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Valorization of Mexican Rambutan Peel through the Recovery of Ellagic Acid via Solid-State Fermentation Using a Yeast

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Abstract: Rambutan (*Nephelium lappaceum* L.) is a tropical fruit that is originally from Southeast Asia and it was introduced to Mexico in the 1960s; the fruit's peel is known to possess ellagitannins such as ellagic acid which give the peel great biological activity; solid-state fermentation has been used to obtain said compounds and rambutan peel can be used as a fermentation support/substrate; this work aims to obtain, identify and quantify ellagic acid obtained via SSF with a strain of yeast. The water-absorption index and the support's maximum moisture were determined. To determine the ideal conditions for ellagic acid accumulation, a Box–Behnken 3^k experimental design was applied using variables such as temperature, moisture and inoculum. The maximum accumulation time of ellagic acid via solid-state fermentation was determined to be 48 h with ideal conditions of 30 °C, 60% moisture and 1.5×10^7 cells/g using *S. cerevisiae*, and high-performance liquid chromatography was used to identify ellagic acid, geraniin and corilagin as the most abundant compounds. The maximum recovery of ellagic acid was 458 ± 44.6 mg/g. HPLC/ESI/MS analysis at 48 h fermentation showed biodegradation of geraniin and corilagin due to ellagic acid. Mexican rambutan peel has been demonstrated to be a suitable substrate for SSF.

Keywords: rambutan; solid-state fermentation; ellagic acid; yeast; ellagitannins

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1. Introduction

Ellagic acid (EA) (2,3,7,8-tetrahydroxy [1]-benzopyranol [5,4,3-cde] benzopyran-5,10-dione) is a highly stable molecule, soluble in water, alcohol and ether [1]; EA can be obtained from hydrolysis of ellagitannins, also named hydrolyzable tannins, which are polyphenolic compounds associated with gallotannins; structurally, they are esters comprised of one or more hexahydroxydiphenic acid (HHDP) units as well as galloyl units attached to a central glucose nucleus possessing molecular weights of up to 300–20,000 Daltons (Da) [2]. HHDP groups are produced via the chemical reaction of hydrolysis using acids and bases that proceed from separating the glycosidic nucleus that subsequently creates lactones spontaneously to obtain EA [3,4].

The attention attracted to EA is due to it is wide range of biological activities, including antioxidant, anticarcinogenic, antimutagenic, anti-inflammatory, antithrombotic and antimicrobial activities [5,6]. Ellagic acid is also known for providing health benefits, improving the process of wound healing, maintaining the skin's elasticity and improving the quality of food by preventing oxidation; EA also has a great potential use in the health sector for medical, cosmetic and pharmaceutical industries [7,8].

Ellagitannins can be obtained from natural sources such as berries, nuts, almonds, raspberries, blackberries, pomegranates, blueberries, mangos, currants and guava [1]. Rambutan (*Nephelium lappaceum* L.) is currently one of the main sources for obtaining EA due to its high content of ellagitannins; the most abundant compound found in rambutan's peel are geraniin, corilagin, rutin, quercetin and ellagic acid [9,10].

Rambutan is a tropical fruit that belongs to the family *Spaindaceae* and that originates in Southeast Asia in tropical countries such as Thailand, Indonesia and Malaysia, which are the main producing and exporting countries. It has an ovoid shape that measures approximately 5 to 8 cm long and 2 to 5 cm wide with a weight of 22.4 and 64.7 g; it is associated with other subtropical fruits such as lychee and longan (Jahurul et al., 2020; Peixoto Araujo et al., 2021) [11,12]. Rambutan is composed of seed, pulp and peel; at the moment of being industrially processed for consumption for the production of sold canned products, juices, liquors, jams or jellies, the seeds and peel are discarded as agro-industrial by-products [13,14]. In Mexico, rambutan is mainly cultivated in the state of Chiapas in the region of Soconusco, but it can also be harvested in other states of Mexico such as Veracruz, Tabasco, Guerrero, Michoacán and Nayarit, where the climate favors the growth of the tree [15].

EA can potentially be obtained through conventional techniques such as maceration and soxhlet extraction. However, the disadvantages of these techniques have been far greater; one of the most important disadvantages is the use of organic solvents which are highly toxic and polluting. On the other hand, new emergent technologies such as ultrasound and microwave-assisted extraction have shown to be efficient and environmentally friendly, but they require high energy consumption and have high market prices [16,17].

To recover EA from a natural source, solid-state fermentation (SSF) is an effective alternative that requires low free-water content [18]; this bioprocess has been implemented using agro-industrial waste as a support and carbon source for obtaining bioactive compounds through the use of microorganisms that are deemed suitable, such as filamentous fungi [19,20]. The metabolism of the microorganism in the substrate depends on environmental conditions such as temperature, water activity, oxygen availability and pH. Appropriate methods are required to recover and purify the product obtained [21]. Yeasts such as *Saccharomyces cerevisiae* have shown the potential to degrade ellagitannins, obtaining EA from pomegranate peel [22].

So far, no studies have been reported on the recovery of EA from Mexican rambutan peel using the SSF bioprocess with yeast. The objective of the present work was to obtain, identify and quantify ellagic acid SSF using a support/substrate based on Mexican rambutan.

2. Materials and Methods

2.1. Raw Material

Fresh rambutan was recollected in the Soconusco region (15°18′56″ N 92°43′35″ O) in the state of Chiapas, Mexico; then the peel was obtained and it was left to dehydrate at 50 °C in a conventional oven for 72 h and grounded to obtain a powder (particle size 2 mm) that was stored in plastic bags protected from light at room temperature

2.2. Microorganisms

Saccharomyces cerevisiae and *Yarrowia lipolytica* yeast strains (obtained from the collection of the Food Research Department of the Autonomous University of Coahuila) were used in this study. The strains were preserved via cryopreservation in a specialized solution (milk powder/glycerol) at a temperature of −20 °C. The cells were activated on potato dextrose agar (PDA-Bioxon, México City, Mexico) and kept in an incubator at a temperature of 30 °C for 3 days.

2.3. Material Characterization

2.3.1. Water-Absorption Index and Maximum Moisture

The water-absorption index was measured using 50 mL centrifuge tubes each with 1.25 g of material and 30 mL of distilled water. The sample was then centrifuged for 30 min at 4900 rpm in a centrifuge model 80-2B (jfLAREN, Guamuchil, Sinaloa, Mexico). The tube with the sample was weighed, discarding the supernatant. The water absorption rate was calculated according to Equation (1) and expressed as grams of gel per gram of dry weight (g/g gel).

$$\text{WAI} = (\text{weight of gel (g)})/(\text{weight of dry support (g)}) \quad (1)$$

The solids and moisture content of the material were determined using a thermobalance (OHAUS, Parsippany, NJ, USA) by weighing 1 g of dry raw material. The maximum support moisture was calculated with the values obtained for solids, moisture content and water-absorption index [23].

General and solid balance:

$$\begin{gathered} M1 + M2 = M3 \\ M1(Xs.1) = M3(Xs.3) \\ Xs.3 = \frac{(M1)(Xs.1)}{M3} \end{gathered} \quad (2)$$

where $Xs.3$ is the maximum moisture of the support (%), $M1$ is the mass of dry material (g), $Xs.1$ is the percentage of total solids and $M3$ is the water-absorption index.

2.3.2. Adaptation of the Microorganisms on Rambutan Peel

A total of 3 g of raw material was weighed and placed in Petri dishes and 6 mL of distilled water was added. As a previous step, *Saccharomyces cerevisiae* and *Yarrowia lipolytica* strains were grown on PDA agar. Once both yeast strains were fully grown, 30 mL of Tween 80 were added for cell collection; subsequently, 1 mL of the solution was placed in Petri dishes with the raw material and water. Growth was measured every 12 h for 48 h and was determined by dry weight (mg/g).

2.3.3. Determination of the Time of Maximum Accumulation of Ellagic Acid via Solid-State Fermentation

SSF was carried out with the selected microorganism to determine the maximum accumulation time of EA. Rambutan-peel powder (1.5 g) was used as a support and placed in polypropylene reactors (5 cm^3) where it was moisturized with a culture medium (3.5 mL to obtain a moisture content of 70%) with the following components: peptone (0.8 g/L), yeast extract (0.8 g/L) and NaCl (0.0184 g/L) and yeast inoculum (2×10^7 cells/g) [22]. The fermented extracts were collected every 12 h for 72 h by manual pressing, adding absolute ethanol/distilled water, 1:1 *v/v* in order to recover EA and were stored under refrigeration (−20 °C) for further analytical determinations and HPLC/ESI/MS analysis. All experiments were performed in triplicate [24].

2.3.4. Selection of the Best Conditions

SSF was evaluated once the maximum EA accumulation was found, with three independent variables—temperature (°C), moisture (%) and inoculum (cells/g)—each at three levels in a response surface methodology (Table 1) using the selected yeast strain. The response variable was EA and the extracts obtained were set to be recovered after 48 h fermentation by manual pressing. Analytical determinations of total polyphenols, antioxidant activity and HPLC/ESI/MS were performed [24].

Table 1. Experimental matrix and evaluated factors of the Box–Behnken 3^k experimental design.

Run	Temperature (°C)	Moisture (%)	Inoculum (Cells/g)
1	−1	−1	0
2	−1	0	−1
3	−1	0	1
4	−1	1	0
5	0	−1	−1
6	0	−1	1
7	0	0	0
8	0	0	0
9	0	0	0
10	0	1	−1
11	0	1	1
12	1	−1	0
13	1	0	−1
14	1	0	1
15	1	1	0
Factors	**Low level (−1)**	**Medium level (0)**	**Maximum level (1)**
Temperature (°C)	25	30	35
Moisture (%)	50	60	70
Inoculum (cells/g)	1×10^7	1.5×10^7	2×10^7

2.3.5. Determination of Total Polyphenols

Hydrolyzable polyphenols and condensed polyphenols were used to measure polyphenolic content. Hydrolyzable polyphenols present in the fermentation extracts in rambutan peel were determined using the Folin–Ciocalteu reagent. Briefly, 400 µL of Folin–Ciocalteu reagent were added to 400 µL of the sample; after 5 min, 400 µL of Na_2CO_3 was added followed by 2.5 mL of distilled water. Gallic acid was used as a standard in the range of 0–500 ppm; the absorbance of the samples was measured at 790 nm by using a spectrophotometer Biomate 3 (Thermo Spectronic, Madison, WI, USA). The experiment was carried out in triplicate, expressing the results as mg/g [25].

Condensed polyphenols in rambutan-peel extracts were determined using HCl-butanol reagent (1:9 *v/v*) and ferric reagent. The catechin reagent was used as a standard in the 0–1000 ppm range. Then, 3 mL of HCl-butanol solution was added to 500 µL of the sample, followed by 100 µL of ferric reagent. The solutions were mixed and taken to a water bath at 100 °C for 1 h. The solutions were allowed to cool at room temperature and the absorbance was measured at 460 nm using a spectrophotometer Biomate 3 (Thermo Spectronic, Madison, WI, USA). The experiment was performed by triplicate, expressing the results as mg/g [26].

2.3.6. Antioxidant Activity

$ABTS^{\bullet+}$ Assay

Determination of antioxidant activity was measured with the ABTS reagent technique which uses a free-radical reagent 2,2-0-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid, No. A1888, Sigma Aldrich, St. Louis, MO, USA) ($ABTS^{\bullet+}$) which was prepared by mixing a solution of 7 mM ABTS and 2.45 mM potassium persulfate each in 5 mL of distilled water and then mixing both solutions. The mixture was then poured into a light-protected container for 12 to 16 h. Once the time passed, the solution was measured at an absorbance of 734 nm to discern the absorbance and dilute it as necessary with ethanol to adjust the absorbance to 0.7. Once the reagent was adjusted, 1 mL of the ABTS solution and 10 µL of the diluted sample (1:50) (fermented rambutan-peel extracts) were poured into an assay tube; then the absorbance was measured at 734 nm using a spectrophotometer

Biomate 3 (Thermo Spectronic, Madison, WI, USA). The results were calculated according to Equation (3) [27].

$$ABTS\ inhibition\ \% = \frac{Ac - As}{Ac}\ (100) \quad (3)$$

where *Ac* represents the control absorbance of ABTS + ethanol and *As* represents the sample absorbance.

DPPH• Assay

To determine the antioxidant activity, the free-radical reagent 2,2-diphenyl-1-picrylhydrazyl (DPPH•, No, D913, Sigma Aldrich, Steinheim, Germany) was prepared in a solution with a concentration of 60 µM in methanol. Then 1475 µL of the DPPH reagent and 25 µL of the diluted (1:50) sample (fermented rambutan-peel extracts) were added and allowed to stand for 30 min at room temperature. Absorbance was measured at 517 nm using a spectrophotometer Biomate 3 (Thermo Spectronic, Madison, WI, USA) and the results were calculated according to Equation (4) [28]

$$DPPH\ inhibition\ \% = \frac{Ac - As}{Ac}(100) \quad (4)$$

where *Ac* represents the control absorbance of DPPH + ethanol and *As* represents the sample absorbance.

2.3.7. Ellagic Acid HPLC/ESI/MS Analysis

The recovered EA was analyzed via HPLC/ESI/MS (High-Performance Liquid Chromatography/Electrospray Ionization/Mass Spectrometry) (Varian Prostar, Santa Clara, CA, USA) using a photodiode array detector. The fermented rambutan husk extracts were filtered (0.45 um). The analyses via Reverse Phase–High Performance Liquid Chromatography were performed on a Varian HPLC system including an autosampler (Varian ProStar 410, USA), a ternary pump (Varian ProStar 230I, USA) and a PDA detector (Varian ProStar 330, USA). A liquid chromatograph ion trap mass spectrometer (Varian 500-MS IT Mass Spectrometer, USA) equipped with an electrospray ion source was also used. Samples (5 µL) were injected into a Denali C18 column (150 mm × 2.1 mm, 3 µm, Grace, Palo Alto, CA, USA). The oven temperature was maintained at 30 °C. The eluents were formic acid (0.2 %, *v/v*; solvent A) and acetonitrile (solvent B). The following gradient was applied: initial, 3% B; 0–5 min, 9% B linear; 5–15 min, 16% B linear; 15–45 min, 50% B linear. The column was then washed and reconditioned. The flow rate was maintained at 0.2 mL/min and elution was monitored at 254, 280, 320, and 550 nm. The whole effluent (0.2 mL/min) was injected into the source of the mass spectrometer, without splitting. All MS experiments were carried out in the negative mode $[M\text{-}H]^-$. Nitrogen was used as nebulizing gas and helium as a damping gas. The ion source parameters were spray voltage of 5.0 kV, and capillary voltage and temperature were 90.0 V and 350 °C, respectively. Data were collected and processed using MS Workstation software (V 6.9). Samples were firstly analyzed in full scan mode acquired in the m/z range 50–2000. MS/MS analyses were performed on a series of selected precursor ions [24].

2.4. Statistical Analysis

For the evaluation of the effect of temperature, moisture and inoculum of fermented rambutan-peel extracts, an experimental matrix of Box–Behnken 3^k was constructed using STATISTICA 7.0 software (Stat Soft, Tulsa, OK, USA) and comparison of means was performed via Tukey's test ($p < 0.05$) using OringPro 2022. All experiments were performed in triplicate and the results were expressed as mean (n = 3) ± standard deviation and subjected to analysis of variance (ANOVA, $p < 0.05$).

3. Results

3.1. Evaluated Parameters of Rambutan Peel

Table 2 shows the results of the evaluated parameters of rambutan peel. Rambutan peel showed a water-absorption index (WAI) of 5.44 g of gel/g of dry weight and maximum support moisture of 83 %.

Table 2. Evaluation parameters of rambutan peel.

Parameters	Results
Moisture (%)	6.72 ± 1.45
WAI (g of gel/g of dry weight)	5.44 ± 0.06
Maximum support moisture (%)	83 ± 0.01

3.2. Growth of Microorganisms on Rambutan Peel

Both strains of yeasts *S. cerevisiae* and *Y. lipolytica* adapted to the substrate within 12 to 24 h (50 and 80 mg/g) of growth. However, at 36 h there was a decrease (33.3 mg/g) and finally at 48 h (40 mg/g) growth increased again; this is due to both microorganisms feeding on new sources that provide energy to regrow on the substrate *S. cerevisiae* and *Y. lipolytica.*

3.3. Solid-State Fermentation

Once the yeast strain (*S. cerevisiae*) was selected, kinetics were performed to determine the maximum EA-accumulation time via SSF under the previously mentioned conditions. The maximum recovery of EA was found at 48 h (136.86 ± 16.06 mg/g), as shown in Figure 1. Based on this, 48 h was defined as the final time for further experiments. In addition, the total polyphenolic content was evaluated using the techniques previously mentioned in the methodology. At 0 h, the initial total polyphenolic content measured 133.25 mg/g. However, at 12 h, it presented a polyphenolic consumption and later after 24 h, it showed the release of these. This shows that the *S. cerevisiae* strain can promote the increase and consumption of phenolic compounds.

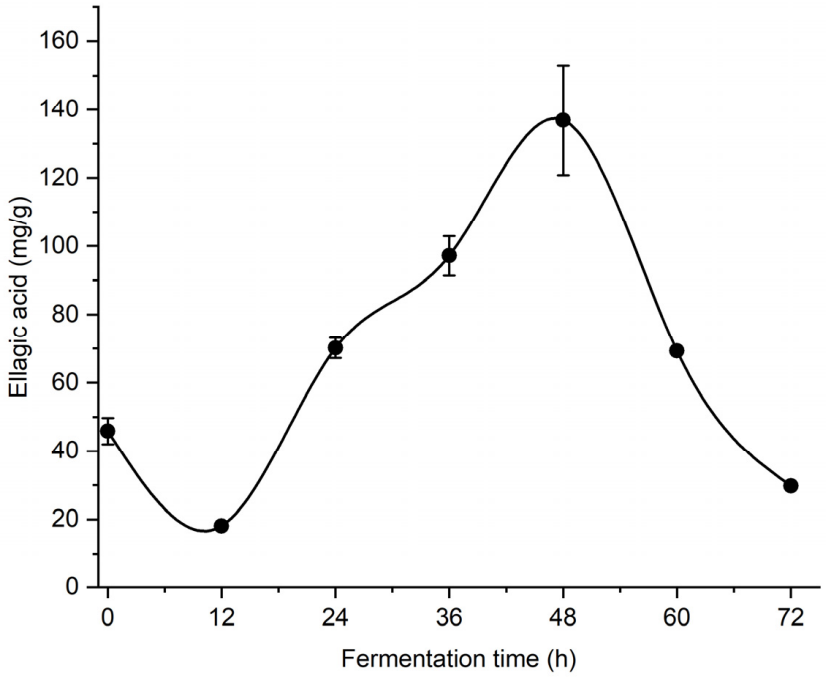


Figure 1. Solid-state fermentation kinetics.

Mexican rambutan peel contains several ellagitannins, the most abundant being geraniin, corilagin and EA. These were quantified at 0 and 48 h. At 0 h, the concentrations of geraniin (357.80 ± 30.40 mg/g) and corilagin (407.59 ± 17.51 mg/g) were higher compared to EA (45.89 ± 3.68 mg/g). However, after 48 h of fermentation, the concentration of geraniin (21.63 ± 2.12 mg/g) and corilagin (44.57 ± 0.59) decreased, giving a higher production of EA (136.86 ± 16.06 mg/g).

3.4. Selection of the Best Conditions and Statical Analysis

The results of the Box–Behnken 3^k experimental design matrix are shown in Table 3, showing treatments and SSF conditions for EA recovery. It was found that treatment 8 accumulated a higher concentration of EA (458.37 ± 44.6 mg/g) using the conditions (30 °C, 60% moisture, 1.5×10^7 cells/g). For all treatments, there were significant differences in EA concentrations.

Table 3. Experimental matrix of the treatments of the Box–Behnken 3^k experimental design. (the letters represent significant differences).

Treatment	Conditions	EA (mg/g)	Total Polyphenols	DPPH (%)	ABTS (%)
1	25 °C/50%/1.5×10^7	14.02 ± 4.4 ^{fg}	58.39 ± 5.14 ^{fg}	62.22 ± 0.62 ^{ab}	95.90 ± 5.90 ^{a}
2	25 °C/60%/1×10^7	72.51 ± 19.12 ^{de}	64.93 ± 6.33 ^{defg}	56.22 ± 7.99 ^{ab}	99.00 ± 1.00 ^{a}
3	25 °C/60%/2×10^7	81.7 ±10.01 ^{d}	67.55 ± 7.45 ^{defg}	63 ± 2.27 ^{a}	94.00 ± 4.83 ^{a}
4	25 °C/70%/1.5×10^7	177.49 ±11.67 ^{b}	80.05 ± 9.58 ^{bcd}	60.86 ± 0.22 ^{ab}	96.95 ± 0.3 ^{a}
5	30 °C/50%/1×10^7	27.84 ± 7.92 ^{efg}	58.05 ± 3.48 ^{fg}	62.35 ± 0.11 ^{ab}	99.38 ± 0.70 ^{a}
6	30 °C/50%/2×10^7	98.88 ± 5.09 ^{cd}	55.33 ± 1.71 ^{g}	62.41 ± 0.4 ^{ab}	100.00 ± 0.52 ^{a}
7	30 °C/60%/1.5×10^7	146.17 ± 5.69 ^{bc}	67 ± 1.16 ^{defg}	62.41 ± 0.73 ^{ab}	94.24 ± 7.18 ^{a}
8	30 °C/60%/1.5×10^7	458.37 ± 44.6 ^{a}	62.06 ± 1.08 ^{efg}	62.41 ± 1.94 ^{ab}	95.33 ± 5.00 ^{a}
9	30 °C/60%/1.5×10^7	90.81 ± 2.58 ^{d}	68.12 ± 1.03 ^{cdefg}	62.41 ± 0.87 ^{ab}	97.14 ± 2.18 ^{a}
10	30 °C/70%/1×10^7	196.30 ± 28.65 ^{b}	75.27 ± 4.75 ^{bcde}	60.93 ± 4.02 ^{ab}	98.71 ± 1.49 ^{a}
11	30 °C/70%/2×10^7	64.13± 14.41 ^{def}	73.13 ± 2.91 ^{cdef}	62.93 ± 0.11 ^{a}	98.19 ± 1.89 ^{a}
12	35 °C/50%/1.5×10^7	88.74 ± 20.12 ^{d}	75.1 ± 4.85 ^{cde}	47.2 ± 14.44 ^{b}	97.38 ± 2.86 ^{a}
13	35 °C/60%/1×10^7	10.19 ± 3.44 ^{g}	90.61 ± 6.85 ^{ab}	57.9 ± 4.41 ^{ab}	94.71 ± 5.32 ^{a}
14	35 °C/60%/2×10^7	9.32 ± 1.67 ^{g}	83.22 ± 9.04 ^{bc}	51.77 ± 7.32 ^{ab}	99.86 ± 0.25 ^{a}
15	35 °C/70%/1.5×10^7	20.11 ± 0.68 ^{fg}	103.66 ± 7.47 ^{a}	52.8 ± 5.07 ^{ab}	99.43 ± 0.74 ^{a}

Antioxidant activity was used to observe an increase in the fermented extracts of Mexican rambutan peel; it was found that ABTS showed results greater than 90% inhibition, while DPPH showed results between 40 and 60%.

Figure 2 shows the Pareto chart where the influence of the factors evaluated in the Box–Behnken design is shown. These factors include temperature, moisture and inoculum. The factors that exceeded the 95% reliability line were temperature and inoculum and they presented a significant positive effect in their quadratic form, so that if the temperature and inoculum level increase, the EA yield will be higher; however, this depends on the microorganism used in the process.

The response surface diagram of the Box–Behnken 3^k experimental design (Figure 3) shows on the X and Y axes the significant factors during the process and on the *Z* axis the response variable, which means that at a temperature of 30 °C (*X* axis, level 0) and using an inoculum of 1.5×10^7 (*Y* axis, level 0) there is a greater accumulation of EA, meaning that the ideal conditions to achieve higher EA production are with the following variables: temperature of 30 °C and an inoculum concentration of 1.5×10^7 cells/g.

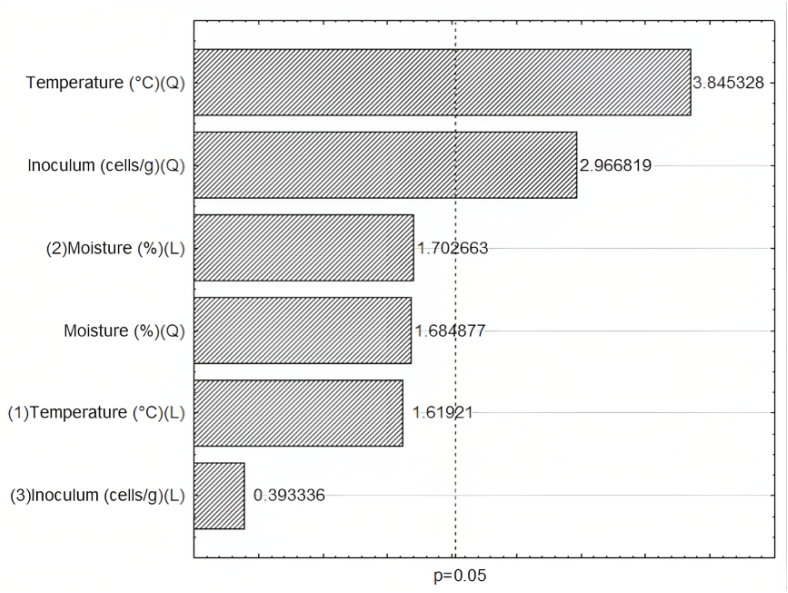


Figure 2. Pareto chart of Box–Behnken 3^k experimental design. L = Lineal (influence at two certain points of the process) Q = Quadratic (influence at three points of the process).

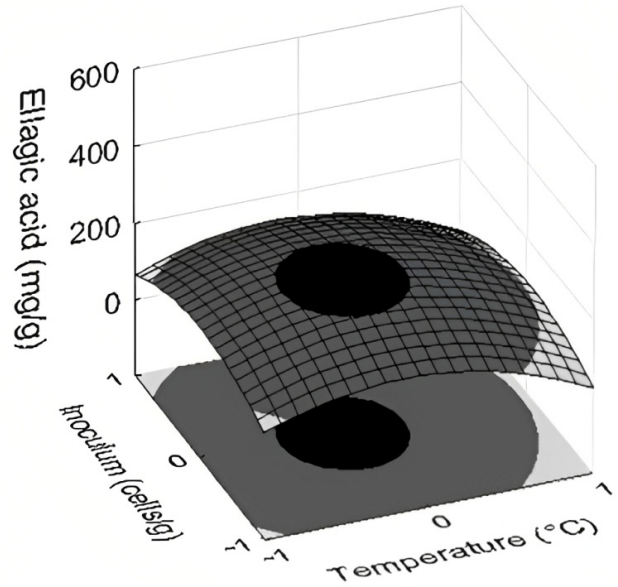
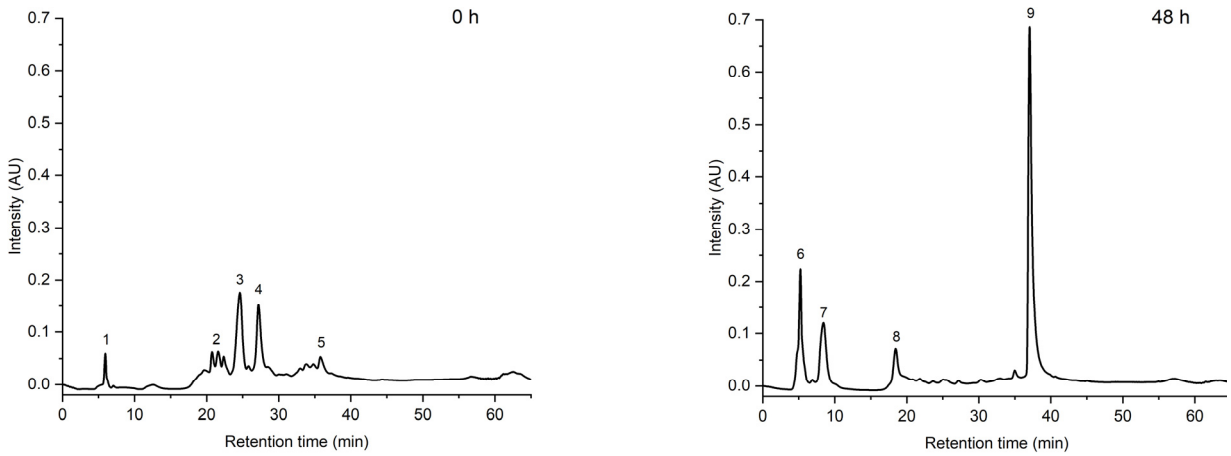


Figure 3. Surface plot of the Box–Behnken 3^k experimental design.

3.5. Identification of Ellagic Acid via HPLC/ESI/MS

The compounds of the fermented extracts of Mexican rambutan peel were identified via HPLC/ESI/MS. Figure 4 shows the chromatograms with the compounds identified in the SSF kinetics (time of maximum accumulation of EA); the major compounds (geraniin and corilagin) were identified at 0 h (unfermented Mexican rambutan peel). Likewise, the compounds present at 48 h were identified, where it was observed that the peaks of geraniin and corilagin disappeared, which shows that biodegradation gave rise to several compounds, among them the EA. Table 4 shows the compounds identified from treatment 8 (30 °C, 60%, 1.5×10^7 cells/g), including EA, after 48 h of fermentation.



(**a**) (**b**)

Figure 4. Chromatograms obtained via HPLC/ESI/MS analysis of the SSF kinetics of rambutan-peel extracts: time 0 h: (**a**) unfermented rambutan peel: (1) caffeic acid 4-O-glucoside; (2) p-coumaric acid 4-O-glucoside and galloyl-HHDP-Hexoside; (3) corilagin; (4) geraniin; (5) ellagic acid; time 48 h: (**b**) extract fermented rambutan peel: (6) gallic acid 4-O-glucoside; (7) gallic acid; (8) catechol; (9) ellagic acid.

Table 4. Compounds identified via HPLC/ESI/MS in treatment 8.

ID	Retention Time (min)	Compound	$[M-H]^-$ (m/z)	MS^2	Group
1	7.03	Gallic acid 4-O-glucoside	331	169, 125	Hydroxybenzoic acids
2	13.22	Gallic acid	169	125	Hydroxybenzoic acids
3	20.75	Cyanidin 3-O-(6″-malonyl-3″-glucosyl-glucoside)	697	565, 393, 271, 209, 147	Anthocyanins
4	39.73	Ellagic acid	301	257, 229, 185	Hydroxybenzoic acid dimers

4. Discussion

The water-absorption index (WAI) and the maximum moisture of the support are both important parameters for carrying out an SSF. Mussatto et al. (2009) [25] describe WAI as the amount of water that can be absorbed by the support and gel-forming capacity of the water macromolecules, allowing the availability of hydrophilic groups. Furthermore, Robledo et al. (2008) [23] mention that materials with high levels of WAI are preferred for carrying out SSF since the microorganism presents a higher growth. In a reported study, Larios et al. (2017) [29,30] evaluated grapefruit residues as support for SSF and the obtained WAI value was 4.30 g gel/g dry weight, which is lower than the WAI value obtained with the Mexican rambutan peel. Likewise, Buenrostro-Figuroa et al. (2023) [18] evaluated the pomegranate peel to be implemented as support in SSF, so they reported a WAI value of 4.38 g gel/g dry weight, which indicated that the pomegranate peel is an ideal support to be applied in an SSF process.

A previous study by Moccia et al. (2019) [22] reported that the time of maximum EA accumulation was in a period of 48 h, obtaining 46 mg/g employing pomegranate peel as substrate and *S. cerevisiae* as microorganism. On the other hand, Cerda-Cejudo et al. (2022) [24] reported the maximum accumulation of EA at 24 h where 13.90 mg/g was obtained using Mexican rambutan peel as substrate and *Aspergillus niger* GH1 as the fermenting microorganism. In both studies, an SSF was performed to obtain the compound of interest. The results obtained in this study were better than those reported in the studies mentioned above; in addition, *S. cerevisiae* proved to be a good microorganism for the biodegradation of ellagitannins present in rambutan peel to obtain EA.

In a study by Sepúlveda et al. (2014) [31], the ideal conditions for EA recovery using *A. niger* GH1 were evaluated in a submerged fermentation system employing a Box–Behnken 3^k experimental design with five treatments; they showed that the appropriate conditions were pH of 5.5, substrate concentration 7.5 g/L and agitation of 150 rpm. With those conditions, a maximum recovery of 20.66 mg/g was achieved. Sepúlveda et al. (2020) [32] used a central-compound design to evaluate parameters like temperature 30 °C, inoculum 2×10^7 and orange-peel polyphenol concentration 6.2 g/L, and managed to obtain a maximum EA concentration of 18.68 mg/g via a submerged state fermentation system. In both studies, the recovery of EA was carried out through submerged fermentation, a different process than that described in the present study. However, the results obtained by both authors were below those reported in this study.

The total polyphenolic content of Mexican rambutan peel was evaluated in a study by Hernández et al. (2017) [10], who reported a total polyphenolic content of 582 mg/g dry matter of Mexican rambutan peel via aqueous extraction; in this study case, the total polyphenol content of the fermented extracts of Mexican rambutan peel presented values ranging from 50 to 100 mg/g in the treatments, which showed a release of phenolic compounds.

Mexican rambutan's peel contains EA, which is a polyphenolic compound known to possess antioxidant activity, as mentioned in a study by Garcia-Niño and Zazueta, (2015) [1]. The antioxidant activity of this compound is due to the presence of the hydroxyl and lactone functional groups, which act as a receptor and donor of hydrogen bonds, allowing EA to eliminate radicals such as $O_2^{\bullet -}$, $HO^{\bullet}$, H_2O_2 and $ONOO^{\bullet}$.

In a study, Muhtadi et al. (2014) [33] evaluated rambutan-peel extracts, employing four different solvents out of which ethyl acetate showed an increase in antioxidant activity. Other polyphenols obtained from an SSF on other kinds of substrates like Castilla rose have shown antioxidant activity; in a study by De León-Medina et al. (2020) [34], polyphenols were recovered from Castilla rose using SSF and *A. niger.* These compounds showed free-radical scavenging activity in ABTS and DPPH with 94.34 and 68.71% inhibition, respectively; this can be directly related to the presence of EA in the polyphenolic extract obtained.

Temperature and inoculum factors are important in SSF. Pandey et al. (2008) [35] mention that the effect of the temperature can vary the inhibition of growth and the production of some secondary metabolites; in addition, each microorganism has a temperature range of adequate growth; also, the heat generated and accumulated in the SSF process reduces microbial growth and can even cause death; likewise, Crafack et al. (2014) [36] mention that if the inoculum in the fermentation process is not in an adequate physiological state for the production of secondary metabolites, it will show a decrease.

Previously, in a study by Estrada-Gil et al. (2022) [25], five compounds were identified in a Mexican rambutan-peel extract obtained by combining ultrasound and microwave technologies. Among them, the three most abundant compounds obtained were geraniin, corilagin and EA, which belong to the ellagitannin family. On the other hand, De León-Medina et al. (2023) [14] extracted the ellagitannins of rambutan peel via microwave/ultrasound, which were isolated via Amberlite XAD-16 column chromatography and implemented in an SSF as a substrate. Via HPLC/MS, it was observed that through SSF, geraniin allowed biodegradation in EA and ellagitanase was the enzyme involved in the biodegradation process with a value of 25.49 U/L in a period of 6–12 h.

5. Conclusions

The solid-state fermentation process has been determined to be a great alternative to obtain compounds of interest from different sources, one of which is Mexican rambutan peel, from which we can obtain ellagitannins with high levels of bioactivity such as EA. The extract obtained from the SSF on Mexican rambutan peel allowed the accumulation and identification of EA using *S. cerevisiae*, which demonstrated the capacity to biodegrade the major compounds such as geraniin and corilagin, in addition to obtaining a greater accumulation of EA in concentrations of 458.37 ± 44.6 mg/g using the selected culture

conditions (30 °C, 60%, 1.5×10^7 cells/g) for SSF. The temperature and inoculum factors were highly significant and influenced the production of EA, as evidenced on the Pareto chart. The EA recovered showed a high inhibitory capacity on ABTS and DPPH radicals, thus exhibiting antioxidant activity of great importance for future applications in the food and pharmaceutical fields.

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