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# Ultrasound-assisted extraction optimization of polyphenols from jambolão (*Syzygium cumini*) fruit and their *in vitro* antioxidant capacity

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**Abstract**
*Syzygium cumini* fruit is a rich source of excellent bioactive compounds, including polyphenols and flavonoids with high antioxidant potential and pharmacological properties. Yet, this plant's improvement of the extraction yield of total polyphenol content (TPC) and antioxidant potential has never been inspected in depth. The objective of this study was to use a $2^3$ factorial design with three repetitions of the central point to investigate the influence of combined parameters such as ultrasonication, solvent concentration, time, and temperature and to employ RSM to optimize the extraction of phenolic compounds from jambolão at three ripeness stages (unripe, mid-ripe and ripe) and maximize their antioxidant activity. The best conditions of the variables for increasing the yield, total phenolic and antioxidant capacity were obtained with 30 % ethanol for 68.4 min, at 39.2 °C for unripe jambolão, with 30 % ethanol for 30 min, at 47.2 °C for mid-ripe and with 90 % ethanol for 30 min, at 60 °C for ripe fruit. The yield, TPC, DPPH, ABTS and FRAP decreased during fruit ripeness. For such optimized conditions of ultrasound-assisted extraction, the highest yield and TPC were experimentally determined for the unripe stage at 9.01 % and 549.16 mg GAE/100g, respectively, with an antioxidant capacity of 45.19 mMol TE/100 g DPPH, 68.20 mMol TE/100 g ABTS and 72.30 mMol TE/100 g FRAP and agreed with the obtained model values. This study showed that it is possible to obtain bioactive-rich extracts from jambolão using experimental design to improve the extraction process.

**Keywords**: Bioactive compounds, ethanol, FRAP, DPPH, ABTS, response surface methodology.

**Graphical Abstract**




The obtaining of bioactive-rich extracts from jambolão can be optimized using the response surface methodology

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## 1. Introduction

Recent developments in the relationship between health and food have led to a renewed interest in natural compounds with antioxidant potential. An antioxidant is a bioactive compound that can inhibit free radicals and oxidation substrate (Ismandari et al., 2020). Also, the inclusion of fruit extract as a colorant and preservative in food is of interest due to increasing reports on the toxicity and adverse effect of synthetic antioxidants and colorants on humans (Azima et al., 2017). Therefore, selecting species/varieties with high contents of bioactive compounds and the ideal harvesting can result in higher yield and content of phytochemical components as well as potential fruit antioxidant capacity (Al-Yafeai et al., 2018; Mahindrakar & Rathod, 2020).

*Syzygium cumini* fruit, popularly known as jambolão, jambolan, black plum, Java plum or jamun, is a plant from the Myrtaceae family originated in tropical Asia, specifically India. Its synonym names are Eugenia cumini and Eugenia jambolana. Jambolão fruits are small, 2–3 cm long, oval in shape with green color when is unripe and a purple-red to black color when ripe, containing a fleshy pink or almost white pulp with an astringent taste. The most important secondary metabolites that have been reported in *Syzygium cumini* species are the phenolic compounds, gallic acid, kaempferol, ellagic acid derivatives, tannins, glycosylated flavonoids, catechin, quercetin, omega-3, omega-6, and omega9 FAs (Bhadange et al., 2022; Faria et al., 2011; Mahindrakar & Rathod, 2020; Rydlewski et al., 2017; Veber et al., 2015).

It is now known that the antioxidant potential is correlated with the high concentration of several phytochemical substances known as bioactive compounds, which, when ingested in significant quantities, are responsible for avoiding cellular aging, promoting human health, and assisting in the reduction of the risk of the incidence of various diseases such as diabetes, cancer, and coronary and neurodegenerative diseases (Ismandari et al., 2020; Mahindrakar & Rathod, 2020).

Therefore, finding the most efficient extraction from natural matrices is a significant challenge for food, pharmaceutical, and cosmetic researchers. The extraction efficiency of bioactive components from plant materials is affected by distinct factors, such as extraction techniques, solvents, time, temperature, solvent-to-plant material ratio and many others. To obtain a higher yield of phytochemical-rich products at a low cost, previous bioactive compounds researchers have applied appropriate strategies for optimizing extraction processes (Cruz et al., 2021; Nekkaa et al., 2021).

From previous studies, the modification of various extraction conditions, such as the applied solvent-to-solid ratio, the temperature, the extraction time, and the solvent composition, among other parameters, significantly influenced the extraction of natural compounds. Time and temperature are essential to minimize the energy costs of the process. In addition, the use of temperature increases the solubility of solute and the diffusion coefficient. Nevertheless, an intense increase in temperature can inevitably cause the loss of phenolic compounds. The polarity plays an essential role in extracting phenolic compounds from plant material. The mixtures between water and alcohol are among the most efficient solvents, and ethanol has advantages regarding its safety for human consumption (Bhadange et al., 2022; Goltz et al., 2018; Žlabur et al., 2020).

Traditional methods, including heat flux solvent extraction, need a lot of organic solvents, energy, and time, which has sparked interest in recent technologies known as “green” or “clean” advanced technologies. The application of ultrasonic waves for the extraction of high- cost medicinally important phytoconstituents is a good alternative due to the cavitational, mechanical and thermal effects that occur in the solvent by the transit of ultrasonic waves, which can enhance the mass transfer by disrupting cell walls and reducing particle size (Bhadange et al., 2022; Goltz et al., 2018; Žlabur et al., 2020). Goltz et al. (2018) verified that ultrasound-assisted extraction using 70 % (v/v) ethanol and 50 % (v/v) acetone, respectively, increased the total phenolic content from Macela (*Achyrolcine satureioides*) by 6.1-fold and the antioxidant capacity by 3.4-fold, compared with conventional extraction.

The literature on the solvent concentration, time, and temperature effect on the extraction of phenolic compounds in jambolão fruits at different ripeness stages is limited and, to some extent, contradictory, presenting no consensus on the best way to extract this fruit. Therefore, this study aims to investigate the influence of combined parameters such as ultrasonication, solvent concentration, time, and temperature and to employ RSM to optimize the

extraction of phenolic compounds from jambolão at three ripeness stages (unripe, mid-ripe and ripe) and maximize their antioxidant activity.

## 2. Material and Methods

### 2.1 Sample preparation

The jambolão (unripe, mid-ripe and ripe, **Fig. 1**) fruits (2 kg each) were collected from trees in Pelotas, Rio Grande do Sul, Brazil (latitude: 31°45′54“S; longitude: 52°28′00“W). After being collected, the intact fruits, free of damage, were selected, manually and visually divided into three ripening stages, 1) unripe: fully green fruits; 2) mid-ripe: partially ripe fruits and 3) ripe: fully ripe fruits, ranging from green/ red to purple-black, washed, blanched at 95 °C for 2 min and dried at room temperature (20 ± 3 °C). They were freeze-dried for 48 h at 300 μmHg followed by grinding (**Fig. 2a**, **2b** and **2c**), packaging, and storing at –20 °C before the extraction experiments.



**Fig. 1** Jambolão fruits at three ripening stages.

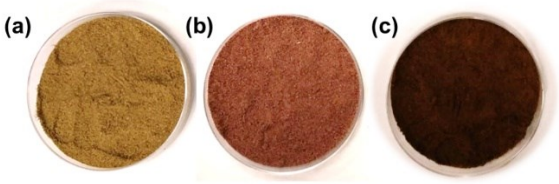


**Fig. 2** Lyophilized powdered jambolão fruits unripe (a), mid-ripe (b) and ripe (c).

### 2.1 Fruit extracts experimental design

The lyophilized powdered fruit extraction followed a $2^3$ factorial design with 8 combinations and three repetitions of the central point comprising a total of 11 runs for each maturity stage (ripe **Table 1**, mid-ripe **Table 2**, and unripe **Table 3**) of jambolão. About 1 g of sample and 10 mL of solvent acidified with 0.1 % 12 N HCl were mixed in 15 mL centrifuge tubes in triplicate. The mixtures were immersed in a 40 kHz (± 6 %) and 100 W ultrasonic water bath (Fisher Scientific, FS30D, Mexico) for a predetermined time. The tested variables and ranges were at the time ($X_1$, 30-90 min), ethanol in hydroalcoholic mixtures ($X_2$, 20–60 %) and temperature ($X_3$, 30-90 °C). The obtained extracts were centrifuged at 1000 rpm (Sorvall BIOS 16, ThermoFisher Scientific, Tewksbury, MA, USA) for 20 min, and the supernatant was recovered and filtrated. The solvent was then evaporated at 35 °C, lyophilized, and the extracted residue gravimetrically quantified (**Fig. 3, 3b** and **3c**). Afterward, it was redissolved in water before analysis (**Fig. 3d, 3e** and **3f**).

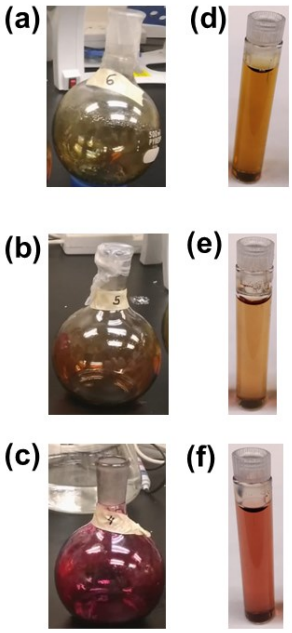


**Fig. 3** Dried (left) and redissolved (right) extracts from jambolão fruits at three ripening stages: unripe (a, d), mid-ripe (d, e) and ripe (c, f)

### 2.4 Total phenolic content (TPC) and antioxidant capacity using DPPH and ABTS radical scavenging assays and ferric reducing antioxidant power (FRAP)

TPC of the extracts was determined using the Folin–Ciocalteu colorimetric method described by Singleton and Rossi (1965), with slight modifications according to Avila et al. (2022). The absorbance was measured at 750 nm in an Epoch microplate spectrophotometer (Synergy-BIOTEK, Winooski, VT,

USA). The results were expressed as mg of gallic acid equivalents (GAE)/100 g of the jambolão. The DPPH radical scavenging capacity was determined according to the method described by Brand-Williams, Cuvelier, and Berset (1995), ABTS radical scavenging capacity according to Re et al. (1999) and the ferric reducing antioxidant power (FRAP) assay based on Benzie and Strain (1996), with adjustments according to Avila et al. (2022) to 96-well microplates. The absorbance of DPPH, ABTS, and FRAP assays was measured at 515, 750 and 593 nm after 30 min of the solution resting in the dark, respectively. The antioxidant capacity was expressed in mMol Trolox equivalent (TE)/100 g of jambolão.

### 2.5 Response surface modeling and statistical optimization of extraction conditions

The main, secondary, and tertiary effects of the variables were assessed by the Response Surface Methodology (RSM), and the quality of the models was evaluated by the variability rate explained by the models ($R^2$) and by the adjusted determination coefficient ($R^2_{adj}$). The experimental data were adjusted to a full cubic model (**Eq. 1**), where E(y) is the predicted function, $b_1$, $b_2$ and $b_3$ are the isolated effect coefficients, $b_{12}$, $b_{13}$, and $b_{23}$ are the quadratic coefficients (binary interactions), $b_{123}$ is the ternary interaction regression coefficient and e $X_1$, $X_2$ and $X_3$ are the variables (ethanol concentration, time, and temperature) utilized for the extractions.

$$E(y) = b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 \quad \textbf{Eq. 1}$$

The variables were optimized using the desirability function, rendering 60 iterations to maximize the yield, TPC, the DPPH, ABTS and FRAP antioxidant assay. Finally, the validation of the optimal point starting from the extraction with the optimized variables and the absolute error was calculated with the model-predicted values.

### 2.6 Statistical Analysis

The results were presented as mean ± standard deviation of triplicate determinations to yield and octuplicate for TPC, DPPH, ABTS and FRAP. The data were analyzed by ANOVA using Statistica software (version 10, StatSoft Inc., Tulsa, OK, USA). Duncan's multiple range tests were used to evaluate the significant differences among the means. The significance level was defined at $p < 0.05$. In addition, Pearson's correlation coefficient (*r*) was determined between phenolic compounds and antioxidant capacity.

## 3 Results and Discussion

The extraction method was applied to the samples with different ripening stages. The responses (dependent variables; Y1 to Y5) observed for the 11 batches of jambolão fruits are shown in **Table 1** for unripe, **Table 2** for mid-ripe and **Table 3** for ripe stages, respectively. The measured yield values (Y1) varied from 5.47 % (batch number 2 to the ripe stage) to 8.83 % (batch number 3 to the unripe stage). Our results are also in agreement with the study performed by Liu et al. (2018).

The highest values for yield were observed at the unripe stage at 20.0% ethanol for 30 min at 90°C. The multiple regression analysis of yield values showed that the model was significant ($p< 0.05$), did not present a lack of fit ($p = 0.36$ to unripe; $p=0.05$ to mid-ripe; $p=0.25$ to ripe), and it could explain 72.82 % for unripe, 68.19 % for mid-ripe and 72.25 % for ripe stage and of all variances in data ($R^2_{adj} = 0.70$; 0.54; 0.60 respectively). The linear regression coefficient of ethanol (X3) was negative and significant. The predicted model can be described by (**Eqs. 2**, **3** and **4**) in coded values. The results suggested that time and temperature had negligible effects on the yield of the extracts.

$$\text{Unripe } Y = 8.824 - 0.011\ X_3 \quad \textbf{Eq. 2}$$

$$\text{Mid-ripe } Y = 8.366 - 0.008\ X_1 - 0.012\ X_3 + 0.001\ X_1X_2X_3 \quad \textbf{Eq. 3}$$

$$\text{Ripe } Y = 6.248 - 0.016\ X_1 - 0.021\ X_3 + 0.001\ X_1X_3 \quad \textbf{Eq. 4}$$

The TPC content of the samples ranged significantly ($p < 0.05$) from 265.20 (batch number 3 to the ripe stage) to 543.15 (batch number 6 to the unripe stage) mg of gallic acid/100 g of jambolão. 60.0 % ethanol for 90 min at 30 °C was the best phenolic extraction combination. The model of phenolic extraction was significant ($p < 0.05$), did not present a lack of fit ($p = 0.12$ unripe; $p = 0.05$ to mid-ripe and ripe), and it could explain 73.38 % for unripe, 98.19 % for mid-ripe and 76.16 % for ripe stage and of all variances in data ($R^2_{adj} = 0.66$; 0.96; 0.67 respectively).

**Table 1** Independent and dependent variables for ultrasound-assisted extraction of polyphenols from unripe jambolão (*Sygium cumini*) fruit.

| Batch No | Time (min) | Ethanol (%) | Temperature (°C) | Yield (%) | TPC (mg GAE/100g) | DPPH (mmol TE/100g) | ABTS (mmol TE/100g) | FRAP (mmol TE/100g) |
|---|---|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $X_3$ | $Y_1$ | $Y_2$ | $Y_3$ | $Y_4$ | $Y_5$ |
| 1 | 30 (-1) | 20 (-1) | 30 (-1) | 8.49 ± 0.04$^{abc}$ | 513.61 ± 64.89$^{abc}$ | 36.85 ± 3.98$^{ef}$ | 75.17 ± 8.28$^{a}$ | 65.32 ± 8.01$^{ef}$ |
| 2 | 90 (1) | 20 (-1) | 30 (-1) | 8.54 ± 0.19$^{abc}$ | 489.13 ± 18.69$^{abcd}$ | 39.46 ± 5.55$^{cde}$ | 65.17 ± 5.11$^{bc}$ | 72.08 ± 9.42$^{de}$ |
| 3 | 30 (-1) | 20 (-1) | 90 (1) | 8.83 ± 0.08$^{a}$ | 378.88 ± 14.83$^{e}$ | 34.77 ± 2.74$^{f}$ | 54.39 ± 9.10$^{de}$ | 59.08 ± 6.10$^{f}$ |
| 4 | 90 (1) | 20 (-1) | 90 (1) | 8.64 ± 0.21$^{ab}$ | 446.86 ± 32.52$^{d}$ | 37.99 ± 3.84$^{def}$ | 52.88 ± 3.89$^{e}$ | 71.66 ± 8.39$^{de}$ |
| 5 | 30 (-1) | 60 (1) | 30 (-1) | 8.20 ± 0.21$^{bc}$ | 519.58 ± 78.45$^{ab}$ | 43.12 ± 2,25$^{bc}$ | 66.14 ± 6.45$^{bc}$ | 81.34 ± 9.76$^{bc}$ |
| 6 | 90 (1) | 60 (1) | 30 (-1) | 8.15 ± 0.19$^{c}$ | 543.15 ± 54.15$^{a}$ | 47.84 ± 1.86$^{a}$ | 55.76 ± 8.83$^{de}$ | 92.49 ± 2.31$^{a}$ |
| 7 | 30 (-1) | 60 (1) | 90 (1) | 8.37 ± 0.21$^{abc}$ | 460.94 ± 20.21$^{cd}$ | 40.73 ± 3.82$^{bcde}$ | 54.29 ± 8.59$^{de}$ | 63.40 ± 9.21$^{f}$ |
| 8 | 90 (1) | 60 (1) | 90 (1) | 8.07 ± 0.17$^{c}$ | 527.24 ± 65.70$^{ab}$ | 44.71 ± 4.86$^{ab}$ | 68.97 ± 5.54$^{b}$ | 86.05 ± 2.49$^{ab}$ |
| 9 | 60 (0) | 40 (0) | 60 (0) | 8.27 ± 0.36$^{bc}$ | 472.48 ± 68.76$^{bcd}$ | 42.53 ± 2.35$^{bc}$ | 64.06 ± 6.09$^{bc}$ | 75.43 ± 6.73$^{cd}$ |
| 10 | 60 (0) | 40 (0) | 60 (0) | 8.39 ± 0.04$^{abc}$ | 501.74 ± 10.65$^{abcd}$ | 41.73 ± 2.94$^{bcd}$ | 60.58 ± 3.55$^{cd}$ | 77.96 ± 7.33$^{cd}$ |
| 11 | 60 (0) | 40 (0) | 60 (0) | 8.45 ± 0.01$^{abc}$ | 477.66 ± 48.52$^{bcd}$ | 42.98 ± 3.93$^{bc}$ | 64.38 ± 5.73$^{bc}$ | 81.31 ± 2.67$^{bc}$ |

Averages followed by different letters on the same column indicate statistical differences according to Duncan's multiple range test ($p \leq 0.05$).

**Table 2** Independent and dependent variables for ultrasound-assisted extraction of polyphenols from mid-ripe jambolão (*Sygium cumini*) fruit.

| Batch No | Time (min) | Ethanol (%) | Temperature (°C) | Yield (%) | TPC (mg GAE/100g) | DPPH (mmol TE/100g) | ABTS (mmol TE/100g) | FRAP (mmol TE/100g) |
|---|---|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $X_3$ | $Y_1$ | $Y_2$ | $Y_3$ | $Y_4$ | $Y_5$ |
| 1 | 30 (-1) | 20 (-1) | 30 (-1) | 8.05 ± 0.14$^{a}$ | 388.42 ± 29.57$^{de}$ | 30.81 ± 4.11$^{ab}$ | 48.07 ± 4.10$^{e}$ | 56.52 ± 4.57$^{def}$ |
| 2 | 90 (1) | 20 (-1) | 30 (-1) | 7.43 ± 0.10$^{c}$ | 449.31 ± 29.86$^{b}$ | 32.05 ± 3.72$^{a}$ | 53.71 ± 4.55$^{abc}$ | 61.46 ± 5.22$^{bcd}$ |
| 3 | 30 (-1) | 20 (-1) | 90 (1) | 8.13 ± 0.04$^{a}$ | 320.33 ± 27.94$^{f}$ | 22.53 ± 3.24$^{e}$ | 44.09 ± 4.66$^{f}$ | 45.32 ± 3.48$^{g}$ |
| 4 | 90 (1) | 20 (-1) | 90 (1) | 7.65 ± 0.07$^{c}$ | 415.11 ± 32.29$^{cde}$ | 28.34 ± 2.14 $^{bcd}$ | 52.85 ± 2.58$^{abcd}$ | 59.92 ± 4.77$^{bcde}$ |
| 5 | 30 (-1) | 60 (1) | 30 (-1) | 7.54 ± 0.24$^{c}$ | 449.85 ± 23.35$^{b}$ | 32.99 ± 1.71 $^{a}$ | 56.31 ± 3.57$^{a}$ | 63.42 ± 8.87$^{b}$ |
| 6 | 90 (1) | 60 (1) | 30 (-1) | 7.40 ± 0.08$^{c}$ | 492.91 ± 34.99$^{a}$ | 30.53 ± 4.20$^{abc}$ | 54.58 ± 5.39$^{ab}$ | 69.12 ± 6.50$^{a}$ |
| 7 | 30 (-1) | 60 (1) | 90 (1) | 7.60 ± 0.05$^{c}$ | 411.49 ± 35.09$^{cde}$ | 27.29 ± 3.12$^{cd}$ | 43.13 ± 3.29$^{f}$ | 62.15 ± 5.63$^{bc}$ |
| 8 | 90 (1) | 60 (1) | 90 (1) | 7.74 ± 0.33b$^{c}$ | 384.66 ± 39.23$^{e}$ | 26.33 ± 2.30$^{d}$ | 53.95 ± 1.58$^{abc}$ | 58.29 ± 3.87$^{bcdef}$ |
| 9 | 60 (0) | 40 (0) | 60 (0) | 7.47 ± 0.08$^{c}$ | 404.91 ± 38.71$^{cde}$ | 30.37 ± 1.19$^{abc}$ | 48.99 ± 2.25$^{de}$ | 57.61 ± 6.82$^{cdef}$ |
| 10 | 60 (0) | 40 (0) | 60 (0) | 7.51 ± 0.02$^{c}$ | 427.27 ± 28.96$^{bc}$ | 29.68 ± 2.97$^{abc}$ | 51.45 ± 3.68$^{bcde}$ | 55.46 ± 2.58$^{ef}$ |
| 11 | 60 (0) | 40 (0) | 60 (0) | 7.39 ± 0.18$^{c}$ | 419.09 ± 20.95$^{bcd}$ | 31.07 ± 2.14$^{ab}$ | 49.79 ± 4.19$^{cde}$ | 54.03 ± 3.88$^{f}$ |

Averages followed by different letters on the same column indicate statistical differences according to Duncan's multiple range test ($p \leq 0.05$).

**Table 3** Independent and dependent variables for ultrasound-assisted extraction of polyphenols from ripe jambolão (*Sygium cumini*) fruit.

| Batch No | Time (min) | Ethanol (%) | Temperature (°C) | Yield (%) | TPC (mg GAE/100g) | DPPH (mmol TE/100g) | ABTS (mmol TE/100g) | FRAP (mmol TE/100g) |
|---|---|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $X_3$ | $Y_1$ | $Y_2$ | $Y_3$ | $Y_4$ | $Y_5$ |
| 1 | 30 (-1) | 20 (-1) | 30 (-1) | 5.47 ± 0.23$^{ab}$ | 332.50 ± 43.58$^{bc}$ | 24.75 ± 3.78$^{abc}$ | 30.06 ± 3.67$^{de}$ | 40.07 ± 1.85$^{e}$ |
| 2 | 90 (1) | 20 (-1) | 30 (-1) | 4.81 ± 0.13$^{c}$ | 334.74 ± 20.26$^{bc}$ | 26.68 ± 3.48$^{a}$ | 33.04 ± 4.92$^{cd}$ | 48.36 ±1.83$^{b}$ |
| 3 | 30 (-1) | 20 (-1) | 90 (1) | 5.64 ± 0.37$^{a}$ | 265.20 ± 18.84$^{d}$ | 18.63 ± 1.40$^{d}$ | 26.02 ± 3.81$^{e}$ | 31.95 ± 0.95$^{f}$ |
| 4 | 90 (1) | 20 (-1) | 90 (1) | 5.02 ± 0.08$^{bc}$ | 304.08 ± 25.85$^{c}$ | 22.71 ±1.12$^{c}$ | 33.54 ± 2.34$^{cd}$ | 42.42 ± 1.48$^{de}$ |
| 5 | 30 (-1) | 60 (1) | 30 (-1) | 4.96 ± 0.28$^{c}$ | 404.51 ± 40.70$^{a}$ | 26.87 ± 3.27$^{a}$ | 41.55 ± 5.13$^{b}$ | 42.56 ± 1.88$^{de}$ |
| 6 | 90 (1) | 60 (1) | 30 (-1) | 4.96 ± 0.17$^{c}$ | 428.65 ± 59.57$^{a}$ | 24.35 ± 2.76$^{abc}$ | 47.01 ± 8.41$^{a}$ | 45.30 ± 6.14$^{bcd}$ |
| 7 | 30 (-1) | 60 (1) | 90 (1) | 5.13 ± 0.03$^{bc}$ | 406.57 ± 57.24$^{a}$ | 25.35 ± 0.77$^{ab}$ | 37.51 ± 5.49$^{bc}$ | 52.14 ± 5.09$^{a}$ |
| 8 | 90 (1) | 60 (1) | 90 (1) | 5.18 ± 0.11$^{bc}$ | 329.38 ± 28.33$^{bc}$ | 24.88 ± 1.51$^{abc}$ | 33.11 ± 2.85$^{cd}$ | 44.29 ± 2.93$^{cd}$ |
| 9 | 60 (0) | 40 (0) | 60 (0) | 5.02 ± 0.03$^{bc}$ | 364.81 ± 15.92$^{b}$ | 24.06 ± 1.10$^{bc}$ | 30.11 ± 3.32$^{de}$ | 47.15 ± 2.02$^{bc}$ |
| 10 | 60 (0) | 40 (0) | 60 (0) | 4.83 ± 0.45$^{c}$ | 349.72 ± 23.54$^{b}$ | 24.05 ± 1.98$^{bc}$ | 33.00 ±3.19$^{cd}$ | 45.47 ± 2.07$^{bcd}$ |
| 11 | 60 (0) | 40 (0) | 60 (0) | 4.99 ± 0.13$^{c}$ | 336.24 ± 33.40$^{bc}$ | 23.70 ± 1.30$^{bc}$ | 31.84 ± 2.15$^{d}$ | 44.27 ± 5.61$^{cd}$ |

Averages followed by different letters on the same column indicate statistical differences according to Duncan's multiple range test ($p \leq 0.05$).

Ethanol concentration ($X_2$) significantly decreased the phenolic extraction at unripe and mid-ripe stages, and interactions of time ($X_1$) and ethanol concentration ($X_2$); time ($X_1$) and temperature ($X_3$); ethanol concentration and temperature ($X_3$) had a significantly positive effect and interactions of time ($X_1$), ethanol concentration (X2) and temperature ($X_3$) had a negative effect (**Eqs. 5**, **6** and **7**):

$$\text{Unripe } Y = 0.548 - 0.002\ X_2 + 0.001\ X_2X_3 \quad \textbf{Eq. 5}$$

$$\text{Mid-ripe } Y = 41.792 - 0.251\ X_2 + 0.003\ X_1X_2 + 0.003\ X_1X_3 + 0.004\ X_2X_3 - 0.001\ X_1X_2X_3 \quad \textbf{Eq. 6}$$

$$\text{Ripe } Y = 28.131 + 0.004\ X_1X_3 + 0.003\ X_2X_3 - 0.001\ X_1X_2X_3 \quad \textbf{Eq. 7}$$

The antioxidant capacity showed a significant variation among the assays used: DPPH range from 18.63 to 47.84 mMol/100 g of dry weight sample, ABTS from 26.02 to 75.17 mMol/100 g and FRAP from 31.95 to 92.49 mMol/100 g. The variation among the results found by in vitro antioxidant assays is due to different mechanisms involved in the determination. DPPH and ABTS radical cations are two stable and colored free radicals with the exact mechanism in which a solution reactant is mixed with the fruit extract that can donate a hydrogen atom. As a result, the reduced form of the radical is generated, followed by loss of color. On the other hand, FRAP is characterized by electron transfer ability, which reduces iron ions in the presence of antioxidant compounds (Zielinski et al., 2016). Using Pearson's correlation, it was possible to verify that total phenolic

compounds had a significant ($P < 0.001$) correlation with antioxidant capacity measured by DPPH ($r$ = 0.876), ABTS ($r$ = 0.879) and FRAP ($r$ = 0.904). A significant correlation ($P < 0.001$) also was observed among all antioxidant methods evaluated, showing a correlation coefficient higher than $r > 0.83$. Among the extracts analyzed, the highest antioxidant capacities and polyphenol contents were detected for unripe jambolão, which was obtained using 60 % ethanol as a solvent for 90 min in 30 °C for TPC, DPPH and FRAP and using 20 % ethanol as a solvent for 30 min in 30 °C for ABTS.

The RSM application on DPPH showed that the model was significant ($p< 0.05$), did not present a lack of fit (p = 0.06 unripe; p = 0.08 mid-ripe; p = 0.25 ripe) and could explain 93.82 % for unripe, 90.72 % for mid-ripe and 99.32 % for a ripe stage of all variances in data ($R^2$adj = 0.89; 0.84; 0.98, respectively). The ethanol concentration ($X_2$) significantly decreased the DPPH levels. Higher temperatures ($X_3$) increased the antioxidant capacity for unripe and ripe stages. Interactions of time ($X_1$) and ethanol concentration ($X_2$); time ($X_1$) and temperature ($X_3$); ethanol concentration and temperature ($X_3$) had a significantly positive effect, and interactions of time ($X_1$), ethanol concentration (X2) and temperature ($X_3$) had a negative effect for ripe stage (**Eqs. 8**, **9** and **10**):

$$\text{Unripe } Y = 36.593 - 0.037\ X_2 + 0.909X_3 + 0.001\ X_1X_3 \quad \textbf{Eq. 8}$$

$$\text{Mid-ripe } Y = 34.743 - 0.208\ X_2 + 0.002\ X_1X_2 + 0.002\ X_2X_3 - 0.001\ X_1X_2X_3 \quad \textbf{Eq. 9}$$

$$\text{Ripe } Y = 26.181 + 0.053\ X_1 - 0.157\ X_2 + 0.053\ X_3 + 0.001\ X_1X_2 - 0.002\ X_1X_3 + 0.002\ X_2X_3 \quad \textbf{Eq. 10}$$

The RSM application on ABTS showed that the model was significant ($p< 0.05$), did not present a lack of fit (p = 0.07 unripe; p = 0.06 mid-ripe; p = 0.08 ripe) and could explain 89.69 % for unripe, 77.22% for mid-ripe and 85.91 % for a ripe stage of all variances in data ($R^2$adj = 0.85; 0.67; 0.79, respectively). The ethanol concentration ($X_2$) significantly decreased the ABTS value for the unripe stage; higher temperature ($X_3$) for the mid-ripe and ripe stage and higher time for the ripe stage increased ABTS antioxidant capacity. Interactions of time ($X_1$) and temperature ($X_3$); ethanol concentration ($X_2$) and temperature ($X_3$) had a significantly positive effect, and interactions of time ($X_1$), ethanol concentration (X2) and temperature ($X_3$) had a negative effect only for ripe stage (**Eqs. 11, 12** and **13**):

$$\text{Unripe } Y = 87.051 - 0.415\ X_2 - 0.007\ X_1X_3 + 0.001\ X_1X_2X_3 \quad \textbf{Eq. 11}$$

$$\text{Mid-ripe } Y = 48.314 + 0.174\ X_3 - 0.004\ X_2X_3 + 0.001\ X_1X_2X_3 \quad \textbf{Eq. 12}$$

$$\text{Ripe } Y = 16.761 + 0.139\ X_1 + 0.365\ X_3 - 0.001\ X_1X_2X_3 \quad \textbf{Eq. 13}$$

The RSM application on FRAP showed that the model was significant ($p< 0.05$), did not present a lack of fit (p = 0.07 unripe; p = 0.05 mid-ripe; p = 0.05 ripe) and could explain 91.96 % for unripe, 78.13 % for mid-ripe and 82.33 % for a ripe stage of all variances in data ($R^2$adj = 0.88; 0.64; 0.70, respectively). The higher temperature ($X_3$) for the ripe stage and higher time for the ripe stage significantly increase the FRAP antioxidant capacity. On the other hand, the ethanol concentration ($X_2$) significantly decreased the FRAP levels. Interactions of time ($X_1$) and ethanol concentration ($X_2$) had a significantly positive effect for the mid-ripe stage, and interactions of ethanol concentration ($X_2$) and temperature ($X_3$) had a significantly negative effect for the unripe stage and positive for the ripe stage; interactions of time ($X_1$), ethanol concentration ($X_2$) and temperature ($X_3$) had a negative effect only for unripe stage (**Eqs. 14**, **15** and **16**):

$$\text{Unripe } Y = 61.318 + 0.544\ X_3 - 0.008\ X_2X_3 + 0.001\ X_1X_2X_3 \quad \textbf{Eq. 14}$$

$$\text{Mid-ripe } Y = 64.693 - 0.457\ X_2 + 0.004\ X_1X_2 + 0.007\ X_2X_3 - 0.001\ X_1X_2X_3 \quad \textbf{Eq. 15}$$

$$\text{Ripe } Y = 33.366 + 0.200\ X_1 - 0.132\ X_2 + 0.006\ X_2X_3 - 0.001\ X_1X_2X_3 \quad \textbf{Eq. 16}$$

Response surface methodology coupled with multiple regression analysis has been used to find the best conditions to perform adequate extraction. The multi-response optimization procedure using the desirability function was conducted with the models to maximize the yield, total phenolic content and antioxidant capacity measured by DPPH, ABTS and FRAP. The effects of parameters on dependent variables are shown in desirability 3D- response surface plots (**Figs. 4a, 4b** and **4c** for unripe; **Figs. 4d, 4e,** and **4f** for mid-ripe and **Figs. 4g, 4h** and **4i** for ripe stage) as a function of time, ethanol concentration and temperature.

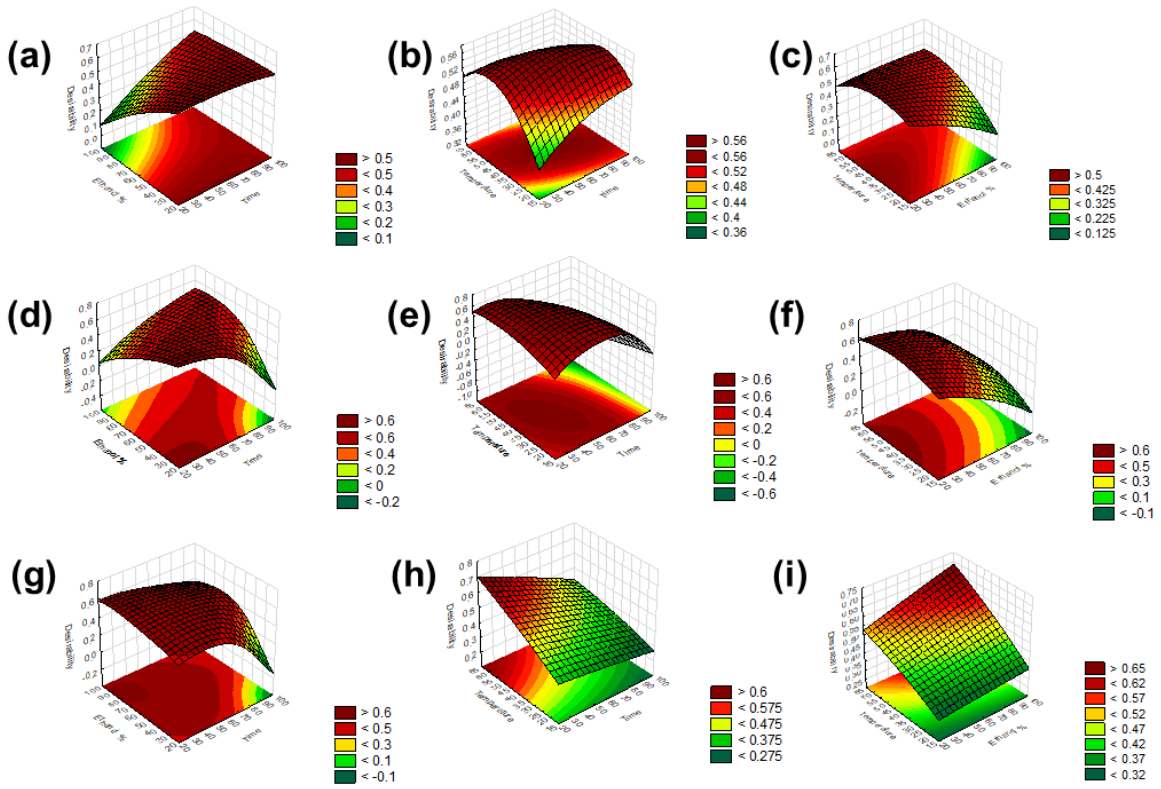


**Fig. 4** Desirability response surface plots of the effects of temperature, time, and ethanol concentrations on the antioxidant activity of unripe (a, b, c), mid-ripe (d, e, f) and ripe (g, h, i) jambolão extracts by DPPH (left chats), ABTS (middle charts), and FRAP (right charts) assays.

The result for this optimization suggested that extraction with 30 % ethanol for 68.4 min, at 39.2 °C for unripe jambolão, with 30 % ethanol for 30 min, at 47.2 °C for mid-ripe fruit stage and extraction with 90 % ethanol for 30 min, at 60 °C for ripe fruit stage were the best solutions for this combination of variables. These new extractions were submitted to the same experimental and analytical procedures as those applied from the beginning of this study. As observed in **Table 4**, the yield and the antioxidant capacity changed significantly during the transition to the last ripening stage after optimized extraction, and no significant variation was observed between TPC at different ripening stages.

**Table 4** The experimental and predicted values and computed absolute errors (ΔE) by the models for ultrasound-assisted extraction from jambolão (*Sygium cumini*) fruit for the analysis of total phenolic compounds (TPC) and antioxidant capacity using DPPH and ABTS radical scavenging assays and Ferric Reducing Antioxidant Power (FRAP) of the optimized extracts.

| | | Unripe | Mid-Ripe | Ripe |
|---|---|---|---|---|
| **Yield (%)** | Experimental value | 9.01 ± 0.45$^{a}$ | 8.39 ± 0.68$^{a}$ | 5.48 ± 0.59$^{b}$ |
| | Predicted value | 8.34 | 7.64 | 5.16 |
| | ΔE % | 8.03 | 9.82 | 6.10 |
| **TPC (mg GAE/100g)** | Experimental value | 546.16 ± 112.99 | 457.15 ± 152.63 | 407.53 ± 25.15 |
| | Predicted value | 515.30 | 431.00 | 401.50 |
| | ΔE % | 5.99 | 6.07 | 1.05 |
| **DPPH (mMol TE/100g)** | Experimental value | 45.19 ± 15.80$^{a}$ | 33.16 ± 1.29$^{ab}$ | 23.85 ± 1.50$^{b}$ |
| | Predicted value | 42.65 | 32.71 | 24.55 |
| | ΔE % | 5.96 | 1.37 | 2.85 |
| **ABTS (mMol TE/100g)** | Experimental value | 68.20 ± 0.71$^{a}$ | 53.04 ± 2.28$^{b}$ | 36.94 ± 1.08$^{c}$ |
| | Predicted value | 64.71 | 53.46 | 36.39 |
| | ΔE % | 5.40 | 0.79 | 1.52 |
| **FRAP (mMol TE/100g)** | Experimental value | 72.30 ± 0.38$^{a}$ | 55.02 ± 2.74$^{b}$ | 43.81 ± 1.71$^{c}$ |
| | Predicted value | 79.86 | 60.17 | 49.27 |
| | ΔE % | 7.56 | 5.15 | 5.46 |

Averages followed by different letters on the same row indicate statistical differences according to Duncan's multiple range test ($p \leq 0.05$).

Our data corroborated with a previous study by Veber et al. (2015), which investigated jambolão at five different maturation stages (green; purplish-green, greenish-purple, half-ripe and fully ripe) and showed that green fruits, 50 % ethanol extraction and hot infusion for 30 minutes presented more significant amounts of phenolic compounds and antioxidant potential (IC50). Furthermore, this confirms the results reported by da Silva et al. (2019) for the TPC, hydroxycinnamic acids, flavonoids and the antioxidant capacity by FRAP and DPPH of the Gala and Lis Gala apple juices showing that decreased from the unripe to ripe stages. The observed and predicted values, along with the computed absolute errors (ΔE), can be observed in table 4. Because of the low absolute error values obtained by comparing observed and predicted values (<10 %), the proposed model could be used to predict the response values.

## 4 Conclusion

The extraction process of polyphenols from jambolão was successfully optimized and established using a $2^3$-factorial response surface design on three parameters (the solvent concentration, extraction time and temperature). The response surface methodology effectively estimated the effect of independent variables on the yield extract, total phenolic content, and antioxidant capacity. A positive correlation was confirmed between the total phenolic content and antioxidant capacity. The value of the variables evaluated (yield, TPC, DPPH, ABTS and FRAP) were proportional to the degree of maturity, and the content decreased during fruit ripeness. The best conditions of the variables for increasing the yield, total phenolic and antioxidant capacity were obtained with 30 % ethanol for 68.4 min, at 39.2 °C for unripe jambolão, with 30 % ethanol for 30 min, at 47.2 °C for mid-ripe and with 90% ethanol for 30 min, at 60 °C for ripe fruit. Jambolão fruits in the three stages of ripeness can be considered a beneficial source of bioactive compounds with antioxidant activity. The ultrasound-assisted method can be considered a simplified, reproducible, inexpensive, and efficient green extraction process without toxic solvents, allowing the application in food, pharmaceutical and cosmetic industries of the optimized extracts with high and viable yields.

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**Authors' Contributions**
S. A. Conceptualization; data curation; formal analysis; investigation; methodology; writing –original draft. P. S. H. Methodology, Writing - review & editing. C. C. H. K. and S. M. R. F. Writing - review & editing.

**Availability of data and materials**
The authors confirm that the data supporting the findings of this study are available within the article.

**Ethics approval and consent to participate.**
Not applicable.

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**Conflicts of Interest**
The authors declare that they have no conflict of interest.

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