Modeling the growth kinetics of cell suspensions of Randia echinocarpa and characterization of their bioactive phenolic compounds.

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Abstract

The fruit of *Randia echinocarpa* is commonly used in the Mexican tradicional medicine to treat different diseases and ailments. So, the establishment of cell suspension cultures (CSC) is a required alternative to obtain bioactive compounds from this plant. Moreover, good kinetic models are necessary good processes control and simulation of plant cell cultures. Therefore, the objective of this study was to model and estimate the growth kinetics of the CSC of *R. echinocarpa*, as well as characterize their phenolic profile by ultra performance liquid chromatography coupled to mass spectrometry. During the 34 d of culture, CSC of *R. echinocarpa* reached a dry cell biomass concentration of 15.16 g/L at day 20. The maximum specific growth rate (μ_{max}) was 0.15 d^{-1}, with a duplication time (t_d) of 4.62 d. The Logistic model proposed adequately predicts the cell growth changes during the culture and the maximum dry cell content the culture medium can sustain (≈ 13.63 g/L). Ten phenolic compounds were identified in the biomass and four in the supernatants. The major phenolic compound in the biomass was chlorogenic acid (CA), with a concentration of 828.6 µg/g at day 20. In the lyophilized supernatant, the major phenolic compound was salicylic acid (SA) with a concentration of 172.7 µg/g at day 30. The production of CA was a growth-dependent process in contrast to the concentration of SA in the media. Our results indicate CSC of *R. echinocarpa* could be a sustainable source for the production of bioactive compounds such as CA and SA.

INTRODUCTION

Randia echinocarpa Sessé et Mociño (Rubiaceae) is a shrub or small tree 2 to 6 m high, distributed along the Pacific Ocean Shore of Mexico. Its fruit, known as “papache” in northwestern Mexico, is used in Mexican traditional medicine to treat diseases (e.g., cancer, malaria, and diabetes) and for organs/systems ailments (e.g., kidney, respiratory, circulatory, and gastrointestinal). Previous studies had reported that the compounds isolated from the fruit of *R. echinocarpa* included mannitol, β-sitosterol, quinovic, oxoquinovic, campesterol, stigmasterol, melanins, ursolic, linoleic, and oleanolic acids (Bye et al., 1991, Cano-Campos et al., 2011, Cuevas-Juarez *et al*., 2014; Montes-Avila et al., 2018). In this context, different organic fractions (hexanic, chloroformic, and ethylacetate) from *R. echinocarpa* fruit containing saponins, terpenes, sterols, flavonoids and tannins showed antioxidant and antimutagenic properties as reported by Cano-Campos et al. (2011).

The high deforestation rates, related with land expansion for agriculture, had resulted in a significant reduction in the wild populations of *R. echinocarpa* in recent years (Valenzuela-Atondo et al., 2020). Therefore, plant cell and tissue culture (PCTC) emerge as a viable biotechnological tool for the production of bioactive compounds at large scale, with a special interest in sustainable conservation and rational use of biodiversity (Días *et al*., 2016; Haida et al., 2019). Among the different techniques of plant cell cultures, cell suspension culture (CSC) is one of the most suitable approaches to produce plant-derived specialized metabolites (Motolinía-Alcántara et al., 2021). Different approaches have been reported to model the growth of plant cells in batch cultures, from structured models to unstructured models like Monod, Gompertz, and Logistic (Villegas et al., 2017).

Valenzuela-Atondo et al. (2020) demonstrated that methanolic extracts from *R. echinocarpa* calli possess significant antioxidant activity. However, these authors did not report the phenolic profile of the methanolic extracts nor established a CSC from the calli. There is scarce information about the phenolic profile of the genus *Randia* (Ojeda-Ayala et al., 2022). Recently, Martínez-Ceja et al. (2022) showed that biomass extracts from *in vitro* culture of *Randia acuelata* had anti-inflammatory and antimicrobial activities but only few phenolic compounds were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). Therefore, the objectives of this study were to establish a CSC of *R. echinocarpa*, characterize its kinetic parameters, and analyze the most abundant bioactive phenolic compounds in the CSC by ultra-high performance liquid chromatography coupled to mass spectrometry (UPLC-MS).

MATERIALS AND METHODS

Plant material and *in vitro* culture

Seeds of *Randia echinocarpa* Moc. & Sessé ex DC. were kindly provided by the Functional Genomics Laboratory of CIIDIR-IPN Sinaloa Unit, in November 2019. Seeds were disinfected, and *in-vitro* germinated according to Valenzuela-Atondo et al. (2020) with some modifications. Inside the laminar flow hood, seeds were immersed in 70% (v/v) ethanol for 1 min, then submerged for 15 min in 3% (v/v) commercial bleach with 0.2% Tween 20 and rinsed five times with sterile distilled water. Disinfected seeds were sown in glass vessels containing full-strength Murashige and Skoog (MS) medium supplemented with 100 mg/L myo-inositol and 30 g/L sucrose. The pH of the MS medium was adjusted to 5.8 and solidified with 2.5 g/L phytagel prior to autoclaving at 121 ºC for 15 min.

For seed germination, vessels were covered with aluminum foil and kept in a plant grow chamber (Caron, 6320, Marietta, OH) at 25 ºC for 2 wk. Afterward, aluminum foil was removed, and vessels were kept in the plant growth chamber at the same temperature and 16:8 h photoperiod (55 µmol/m^2s irradiance) for 4 wk. At the end of this period, the percentage of seed germination was determined.

Callus culture and cell suspensions establishment

Cotyledons (1 cm^2) and hypocotyls (1 cm) from 6 wk old germinated plantlets were cultured in glass vessels with MS medium containing 1-naphthalene acetic acid (NAA; 2 mg/L; Sigma-Aldrich) and 6-benzylaminopurine (BAP; 1 mg/L; CaissonLabs) as plant growth regulators (PGRs) were used for callus induction. Cultures were maintained at the same conditions for *in vitro* seed germination for 6 wk and subculture onto fresh medium was made every 2 wk. The percentage of explants with calli and the color and texture of the calli were determined at the end of this period.

For CSC, cell aggregates consisting of friable callus (2 g) were excised and transferred in 125 mL flasks containing 20 mL of MS liquid medium with the same PGRs concentration as described above. The cultures were maintained in the dark at 25 ºC and with constant shaking at 120 rpm. After 2 wk, the big aggregates were removed from the cultures and fresh medium was added to multiply the cellular

suspensions. To obtain fine cell suspensions, a total of six subcultures were done every 2 wk, by transferring 2 g of fresh cell biomass as inoculum into 20 mL fresh MS liquid medium with PGRs.

Cell growth kinetics

To perform the growth curves of *R. echinocarpa* cell suspensions, several flasks of 125 mL containing 20 mL of MS medium were inoculated with 2 g of cell biomass and incubated under the same conditions described above. The cellular suspensions contained in three flasks were harvested every 3 or 4 days for 34 days and filtered on Whatman paper No. 1. Supernatants were recovered and stored at -70°C until used for quantification of residual sucrose, ammonia and phenolics. For dry cell weight (DCW), the samples were lyophilized for 24 to 48 h and stored (-70°C) for further analyses.

Quantification of residual sucrose and ammonia

The quantification of residual sucrose was determined according to Dubois et al. (1956). In brief, 2 mL of samples previously diluted (1:1000) were transferred in test tubes with caps, then 50 µL of 80% phenol (w/v) was added followed by the addition of 5 mL of concentrated sulfuric acid (98%). Samples were kept at room temperature for 10 min, with a subsequent incubation in a water bath (25 ºC) for 10 min. Samples were analyzed in a spectrophotometer (Perkin Elmer, Lambda 25) at 490 nm. For residual ammonia, concentration was determined using the phenol-hypochlorite reaction described by Weatherburn (1967) with some modifications. In a test tube with cap, 10 µL of the previously diluted (1:10) sample was added followed by 2.5 mL of Reagent A (1 g phenol, 5 mg sodium nitroprusside, 100 mL distilled water). The mixture was homogenized using a vortex and 2.5 mL of Reagent B (0.5 g NaOH, 0.84 mL NaOCl, 100 mL distilled water) was added. The reaction mixture was incubated for 20 min at 37 ºC, then, the absorbance was measured at 625 nm. The content of both residual sucrose and ammonia was calculated using a calibration curve from 0 to 35 µg/mL for sucrose, and 0 to 400 µg/mL for ammonia.

Extraction and quantification of phenolic compounds

Total phenolics content from the cell biomass was determined according to Waterhouse (2002) using the Folin-Ciocalteu reagent. In a 96 well plate, 25 µL of the methanolic extracts were mixed with 25 µL of Folin-Ciocalteu (1:8) reagent and 200 µL of distilled water. The reaction mixture was incubated for 3 min. Then, 25 µL of a saturated sodium carbonate solution (10.6 g/100 mL) was added to the mixture and the plate was carefully shaken for 20 s. Afterwards, the mixture was incubated for 2 h min in the dark at room temperature and the absorbance was measured at 765 nm using a microplate reader (Cytation 5, BioTek, Santa Clara, CA). The total phenolic content was expressed as micromole of gallic acid equivalents (GAE) per gram of DCW.

The extraction of phenolic compounds was carried out according to Juárez-Trujillo et al. (2018). Lyophilized tissue (0.3 g) was homogenized in 7 mL of methanol (99%, HPLC grade, Sigma-Aldrich, St. Louis, MO) and sonicated 4 times for 10 min (Cole-Parmer 08895-91, 40 kHz). After sonication, samples

were centrifuged (3,500 rpm, 4°C, 15 min) and supernatants were recovered, filtered (Phenomenex, 0.2 µm) and collected in glass vessels. Supernatants were evaporated to dryness under vacuum at 42°C and the dried extracts (30 mg) were resuspended in methanol (1 mL) with 0.1% formic acid (MS grade, Sigma-Aldrich, St. Louis, MO).

The phenolic compound profile of the cell suspensions, from both dried cell biomass and culture broth, was determined by UPLC (Agilent 1290 series, Agilent Technologies, St. Clara, CA) coupled to a triple quadrupole mass spectrometer (Agilent 6460) with a dynamic multiple reaction monitoring (dMRM) as acquisition method following the protocol described by Juárez-Trujillo et al. (2018), which consider a co-analysis with a total of 60 phenolic standards. A ZORBAX SB-C18 column (1.8 µm, 2.1×50 mm; Agilent Technologies, Santa Clara, CA) was used to carry out the chromatographic analysis with the column oven temperature at 40°C. Water (A) and acetonitrile (B) (both MS grade with 0.1% formic acid) were used as mobile phase. The gradient conditions of the mobile phase were: 0/1, 40/40, 42/90, 44/90, 46/1, 47/1 (min / % phase B). The flow rate was 0.1 mL/min, and 5 µL was the sample injection volume. The ESI source was operated in positive and negative ionization modes. The desolvation temperature was 300°C, the cone gas (N_2) flow was 5 L/min, the nebulizer pressure was 45 psi, the sheath gas temperature was 250°C, the sheath gas flow was 11 L/min, the capillary voltage (positive and negative) was 3500 V, and the nozzle voltage (positive and negative) was 500 V. The fragmentor voltage was 100 V and the cell accelerator voltage was 7 V for all compounds. The identity was confirmed by co-elution with standards under the same analytical conditions described above for each compound. For quantitation of each phenolic compound a calibration curve in a concentration range of 1–9 µM was prepared (r^2 values ≥ 0.97 were considered for the linearity range). The data were processed using the MassHunter Workstation Software, version B.06.00 (Agilent Technologies, Santa Clara, CA). The results were expressed as µg/g of dry cell biomass for intracellular phenolics, or as µg/g of dry weight for extracellular phenolics.

Mathematical model

The model describing cell biomass growth, substrate compsumption and phenolic compounds production, was established in accordance with the characteristics of the plant cells. First, cell biomass is viable while the limiting substrate is available in the culture medium. Thus, changes in the biomass concentration (x) will occur according to Eq. 1 (Eq. 1):

$$\frac{dx}{dt} = \mu x \quad \text{(Eq. 1)}$$

where μ is the specific growth, which was calculated during the exponential growth phase. As a first approach for modelling the growth kinetics of *R. echinocarpa*, the Logistic equation (Eq. 2) was used, since this equation considers the specific growth rate dependency only on maximum population size allowed by the culture system (Zhang et al., 2013),

$$\mu = \mu_{max}\left(1 - \frac{x}{x_{max}}\right) \quad \text{(Eq. 2)}$$

where μ_{max} is the maximum specific growth rate and x_{max} is the maximum population size. The parameters of the mathematical model describing the plant cells kinetics, cultured in shaken flasks, were sequencially estimated. The maximun specific growth rate (μ_{max}) was obtained during exponential growth phase and the maximum dry cell biomass (x_{max}) was determined experimentally.

Since there are few studies reporting the use of unstructured models for the description of cell growth kinetics of plant cell suspensions in batch cultures (Omar et al., 2006; Zhang et al., 2013), and to our knowledge, there are no studies reporting the growth kinetics of *R. echinocarpa* CSC; it was assumed two possible limiting substrates: sucrose (s) and ammonia (NH_4). In this context, the use of the carbon (sucrose) and nitrogen (ammonia) sources were divided in two: the carbon and ammonia used for cell growth, and the carbon and ammonia used to maintain the existing biomass. The coefficients $Y_{x/s}$ and Y_{x/NH_4} represent the conversion efficiency of carbon and ammonia, respectively, based on dry weight biomass, while m_s and m_{NH_4} are the carbon and ammonia necessary to sustain endogenous metabolism. These assumptions are established by the equations (3) and (4), respectively.

$\frac{ds}{dt} = -\frac{\mu x}{Y_{x/s}} - m_s x$ (Eq. 3)

$\frac{dNH_4}{dt} = -\frac{\mu x}{Y_{X/NH_4}} - m_{NH_4} x$ (Eq. 4)

Equation (5) is regarded with the production (p) of phenolic compounds. Where α is the growth-associated coefficient and β is the non-growth-associated coefficient (Luedeking & Piret, 1959).

$\frac{dp}{dt} = \alpha\mu x + \beta x$ (Eq. 5)

Kinetic parameters estimation

The cell biomass yield coefficients for both sucrose and ammonium ($Y_{x/s}, Y_{x/NH_4}$) were calculated during each growth phase; one for exponential growth phase and the other for steady state (stationary) phase. During the first one, the yields were obtained as:

$Y_{x/s} = -\frac{dx}{ds}$ (Eq. 6)

$Y_{x/NH_4} = -\frac{dx}{dNH_4}$ (Eq. 7)

During stationary phase, the sucrose and ammonia consumption were determined as follow:

- $-\frac{1}{x_{ss}}\frac{ds}{dt} = m_s$ (Eq. 8)
- $-\frac{1}{x_{ss}}\frac{dNH_4}{dt} = m_{NH_4}$ (Eq. 9)

Where x_{ss} is the average of the dry cell biomass during the steady state phase. Analogously, the non-growth-associate coefficient of phenolic compounds production was calculated as follow:

$-\frac{1}{x_{ss}}\frac{dp}{dt} = \beta$ (Eq. 10)

Statistical analysis

Three independent experiments were performed and results are expressed as means ± standard deviation. The means values were subjected to ANOVA followed by a *post hoc* Tukey multiple comparison test ($p < 0.05$). To analyze the correlation between variables, a multivariate principal components analysis of ($p > 0.05$) and Spearman ρ non-parametric correlation analysis were performed using JMP 16.0 (SAS Institute Inc. Cary, NC).

RESULTS AND DISCUSSION

Seed germination and callus induction

Valenzuela-Atondo et al. (2020) compared different germination media for *Randia echinocarpa* and the highest germination percentage (> 98.5%) was observed when seeds were cultured in half-strength MS with 15 g/L sucrose medium for 6 wk. In our study, 95% of seed germination was obtained after the same time of culture but using full-strength MS medium with 30 g/L sucrose. Other study carried out by Martínez-Ceja et al. (2022) reported 85% germination yield of *R. aculeata* L. seeds after 2 wk of culture. Differences in the germination percentages are dependent to the plant genotype, disinfection procedures, medium composition, gelling agents, and PGRs used (Hesami et al., 2021).

Callus can grow in either a compact or friable form. Friable calli are preferred since they can be used to obtain single-cell cultures (Efferth, 2019). In this study, the addition of NAA (2 mg/L) and BAP (1 mg/L) was efficient in inducing “green-creamy” friable callus after 6 wk of culture. Valenzuela-Atondo et al. (2020) reported the highest biomass (2.9 ± 1.4 g fresh weight) and friable callus after 12 wk of culture, when explants were cultured in MS medium with 1 mg/L of BAP and indoleacetic acid (IAA). On the other hand, Martínez-Ceja et al. (2022) reported high biomass in callus cultured for 30 d in MS medium supplemented with NAA and kinetin (KIN) at 2 mg/L and 0.5 mg/L, respectively. Differences in callus induction among plant species can be attributed to the type and concentration of PGRs, type of explant, as well as variations in the *in vitro* culture conditions (Tuskan et al., 2018).

Cell growth kinetics and mathematical model

Cell suspensions for *R. echinocarpa* cultured for 34 days showed a typical sigmoidal growth curve (Fig. 1a). The lag phase of growth was not apparent, indicating that cultures entered to exponential growth phase from the begining, while the stationary phase occurred from day 20 to day 34 of culture. The maximum specific growth rate (μ_{max}) during the exponential phase was 0.15 d^{-1} with a duplication time (t_d) of 4.62 d (Table 1). The CSC reached a dry cell biomass concentration of 15.16 ± 2.48 g/L at day 20, thereafter, remained without significant changes ($p < 0.05$). The model predicted that the maximum dry cell biomass (x_{max}) the culture medium can sustain was 13.63 g/L. The logistic equation

(Eq. 2) was useful to predict the maximum population (biomass) a system can sustain (Kawano et al., 2020). The Logistic model has been previously used to predict the cell growth of some plant species. Omar et al. (2006) used the Monod, Gompertz and Logistic equations to adequately describe the cell growth of *Centella asiatica* cells suspensions cultured in both shake flasks and bioreactor systems. On the other hand, Zhang et al. (2013) and Pan et al. (2020) used a Logistic model to predict the kinetic parameters of *Taxus media* and *Bletilla striata* cell suspensions, respectively.

Previous studies reported the maximum cell biomass when the most of the sucrose concentration in the culture medium was consumed by the cells, or affected by the initial sucrose concentration (Rho & André, 1991; Sánchez-Ramos et al., 2020; Bernabé-Antonio et al., 2021). Interesting, *R. echinocarpa* CSC reached the maximum cell biomass without depleting the sucrose content in the medium (Fig. 1a,b), indicating endogenous metabolism, which was in accordance with the m_s estimated value (Table 1). During the exponential phase, *R. echinocarpa* cells showed a substrate yield of $Y_{x/s} = 0.88$ g/g, while global yield value was 0.41 g/g. The $Y_{x/s}$ value during the exponential phase indicates the cell culture was efficient in converting the sucrose into cell biomass, a similar $Y_{x/s}$ value for biomass yield convertion from sucrose was reported by Guardiola et al. (1995). The global value of $Y_{x/s}$ was more in accordance with those reported in the literature (Srinivasan et al., 1995). The m_s value was higher than those previously reported for *C. roseus* and *N. tabacum* by Van Gulik et al. (1992).

Table 1
Kinetic parameters estimated by the experimental data.

Parameter	Value	Dimension
μ_{max}	0.15	1/d
x_{max}	15.16	g/L
t_d	4.62	d
$ExpY_{x/s}$	0.88	g/g
$GlobalY_{x/s}$	0.41	g/g
Y_{x/NH_4}	3.63	g/g
m_s	0.012	g/g • h
α	0.029	g/g

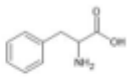
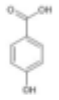
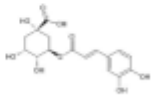
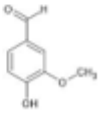
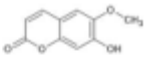
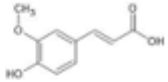
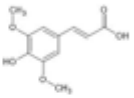
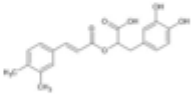
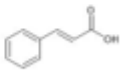
Regarding the ammonia consumption (Fig. 1c), its content in the medium decreased from 3.71 g/L to 1.58 during the first 13 days of culture, then remained unchanged. Plant cells regularly require another nitrogen source for growth and endogenous metabolism (Behrend & Mateles, 1975). This could explain the absence of changes in the ammonia concentration from day 13 to the end of the culture.

The increment in phenolic compounds concentration was correlated (ρ = 0.753) with the increment of the cell biomass, and remained unchanged after culture reached the maximum cell biomass at day 20 (Fig. 1d). Moreover, their production was also related with the consumption of sucrose and ammonia during the exponential phase. Therefore, the production of phenolic compounds was a growth-associated process with an α value of 0.025 g/g.

Profile of phenolic compounds

In the biomass, chlorogenic acid (CA) was the most abundant compound reaching the highest concentration (828.6 ± 218.5 µg/g DCW) at day 20 of culture (Table 2). Juarez-Trujillo *et al*. (2018) also reported CA as the most abundant phenolic compound in the aqueous, methanolic and ethanolic extracts from fruit pulp and seeds of *R. monantha* Benth. In another study, CA was identified in extracts from *R. ferox* (Cham & Schltdl) DC. (Pappis *et al*., 2021).

Table 2. Phenolic compounds identified in the dried cell biomass (DCW) and dried supernatants (DS) of *Randia echinocarpa* culture from days 16 to 34.

Compound	Chemical structure	DCW concentration (µg/g)	DS concentration (µg/g)
Phenylalanine		18 – 75.8	2.3 – 19.7
4-Hydroxybenzoic acid		0 – 0.7	N/D
Vanillic acid		0 – 2.5	N/D
Chlorogenic acid (3-O-CQA)		18.7 – 828.6*	0.9 – 6.7
Vanillin		0.1 – 1.1	N/D
Scopoletin		0.5 – 8.8	6.3 – 28.8
Ferulic acid		0 – 7	N/D
Sinapic acid		0 – 6.3#	N/D
Rosmarinic acid		2 – 10.1*	N/D
Trans-cinnamic acid		0 – 1.6*	N/D
Salicylic acid		N/D	20.3 – 172.7+

*Significantly higher at 20 days of culture.

#Only detected at 20 days of culture.

+Significantly higher at 30 days of culture.

N/D Not detected.

Rosmarinic acid (RA) in the *R. echinocarpa* DCW reached its highest concentration (10.1 ± 2.8 µg/g DCW) at day 20. RA has not been identified previously in plants or fruits from the *Randia* genus. This compound is distributed in 39 plant families, and it is commonly found in the Boraginaceae family and the Nepetoideae subfamily of Lamiaceae (Kim et al., 2015). All forms of *in vitro* cultures (shoot, root, hairy root, callus, and suspension cultures) may contain RA, and often in higher amounts than those in natural plants (Petersen, 2013). The presence of both CA and RA in the CSC of *R. echinocarpa* can be related to the pharmacological properties reported in the fruit. Both CA and RA possess a wide range of biological

activities including antioxidant, hepatoprotective, neuroprotective, anti-diabetes, anti-inflammatory, antiviral, and anti-microbial, among others (Naveed et al., 2018; Nadeem et al., 2019; Lu et al., 2020; Guan et al., 2022).

The concentration of CA was strongly correlated (ρ = 0.8702) with that of RA (Fig. 2). CA is biosynthesized from the phenylpropanoid pathway, using 4-coumaryl-CoA as precursor (de la Rosa et al., 2019), while the biosynthesis of RA also requires 3,4-dihydroxyphenyllactic acid that comes from L-tyrosine (Marchev et al., 2021). Interestingly, the highest concentration of both CA and RA was reached at day 20, where the maximum DCW was also obtained. Another compound, such as sinapic acid (6.3 ± 0.9 µg/g DCW), was only detected at day 20. The concentration of CA in the dry biomass at day 30 significantly decreased as compared with its content at day 20. In this experiment, the production of phenolic compounds was associated with growth; thus, the reduced concentration of these compounds at day 30 can be associated with the absence of cell biomass formation. Some strategies have been proved to increase the concentration of CA in CSC. In *Carthamus tinctorius*, using elicitors such as methyl jasmonate, salicylic acid (SA) and yeast extract, and precursors like phenylalanine, tyrosine or cinnamic acid significantly increased the concentration of different chlorogenic acids (Liu et al., 2023).

The presence of other phenolic compounds in the species of *Randia* genus, such as ferulic and vanillic acids, has been previously reported by Martínez-Ceja et al. (2022). The concentration of these compounds in dichloromethane extracts of CSC from *R. acuelata* L. was very low and not significantly different during the fermentation time. The low concentrations of these compounds can be attributed to the preference for the biosynthesis of CA, since these compounds share 4-coumaryl-CoA as precursor (Kundu *et al.*, 2017).

Only four compounds were identified in the dry supernatant (DS): phenylalanine, CA, scopoletin and salicylic acid (SA; Table 2). As in biomass, the highest concentration of CA (6.7 µg/g DW) was detected at day 20. In contrast, the highest concentration of SA was detected at day 30 of culture. The biosynthesis of SA uses either isochorismate or benzoic acid as precursors. Moreover, the biosynthesis of benzoic acid uses *trans*-cinnamic acid, a precursor also required for the biosynthesis of CA (Dempsey, et al., 2011; Lefevere et al., 2020). Thus, the increment in the SA content was due to a preference for its biosynthesis instead for that of CA.

CONCLUSIONS

Cell suspension cultures of *R. echinocarpa* were successfully established. The Logistic model proposed was sufficient to describe cell growth, sucrose and ammonia consumption, and the production of phenolic compounds. This model prediction could be useful for process controlling and optimization, necessary for the cultivation of plant cell in larger culture systems, like bioreactors. *R. echinocarpa* cell cultures could serve as an important source of CA and SA, important phenolic compounds with a wide range of beneficial effects in human health.

Declarations

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AUTHOR CONTRIBUTION

Miguel Aguilar-Camacho: Conceptualization, experimental work, data analysis, writing - original draft. Carlos Eduardo Gómez-Sanchez, Abraham Cruz-Mendívil, José A Guerrero-Analco, Juan L. Monribot-Villanueva: support during experimental work and formal analysis. Janet A. Gutiérrez-Uribe: conceptualization, funding acquisition, experimental design, project administration, supervision, manuscript review and edition.

ETHICAL STATEMENT

The authors have no competing interests to declare that are relevant to the content of this article.

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Figures

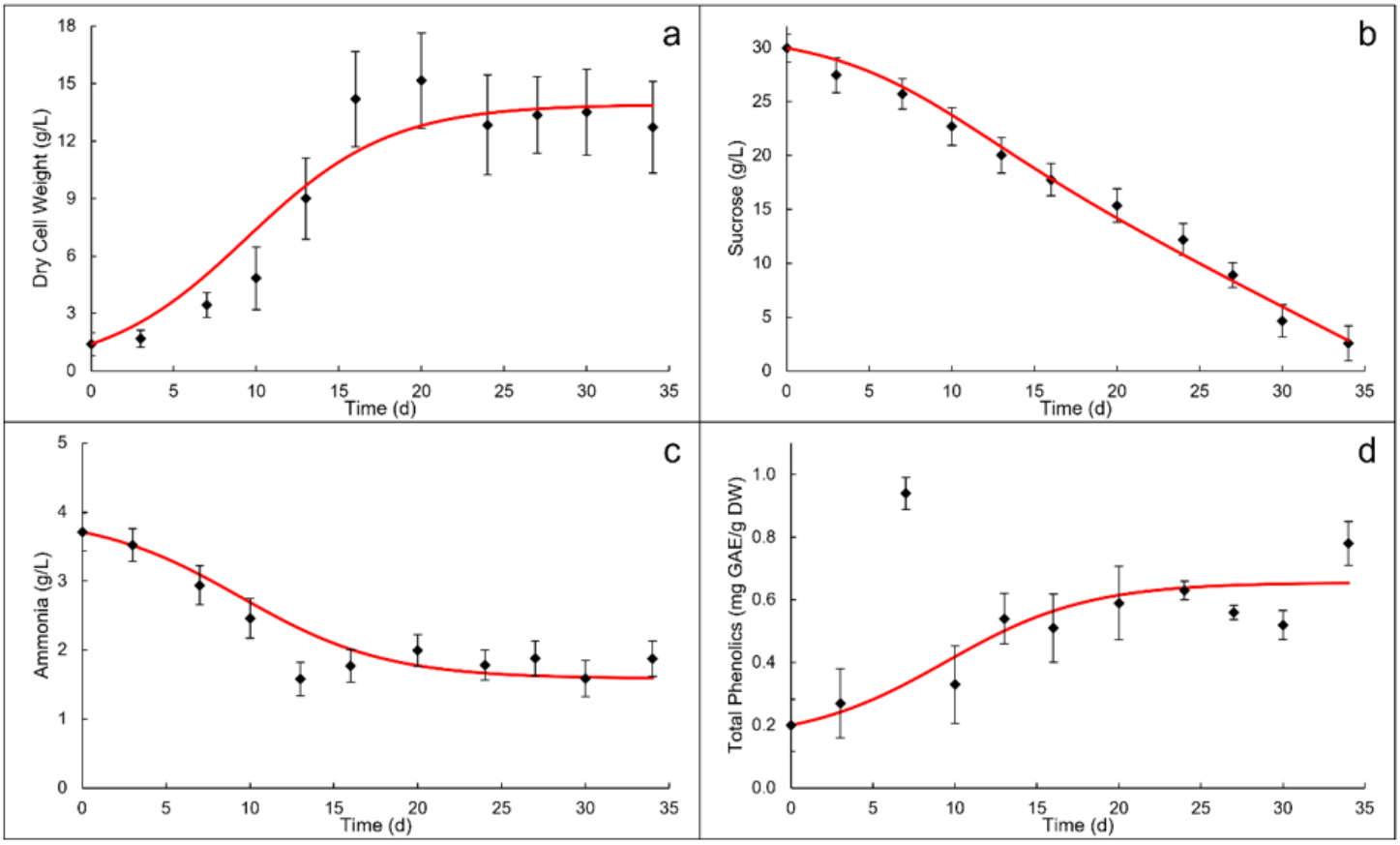

Fig. 1. Comparison of the experimental and model-calculated data for cell growth (a), sucrose consumption (b), ammonia consumption (c), and phenolics production (d). The symbol (♦) corresponds to experimental results and the lines (—) are the model prediction results. Bars represent ± standard deviation.

Figure 1

See image above for figure legend

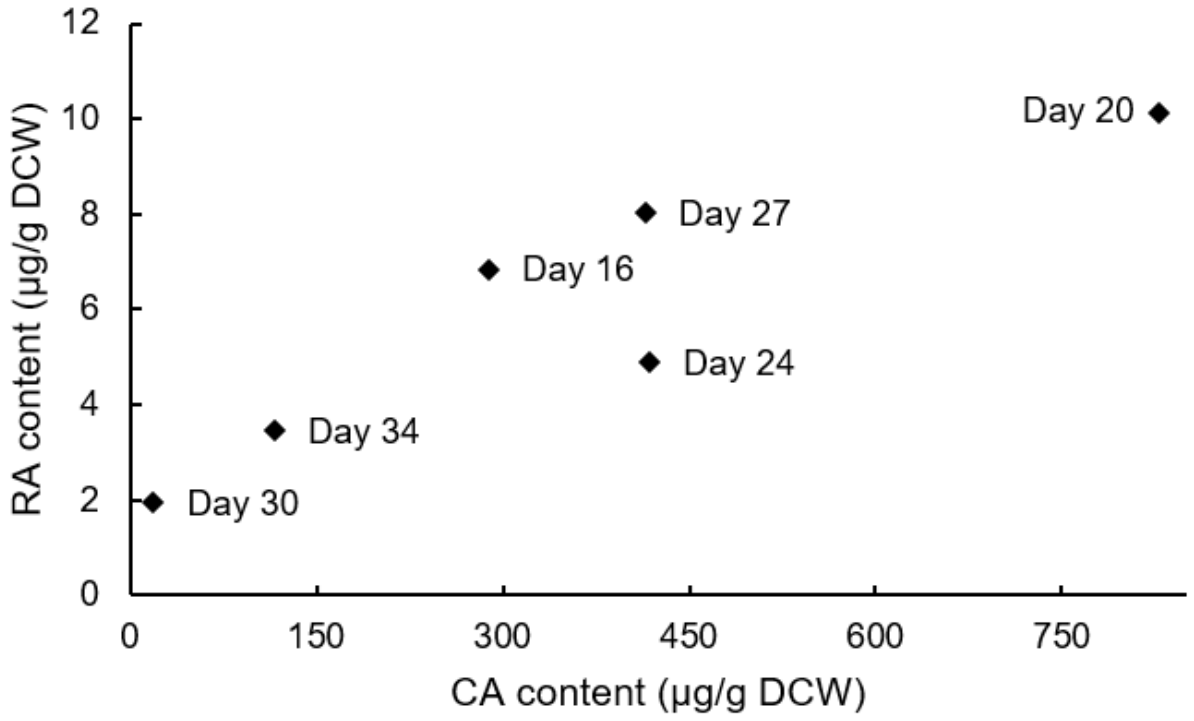

Figure 2

Changes in the concentrations of chlorogenic acid (CA) and rosmarinic acid (RA) in the dry cell weight (DCW) from day 16 to day 34 of culture. The production of CA was correlated ($\rho = 0.8702$) with the production of RA. Values represent the means of three replicates.