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Enhancing the Release of Ellagic Acid from Mexican Rambutan Peel Using Solid-State Fermentation

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Abstract: This work describes research focused on the recovery of ellagic acid (EA) using solid-state fermentation-assisted extraction (SSF) with *Aspergillus niger* GH1 and Mexican rambutan peel as support. Several culture conditions (temperature, initial moisture, levels of inoculum, and concentration of salts) were evaluated using a Placket–Burman design (PBD) for screening culture factors followed by a central composite design (CCD) for enhancing the EA. Antioxidant activity and polyphenol content were evaluated in SSF. Temperature (28.2 °C), inoculum (2×10^7 spores/g), and $NaNO_3$ (3.83 g/L) concentration were identified as a significant parameter for EA in SSF. This enhancing procedure resulted in an increase in EA recovery [201.53 ± 0.58–392.23 ± 17.53 mg/g] and, with two steps of purification, [396.9 ± 65.2 mg/g] of EA compound was recovered per gram of recovered powder. Fermentation extracts reflect inhibition of radicals and the presence of polyphenol content. This work proposes to identify the ideal conditions of fermentation in order to obtain a higher yield high-quality compound from agro-industrial wastes through SSF.

Keywords: ellagitannins; *Aspergillus niger* GH1; bioprocess; agro-industrial waste; ellagic acid

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1. Introduction

Solid-state fermentation (SSF) is a procedure used for the generation of microbial metabolites, where the process is performed with low moisture content and a solid substrate, which can be from a natural source or synthetic [1]. This kind of bioprocess possesses the assets of a high product concentration and recovery with relatively low energy requirements, less water consumption, easy gaseous transportation, the use of smaller reactors, and the usage of agro-industrial wastes [2]. Although this method of recovery has many advantages, it also has a great disadvantage, which is the difficulty of upscaling it to large-scale industrial processing [2].

This bioprocess has been used to recover compounds of interest, like polyphenols, which are secondary metabolites present in plants. Polyphenols can be categorized in several types, but this study will focus on hydrolyzable polyphenols, especially in ellagitannins. The ellagitannins are composed of hexahydroxydipernic acid molecules (HHDP groups) attached to a central sugar core. When they are hydrolyzed, these molecules are released. Since the HHDP groups are not steady, they are rapidly lactonized leading to the formation of ellagic acid, which is a dilactone [3].

Ellagic acid (EA) is a derivative of ellagitannins found in several sources such as pomegranate, strawberries, rambutan, nuts, and green tea among others, and also in agro

industrial wastes, like peels or seeds [4]. This compound has been increasingly studied due to its relevance in improving human health. EA has been reported to be an antiproliferative agent against several cancers, including breast cancer, and also has been reported to exhibit biological activities such as antibacterial, cardio-, and gastro-protective, antioxidant, antiviral, and anti-inflammatory [5–8]. Although polyphenols are present in all plants, the kind and amount of these bioactive compounds vary depending on the plant, the ambience conditions, and their genetic factors. Also, their recovery by SSF depend on the factors that affect this bioprocess, like the size and type of bioreactors, the temperature, substrate, moisture, inoculum and microorganism, pH, moisture, and water capacity [2,9–11]. The modification of these factors will affect the production and extraction of bioactive compounds. Therefore, this study was aimed at evaluating the ideal SSF conditions using *Aspergillus niger GH1* and Mexican rambutan peel as the substrate and only carbon source, due to its importance as agro-industrial waste rich in ellagitannins, in EA recovery.

2. Materials and Methods

2.1. Microorganism and Plant Material

The strain GH1 of *A. niger* belonging to the DIA-UadeC collection and deposited in the Micoteca of the University of Minho (MUM 23.16) was used. A cryoprotective solution (skimmed milk/glycerol 9:1 v/v) at −55 °C was implemented for the conservation of the fungal strain. A potato dextrose agar (PDA-Bioxon®, Mexico City, Mexico) was used to activate the spores at a temperature of 30 °C for 5 days. A solution of 0.01% Tween 80 was used to recover the spores and a Neubauer chamber was used to count the spores/mL. Rambutan peel was collected in the region of Soconusco, in the state of Chiapas, Mexico; then, the peel was dehydrated at 50 °C for 72 h in a conventional oven, and ground to a fine powder of 150 µM particles. It was then protected from light and stored in plastic bags at room temperature.

2.2. Water Absorption Capacity (WAC) and Maximum Moisture

An amount of 2.5 g of plant material and 30 mL of distilled water were mixed and incubated at 30 °C for 15 min in centrifuge tubes of 50 mL and, after heating, the sample was stirred. The sample was centrifuged for 10 min at 4000 rpm, the remaining gel was weighted, and the supernatant of the sample was discarded. Water absorption capacity (WAC) was calculated and expressed as gel grams per gram of dry weight (gel g/g) [12].

$$WAC = \frac{weigth\ of\ gel\ (g)}{weigth\ of\ dry\ suppot\ (g)}$$

In a thermo-balance (OHAUS, Parsippany, NJ, USA), the moisture and solids content of the material were determined by weighing 1.0 g of plant material. The solids, moisture content, and WAC values were used to calculate the maximum material moisture [12].

$$M1 + M2 = M3$$

$$Solids\ Balance = M1Xs, 1 = M3\ Xs, 3$$

$$Maximum\ material\ moisture = \frac{(Dry\ sample\ (g))(\%\ Solids)}{(\%\ Solids)}$$

2.3. Screening of SSF Conditions

SSF was performed on tray reactors (polypropylene, 125 cm^3) with Mexican rambutan peel as support. A minimal culture Czapek-Dox medium was prepared and inoculated with spores of *A. niger* GH1. Mexican rambutan peel was impregnated with the culture medium inoculated at an initial moisture content according to Table 1. The bioreactors were maintained at defined temperatures for 24 h [12]. A mix of ethanol–water solution (50:50 v/v) was used to recover the extracts, then these were manually filtered and stored in an amber flask protected from light. All fermentations were performed in triplicate.

Table 1. The PBD matrix used to determine the influence of different variables (A, B, C, D, E, F, and G) on EA (mg/g) from Mexican rambutan peel powder by *A. niger* GH1 under SSF conditions.

Treatment	A	B	C	D	E	F	G	EA (mg/g) *
1	−1	−1	−1	1	1	1	−1	97.5 ± 1.9^{c}
2	1	−1	−1	−1	−1	1	1	18.3 ± 5.3^{e}
3	−1	1	−1	−1	1	−1	1	132.5 ± 7.3^{b}
4	1	1	−1	1	−1	−1	−1	24.7 ± 5.2^{d}
5	−1	−1	1	1	−1	−1	1	91.1 ± 10.1^{c}
6	1	−1	1	−1	1	−1	−1	91.3 ± 6.4^{bc}
7	−1	1	1	−1	−1	1	−1	201.5 ± 0.6^{a}
8	1	1	1	1	1	1	1	80.5 ± 11.1^{bc}
Code		Factors				High level		Low level
A		Temperature (°C)				30		25
B		Moisture (%)				70		60
C		Inoculum (spores/g)				2×10^{7}		1.5×10^{7}
D		$NaNO_3$ (g/L)				7.65		3.83
E		KCl (g/L)				5.08		2.54
F		$MgSO_4$ (g/L)				3.04		1.52
G		KH_2PO_4 (g/L)				3.04		1.52

* Different letters mean no significant differences among treatments (Tukey $\alpha = 0.05$).

2.4. Enhancement of EA Using a Central Composite Design

A central composite design (CCD) was used based on PBD results, to find the conditions of maximal EA recovery. Hence, three independent variables were coded at three levels (−1, 0, and 1) with two axial points ($-\alpha$ and α) and six central points (Table 2).

Table 2. Condensed matrix from CCD used to optimize EA recovery from Mexican rambutan peel by *A. niger* GH1.

	A	B	C		
Treatment	**Temperature (°C)**	**Inoculum (Spores/g)**	**$NaNO_3$ (g/L)**	**EA Recovered (mg/g)**	
1	−1	−1	−1	27.9 ± 0.8^{h}	
2	−1	−1	1	96.9 ± 8.5^{fg}	
3	−1	1	−1	101.5 ± 5.4^{fg}	
4	−1	1	1	102.4 ± 2.0^{fg}	
5	1	−1	−1	121.9 ± 13.3^{ef}	
6	1	−1	1	117.9 ± 5.7^{efg}	
7	1	1	−1	148.5 ± 4.5^{de}	
8	1	1	1	255.8 ± 27.8^{b}	
9	$-\alpha$	0	0	280.5 ± 15.6^{b}	
10	α	0	0	392.2 ± 17.5^{a}	
11	0	$-\alpha$	0	203.5 ± 10.5^{c}	
12	0	α	0	83.2 ± 4.3^{g}	
13	0	0	$-\alpha$	148.3 ± 2.1^{de}	
14	0	0	α	257.3 ± 13.7^{b}	
15C	0	0	0	163.7 ± 11.8^{d}	
16C	0	0	0	178.9 ± 7.2^{cd}	
			Levels		
Factors	α	1	0	−1	$-\alpha$
Temperature (°C)	28.2	27	25	23	21.8
Inoculum (spores/g)	2.8×10^{7}	2.5×10^{7}	2×10^{7}	1.5×10^{7}	1.2×10^{7}
$NaNO_3$ (g/L)	6.87	5.75	3.83	1.915	0.766

* Different letters mean no significant differences among treatments (Tukey $\alpha = 0.05$).

2.5. Analytical Analysis

2.5.1. Determination of Total Polyphenols

Total polyphenolic content was determined by condensed polyphenols and hydrolysable polyphenols content. Condensed polyphenols were determined using ferric reagent and HCl–butanol assay (1:9 v/v) and using a curve of catechin (1000 ppm) [13]. The resulting reaction was then measured at 460 nm using a Biomate 3 spectrophotometer (Thermo Spectronic, Madison, WI, USA). Hydrolysable polyphenols were determined using Folin–Ciocalteu's essay, using a mixture of distilled water, sodium carbonate, and Folin–Ciocalteu's reagent (Sigma-Aldrich, México City, Mexico), compared to a curve of gallic acid [1000 ppm]. Using a Biomate 3 spectrophotometer (Thermo Spectronic, Madison, WI, USA) the reaction was then measured at 790 nm. The results show the mean of three replicates, expressed as mg/g for each analysis [13,14].

2.5.2. ABTS Antioxidant Assay

In a dark room, by mixing ABTS solution 7 mM (1:2 v/v) and potassium persulfate 2.45 mM the 2,2-0-Azino-bis(3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS) free radical reagent was prepared. The mixture was left to rest for 12–16 h protected from light. The absorbance of the solution was measured at 734 nm and adjusted at 0.7 by the addition of ethanol after this time. The reaction was conducted by mixing 1 mL of ABTS solution with 10 µL of the sample (fermented rambutan peel extracts); then, using a Biomate 3 spectrophotometer (Thermo Spectronic, Madison, WI, USA) the absorbance was measured at 734 nm. The trolox reagent curve was used as a control. The results were calculated and reported as mEq trolox per gram [15].

2.5.3. DPPH Antioxidant Assay

The 2,2-diphenyl-1-picrylhydracyl (DPPH) free radical reagent was prepared at a 60 µM concentration in methanol. Then, at room temperature, 7 µL of the sample (fermented rambutan peel extracts) was mixed with 193 µL of DPPH–methanol solution in a 96-well microplate which was allowed to rest for 30 min. To determine the absorbance of the solutions, a microplate reader at 517 nm (TECAN Sunrise, Männedorf, Switzerland) was used. The results were calculated and reported as mEq/trolox per gram. A DPPH–methanol reagent was used as a control, and the results were calculated and reported as mEq/trolox per gram [15,16].

2.6. Ellagic Acid HPLC-ESI-MS Analysis

For the analysis of the recovered EA of both fermentations, high-performance liquid chromatography (HPLC) (Varian ProStar 410, Palo Alto, CA, USA) was used using a photodiode array detector at 253 nm. Fermented fractions and EA standard (Sigma-Aldrich®) were filtered (0.45 µm) and injected at a ratio of (1:20 v/v) of rambutan extracts and solvent etanol–water (50:50 v/v). With a Denali C18 column (150 × 2.1 mm, 3 µm, Palo Alto, CA, USA), separation was performed at 30 °C in samples of 1 mL. The mobile phase (wash phase) was methanol, using acetonitrile (Solvent B) and acetic acid solution (3%, Solvent C) as eluents. Initially, the gradient program was 3% B and 97% C, 0–5 min 9% B and 91% C, 5–15 min 16% B and 84% C, 15–30 min 33%, and 67% C, 30–33 min 90% B and 10% C, 33–35 min 90% B and 10% C, 35–42 min 3% B and 97% C.

The flow rate was 1.2 mL/min with a volume of sample injection of 10 µL. The EA standard and the fermented fractions were analyzed directly using a Varian 500-MS ion tramp instrument equipped with an electrospray ionization (ESI) interface. The capillary voltage was 90 v. The spectra were recorded in a negative ion mode between m/z 100 and m/z 2000. The EA content was reported as mg of EA per gram of Mexican rambutan peel (mg/g) [12,17].

2.7. Statistical Analysis

To evaluate the effect of these parameters on EA recovery from the fermented extracts of rambutan peel and to evaluate the ideal solid-state fermentation parameters in the recovery of EA, a Placket–Burman design (PBD) (Table 1) and a central composite design (CCD) (Table 2) were used.

On the one hand, on a Placket–Burman design (PBD), seven variables (inoculum, temperature, moisture, Czapek-Dox medium salts, $NaNO_3$, KCl, $MgSO_4$, and KH_2PO_4) were evaluated to identify the variables with a significant effect on EA recovery (Table 1).

The central composite design (CCD) was completed to find the variable value levels for higher EA recovery (Table 2) based on the PBD results.

Three independent variables (Table 2) were coded at three value levels (−1, 0, and 1) and at two axial points ($-\alpha$ and α).

The experimental designs (PBD and CCD) were carried out in triplicate and the results were expressed as the mean (n = 3) ± standard deviation in which data were submitted to an analysis of variance (ANOVA). Using the Statistica 7.0 software (Stat Soft, Tulsa, OK, USA), the data analysis and model were designed and analyzed [18].

The outcome results were visualized in a Pareto chart with the absolute value of the magnitude of the variables' level in increasing order and compared to the minimum magnitude of statistically significant factors.

Ideal conditions were estimated by means of the regression coefficient generated for each assayed term and its combination for the CCD, their significance was obtained by α = 0.05. Then, the experimental data and regression coefficients were adjusted with the empiric polynomial model; the regression coefficients were obtained by the multiple lineal regression equation Equation (1):

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_0 X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \quad (1)$$

where Y represents the predicted response, (EA expressed in mg/g); X_i and X_j represent the independent variables; k is the number of variables evaluated; b_0, b_i, b_{ii}, and b_{ij} are the regression coefficient for the intercept, lineal, quadratic, and interaction effect terms, respectively, and mg/g means milligrams per grams [18].

2.8. Validation of the Model

The ideal conditions for EA recovery from Mexican rambutan peel (temperature, inoculum, moisture, Czapeck-Dox salts, $NaNO_3$, KH_2PO_4, $MgSO_4$, and KCl) were obtained from the predictive surface response equation. For the validation of the model, experimental and predicted values were statistically compared [18].

2.9. Separation and Partial Purification of EA Using Amberlite XAD-16

Once the ideal conditions of SSF in EA recovery were selected, a higher volume SSF was performed to obtain a higher phenolic extract quantity [17]. Through a column chromatography with Amberlite XAD-16, the phenolic extracts were processed. Two eluents were used to obtain the fraction of interest, the first one being distilled water which was selected to discard any undesirable compounds like carbohydrates and other impurities, and, as second eluent, ethanol was used to recover molecules of interest retained in Amberlite XAD-16. In an oven at 50 °C, the phenolic fraction was evaporated and recovered as a fine powder [19].

2.10. Separation and Isolation of EA by Preparative HPLC

The soluble polyphenol of the fermented fractions was separated by high-resolution preparative-scale chromatography for the purification of the extracts using the method described by Aguilar-Zárate et al. (2017) [20]. An amount of 300 mg of polyphenols was weighed and prepared in a solution of 10% ethanol and 50% water, which was filtered with 0.45 µm membranes. Using liquid chromatography equipment (Varian ProStar 3300, Varian, Palo Alto,

CA, USA) and a Dynamax Microsorb300 C18 column (250 mm × 21.4 mm, 10 µm) the extracts were separated. A flow rate of 8 mL/min was used and the conditions were as follows: as a mobile phase, (A) CH_3COOH (3% *v*/*v* in water) and (B) methanol. The method used for the separation of the molecules was isocratic: 5% initial B; 0–45 min, 5–90% B; 45–50 min, 90% B; 50–70 min, 90–5% B; 70–95 min. At 280 nm, the elution of the compounds (ellagitannins) was monitored. With 90% methanol (45–60 min) the column was washed and reconditioned to the initial conditions (60–80 min). By HPLC/ESI/MS analysis, the fractions were recovered and characterized [19].

3. Results

3.1. Water Absorption Capacity and Maximum Moisture

For the WAC of Mexican rambutan peel, Table 3 exhibits the results obtained for solids of the dry plant and moisture. The maximum moisture that the rambutan peel can accumulate was 84%; meanwhile the WAC obtained was 5.4 g gel/g of dry weight.

Table 3. Data obtained regarding water absorption capacity of Mexican rambutan peel (in dry base).

Parameters	Results
Maximum moisture of the support/substrate (%)	84.0 ± 2.0
Water absorption capacity (g gel/g of dry weight)	5.4 ± 0.2
Moisture (%)	6.0 ± 0.0
Solids (%)	94.0 ± 0.0

3.2. Identification of Parameters Affecting EA Using PBD

To determine which factors affect EA recovery in SSF with a 24 h incubation time, a PBD was performed. Table 1 shows that the highest EA recovery was achieved by Treatment 7, which produced 201.5 ± 0.6 mg/g.

Figure 1 shows the chromatograms of unfermented rambutan peel and the chromatogram of Treatment 7 with PBD. In chromatogram (b) it can be seen that the signals for gerannin and corilagin diminish when the peel goes through a fermentation process compared to the unfermented peel extract which has a bigger signal for corilagin.

Table 4 presents the estimated effect values of each factor on EA, where all evaluated factors had a significant effect on EA recovery ($\alpha = 0.05$); where parameters have a positive effect, as their level value increases, the EA recovery also increases. Conversely, a parameter that presented a negative effect, refers to any increase in this parameter, hence EA recovery decreases. Therefore, lower values are preferred to increase EA recovery.

Table 4. Estimated effect values of each factor affecting EA recovery.

Parameters	Significant Effect *
Temperature (°C)	(−)
Inoculum (spores/g)	(+)
$NaNO_3$ (g/L)	(−)
Moisture (%)	(+)
$MgSO_4$ (g/L)	(+)
KCl (g/L)	(+)
KH_2PO_4 (g/L)	(−)
* Tukey ($\alpha = 0.05$)	

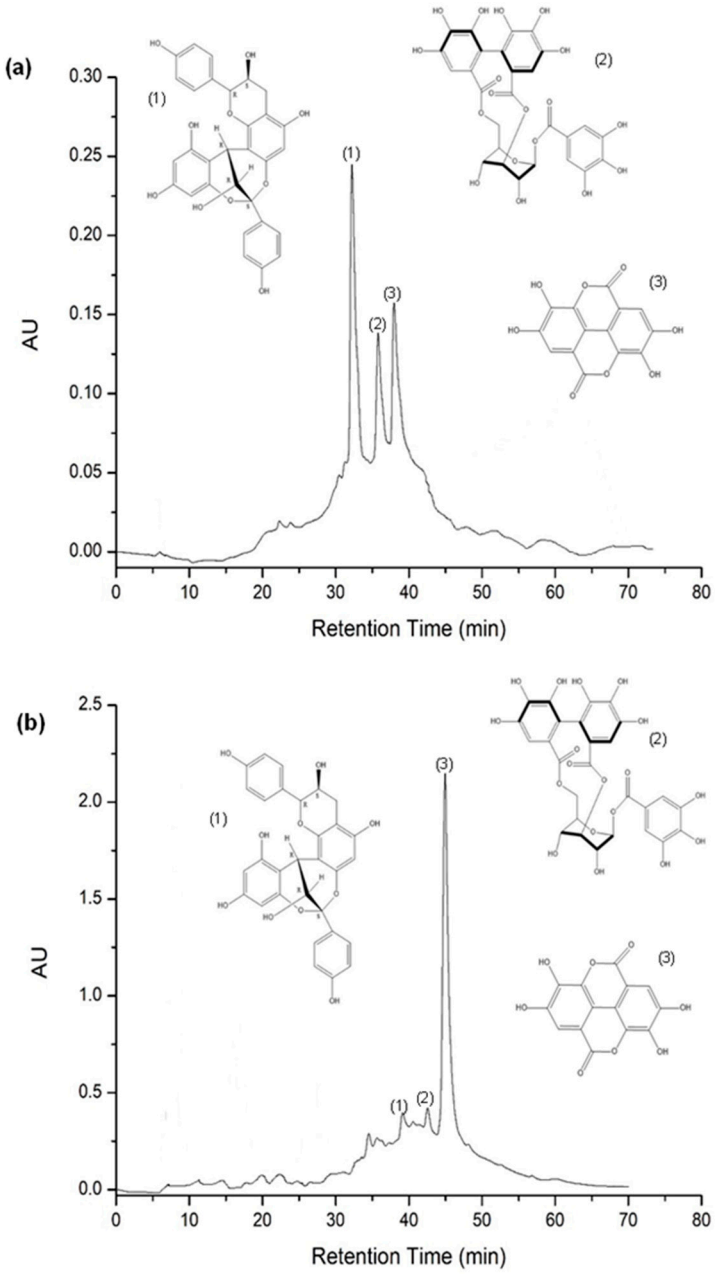


Figure 1. Chromatograms of Mexican rambutan peel (**a**) and Treatment 7 (**b**) with the best conditions for the recovery of EA. Compound (**1**) gerannin, compound (**2**) corilagin, and compound (**3**) ellagic acid.

3.3. Effects of Ideal SSF Conditions for the Maximum Recovery of EA

To evaluate the influence of inoculum, temperature, and $NaNO_3$ on maximal EA recovery from Mexican rambutan peel by-products, using SSF, an experimental design was established. Values and coded levels of the variables studied in CCD as well as EA recovery are shown in Table 2.

Analytical analysis, determination of total polyphenols and antioxidant activity, was performed in order to monitor *A.niger* GH1 behavior relative to SSF activity in EA recovery. According to CCD, the highest EA recovery was achieved by Treatment 10 with (392.23 ± 17.53 mg/g), which presented a total content of polyphenols of (73.18 ± 0.29) mg/g and (98.44 ± 0.14%) and (74.73 ± 0.11%) inhibition of ABTS and DPPH radicals; meanwhile Treatment 1 showed the minimum value of EA recovered of (27.94 ± 0.87 mg/g).

From the CCD analysis, the regression coefficients in linear (L) and quadratic (Q) effects and the interaction between factors were obtained (Figure 2).

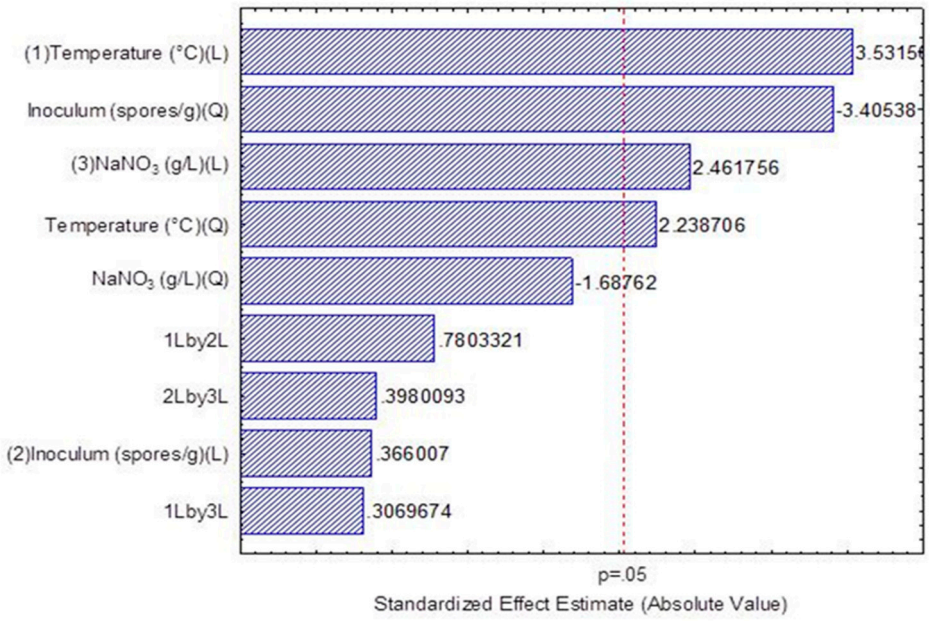


Figure 2. Pareto chart: independent variables significant for EA recovery.

The linear term of temperature and $NaNO_3$ were significant in the process at a level of $p \leq 0.05$. For quadratic terms, inoculum and temperature were significant; meanwhile none of the interaction factors were significant.

In order to analyze the ideal conditions (inoculum, temperature, and $NaNO_3$) for the highest EA recovery, a second-order polynomial model (Equation (1)), experimental data, and regression coefficients were obtained by multiple linear regressions (Equation (2)) as follows:

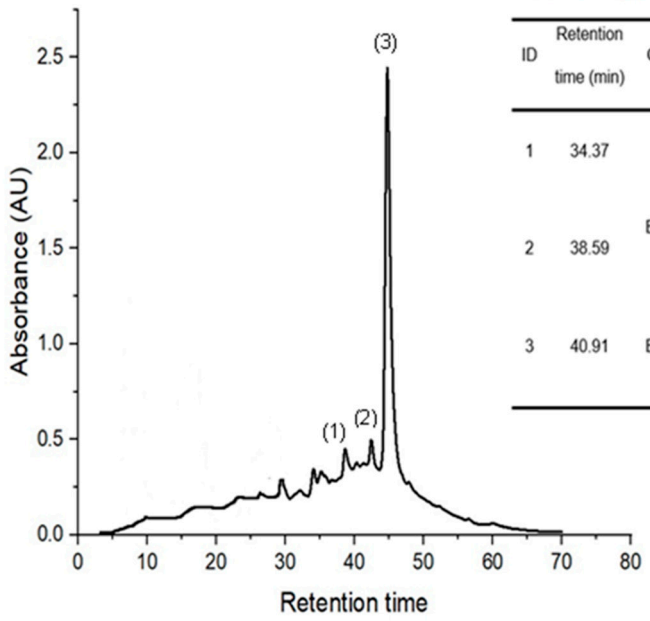
$$EA = 4194.87 + 4.4X10^{-7}X_1^2 - 1.8X10^{-12}X_2 - 1X10^{-6}X_1X_2 + 28.2X_2X_3 \quad (2)$$

The simplified model predicted a maximal yield of EAmax = 234.95 mg/g of EA under ideal conditions; meanwhile, the experimental value obtained was 392.22 ± 17.53 mg/g of EA; where the results obtained for EA released using SSF only fits 55% according to the mathematical model for this study. There was a lot of variability in the results obtained for ellagic acid recovery due to the same bioprocess (Table 2); where a statistical model fits the data well if the differences between observed values and the model-predicted values are small and unbiased.

According to these results, it is not possible to indicate this study as an optimization of the SSF; however, the SSF conditions analyzed and the results on EA release in this work expand the landscape for new studies in SSF in order to search for the optimal conditions for EA release.

3.4. Identification of Phenolic Compounds

Figure 3 represents the chromatogram of the SSF at a higher volume using the ideal conditions of SSF reported for Treatment 10 after EA purification using Amberlite XAD-16, preparative HPLC, and quantification by HPLC-ESI-MS with a concentration of mg/g of EA per gram of recovered powder.



ID	Retention time (min)	Compound	[M-H]⁻ (m/z)	MS²	Group
1	34.37	Corilagin	632.9	481, 301, 275	Ellagitannins
2	38.59	Ellagic acid hexoxide	432.9	257, 229, 185	Ellagitannins
3	40.91	Ellagic acid	300.9	257, 229, 185	Ellagitannins

Figure 3. Chromatogram of Treatment 10 with the best conditions for the recovery of EA. Compound (**1**) corilagin, compound (**2**) ellagic acid hexoxide, and compound (**3**) ellagic acid.

Signal (1) corresponds to corilagine, signal (2) to ellagic acid hexoxide, and signal (3) corresponds to EA; the signal of gerannin is not present anymore, which can be compared to Figure 1 in the unfermented peel, where this signal is at its highest compared to the EA signal.

4. Discussion

Substrates with higher moisture values are better for usage in SSF. This is due to the water activity which allows microorganisms to grow and develop in said material. However, when filamentous fungi are used, particle agglomeration, the development of microorganisms like bacteria, and oxygen transfer difficulties can occur if moisture values are raised [21]. Larios et al. (2017) [22] reported a WAC of 4.30 g/g of dry weight in grapefruit residues used as support in SSF. The result of the WAC mentioned is lower compared to the one obtained for Mexican rambutan peel, which turned out to be an excellent material to be used as a support in SSF.

In SSF, water content can change the whole substrate matrix by respiration and evaporation, different states (free, bound, and immobilized) of water migration and internal distribution transfer over the substrate matrix due to gradients can affect the water dynamic [23].

The results obtained in this study can be compared with the literature and make rambutan peel an adequate substrate to be used in SSF.

Inoculum preparation plays an important role due to the fact that certain mediums possess conditions, like viscosity or pH, that affect the morphology and physiology of microorganisms [24]. Also, there are other important factors that interfere in microbial growth, like water dynamics, which, when controlled, monitored, and optimized in SSF, are key to the regulation of microbial growth [25]. Temperature is an important factor that influences the production or could potentially stop the production of metabolites and enzymes [26]. In addition, the culture medium should be formulated to promote fast microbial growth, and this will depend on the microbial strain used in SSF [27]. Nitrogen supplementation influences the level of amino acid products and proteins. Phosphorus (phosphates), where its function is the transduction of energy, is essential for the biosynthesis of fungal nucleic acids and membrane structures. Additionally, the salts of potassium and magnesium help in terms of the enzyme activity, the ionic balance, and the cell structure [28].

The fungal growth temperature has a wide range and can vary depending on different attributes such the genetic background of the species or the nutrients available. Most fungal

species are able to grow in a temperature of 25 °C and, as it is important to notice, fungi do not have the means to regulate their internal temperature, which means that the higher the temperature, the greater the cellular damage caused by heat (and structurally, as organic amino nitrogen forms in proteins and enzymes) [29].

EA recovery is a function of the parameters that help fungal physiology, meaning that the metabolism, nutrition, growth, reproduction, and death of fungal cells are vital for the production of EA.

Fungi possess simple nutritional needs, including macronutrients from sources of carbon, which can be obtained from sugar and provides the cell with energy, and nitrogen, from NH_4^+ and amino acids, which acts functionally and structurally as organic amino nitrogen in proteins and enzyme [29].

The production of molecules of interest can be associated with the formation of spores, which can be regulated by media design. Low levels of nitrogen induce spore formation [30]. The preparation of inoculum is crucial for SSF; it must be healthy and active to be optimized, it should adapt quickly to the environmental conditions in the substrate and culture media, and, to obtain a proper efficiency in the optimization, microbiological techniques of inoculation must be carefully handled [31].

As a result, all of the factors interfere in a significant way with the recovery of EA; however, inoculum, temperature, and the salt of $NaNO_3$ from the Czapek-Dox culture medium were the conditions selected to be implemented in the search for the ideal SSF conditions for EA recovery. The results in Treatment 10, in terms of EA, are notably superior compared to those reported by Cerda-Cejudo et al. 2022 [32], where the authors declared (37.09 $\pm$ 3.15 mg/g) of EA recovery using SSF. Even though the same microorganism, substrate, and time of fermentation were used in both studies, the analysis of the effect of the salts in the medium and different experimental designs were used. Likewise, in a study by De La Rosa et al. (2023) [33], the ideal conditions for EA recovery using 1.5×10^7 cell/g of *Saccharomyces cerevisiae* in SSF at 30 °C and 60% of moisture using Mexican rambutan peel as support, employing the Box–Behnken 3^k experimental design, achieved a maximum EA recovery of (458.37 $\pm$ 44.6 mg/g).

Factors like microorganisms and temperature in these cases, where the substrate is the same, perform an important role in SSF. The effect of temperature can fluctuate the inhibition of growth and the production of secondary metabolites; also, each kind of microorganism has its temperature range for optimal growth. In addition, when it comes to fermentation, if the microorganism does not have an adequate physiological state for the production of secondary metabolites, it will decrease [34,35].

Microorganisms in SSF must be carefully overhauled due to the requirements that their metabolism demands. Although filamentous fungi and yeasts are the most employed microorganisms in SSF due to their environmental adaptation, in Treatment 10, *A. niger* GH1 demonstrates the capacity, under ideal conditions of temperature, moisture, and salts in the medium, to biodegrade gerannin and corilagin and to produce secondary metabolites such as EA [35–37].

The relevance of the recovery of biocompounds of interest, such as EA, through the use of SSF makes it possible to explore new and innovative opportunities in this scientific field.

5. Conclusions

In the present research, the factors influencing EA recovery through SSF using Mexican rambutan peel were recognized and controlled. A significant amount of EA was gathered after the use of two consecutive experimental designs, PBD and CCD. Ellagic acid recovery reached 392.23 $\pm$ 17.53 mg/g after 24 h of fermentation, where this valuable compound was successfully recovered, partially purified, and quantified obtaining 396.94 $\pm$ 65.26 mg/g of EA per gram of recovered powder.

Even though this study is not an optimization work, it demonstrated that controlled values of temperature and $NaNO_3$ salt concentration, as well as controlled values such as the concentration of inoculum, are the ideal conditions for SSF that should be used in

order to achieve higher EA recovery. Biotechnological alternatives like SSF in the recovery of molecules of interest, like EA, would offer environmental and economic benefits over emergent extraction techniques and traditional chemical methods.

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