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Solid-State Fermentation as a Sustainable Tool for Extracting Phenolic Compounds from Cascalote Pods

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Abstract: Cascalote pods are an important source of phenolic compounds, mainly recovered using solvent extraction methods. To find a sustainable alternative to these methods, this study aimed to evaluate solid-state fermentation (SSF) in order to enhance the extractability of total phenolic compounds (TPC) with antioxidant activity (AA) from cascalote pods. *Aspergillus niger* GH1 was selected based on the amount of TPC released and AA measured (ABTS, DPPH, FRAP) in a short period of time. Moreover, moisture, temperature, inoculum size, and mineral composition were evaluated. The largest amount of TPC released was 124.17 mg/gdw (g of dry weight) after 12 h of culture, which significantly correlated with the AA (Pearson's R = 0.94). Moisture and KH_2PO_4 concentration were the main influencing factors of TPC release. Treatment 6 (1×10^7 spores/gdw, 30 °C, 60% moisture, mineral composition (g/L): KH_2PO_4, 1.52; $NaNO_3$, 7.65; and $MgSO_4$, 1.52) was selected due to the highest values of both TPC and AA. SSF-assisted extraction allowed for an increase of 118% and 93% in TPC and AA values, respectively. Corilagin, lagerstannin, geraniin, and ellagic acid were the main phenolic compounds identified by RP-HPLC-ESI-MS in the cascalote extracts. The results obtained demonstrate the feasibility of SSF-assisted extraction as a biotechnological alternative for the recovery of important bioactive molecules from this underutilized material.

Keywords: condensed tannins; hydrolysable tannins; ABTS; DPPH; solid-state fermentation; improved extraction; RP-HPLC-ESI-MS

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1. Introduction

Caesalpinia coriaria is a plant found in the Pacific coastal plains of Central America, commonly called "cascalote" or "divi-divi", and it is used to obtain firewood, charcoal, poles, and beams. This plant produces pods 3–7 cm long, with a green color when unripe and dark brown when ripe. According to Palma García [1], one cascalote tree can produce up to 150 kg of pods, which indicates a large production.

These pods are commonly used in leather tanning and fodder and are traditionally used as a treatment for infectious skin problems [2]; however, most of the pods produced are underutilized and, consequently, not fully exploited. Additionally, economic activity derived from harvesting has declined. Cascalote pods are astringent due to their high content of phenolic compounds—important molecules that are of great interest in health studies, mainly due to their biological activities such as antimicrobial, antioxidant, and anti-inflammatory [3,4]. The main phenolic compounds that have been reported in cascalote fractions with biological activity are gallic acid, ethyl gallate, stigmasterol, tannic acid, and corilagin [2,5].

Phenolic compounds are commonly recovered using solvents and several extraction techniques, such as maceration, microwave, and ultrasound, among others; however, their use involves expensive equipment as well as environmental pollution and toxicological safety concerns regarding the use of solvents [6,7]. As a biotechnological alternative, the use of fermentative or enzymatic methods for the assisted extraction of biomolecules from vegetal matrices has been reported, which includes solid-state fermentation (SSF) [8].

SSF does not require the use of solvents and promotes high yields and the easy recovery of bioactive compounds. During the SSF process, polyphenols could be biosynthesized or biotransformed into simpler molecules using the activity of microbial enzymes [9,10], which degrade the wall cell components, thus increasing the extraction of free and bound polyphenols [11]. Recently, SSF has been successfully used to obtain bioactive compounds from rambutan peel [12], orange peel [13], pomegranate husk [14], and pineapple waste [15], among others; however, the use of SSF in cascalote pods as a substrate to obtain bioactive compounds has not been studied. Based on responsible production and consumption, this work aimed to develop an eco-friendly bioprocess, SSF, for the extraction, recovery, and identification of compounds with antioxidant capacity from cascalote pods.

2. Materials and Methods

2.1. Raw Material

Cascalote pods were collected in San Miguel Totolapan, Guerrero, México (18.152094485006664, −100.36604425470092), and transported to the laboratory of Biotechnology and Bioengineering in Delicias, Chihuahua, Mexico. They were dried at 50 °C for 24 h (Shel-Lab 1380FX Forced-Air Oven, Cornelius, OR, USA), pulverized (PULVEX Mini 100, Mexico City, Mexico) to a particle size of 1 mm, and stored in a hermetic black polyethylene bottle at room temperature (30 °C) until use.

2.2. Physicochemical Characterization of Cascalote Pods

Protein, fat, carbohydrate, fiber, moisture, and ash content were determined according to the procedures established by the Association of Official Agricultural Chemists (AOAC) [16]. The critical humidity point (CHP) and water absorption capacity (WAC) were obtained according to Orzua et al. [17]. The maximum material moisture was obtained using solids, moisture content, and WAC values [12].

2.3. Microorganisms

For this study, the following strains were used: *Aspergillus niger* GH1, *Aspergillus awamori*, and *Aspergillus niger HT4* (belonging to the DIA-UAdeC collection). The GH1 strain of *A. niger* was deposited in the Micoteca of the University of Minho with the number MUM:23.16. Fungal strains were cryopreserved at −55 °C in a skim milk/glycerol (9:1 v/v) solution. Fungal spores were activated on potato dextrose agar (PDA-Bioxon™) plates at 30 °C for 5 days. For the inoculum preparation, fungal spores were collected with a sterile solution of Tween-80 (0.01% v/v) and counted in a Neubauer chamber.

2.4. Solid-State Fermentation (SSF)

Cascalote pods mixed with vermiculite (50:50 w/w) were used as support and as the sole carbon source for fungal growth. SSF was performed in column reactors (31.5 × 180 mm) packed aseptically with a homogeneous mixture containing 6 g of fermentable mass with an initial moisture of 50% (3 g of support impregnated with 3 mL of Czapek–Dox saline solution, previously inoculated with 1×10^6 spores/g of support). A saline solution was prepared to contain (g/L): KCl (1.51), $MgSO_4$ (1.52), $NaNO_3$ (7.65), and K_2HPO_4 (3.04). SSF was monitored at 30 °C for 72 h, withdrawing samples every 12 h to determine the total phenolic content and antioxidant activity. To obtain the fermented extracts, 12 mL of 50% ethanol was added to each reactor. Samples were shaken in a vortex and filtered through cotton and filter paper (0.45 µm) before being poured into 2 mL vials and stored at −18 °C until analysis.

2.5. Evaluation of the SSF Factors for the Total Polyphenol Extraction

Once the fungal strain had been selected, the time of the maximum release of phenolic compounds and the antioxidant activity were selected as well. Other SSFs were conducted in parallel to identify the effects of inoculum, temperature, and moisture, and the concentrations of KCl, $MgSO_4$, $NaNO_3$, and K_2HPO_4, according to Table 1. The extracts were obtained after 12 h of SSF with *A. niger* GH1 and used to determine the TPC and the activity in antioxidant assays.

Table 1. Treatment matrix of the experimental design by Box, Hunter, and Hunter.

Treatment	A	B	C	D	E	F	G
1	−1	−1	−1	1	1	1	−1
2	1	−1	−1	−1	−1	1	1
3	−1	1	−1	−1	1	−1	1
4	1	1	−1	1	−1	−1	−1
5	−1	−1	1	1	−1	−1	1
6	1	−1	1	−1	1	−1	−1
7	−1	1	1	−1	−1	1	−1
8	1	1	1	1	1	1	1

Code	Factor	Low level (−1)	High level (1)
A	Inoculum (spores/gdw)	1×10^6	1×10^7
B	Temperature (°C)	30	40
C	Moisture (%)	50	60
D	KH_2PO_4 (g/L)	1.52	3.04
E	$NaNO_3$ (g/L)	3.04	7.65
F	$MgSO_4 \bullet 7H_2O$ (g/L)	1.52	3.04
G	KCl (g/L)	1.52	3.04

2.6. Analytical Analysis

2.6.1. Determination of Polyphenol Content

The hydrolysable polyphenols (HP) assay was carried out according to Wong-Paz et al. [18]. In a 96-well microplate, 20 µL of the sample, 20 µL of the Folin–Ciocalteu reagent (2 N), 20 µL of a 20% sodium carbonate solution, and 125 µL of distilled water were mixed. After 5 min of incubation, the absorbance was measured at 790 nm in a microplate reader (Multiskan GO, Thermo Scientific, Waltham, MA, USA). The results were expressed as mg of gallic acid equivalents/g of dry weight (mgGAE/gdw) using a calibration curve (0.04–0.20 mg/mL; $R^2 = 0.99$).

The condensed polyphenols (CP) were determined using a ferric reagent and HCl-butanol (1:9 *v*/*v*) [12]. The sample (250 µL) was mixed with 1500 µL of HCl-butanol, and 50 µL of the ferric reagent in 20% HCl was added. The mixture was sealed hermetically and boiled for 40 min. Finally, the samples were cooled, and 200 µL was placed in a 96-well microplate; the absorbance was recorded at 460 nm using a microplate reader (Multiskan GO, Thermo Scientific). The results were expressed as mg of catechin equivalents/g of dry matter (mgCE/gdw) using a calibration curve (0.2–1 mg/mL; $R^2 = 0.99$). The TPC was determined by adding the values of the hydrolysable and condensed polyphenols and expressed as mg/g of dry weight (mg/gdw).

2.6.2. Antioxidant Activity

The antioxidant activity of the extracts was evaluated based on DPPH, ABTS, and FRAP assays. For the DPPH assay, 60 µM DPPH (2,2-diphenyl-1-picrylhydracyl (Sigma-Aldrich®, Naucalpan de Juarez, México)) stock solution (2.365 mg dissolved in 100 mL absolute ethanol) was prepared. The sample (7 µL) and DPPH stock solution (193 µL) were placed in a 96-well microplate, mixed, and kept in darkness for 30 min at room

temperature [19]. A microplate reader (Multiskan GO, Thermo Scientific) was used to measure the absorbance at 517 nm.

The ABTS decolorization assay was carried out using the protocol adapted to a microplate [20,21]. The $ABTS^{\bullet+}$ was prepared by mixing 7 mM ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate)) (Sigma-Aldrich®) solution (0.3602 g in 100 mL distilled water) with 2 mM potassium persulfate (2.45 µM, 0.0662 g in 100 mL distilled water). The mixture was kept in darkness for 12–16 h at room temperature. After that, the absorbance of $ABTS^{\bullet+}$ was adjusted to 0.70 at 734 nm. The sample (10 µL) and $ABTS^{\bullet+}$ (190 µL) were mixed, and after one minute, the absorbance was measured using a microplate reader (Multiskan GO, Thermo Scientific). The results for the DPPH and ABTS assays were expressed as mg of Trolox equivalents/g of dry weight (mgTE/gdw) using a calibration curve with 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox; 0.04–0.2 mg/mL; R^2 = 0.98 and 0.99, respectively).

The FRAP (ferric reducing antioxidant power) assay was carried out according to Pulido et al. [22], adapted to a 96-well microplate. The FRAP reagent (0.3 M acetate buffer (pH 3.6), 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TTPZ; Sigma-Aldrich®) solution in 10 mM HCl, and 20 mM $FeCl_3.6H_2O$; 10:1:1 *v*/*v*) was prepared daily and incubated at 37 °C for 30 min. For the reaction, 6 µL of the sample, 18 µL of distilled water, and 180 µL of the FRAP reagent were mixed. The resulting solution was incubated at 37 °C for 60 min, and the absorbance was measured using a microplate reader (Multiskan GO, Thermo Scientific) at 595 nm. The results obtained were expressed as mg of Fe^{+2} equivalents/g of dry weight ($mgFe^{+2}$/gdw), using a calibration curve with Fe_2SO_4 (Sigma-Aldrich®) in 80% methanol (0.1–1 mg/mL; R^2 = 0.99).

2.6.3. Identification of Phenolic Compounds

The phenolic profile of the fermented extract was obtained by reverse-phase high-performance liquid chromatography/electrospray ionization/mass spectrometry (RP-HPLC/ESI/MS) according to [14], using a Varian HPLC system, including an autosampler (Varian ProStar 410, Palo Alto, CA, USA), a PDA detector (Varian ProStar 330, Palo Alto, CA, USA), and a ternary pump (Varian ProStar 230I, Palo Alto, CA, USA). The separation was carried out at 30 °C in a Denali C18 column (150 mm × 2.1 mm, 3 µm, Grace, Palo Alto, California, USA) in 5 µL samples. The mobile phase (wash phase) was methanol, and the eluents were formic acid (0.2%, *v*/*v*) and acetonitrile (solvent A and B, respectively). The elution gradient applied was: initial, 3% B; 0–5 min, 9% B linear; 5–15 min, 16% B linear; 14–45 min, 50% B linear. A mass spectrometer (Varian 500-MS IT Mass Spectrometer, Palo Alto, CA, USA) equipped with an electrospray ionization source, operated in negative mode $[M\text{-}H]^{-1}$, was used for the LC-ESI-MS analysis. Data were collected and processed using the MS Workstation software (V 6.9). Samples were initially analyzed in full-scan mode, acquired in the m/z range of 50–2000.

2.7. Experimental Design

To select the best strain and time for the largest TPC content and AA, a completely randomized design with factorial arrangement was established. The factors evaluated were fungal strain (*A. niger* GH1, *A. awamori*, and *A. niger* HT4) and culture time (0, 12, 24, 36, 48, 60, and 72 h) in triplicate (n = 63).

After that, a 2^k Box, Hunter, and Hunter (BHH) design was set up to identify the factors with a significant effect on the TPC extraction and AA. For this purpose, six factors (inoculum size, temperature, moisture, $MgSO_4$, $NaNO_3$, and K_2HPO_4) at two levels each were used to construct a condensed matrix with eight treatments (Table 1). All treatments were performed in triplicate and expressed as a mean (n = 3) ± standard deviation. Data were submitted to Analysis of Variance (ANOVA) and Tukey´s test using the Statistica 7.0 software (Stat Soft, Tulsa, OK, USA). A p-value ≤ 0.05 was considered to indicate a significant difference. Correlations between phenolic compound content (HP, CP, and TPC)

and the activity in antioxidant assays (ABTS, DPPH, and FRAP) were determined using Pearson´s correlation coefficient ($p < 0.01$).

3. Results

3.1. Physicochemical Characterization of Cascalote Pods

The chemical composition of cascalote pods (Table 2) was determined in order to evaluate their nutritional feasibility to be used as a substrate for fungal growth in an SSF process. The main components of cascalote pods are carbohydrates and fiber, whereas proteins constitute both elemental carbon and nitrogen sources for fungal growth and enzyme production [15]. The physicochemical values for the water absorption capacity (WAC), critical humidity point (CHP), and maximum moisture of the substrate (see Table 2) indicate the potential of any material to be used as a substrate in an SSF process.

Table 2. Physicochemical characterization of cascalote pods.

Component	Content
Moisture (% *w*/*w*)	3.36 ± 0.15
Fat (g/100 gdw)	0.65 ± 0.09
Fiber (g/100 gdw)	6.54 ± 0.25
Protein (g/100 gdw)	3.44 ± 0.13
Ash (g/100 gdw)	2.13 ± 0.21
Carbohydrates (g/100 gdw)	87.24 ± 0.96
Water absorption capacity (g of gel/gdw)	2.97 ± 0.07
Critical humidity point (%)	3.75 ± 0.29
Maximum moisture of cascalote pods (%)	79.33 ± 2.08

gdw: gram of dry weight.

3.2. Kinetics of TPC Extraction and AA Using Aspergillus Strains

The TPC release and AA assays (DPPH, ABTS, and FRAP), in relation to fermentation time, are shown in Figure 1. All of the evaluated strains could use cascalote pods as a substrate-support for their growth. TPC release was fast at the beginning, reaching a maximal value after 12 h of fermentation for all of the strains. At 12 h, the amount of TPC released was 70.22, 102.21, and 117.69 mgGAE/gdw for *A. niger* HT4, *A. awamori*, and *A. niger* GH1, respectively. After that time, TPC started to decrease, attaining a concentration of 55 mgGAE/gdw for all of the strains. Similar patterns were observed in the AA assays, DPPH (Figure 1b), ABTS (Figure 1c), and FRAP (Figure 1d), which achieved the largest values at 12 h for all of the strains. Based on the obtained results, *A. niger* and 12 h of fermentation time were selected for further studies.

Data from the extraction kinetics were used to estimate the relation between the phenolic compounds and antioxidant activity of the fermented extracts on the cascalote pods through the Pearson correlation coefficient. As shown in Table 3, the phenolic compound content (HP, CP, and TPC) was significantly correlated with the in vitro antioxidant activity measured by ABTS, DPPH, and FRAP.

3.3. Significant Factors for TPC Release by SSF

Experimental design is a useful tool for defining the conditions necessary to be able to perform a minimal number of experiments. The BHH design was performed to identify the main factors that influence TPC release from cascalote pods by SSF. For that, an eight-treatment experimental matrix was constructed to evaluate three process parameters (inoculum size, temperature, and moisture) and three media components (KH_2PO_4, $NaNO_3$, and $MgSO_4$) in the hydrolyzed, condensed, and total polyphenol content, as well as the antioxidant activity against DPPH and ABTS radicals in the cascalote pods fermented with *A. niger* GH1; the results are shown in Table 4. Maximal values were obtained in treatment 6, without significant differences in comparison to treatment 7, which were 0.98-,

0.79-, and 1.1-fold higher than those obtained in treatment 2 for the TPC, ABTS, and DPPH values, respectively.

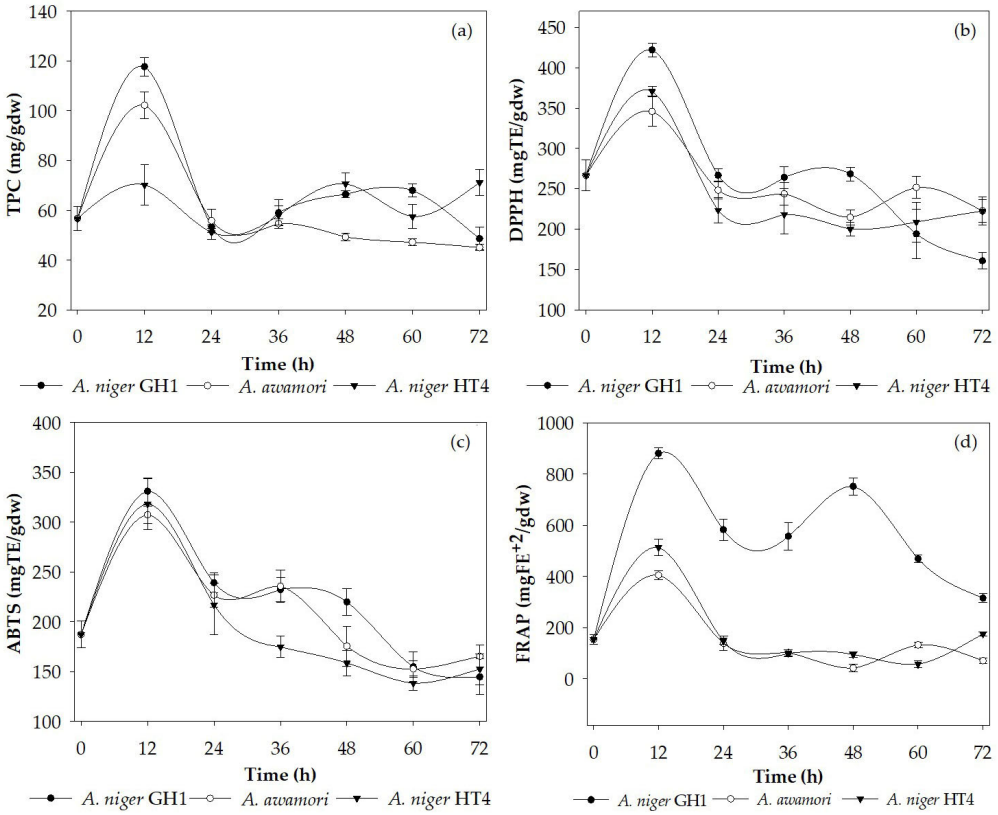

Figure 1. Kinetics of: (**a**) total polyphenol content, (**b**) antioxidant activity by DPPH assay, (**c**) antioxidant activity by ABTS assay, and (**d**) antioxidant activity by FRAP assay, for the fermented extracts of *Aspergillus* strains on cascalote pods.

Table 3. Pearson's correlation coefficients between phenolic compounds (HP, CP, and TPC) and activity in antioxidant assays (ABTS, DPPH, and FRAP).

Variables	HP	CP	TPC	ABTS	DPPH	FRAP
HP	1	0.94 *	0.98 *	0.94 *	0.94 *	0.82 *
CP		1	0.98 *	0.93 *	0.94 *	0.84 *
TPC			1	0.95 *	0.95 *	0.85 *
ABTS				1	0.95 *	0.87 *
DPPH					1	0.95 *
FRAP						1

* Significant correlations ($p < 0.01$).

To evaluate the influence of these factors, the absolute values of standardized effects were estimated; their behaviors are shown in Figure 2. Moisture was the most influential ($p < 0.05$) and had a positive effect, indicating that as the factor level increased, the expected response also increased (Figure 2a). In contrast, TPC release was negatively affected by KH_2PO_4, $MgSO_4$, and KCl concentrations (Figure 2b–d), revealing that an increase in these factors harms the TPC release. Temperature and $NaNO_3$ showed no significant effect ($p > 0.05$). Therefore, any level of these factors could be used for evaluation. Media components that show a negative (KH_2PO_4, $MgSO_4$, and KCl concentration) or non-significant (temperature and $NaNO_3$) effect are recommended for use in low levels (see Table 1). Therefore, treatment 6 was selected due to the highest values of TPC release and higher activity in antioxidant assays (DPPH, ABTS, and FRAP).

Table 4. Treatment matrix of the experimental design by Box, Hunter, and Hunter used to determine the influence of independent factors (A, B, C, D, E, F, and G) on the TPC and AA (ABTS, DPPH, FRAP) of extracts obtained by SSF with *A. niger* GH1 on the cascalote pods.

Treatment	A	B	C	D	E	F	G	TPC (mg/gdw)	ABTS (mgTE/gdw)	DPPH (mgTE/gdw)	FRAP ($mgFE^{+2}$/gdw)
1	−1	−1	−1	1	1	1	−1	71.62 ± 2.23 ^{f}	271.46 ± 3.15 ^{de}	237.32 ± 5.51 ^{f}	735.78 ± 12.85 ^{d}
2	1	−1	−1	−1	−1	1	1	62.95 ± 3.51 ^{g}	247.90 ± 15.01 ^{e}	226.25 ± 7.39 ^{f}	737.49 ± 15.09 ^{d}
3	−1	1	−1	−1	1	−1	1	99.88 ± 1.96 ^{c}	380.68 ± 19.01 ^{b}	365.92 ± 4.54 ^{c}	945.92 ± 55.67 ^{c}
4	1	1	−1	1	−1	−1	−1	79.48 ± 0.52 ^{e}	294.85 ± 8.28 ^{cd}	297.58 ± 6.97 ^{e}	800.07 ± 53.24 ^{d}
5	−1	−1	1	1	−1	−1	1	92.55 ± 1.45 ^{d}	384.55 ± 15.28 ^{b}	345.96 ± 9.63 ^{cd}	925.42 ± 39.09 ^{c}
6	1	−1	1	−1	1	−1	−1	124.17 ± 1.94 ^{a}	447.64 ± 5.81 ^{a}	498.46 ± 13.15 ^{a}	1251.13 ± 29.45 ^{a}
7	−1	1	1	−1	−1	1	−1	114.23 ± 2.67 ^{b}	438.81 ± 14.00 ^{a}	452.64 ± 3.94 ^{b}	1121.07 ± 44.45 ^{b}
8	1	1	1	1	1	1	1	71.84 ± 2.78 ^{f}	320.19 ± 14.71 ^{c}	329.10 ± 15.32 ^{d}	1019 ± 52.78 ^{bc}

Code	Factor	Low level (−1)	High level (1)
A	Inoculum (spores/gdw)	1×10^{6}	1×10^{7}
B	Temperature (°C)	30	40
C	Moisture (%)	50	60
D	KH_2PO_4 (g/L)	1.52	3.04
E	$NaNO_3$ (g/L)	3.04	7.65
F	$MgSO_4 \bullet 7H_2O$ (g/L)	1.52	3.04
G	KCl (g/L)	1.52	3.04

Different lowercase letters indicate no significant differences among treatments (Tukey's test; $p \leq 0.05$).

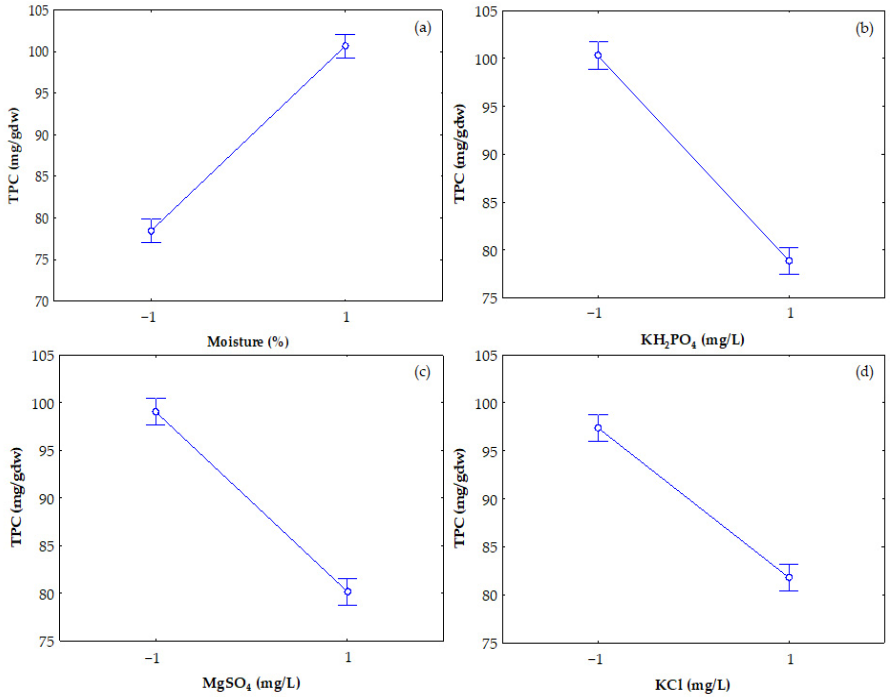

Figure 2. Effect of the significant factors (**a**) Moisture, (**b**) KH_2PO_4, (**c**) $MgSO_4$ and (**d**) KCl on TPC release by SSF with *A. niger* GH1.

3.4. Effect of SSF on Chemical Composition, Tannin Content, and Antioxidant Activity

The chemical composition, tannin content, and antioxidant activity of treatment 6 were determined and compared to control; no fermentation was conducted (Table 5). Significant changes were observed for all of the parameters ($p \leq 0.05$), including an increase in moisture, fat, protein, and ash content. A reduction in the fiber and carbohydrate contents as an effect of SSF was observed. Concerning tannin content, SSF by *A. niger* GH1 increased the content

of hydrolyzed, condensed, and total polyphenols, reaching values that were 66%, 337%, and 118%, respectively, higher than the control (without fermentation). The activity of the fermented extracts in the DPPH, ABTS, and FRAP assays was improved by 66.76%, 93%, and 55.67%, respectively, compared to the control.

Table 5. The SSF of cascalote pods (mixed with vermiculite; 50:50 *w*/*w*) using *A. niger* GH1 and its effect on chemical composition, tannin content, and antioxidant activity.

Parameter/Treatment	Control (0 h)	Treatment 6 (12 h)
Moisture (g/100 gdw)	4.90 ± 0.04 ^{b}	5.40 ± 0.20 ^{a}
Fat (g/100 gdw)	0.23 ± 0.05 ^{b}	0.34 ± 0.10 ^{a}
Fiber (g/100 gdw)	5.54 ± 0.23 ^{a}	1.77 ± 0.12 ^{b}
Protein (g/100 gdw)	3.24 ± 0.15 ^{b}	3.59 ± 0.10 ^{a}
Ash (g/100 gdw)	52.88 ± 0.95 ^{b}	59.28 ± 0.93 ^{a}
Carbohydrates (g/100 gdw)	38.11 ± 1.95 ^{a}	35.02 ± 1.76 ^{b}
Hydrolyzed polyphenols (mgGAE/gdw)	45.76 ^{b}	76.22 ^{a}
Condensed polyphenols (mgCE/gdw)	10.97 ^{b}	47.95 ^{a}
Total polyphenol content (mg/gdw)	56.73 ^{b}	124.17 ^{a}
Antioxidant activity:		
DPPH (mgTE/gdw)	266.63 ^{b}	444.64 ^{a}
ABTS (mgTE/gdw)	258.18 ^{b}	498.46 ^{a}
FRAP ($mgFe^{+2}$/gdw)	354.03 ^{b}	551.13 ^{a}

Different letters indicate no significant differences between treatments (Tukey $\alpha = 0.05$).

3.5. Identification of Phenolic Compounds by RP-HPLC-ESI-MS

The extracts obtained at 0 and 12 h of SSF under the conditions of treatment 6 (see Table 1) were characterized by RP-HPLC-ESI-MS. A total of nine phenolic compounds were detected (Table 6), mainly belonging to the family of hydroxybenzoic acids and ellagitannins; however, two of them were unidentified according to the existing database, but according to the phenolic compound profiles and the weight of unidentified compounds, they are closely related to other ellagitannins. These compounds will be further elucidated using other procedures (i.e., Nuclear Magnetic Resonance).

Table 6. Phenolic compound profiles on cascalote pods fermented by *A. niger* GH1 and identified using the RP-HPLC-ESI-MS technique.

#	RT	$[M-H]^{-}$	Bioactive Compound	Molecular Formula	Family	0 h	12 h SSF
						UA	UA
1	10.41	342.5	5-O-Galloylquinic acid	$C_{14}H_{16}O_{10}$	Hydroxybenzoic acids	0.026	0.343
2	15.02	798.4	Ellagic acid derivate		Hydroxybenzoic acids	0.136	1.539
3	17.64	494.7	Unidentified			0.373	2.479
4	18.51	1118.1	Unidentified			0.384	2.451
5	20.51	632.6	Corilagin	$C_{27}H_{22}O_{18}$	Ellagitannins	0.747	2.501
6	24.18	782.4	Gallagyl-hexoside	$C_{34}H_{22}O_{22}$	Ellagitannins	0.338	ND
7	24.62	968.1	Lagerstannin B derivate		Ellagitannins	ND	2.458
8	25.79	952.2	Geraniin	$C_{41}H_{28}O_{27}$	Ellagitannins	0.377	2.307
9	28.84	300.6	Ellagic acid	$C_{14}H_{6}O_{8}$	Hydroxybenzoic acid dimers	0.247	2.155

RT = retention time; UA = unit of absorbance; ND = not detected.

4. Discussion

A culture medium is characterized by a mixture of nutrients that, in adequate concentrations and under optimal physical conditions, allows microbial growth and metabolic

processes to occur [14]. The obtained results (Table 2) are in the range of the values reported in the literature for moisture (3%), protein (3.85–5.34%), ash (1.87–2.58%), fat (0.19–3.35%), and carbohydrate (71.62–88.82%) content [1,23].

In the SSF process, WAC, CHP, and maximum moisture are important parameters for the evaluation of the vegetal material's suitability as a solid support for fungal growth and metabolite production. WAC indicates the amount of water that can be absorbed by the substrate, and it is related to the hydroxyl groups present on it and allows additional water interactions by hydrogen bonding [12,24]. Thus, a high WAC value is convenient for the SSF process because moisture content can be modified, thus allowing microbial growth. Cascalote pods presented a WAC value of 2.97 g gel/gdw, which is in the range reported (2.16–3.4 g gel/gdw) for agro-industrial byproducts considered as suitable supports for SSF such as grape waste [25], corn cobs [26], candelilla stalks [26], rambutan peels [12], and fig byproducts [27]. The CHP represents the water linked to the support that cannot be used for the biological functions of the microorganism. Microbial growth is promoted at low CHP values since high values affect it in a big proportion of the water linked to the substrate; therefore free water content is low [15]. The CHP of the cascalote pods was 3.75%, which is lower than those reported for fig byproducts (4.63%), sugarcane bagasse (9.46%), pomegranate peel (10.13%), coconut husk (16%), corn cobs (27%), grape waste (53.33%), pineapple waste (55.6%), and mango seeds (56.5%)—materials successfully used as substrate supports in SSF [11,14,15,25–27]. Additionally, cascalote pods could be used as a substrate in the SSF process at high moisture levels due to the maximum moisture level that they obtained (79.33%); however, it is recommended to work at a moisture level below 70% in order to avoid oxygen transfer limitations, particle agglomeration, and bacterial growth [12,28]. Based on their physicochemical characterization, cascalote pods are suitable for use as a substrate support for SSF.

Filamentous fungi are the most used microorganism in SSF, and they have great potential to release bioactive compounds from vegetal matrices [8]. Fungi can assimilate a wide variety of carbon sources, thus being able to synthesize, degrade, and transform several phenolic compounds and other aromatic compounds [29,30]. During SSF, fungi produce several enzymes that degrade the cell wall components, resulting in an enhanced phenolic compound extraction [15]. *A. niger* GH1 has been previously reported as a potential fungus for the degradation of lignocellulosic materials and the release of phenolic compounds [11,12,14]. In this study, the use of *A. niger* GH1 increased the TPC release 1.67-fold and the AA 2.17-fold as compared to the other *Aspergillus* strains evaluated.

The TPC release was positively correlated with AA (0.95; $p < 0.01$), confirming that the increase in AA values was due to the increase in TPC release during the SSF process. This correlation is in accordance with the previous studies published by Paz-Arteaga et al. [15], Jericó-Santos et al. [31], and Buenrostro-Figueroa et al. [14] on the SSF of pineapple, tamarind, and pomegranate byproducts, which reported correlation values between TPC and DPPH of 0.63, 072, and 0.86, respectively. Increments in the TPC released are associated with the fungal enzymes produced during the microbial growth phase. These enzymes (xylanases, pectinases, proteases, and glucosidase, among others) are responsible for the oxidative degradation of lignin and the breakdown of the links between the cell wall matrix and phenolic compounds, which results in their release [15,32].

Exploring the effects of nutritional and physical parameters in SSF influences microbial growth and their metabolic processes. Moisture plays an important role in fungal growth. SSF requires the close control of water content; depending on the microbial strain used, a specific moisture content is needed to ensure growth and metabolite production [33]. In this study, TPC release was favored at high levels of moisture (60%), similar to the results obtained by Buenrostro-Figueroa et al. [27] in the SSF of fig byproducts using *A. niger* GH1.

Magnesium is necessary for fungal nutrition, and it has an important role in hyphae development. An optimal concentration of this mineral improves the sporulation rate, thus promoting an efficient enzyme synthesis and consequently, fungal biomass proliferation and phenolic compound release [14,34]. Using *A. niger* GH1 and pomegranate husk to obtain

ellagic acid and total phenolic compounds by SSF, Sepulveda et al. [34] and Buenrostro-Figueroa et al. [14] found that increments in $MgSO_4$ levels improved the release of ellagic acid and total phenolic compounds, respectively. Furthermore, those authors reported that the best phenolic compound release was attained at high levels of KH_2PO_4 and KCl. In the present study, the same KH_2PO_4, KCl, and $MgSO_4$ levels were used; however, a contrary effect was observed. This may be due to the substrate support (cascalote pods) itself containing sufficient amounts of minerals (Mg, P, and K) needed for both microbial growth and TPC release. The addition of higher amounts of minerals could have affected the enzyme production [35]. The cell wall of *A. niger* contains carbohydrates, proteins, ash, and lipids [36]. Increases in protein and lipid content are related to the fungal biomass present in fermented cascalote pods. During fungal growth, the available nutrients are used to synthesize lipids as mycelium components. Reduction in carbohydrate and fiber contents are associated with fungal growth since these components are used by the fungi as a carbon source for its development and production of cell wall-degrading enzymes [15,32].

Out of all the treatments evaluated, the best values for the response variables (TPC and AA) were obtained in treatment 6, which may have been due to these conditions (30 °C, 60% moisture, and a concentration of mineral salts in the medium (g/L) of: KH_2PO_4: 1.52, $NaNO_3$: 7.65, $MgSO_4 \bullet 7H_2O$: 1.52, and KCl: 1.52) promoting the better growth and metabolic processes of *A. niger* GH1. SSF substantially improved the amount of phenolic compounds released from cascalote pods as well as the antioxidant activity. This is explained by the fact that several enzymes with important hydrolytic activities participate in SSF, inducing the release of phenolic compounds from polymeric matrices [15,37].

Based on the above results, SSF was successfully carried out by *A. niger* GH1 on cascalote pods, with 12 h as the best time for TPC release. This is the first report to be conducted for cascalote pods under the conditions described. Using extraction by maceration with methanol, Sánchez et al. [38] reported values of 21.71 mg/gdw for condensed tannins and 32.06 mg/gdw for total tannins. Rojas-Morales et al. [39] reported values for condensed and total tannins of 13 and 34 mg/gdw, respectively. A total condensed tannins content of 7.72 mg/gdw was reported by Pineda-Peña et al. [40]. The values for condensed and total polyphenols released from cascalote pods in the present work are 120–521% and 265–288% higher than the values previously reported [38–40].

In addition, the cascalote pods exhibited strong antioxidant activity by reducing the agents for ferric ions and scavenging free radicals. According to the ABTS assay, SSF provided an increase of up to 93% in the AA of the extract (498.46 mgTE/gdw) as compared to the value before SSF (258.18 mgTE/gdw). Ethanolic extract from cascalote was evaluated against DPPH and ABTS antioxidant assays [41]. At 500 mg/L, the inhibition rate was higher than 90% in both cases. Based on the strongly positive and highly significant correlation between phenolic compound content and activity in antioxidant assays (DPPH, ABTS, and FRAP), the increase in AA can be attributed to the amount and type of phenolic compounds released by the SSF. These results show the feasibility of obtaining TPC with AA from vegetal matrices via SSF in comparison to chemical processes or the use of commercial enzymes.

The RP-HPLC-ESI-MS of cascalote extracts showed that the main compounds were 5-O-galloylquinic acid, ellagic acid derivatives, corilagin, gallagyl-hexoside, lagerstannin B derivative, geraniin, and ellagic acid. There have been no reports indicating the identification of phenolic compounds obtained from cascalote pods by SSF; however, some of these compounds have been previously reported from the hydroalcoholic extracts of *C. coriaria* [40,42]. In addition, other compounds, such as ethyl gallate, methyl gallate, gallic acid, tetragalloylglucose, pentagalloylglucose, valoneic acid dilactone, digalloylshikimic acid, and other phenolic compound derivatives, have been cited [2,4,43].

From the nine compounds detected, seven of them increased in concentration at 12 h of SSF (in terms of units of absorbance), with 5-*o*-galloyquinic acid, corilagin, geraniin, and ellagic acid having values 12.19-, 2.34-, 5.11-, and 7.72-fold higher than those obtained at 0 h of SSF. The lagerstannin B derivative was only found at 12 h of SSF, while gallagyl-hexoside

was only detected at 0 h. The differences between the phenolic profile and absorbance values might be due to the phenolic compounds being in free or conjugated form (esterified). During SSF, these bonds are broken by the action of microbial enzymes, which facilitates the partial or complete release of the phenolic compounds, therefore improving their solubility or producing new molecules [15].

Identified polyphenol molecules in cascalote pods have different biological activities, such as antioxidant effects, hepatoprotective effects [41], anthelmintic effects [39,44], arginase inhibitory activity [45], antimicrobial activity [4,43,46], and gastroprotective effects [40]. These benefits provide a wide range of possible applications in the food, pharmaceutical, and cosmetic industries.

Accurate data related to cascalote production were not found; however, a yield of 150 kg of pods per tree has been reported [1]. Using the bioprocess described here, up to 18.6 kg of TPC per tree of cascalote pods could be obtained (124 kg of TPC/ton). Considering the commercial prices on the Sigma-Aldrich website (https://www.sigmaaldrich.com/US/en/life-science/sigma-aldrich. accessed on 31 August 2023) for corilagin (SKU: 75251), geraniin (SKU: PHL80994), and ellagic acid (SKU: PHL89653) (594 USD/10 mg, 501 USD/10 mg, and 271 USD/50 mg, respectively), the SSF extraction process represents a profitable and sustainable alternative for the acquisition of valuable compounds with industrial applications. These results confirm that SSF permits the recovery of larger amounts of high-value molecules through a process that involves a shorter amount of time. With particular focus on the highly desired circular economy model, an alternative to the diversification of cascalote pods has thus been reported.

5. Conclusions

A. niger GH1 utilized cascalote pods as a carbon source and released phenolic compounds with antioxidant activity. The factors that most influenced the TPC release were moisture and KH_2PO_4 concentration. The higher TPC release and antioxidant activity were obtained at the following SSF conditions: *A. niger* GH1 (1×10^7 sp/gdw) at 12 h of culture, 30 °C, 60% moisture, and a concentration of mineral salts in the medium (g/L) of: KH_2PO_4: 1.52, $NaNO_3$: 7.65, $MgSO_4 \bullet 7H_2O$: 1.52, and KCl: 1.52. Corilagin, lagerstannin, geraniin, and ellagic acid were the main phenolic compounds identified by RP-HPLC-ESI-MS in the cascalote extracts. The development of a bioprocess such as the SSF-assisted extraction conducted in this study allowed for the significant increase in TPC release and exhibited high AA, thus offering an alternative use for this underutilized material—the cascalote pods. The recovered molecules are of great interest in the cosmetic, pharmacy, and food industries and are considered to be high added-value products.

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References

1. Palma García, J.M. Caesalpinia coriaria (Jacq.) Wild. In *Recursos Arboreos y Arbustivos Tropicales*; Palma García, J.M., González-Reveles, C., Eds.; Universidad de Colima: Colima, México, 2018; pp. 30–34. Available online: http://ww.ucol.mx/content/publicacionesenlinea/adjuntos/Recursos-arboreos-y-arbustivos-tropicales_462.pdf (accessed on 17 July 2023).
2. Sánchez-Carranza, J.N.; Alvarez, L.; Marquina-Bahena, S.; Salas-Vidal, E.; Cuevas, V.; Jiménez, E.W.; Veloz G, R.A.; Carraz, M.; González-Maya, L. Phenolic Compounds Isolated from *Caesalpinia coriaria* Induce S and G2/M Phase Cell Cycle Arrest Differentially and Trigger Cell Death by Interfering with Microtubule Dynamics in Cancer Cell Lines. *Molecules* **2017**, *22*, 666. [CrossRef]
3. Jeeva, K.; Thiyagarajan, M.; Elangovan, V.; Geetha, N.; Venkatachalam, P. *Caesalpinia coriaria* leaf extracts mediated biosynthesis of metallic silver nanoparticles and their antibacterial activity against clinically isolated pathogens. *Ind. Crops Prod.* **2014**, *52*, 714–720. [CrossRef]
4. Olmedo-Juárez, A.; Briones-Robles, T.I.; Zaragoza-Bastida, A.; Zamilpa, A.; Ojeda-Ramírez, D.; Mendoza de Gives, P.; Olivares-Pérez, J.; Rivero-Perez, N. Antibacterial activity of compounds isolated from *Caesalpinia coriaria* (Jacq) Willd against important bacteria in public health. *Microb. Pathog.* **2019**, *136*, 103660. [CrossRef] [PubMed]
5. Li, X.; Deng, Y.; Zheng, Z.; Huang, W.; Chen, L.; Tong, Q.; Ming, Y. Corilagin, a promising medicinal herbal agent. *Biomed. Pharmacother.* **2018**, *99*, 43–50. [CrossRef] [PubMed]
6. Makris, D.P.; Boskou, G.; Andrikopoulos, N.K. Recovery of antioxidant phenolics from white vinification solid by-products employing water/ethanol mixtures. *Bioresour. Technol.* **2007**, *98*, 2963–2967. [CrossRef] [PubMed]
7. Alara, O.R.; Abdurahman, N.H.; Ukaegbu, C.I. Extraction of phenolic compounds: A review. *Curr. Res. Food Sci.* **2021**, *4*, 200–214. [CrossRef] [PubMed]
8. Cano y Postigo, L.O.; Jacobo-Velázquez, D.A.; Guajardo-Flores, D.; Garcia Amezquita, L.E.; García-Cayuela, T. Solid-state fermentation for enhancing the nutraceutical content of agrifood by-products: Recent advances and its industrial feasibility. *Food Biosci.* **2021**, *41*, 100926. [CrossRef]
9. Huynh, N.T.; Van Camp, J.; Smagghe, G.; Raes, K. Improved Release and Metabolism of Flavonoids by Steered Fermentation Processes: A Review. *Int. J. Mol. Sci.* **2014**, *15*, 19369–19388. [CrossRef]
10. Martins, S.; Mussatto, S.I.; Martínez-Avila, G.; Montañez-Saenz, J.; Aguilar, C.N.; Teixeira, J.A. Bioactive phenolic compounds: Production and extraction by solid-state fermentation. A review. *Biotechnol. Adv.* **2011**, *29*, 365–373. [CrossRef]
11. Torres-León, C.; Ramírez-Guzmán, N.; Ascacio-Valdés, J.; Serna-Cock, L.; dos Santos Correia, M.T.; Contreras-Esquivel, J.C.; Aguilar, C.N. Solid-state fermentation with *Aspergillus niger* to enhance the phenolic contents and antioxidative activity of Mexican mango seed: A promising source of natural antioxidants. *LWT* **2019**, *112*, 108236. [CrossRef]
12. Cerda-Cejudo, N.D.; Buenrostro-Figueroa, J.J.; Sepúlveda, L.; Torres-Leon, C.; Chávez-González, M.L.; Ascacio-Valdés, J.A.; Aguilar, C.N. Recovery of ellagic acid from mexican rambutan peel by solid-state fermentation-assisted extraction. *Food Bioprod. Process.* **2022**, *134*, 86–94. [CrossRef]
13. Xue, P.; Liao, W.; Chen, Y.; Xie, J.; Chang, X.; Peng, G.; Huang, Q.; Wang, Y.; Sun, N.; Yu, Q. Release characteristic and mechanism of bound polyphenols from insoluble dietary fiber of navel orange peel via mixed solid-state fermentation with *Trichoderma reesei* and *Aspergillus niger*. *LWT* **2022**, *161*, 113387. [CrossRef]
14. Buenrostro-Figueroa, J.J.; Nevárez-Moorillón, G.V.; Chávez-González, M.L.; Sepúlveda, L.; Ascacio-Valdés, J.A.; Aguilar, C.N.; Pedroza-Islas, R.; Huerta-Ochoa, S.; Arely Prado-Barragán, L. Improved Extraction of High Value-Added Polyphenols from Pomegranate Peel by Solid-State Fermentation. *Fermentation* **2023**, *9*, 530. [CrossRef]
15. Paz-Arteaga, S.L.; Ascacio-Valdés, J.A.; Aguilar, C.N.; Cadena-Chamorro, E.; Serna-Cock, L.; Aguilar-González, M.A.; Ramírez-Guzmán, N.; Torres-León, C. Bioprocessing of pineapple waste for sustainable production of bioactive compounds using solid-state fermentation. *Innov. Food Sci. Emerg. Technol.* **2023**, *85*, 103313. [CrossRef]
16. AOAC, I.; Latimer, G.W. *Official Methods of Analysis of AOAC*, 19th ed.; AOAC International: Gaithersburg, MD, USA, 2012.
17. Orzua, M.C.; Mussatto, S.I.; Contreras-Esquivel, J.C.; Rodriguez, R.; de la Garza, H.; Teixeira, J.A.; Aguilar, C.N. Exploitation of agro industrial wastes as immobilization carrier for solid-state fermentation. *Ind. Crops Prod.* **2009**, *30*, 24–27. [CrossRef]
18. Wong-Paz, J.E.; Muñiz-Márquez, D.B.; Aguilar-Zárate, P.; Rodríguez-Herrera, R.; Aguilar, C.N. Microplate Quantification of Total Phenolic Content from Plant Extracts Obtained by Conventional and Ultrasound Methods. *Phytochem. Anal.* **2014**, *25*, 439–444. [CrossRef]
19. Meléndez, N.P.; Nevárez-Moorillón, V.; Rodriguez-Herrera, R.; Espinoza, J.C.; Aguilar, C.N. A microassay for quantification of 2,2-diphenyl-1-picrylhydracyl (DPPH) free radical scavening. *Afr. J. Biochem. Res.* **2014**, *8*, 14–18. [CrossRef]
20. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef] [PubMed]
21. Rojo-Gutiérrez, E.; Carrasco-Molinar, O.; Tirado-Gallegos, J.M.; Levario-Gómez, A.; Chávez-González, M.L.; Baeza-Jiménez, R.; Buenrostro-Figueroa, J.J. Evaluation of green extraction processes, lipid composition and antioxidant activity of pomegranate seed oil. *J. Food Meas. Charact.* **2021**, *15*, 2098–2107. [CrossRef]
22. Pulido, R.; Bravo, L.; Saura-Calixto, F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agric. Food Chem.* **2000**, *48*, 3396–3402. [CrossRef]

23. Mora-Santacruz, A.; Roman-Miranda, M.L.; González-Cueva, G.; Barrientos-Ramírez, L. Chemical composition of cascalote *Caesalpinia coriaria* (Jacq.) Willd. and diversity of uses in the rural areas of dry tropics. *Rev. Investig. Desarro.* **2018**, *4*, 24–28. Available online: https://www.ecorfan.org/spain/researchjournals/Investigacion_y_Desarrollo/vol4num12/Revista_de_Investigacion_y_Desarrollo_V4_N12_4.pdf (accessed on 20 July 2023).
24. Martins, Z.E.; Pinho, O.; Ferreira, I.; Jekle, M.; Becker, T. Development of fibre-enriched wheat breads: Impact of recovered agroindustrial by-products on physicochemical properties of dough and bread characteristics. *Eur. Food Res. Technol.* **2017**, *243*, 1973–1988. [CrossRef]
25. Martínez-Ávila, G.C.; Aguilera-Carbó, A.F.; Rodríguez-Herrera, R.; Aguilar, C.N. Fungal enhancement of the antioxidant properties of grape waste. *Ann. Microbiol.* **2012**, *62*, 923–930. [CrossRef]
26. Buenrostro-Figueroa, J.; Ascacio-Valdés, A.; Sepúlveda, L.; De La Cruz, R.; Prado-Barragán, A.; Aguilar-González, M.A.; Rodríguez, R.; Aguilar, C.N. Potential use of different agroindustrial by-products as supports for fungal ellagitannase production under solid-state fermentation. *Food Bioprod. Process.* **2014**, *92*, 376–382. [CrossRef]
27. Buenrostro-Figueroa, J.J.; Velázquez, M.; Flores-Ortega, O.; Ascacio-Valdés, J.A.; Huerta-Ochoa, S.; Aguilar, C.N.; Prado-Barragán, L.A. Solid state fermentation of fig (*Ficus carica* L.) by-products using fungi to obtain phenolic compounds with antioxidant activity and qualitative evaluation of phenolics obtained. *Process Biochem.* **2017**, *62*, 16–23. [CrossRef]
28. Gowthaman, M.K.; Krishna, C.; Moo-Young, M. Fungal solid state fermentation—An overview. In *Applied Mycology and Biotechnology*; Khachatourians, G.G., Arora, D.K., Eds.; Elsevier: Amsterdam, The Netherlands, 2001; Volume 1, pp. 305–352. [CrossRef]
29. Stoilova, I.; Krastanov, A.; Yanakieva, I.; Kratchanova, M.; Yemendjiev, H. Biodegradation of mixed phenolic compounds by *Aspergillus awamori* NRRL 3112. *Int. Biodeterior. Biodegrad.* **2007**, *60*, 342–346. [CrossRef]
30. Vargas-Sánchez, R.D.; Torres-Martínez, B.M.; Torrescano-Urrutia, G.R.; Sánchez-Escalante, A.; Esqueda, M. Extraction of phenolic compounds from agro-industrial by-products by fungal fermentation with potential use as additives for meat and meat products. A review. *Biotecnia* **2021**, *23*, 66–77. [CrossRef]
31. Jericó Santos, T.R.; Santos Vasconcelos, A.G.; Lins de Aquino Santana, L.C.; Gualberto, N.C.; Buarque Feitosa, P.R.; Pires de Siqueira, A.C. Solid-state fermentation as a tool to enhance the polyphenolic compound contents of acidic *Tamarindus indica* by-products. *Biocatal. Agric. Biotechnol.* **2020**, *30*, 101851. [CrossRef]
32. da Costa Maia, I.; Thomaz dos Santos D'Almeida, C.; Guimarães Freire, D.M.; d'Avila Costa Cavalcanti, E.; Cameron, L.C.; Furtado Dias, J.; Simões Larraz Ferreira, M. Effect of solid-state fermentation over the release of phenolic compounds from brewer's spent grain revealed by UPLC-MSE. *LWT* **2020**, *133*, 110136. [CrossRef]
33. Krishna, C. Solid-State Fermentation Systems—An Overview. *Crit. Rev. Biotechnol.* **2005**, *25*, 1–30. [CrossRef]
34. Sepúlveda, L.; Aguilera-Carbó, A.; Ascacio-Valdés, J.A.; Rodríguez-Herrera, R.; Martínez-Hernández, J.L.; Aguilar, C.N. Optimization of ellagic acid accumulation by *Aspergillus niger* GH1 in solid state culture using pomegranate shell powder as a support. *Process Biochem.* **2012**, *47*, 2199–2203. [CrossRef]
35. Sirisansaneeyakul, S.; Jitbanjongkit, S.; Prasomsart, N.; Luangpituksa, P. Production of β-Fructofuranosidase from *Aspergillus niger* ATCC 20611. *Agric. Nat. Resour.* **2000**, *34*, 378–386.
36. Garcia-Rubio, R.; de Oliveira, H.C.; Rivera, J.; Trevijano-Contador, N. The Fungal Cell Wall: *Candida*, *Cryptococcus*, and *Aspergillus* Species. *Front. Microbiol.* **2020**, *10*, 2993. [CrossRef] [PubMed]
37. Espitia-Hernández, P.; Ruelas-Chacón, X.; Chávez-González, M.L.; Ascacio-Valdés, J.A.; Flores-Naveda, A.; Sepúlveda-Torre, L. Solid-State Fermentation of Sorghum by *Aspergillus oryzae* and *Aspergillus niger*: Effects on Tannin Content, Phenolic Profile, and Antioxidant Activity. *Foods* **2022**, *11*, 3121. [CrossRef]
38. Sánchez, N.; Mendoza, G.D.; Martínez, J.A.; Hernández, P.A.; Camacho Diaz, L.M.; Lee-Rangel, H.A.; Vazquez, A.; Flores Ramirez, R. Effect of *Caesalpinia coriaria* Fruits and Soybean Oil on Finishing Lamb Performance and Meat Characteristics. *BioMed Res. Int.* **2018**, *2018*, 9486258. [CrossRef] [PubMed]
39. Rojas-Morales, D.; Cubides-Cárdenas, J.; Montenegro, A.C.; Martínez, C.A.; Ortíz-Cuadros, R.; Rios-de Álvarez, L. Anthelmintic effect of four extracts obtained from *Caesalpinia coriaria* foliage against the eggs and larvae of *Haemonchus contortus*. *Rev. Bras. Parasitol. Vet.* **2021**, *30*, e002521. [CrossRef]
40. Pineda-Peña, E.A.; Capistran-Amezcua, D.; Reyes-Ramírez, A.; Xolalpa-Molina, S.; Chávez-Piña, A.E.; Figueroa, M.; Navarrete, A. Gastroprotective effect methanol extract of *Caesalpinia coriaria* pods against indomethacin- and ethanol-induced gastric lesions in Wistar rats. *J. Ethnopharmacol.* **2023**, *305*, 116057. [CrossRef]
41. Pájaro González, Y.; Méndez Cuadro, D.; Fernández Daza, E.; Franco Ospina, L.A.; Redondo Bermúdez, C.; Díaz Castillo, F. Inhibitory activity of the protein carbonylation and hepatoprotective effect of the ethanol-soluble extract of *Caesalpinia coriaria* Jacq. *Orient. Pharm. Exp. Med.* **2016**, *16*, 225–232. [CrossRef]
42. García-Hernández, C.; Rojo-Rubio, R.; Olmedo-Juárez, A.; Zamilpa, A.; Mendoza de Gives, P.; Antonio-Romo, I.A.; Aguilar-Marcelino, L.; Arece-García, J.; Tapia-Maruri, D.; González-Cortazar, M. Galloyl derivatives from *Caesalpinia coriaria* exhibit in vitro ovicidal activity against cattle gastrointestinal parasitic nematodes. *Exp. Parasitol.* **2019**, *200*, 16–23. [CrossRef]
43. Rangel-López, L.; Rivero-Perez, N.; Valladares-Carranza, B.; Olmedo-Juárez, A.; Delgadillo-Ruiz, L.; Vega-Sánchez, V.; Hori-Oshima, S.; Nassan, M.A.; Batiha, G.E.; Zaragoza-Bastida, A. Antibacterial Potential of *Caesalpinia coriaria* (Jacq) Willd Fruit against *Aeromonas* spp. of Aquaculture Importance. *Animals* **2022**, *12*, 511. [CrossRef]

44. García-Hernández, C.; Rojo-Rubio, R.; Gives, P.M.-d.; González-Cortazar, M.; Zamilpa, A.; Mondragón-Ancelmo, J.; Villa-Mancera, A.; Olivares-Pérez, J.; Tapia-Maruri, D.; Olmedo-Juárez, A. In vitro and in vivo anthelmintic properties of *Caesalpinia coriaria* fruits against *Haemonchus contortus*. *Exp. Parasitol.* **2022**, *242*, 108401. [CrossRef] [PubMed]
45. Wulansari, A.; Elya, B.; Noviani, A. Arginase Inhibitory and Antioxidant Activities of *Caesalpinia coriaria* (Jacq.) Willd. Bark Extract. *Pharmacogn. J.* **2018**, *10*, 1174–1179. [CrossRef]
46. Pitre-Ruiz, L.; Galván-Ayala, D.; Castro-Uriana, O.; Ávila Mendez, K.J.; Lopez-Pazos, S.A. In vitro antimicrobial activity of *Caesalpinia coriaria* (Jacq.) Willd extracts on *Streptococcus pyogenes* and *Candida albicans*. *Vitae* **2021**, *28*, 1–15. [CrossRef]