	X ₂ (L)	11.747	0.690	17.088	<0.001	10.336	13.234
	Adjusted R ²	0.988					
	p-value (lack of fit)	0.167					

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TFC=total flavonoid content, TPC=total phenolic content, TC=total carotenoids, QE=quercetin equivalents, GAE=gallic acid equivalents, TE= Trolox equivalents. X_1 =temperature (°C), X_2 =extraction time (min), L=linear effect, Q=quadratic effect, CI=confidence interval. Only coefficients with $p<0.05$ were retained in the models

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Table 4. Comparison between predicted and experimental values of bioactive compounds and antioxidant activity in optimized *Butia eriospatha* extracts, considering individual and global responses

Response	w(TFC as QE)/(mg/g)	w(TPC as GAE)/(mg/g)	w(ABTS as TE)/(mg/g)	w(TC as β -carotene)/(mg/100 g)
Predicted (individual)	8.4	167.6	141.5	93.6
95 % CI lower	7.9	163.8	137.3	90.6
95 % CI upper	8.9	171.3	145.8	96.6
Experimental (individual)	8.3 $\pm$ 0.1	166.1 $\pm$ 0.8	139.9 $\pm$ 2.1	124.3 $\pm$ 2.5
Predicted (global)	8.4	125.3	138.4	65.6
95 % CI lower	7.9	121.5	134.7	62.5
95 % CI upper	8.9	129.2	142.2	68.7
Experimental (global)	8.1 $\pm$ 0.1	129.0 $\pm$ 1.6	135.0 $\pm$ 1.6	63.8 $\pm$ 1.5

Experimental values are expressed as mean $\pm$ S.D. (dry basis). Individual=optimization of each response separately, global=simultaneous optimization of all responses. TFC=total flavonoid content, TPC=total phenolic content, TC=total carotenoids, QE=quercetin equivalents, GAE=gallic acid equivalents, TE= Trolox equivalents, CI=confidence interval

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Table 5. Total phenolic content (TPC) of the extract obtained under optimal extraction conditions after heating under different temperatures and exposure times

Temperature/°C	t/min	w(TPC as GAE)/(mg/g)	TPC/TPC ₀
60	0	(166.1±0.8) ^a	1.000
60	15	(165.4±1.4) ^a	0.996
60	30	(162.1±6.0) ^a	0.976
60	60	(165.3±1.0) ^a	0.996
60	120	(166.2±1.8) ^a	1.001
70	0	(166.1±0.8) ^a	1.000
70	15	(167.8±0.9) ^a	1.010
70	30	(166.8±0.6) ^a	1.004
70	60	(167.2±1.0) ^a	1.007
70	120	(166.1±1.9) ^a	1.000
80	0	(166.1±0.8) ^a	1.000
80	15	(166.7±1.4) ^a	1.004
80	30	(167.1±1.5) ^a	1.006
80	60	(164.3±2.3) ^a	0.989
80	120	(163.7±1.9) ^a	0.986

Values are expressed as mean±S.D. (dry basis). TPC=total phenolic content, GAE=gallic acid equivalents. The phenolic content at time zero (t_0) corresponds to the mean value obtained at the optimal extraction conditions, used as the initial reference for calculating TPC/TPC₀. Different letters in the same column indicate significant differences among experiments ($p \leq 0.05$)

Table 6. Kinetic and thermodynamic parameters of thermal degradation of total phenolic content between 60 and 80 °C

Temperature/°C	T/K	k/h ⁻¹	$t_{1/2}$ /h	E_a /(kJ/mol)	ΔH /(kJ/mol)	ΔG /(kJ/mol)	ΔS /(J/(mol·K))
60	333.15	0.0078	88.9	19.7	16.9	118.0	-303.5
70	343.15	0.0048	144.0	19.7	16.8	123.0	-309.5
80	353.15	0.0118	58.7	19.7	16.7	124.1	-303.9

k =degradation rate constant, $t_{1/2}$ =half-life, E_a =activation energy, ΔH =enthalpy change, ΔG =Gibbs free energy, ΔS =entropy change

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Table 7. Total phenolic content (TPC) of the extract obtained under optimal extraction conditions after light exposure at different storage times

t/h	w(TPC as GAE)/(mg/g)	TPC/TPC ₀
0	(163.9±1.6) ^a	1.000
3	(163.5±1.5) ^a	0.998
19	(163.4±1.1) ^a	0.997
24	(164.6±0.6) ^a	1.004
48	(164.6±0.7) ^a	1.004
72	(164.4±0.9) ^a	1.003
96	(164.0±1.6) ^a	1.001

Values are expressed as mean±S.D. (dry basis). TPC=total phenolic content, GAE=gallic acid equivalents. Extracts were stored under light exposure at 24 °C. Different letters within the same column indicate significant differences ($p \leq 0.05$)

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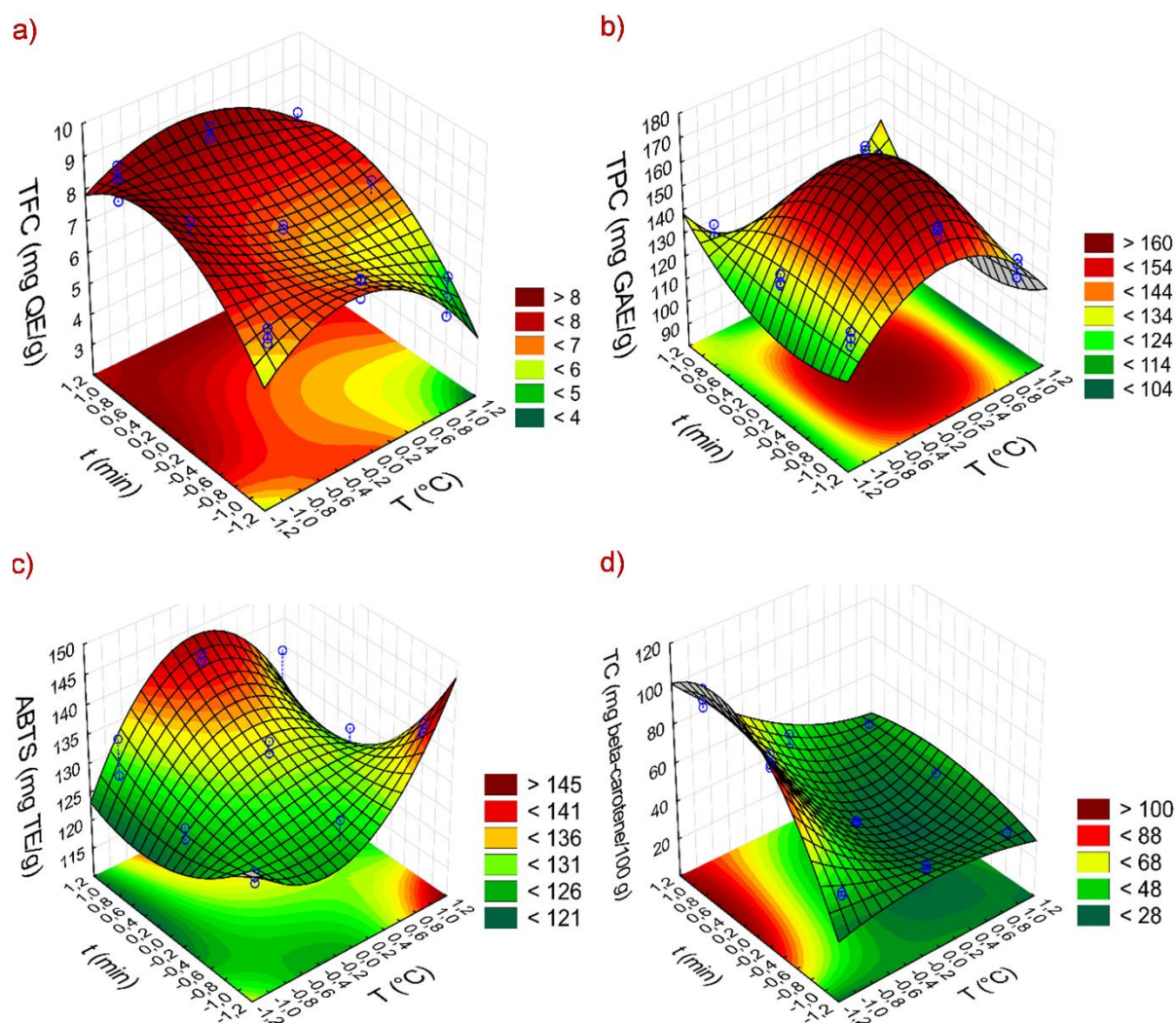


Fig. 1. Response surface plots and contour maps describing the effects of temperature and extraction time on bioactive compounds and antioxidant activity in *Butia eriospatha* extracts obtained with NADES: a) TFC=total flavonoid content, b) TPC=total phenolic content, c) ABTS radical scavenging activity, d) TC=total carotenoids. QE=quercetin equivalents, GAE=gallic acid equivalents, TE= Trolox equivalents