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Evaluation of Solid-State Fermentation Conditions from Pineapple Peel Waste for Release of Bioactive Compounds by *Aspergillus niger* spp.

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Abstract: Currently, agroindustrial waste can be used to obtain bioactive compounds. The solid-state fermentation is an alternative for the valorization of these waste and to be able to release bioactive compounds that may be of interest to different industrial sectors. The aim of this study was to evaluate solid-state fermentation conditions using pineapple peel waste as the substrate with *Aspergillus niger* spp., to release bioactive compounds using a Plackett–Burman exploratory design. Temperature, humidity, inoculum, $NaNO_3$, $MgSO_4$, KCl, and KH_2PO_4 conditions in the fermentation process were evaluated. The antioxidant capacity was determined, and the main compounds of the fermentation extracts were identified. The results revealed that the *Aspergillus niger* HT3 strain reached a hydrolyzable tannin release of 10.00 mg/g, While *Aspergillus niger* Aa20 reached a condensed tannin release of 82.59 mg/g. The KH_2PO_4 affects the release of condensed tannins with *A. niger* Aa20, and $MgSO_4$ affects the release of hydrolyzable tannins with *A. niger* HT3. In addition, a positive antioxidant activity was demonstrated for the DPPH, ABTS, and FRAP technique. The main compounds in the fermented pineapple peel were 3-feruloylquinic acid, caffeic acid, lariciresinol, and 3-hydroxyphloretin 2′-O-xylosyl-glucoside, among others. The solid-state fermentation process is a biotechnological alternative for the release of bioactive compounds.

Keywords: condensed tannins; hydrolyzable tannins; antioxidant; Plackett–Burman; DPPH; FRAP

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1. Introduction

Currently, bioactive compounds are gaining great interest due to the importance of their benefits to human health and their applications in the food, pharmaceutical, and cosmetic industries, among others. These compounds are called tannins; they are secondary metabolites found in plants and fruits [1]. Tannins have been classified into several groups; among them, there are condensed ones, which are oligomers or polymers of flavan-3-ols and flavan-3,4-diols [2]. Inside these groups, there are also proanthocyanidins, and among the most common are epicatechin, gallocatechin, catechin, and epigallocatechin. Furthermore, hydrolyzable tannins are divided into two large groups: gallotannins, which are esters of gallic acid, and ellagitannins, which are esters of ellagic acid [3,4].

Tannins have been of great interest due to their biological activities such as their antioxidant, antimicrobial, anti-inflammatory, chemotherapeutic, antiglycemic, and other activities. They can also be applied in medicines, foods, supplements, dermatological products, and cosmetics [3,5]. Bioactive compounds have been found in waste peel fruits

such as grape, orange, pomegranate, and rambutan. According to the Food and Agriculture Organization (FAO), the world pineapple production in 2022 was 29,361,138 tonnes [6]. The producing countries include Costa Rica, the Philippines, Brazil, Thailand, and India. Pineapple waste can vary between 60–80% (depending on the variety) and corresponds to the crown, peel, leaves, core, and stems [7]. For this reason, alternatives are being sought to use this type of waste; among the possible uses are the manufacture of textiles, paper, and animal feed [8], bioethanol production [9], the production of recycled packaging [10], and the preparation of natural pigments [11], among others.

However, it has been shown that pineapple peel waste are also a potential source for obtaining tannins [12]. These wastes are rich in minerals (magnesium, potassium, zinc, and sodium, among others), different vitamins such as A, C, K, and E [13], and aromatic compounds such as limonene, ethyl hexanoate, butyl acetate, 1-butanol, and furfural, among others [14]. Several conventional and emerging technologies have been used to obtain this type of compound, including soxhlet-assisted extraction, liquid–liquid extraction, vapor extraction, infusion, ultrasound extraction [15], autohydrolysis [16], microwave, and supercritical fluid extraction [17].

Currently, new environmentally friendly alternatives are being sought to obtain tannins; solid-state fermentation is a bioprocess that occurs at low water levels. The use of yeasts and filamentous fungi has mainly been reported [18]. It is very important to study the factors that affect the solid-state fermentation process such as temperature, humidity, and pH, among others [19].

The objective of this work was to evaluate the conditions of the solid-state fermentation process using *Aspergillus niger* spp. for the release of polyphenolic compounds from pineapple peel waste and identify the main compounds with antioxidant activity.

2. Materials and Methods

2.1. Obtaining Pineapple Peel Waste and Reagents

Pineapple peel waste was collected from a local market located in the center of the city of Saltillo, Coahuila. The waste was cut into small pieces, washed, and placed in containers and dried in an oven (Luzeren®, model WGL-65B, Shanghai, China) at 60 °C for 72. Once dried, it was ground in a blender, finally obtaining a powder.

Gallic acid (CAS 149-91-7), catechin (CAS 18829-70-4), trolox (CAS 53188-07-1), DPPH (CAS 1898-66-4), ABTS (CAS 30931-67-0), and TPTZ (CAS 3682-36-7) were from Sigma-Aldrich® (St. Louis, MI, USA). Phenol (CAS 108-95-2), sodium nitrate (CAS 7631-99-4), potassium chloride (CAS 7447-40-7), magnesium sulfate (CAS 10034-99-8), sodium acetate (CAS127-09-3), potassium persulfate (7727-21-1), and iron sulphate (CAS 7782-63-7) were from FAGA®Lab. Sulfuric acid (CAS 7664-93-9), hydrochloric acid (CAS 7647-01-0), monopotassium phosphate (CAS 7778-77-0) from Analytyka®. Ethanol (CAS 64-17-5), sodium carbonate (CAS 497-19-8), isobutanol (CAS 78-83-1), methanol (CAS 67-56-1), and dextrose (50-99-7) were from Jalmek® (Nuevo Leon, Mexico). Acetic acid (CAS 64-19-7) was from Macron Fine Chemicals™. Potato dextrose agar was from BD Bioxon™ (Kowale, Poland). Folin reagent was from Godel Bell™ Reagents. Distilled water was provided by the Faculty of Chemical Sciences in Autonomous University of Coahuila.

2.2. Physicochemical Properties of Pineapple Peel Waste

For substrate-support characterization, pineapple peal waste was subjected to two analyses, which were water absorption index (WAI) and critical moisture point (CMP). These analyses were determined according to the methodology reported by Buenrostro-Figueroa et al. [20]. For WAI, 1.5 g of dry matter were placed in a 50 mL centrifugation tube with 15 mL of distilled water, manually shaken for 1 min at room temperature, and then centrifuged at 3000× *g* for 10 min (Premiere ®, Model XC-2450, Muskogee, OK, USA). The supernatant was discarded, and the WAI was calculated from the remaining weight of the gel expressed in g gel/g dry weight. The CMP was estimated by the addition of 1 g of sample on a thermobalance (OHAUS, Model MB23, Greifensee, Switzerland)

where weight loss by dehydration was monitored until inflection point was reached for parameter determination.

2.3. Proximate Analysis of Pineapple Peel Waste

The proximate analysis consisted of the determination of moisture, ash, and fat content according to the AOAC methodology according Polania-Rivera et al. [20]. Total carbohydrate content was determined by the phenol-sulfuric method according to Polonia-Rivera et al. [21] with some modifications where a standard curve was performed with dextrose with a concentration of 0–140 ppm. Reducing sugars were determined according to Selvanathan and Masngut [22]. The protein content was carried out according to the Lowry spectrophotometric method according to the methodology reported by Redmile-Gordon et al. [23] Fiber determination was determined according to that reported by Púa et al. [24].

2.4. Evaluation of Fungal Strains with Invasive Capacity on Pineapple Peel Waste

Five fungal strains from the Food Research Department of the Autonomous University of Coahuila, which were previously identified and characterized (*Aspergillus* HT3, *Aspergillus niger* GH1 [MUM 23.16], *Aspergillus niger* Aa20, *Aspergillus* Aa120, and *Aspergillus oryzae*) were evaluated. The strains were reactivated on potato dextrose agar (PDA) at 30 °C for 5 days. The invasive growth capacity of the micro-organism was evaluated using ground pineapple peel waste as substrate. Growth kinetics was performed by taking measurements every 24 h for 5 days and determining the growth rate [25]. Subsequently, extractions were performed by recovering with 15 mL of ethanol (50%), for subsequent analysis.

2.5. Quantification of Polyphenolic Compounds

Hydrolyzable tannins (HTs) were determined by using the Folin–Ciocalteu's method described by Zaki et al. [26] with some modifications. An aliquot of 20 µL of fermentation extract (samples were diluted at 1:100) were placed in a microplate well, and then 20 µL of Folin–Ciocalteu's reagent were added and allowed to stand for 5 min, and then 20 µL of sodium carbonate (0.01 M) were added and allowed to stand for 5 min, and finally diluted in 125 µL of distilled water and read at 750 nm in a microplate reader (Thermo Scientific, Waltham, MA, USA). Values were calculated using a gallic acid calibration curve (0–200 ppm). Hydrolyzable tannin content was expressed as mg/g pineapple peel weight. Condensed tannins (CTs) were determined according to the methodology described by Palacios et al. [27], with some modifications. The HCl-Isobutanol was prepared by dissolving 70 mg of $FeSO_4.7H_2O$ in 10 mL of HCl 36% and the solution reached up to 200 mL with isobutanol. Aliquots of 333 µL of extracts were added to 2 mL of HCl-Isobutanol reagent in a screw-cap glass tube at 100 °C for 1 h. After cooling at room temperature, 180 µL was pipetted to microplate wells and read at 460 nm in a microplate reader (Thermo Scientific, Waltham, MA, USA). Values were calculated using a catechin calibration curve (0–1500 ppm). The CT content was expressed as mg/g of pineapple peel weight.

2.6. Evaluation of Solid-State Fermentation (SSF) Conditions Using Pineapple Peel Waste for the Release of Bioactive Compounds

An exploratory Plackett–Burman design for the release of polyphenols was used to evaluate the SSF conditions. The design used two levels (+1, −1): 7 factors which were temperature, humidity, inoculum, $NaNO_3$, $MgSO_4$, KCl, and KH_2PO_4, and 8 treatments, which were performed in triplicate; the factors and treatment are shown in Table 1. The response variable was the amount of HT and CT; its quantification was performed according to the methodologies of Section 2.5. The extracts were recovered by adding 15 mL of an ethanol: water mixture (50% *v*/*v*), and then filtered and stored for further analysis.

Table 1. Condensed matrix of Plackett–Burman design treatments and factor levels for polyphenol release.

Treatments	Temperature (°C)	Humidity (%)	Inoculum (spores/g)	$NaNO_3$	$MgSO_4$	KCl	KH_2PO_4
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

Factors	Levels	
	+1	−1
Temperature (°C)	30	25
Humidity (%)	80	70
Inoculum (spores/g)	1×10^7	1×10^6
$NaNO_3$ (g/L)	15.6	7.65
$MgSO_4$ (g/L)	3.04	1.52
KCl (g/L)	3.04	1.52
KH_2PO_4 (g/L)	6.08	3.04

2.7. Analysis of the Polyphenolic Content of the Fermentation Extracts by RP-HPLC-ESI-MS

For the identification of the polyphenolic content of the fermentation extracts, they were filtered with a 0.45 µm nylon membrane; then, 1.5 mL was taken and placed in a vial for chromatography. The identification was carried out according to the methodology reported by Diaz-Herrera et al. [28]. The analyses by Reverse Phase–High Performance Liquid Chromatography were performed on a Varian HPLC system including an autosampler (Varian ProStar 410, Palo Alto, CA, 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 also was used.

2.8. Determination of Antioxidant Capacity

The DPPH assay was performed using the methodology described by Sepúlveda et al. [16], with some modifications. The DPPH radical was prepared at a concentration of 60 µM in methanolic solution. Then, 193 µL of DPPH-Methanol solution and 7 µL of fermentation extract were mixed and placed in a microplate. Methanol was used as blank and DPPH-Methanol solution was used as control absorbance. A standard solution of Trolox was prepared from 0 to 200 ppm. The samples were read at 540 nm in a microplate reader (Thermo Scientific, Waltham, MA, USA). The radical scavenging activity was calculated according to Equation (1):

$$RSA\ (\%) = \frac{(Ac - Am)}{Ac} \times 100 \quad (1)$$

where *Ac* is the control absorbance and *Am* is the absorbance of the sample.

The ABTS assay was performed using the methodology by Sepulveda et al. [15], with some modifications. A solution of ABTS (7 mM) was prepared and mixed with a solution of $K_2S_2O_8$ (2.45 mM). It was allowed to stand in the dark for 12 h at room temperature. An aliquot of 193 µL of ABTS solution and 7 µL of fermentation extract were mixed and placed in a microplate. Ethanol was used as the reading blank and ABTS-Ethanol as the control absorbance. A standard solution of Trolox was prepared from a range of 0 to 200 ppm. The samples were read at 750 nm in a microplate reader (Thermo Scientific, Waltham, MA, USA). The results were expressed according to Equation (1).

The FRAP assay was carried out according to Mala et al. [29] with some modifications. FRAP reagent was prepared daily, and maintained at 37 °C, by mixing acetate buffer (0.3 M pH 3.6) with a 10 mM solution of TPTZ in 40 mM HCl, and a 20 mM solution of $FeCl_3.6H_2O$, in a 10:1:1 ratio. An aliquot of 24 µL of sample was mixed with 180 µL of FRAP reagent solution and incubated in the dark for 30 min at room temperature. A standard solution of Trolox was prepared from a range of 0 to 500 ppm. The samples were read at 595 nm in a microplate reader (Thermo Scientific, Waltham, MA, USA). The polyphenolic content was analyzed according to the methodology of Section 2.7, of the treatments with the best antioxidant activity.

3. Results

3.1. Physicochemical Characterization of Pineapple Peel

In order to carry out the SSF process, it is necessary to know certain parameters, such as the water absorption index (WAI), which is the amount of water absorbed by the support [30], and the critical moisture point (CMP), which is the water bound to the support [31]. In the present study, pineapple peel obtained values of 5.42 ± 0.47 g gel/g dry peel and 4.60 ± 1.03% of WAI and CMP, respectively. The results of the proximate analysis of pineapple peel has a moisture of 8.90 ± 0.86%, ash of 1.62 ± 0.99%, fat of 3.78 ± 0.46%, protein of 6.63 ± 0.45%, and fiber content of 20.90 ± 1.53% were obtained. Total sugars achieved a value of 39.63 ± 6.04%, and reducing sugars achieved values of 34.88 ± 1.26%.

3.2. Evaluation of Fungal Strains with Invasive Growth Capacity

For the radial growth evaluation, six *Aspergillus* strains were used (*A. niger* HT3, *A. niger* Aa210, *A. niger* Aa20, *A. niger* GH1, and *A. oryzae*). According to Figure 1, it is observed that there is no significant difference between the *Aspergillus* strains. However, determining the growth velocity, *A. niger* A20 and *A. niger* HT3 were the ones that adapted better to the substrate since a result of 0.039 mm/h and 0.038 mm/h was obtained, respectively. Compared to the others, a lower growth rate was obtained, having as a result 0.036 mm/h, 0.033 mm/h, and 0.033 mm/h, from the strains of *A. niger* Aa210, *A. niger* GH1, and *A. oryzae*, respectively.

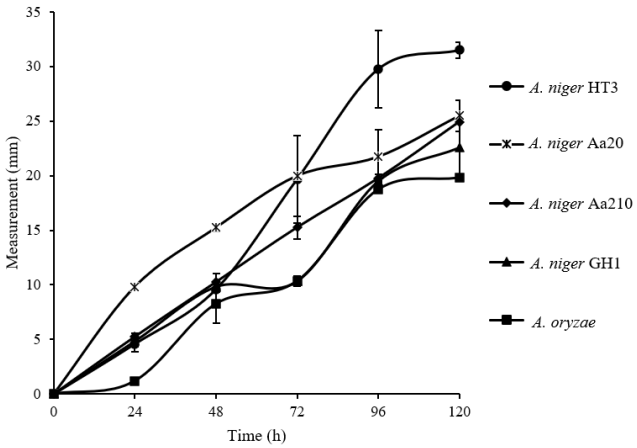


Figure 1. Radial growth for the evaluation of fungal strains with invasive capacity on pineapple peel waste.

3.3. Quantification of Tannins from Fermentation Kinetics

The *A. niger* Aa20 and *A. niger* HT3 strains were used for the quantification of HT and CT. Figure 2A shows the quantification of HT with *A. niger* Aa20 and *A. niger* HT3. The strain of *A. niger* Aa20 released up 6.02 mg/g of HT at 48 h. However, *A. niger* HT3, released up 10.00 mg/g of HT at 72 h. According to these results, *A. niger* HT3 was the strain that favored the release of HT. Figure 2B shows the quantification of CT with *A. niger* Aa20 and *A. niger* HT3. The strain of *A. niger* Aa20 released up 82.59 mg/g of CT at 48 h.

On the other hand, *A. niger* HT3 released up 78.71 mg/g of CT at 72 h. Both strains favored the release of CT; however, the strain of *A. niger* Aa20 was the one with the highest release of CT.

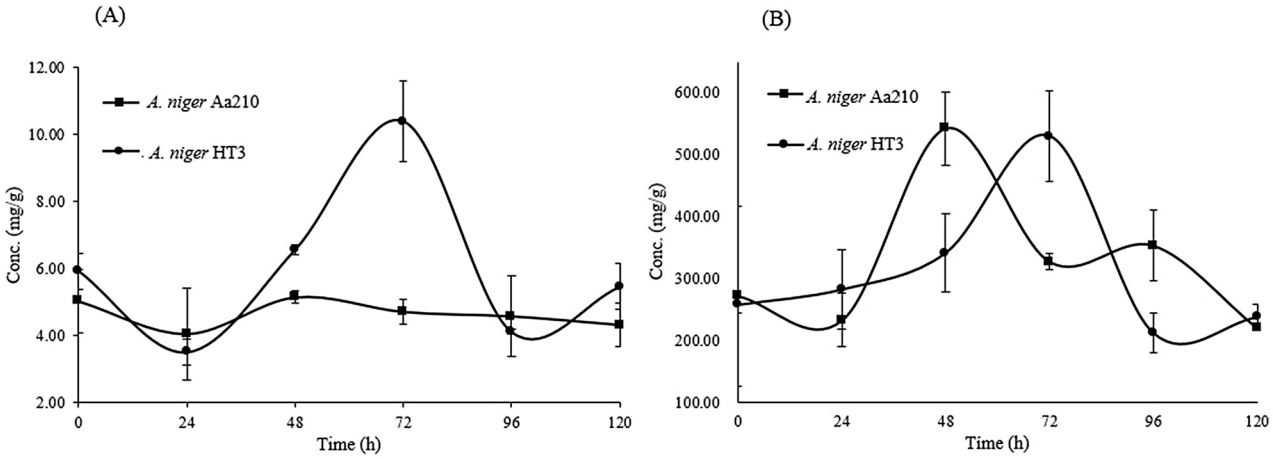


Figure 2. Kinetics of quantification of (**A**) HT and (**B**) CT of *A. niger* Aa20 and *A. niger* HT3.

3.4. Evaluation of the SSF Conditions for the Release of HT and CT

Figure 3A shows the treatments used for the release of HT; for *A. niger* HT3, a fermentation time of 72 h was used, and treatment 6 had the highest release of HT with a concentration of 17.46 ± 2.35 mg/g of the dry sample of pineapple peel. The conditions of treatment 6 were a temperature of 30 °C, 70% humidity, inoculum of 1×10^7, and concentration of salts of $NaNO_3$ (7.65 g/L), $MgSO_4$ (3.04 g/L), KCl (1.52 g/L), and KH_2PO_4 (3.04 g/L). Compared to the non-fermented sample (SWF), fermentation favors the release of polyphenolic compounds up to 2.8 times. Figure 3B shows the treatments used for the release CT; for *A. niger* Aa20, a fermentation time of 48 h was used, and treatment 4 had the best release of CT with a concentration of 92.03 ± 11.23 mg/g of the dry sample of pineapple peel. The conditions of treatment 4 were a temperature of 30 °C, 80% humidity, inoculum of 1×10^6, and concentration of salts of $NaNO_3$ (15.6 g/L), $MgSO_4$ (1.52 g/L), KCl (1.52 g/L), and KH_2PO_4 (3.04 g/L). Compared to the SWF, fermentation favors the release of polyphenolic compounds up to 2.1 times.

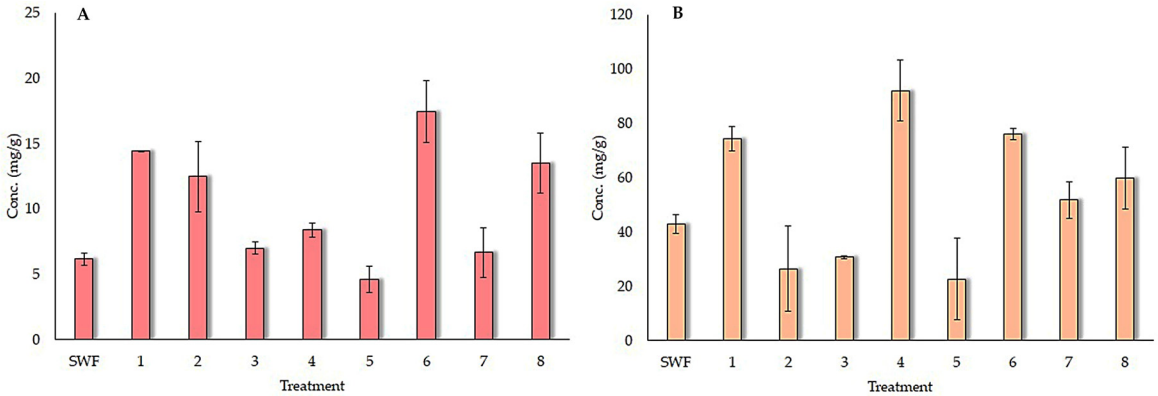
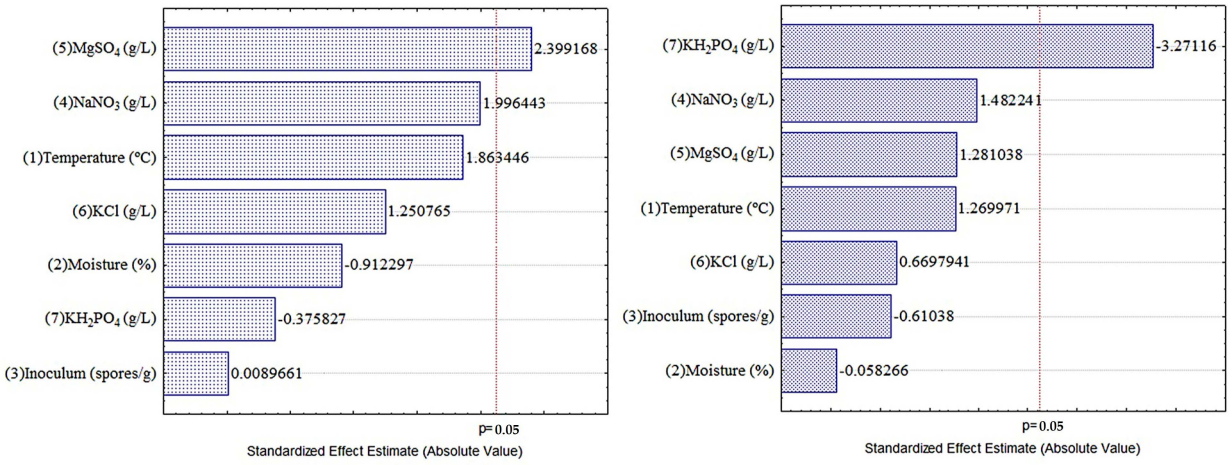


Figure 3. Concentration of polyphenols found in the fermentation process: (**A**) HT concentrations of *A. niger* HT3 treatments; and (**B**) CT concentrations of *A. niger* Aa20 treatments.

Figure 4A shows the standardized effect of factors affecting HT release with the *A. niger* HT3 strain. $MgSO_4$ directly affects the release of HT. The rest of the factors that do not exceed the dotted line have no effect on the release of HT. Figure 4B shows the standardized effect of factors affecting CT release with the *A. niger* Aa20 strain. KH_2PO_4 directly affects

the release of CT. The rest of the factors that do not exceed the dotted line have no effect on the release of CT.



(**A**) (**B**)

Figure 4. Pareto chart of the variables that affect the fermentation process: (**A**) HT for *A. niger* HT3; and (**B**) CT for *A. niger* Aa20.

3.5. Identification of HT and CT by HPLC-MS

The polyphenolic content (Table 2) of the unfermented sample and of the SSF extracts of pineapple peel with *A. niger* Aa20 (Treatment 4) and *A. niger* HT3 (Treatment 6) were determined by HPLC-MS. In the unfermented sample, 10 compounds belonging to the families of hydroxycinnamic acids, methoxyflavonols, stilbenes, hydroxybenzoic acids, flavones, and isoflavones were identified. In treatment 4 with *A. niger* Aa20, five compounds belonging to the families of curcuminoids, hydroxycinnamic acids, lignans, methoxycinnamic acids, and flavonols were identified. In treatment 6 with *A. niger* HT3, 10 compounds belonging to the families of lignans, hydroxycinnamic acids, methoxyflavonols, catechins, flavanones, methoxycinnamic acids, hydroxybenzoic acids, and anthocyanins were identified.

Table 2. Polyphenolics compounds identified in treatment 6 with *A. niger* HT3 and treatment 4 with *A. niger* Aa20.

Unfermented Sample				
No.	Molecular weight	Chemical formula	Molecule	Family
1	341.0	$C_{15}H_{18}O_9$	Caffeic acid 4-*O*-glucoside	Hydroxycinnamic acids
2	314.9	$C_{16}H_{12}O_7$	Rhamnetin	Methoxyflavonols
3	389.0	$C_{20}H_{22}O_8$	Resveratrol 3-*O*-glucoside	Stilbenes
4	322.9		Gallic acid 3-*O*-gallate	Hydroxybenzoic acids
5	252.9	$C_{15}H_{10}O_4$	7,4′-Dihydroxyflavone	Flavones
6	352.8	$C_{16}H_{18}O_9$	1-Caffeoylquinic acid	Hydroxycinnamic acids
7	370.8	$C_{20}H_{20}O_7$	Sinensetin	Methoxyflavones
8	336.8	$C_{16}H_{18}O8$	3-*p*-Coumaroylquinic acid	Hydroxycinnamic acids
9	622.8	$C_{28}H_{32}O_{16}$	Isorhamnetin 3-*O*-glucoside 7-*O*-rhamnoside	Methoxyflavonols
10	414.9	$C_{21}H_{20}O_9$	Daidzin	Isoflavones

Table 2. *Cont.*

Treatment 4 of *A. niger* Aa20 strain				
No.	Molecular weight	Chemical formula	Molecule	Family
1	368.8	$C_{21}H_{20}O_6$	Curcumin	Curcuminoids
	352.9	$C_{16}H_{18}O_9$	1-Caffeoylquinic acid	Hydroxycinnamic acids
2	359.8	$C_{20}H_{24}O_6$	Lariciresinol	Lignans
3	366.8	$C_{17}H_{20}O_9$	3-Feruloylquinic acid	Methoxycinnamic acids
4	380.5	$C_{15}H_{10}O_{10}S$	Quercetin 3′-sulfate	Flavonols
5	300.8	$C_{15}H_{10}O_7$	Quercetin	Flavonols
Treatment 6 of *A. niger* HT3 strain				
No.	Molecular weight	Chemical formula	Molecule	Family
1	358.6	$C_{20}H_{24}O_6$	Lariciresinol	Lignans
2	352.9	$C_{16}H_{18}O_9$	1-Caffeoylquinic acid	Hydroxycinnamic acids
3	283.9	$C_{16}H_{12}O_5$	Methylgalangin	Methoxyflavonols
4	306.8	$C_{15}H_{14}O_7$	(+)-Gallocatechin	Catechins
5	256.7	$C_{15}H_{12}O_4$	Pinocembrin	Flavanones
6	311.9	$C_{13}H_{12}O_9$	Caffeoyl tartaric acid	Hydroxycinnamic acids
7	588.9	$C_{39}H_{58}O_4$	Schottenol ferulate	Methoxycinnamic acids
8	600.9	$C_7H_6O_5$	Gallagic acid	Hydroxybenzoic acids
9	289.0	$C_{15}H_{14}O_6$	(+)-Catechin	Catechins
10	286.6	$C_{15}H_{11}O_6^+$	Cyanidin	Anthocyanins

3.6. Antioxidant Activity of the Fermentation Extracts

Figure 5 shows the treatments of each of the strains used in comparison with the unfermented sample for DPPH activity. Treatment 6, which corresponds to *A. niger* HT3, obtained a higher percentage of inhibition, reaching up to 60.28 ± 1.74%. This result compared to the unfermented sample represents an improvement of up to 1.5 times in DPPH activity. Treatment 6, which corresponds to *A. niger* Aa20, obtained a higher percentage of inhibition, reaching up to 57.87 ± 0.46%. This result compared to the unfermented sample represents an improvement of up to 1.5 times in DPPH activity.

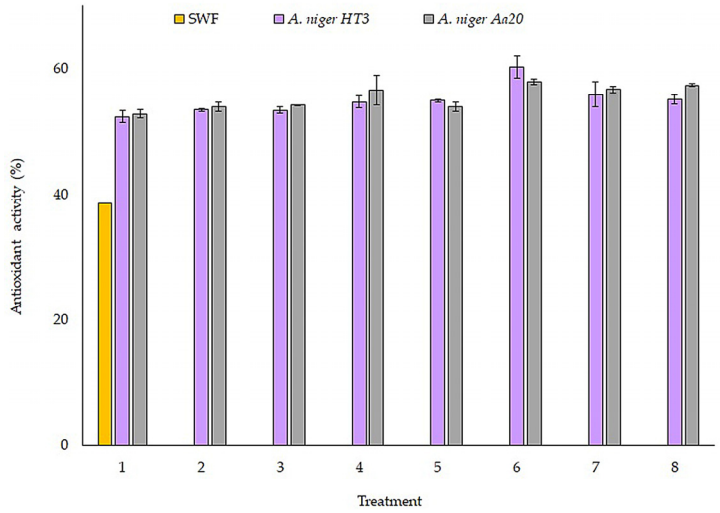


Figure 5. Antioxidant activity of pineapple peel waste of fermentation extracts for DPPH.

Figure 6 shows the treatments of each of the strains used in comparison with the unfermented sample for ABTS activity. Treatment 7, which corresponds to *A. niger* HT3, obtained a higher percentage of inhibition, reaching up to 77.38 ± 6.64%. This result compared to the unfermented sample represents an improvement of up to 2.6 times in ABTS activity. Treatment 6, which corresponds to *A. niger* Aa20, obtained a higher percentage of

inhibition, reaching up to 81.41 ± 4.06%. This result compared to the unfermented sample represents an improvement of up to 2.8 times in ABTS activity.

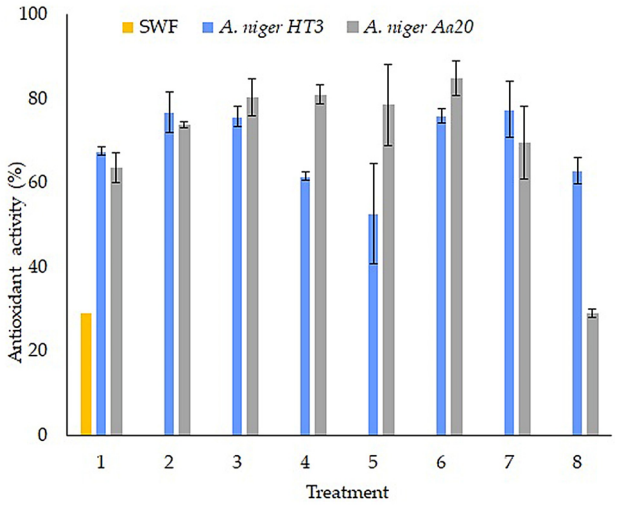


Figure 6. Antioxidant activity of pineapple peel waste of fermentation extracts for ABTS.

Figure 7 shows the treatments of each of the strains used in comparison with the un-fermented sample for FRAP activity. Treatment 8, which corresponds to *A. niger* HT3, obtained a higher power antioxidant, reaching up to 176.64 ± 27.81 mEq Trolox/g. This result compared to the unfermented sample represents an improvement of up to 2.9 times in FRAP activity. Treatment 4, which corresponds to *A. niger* Aa20, obtained a higher power antioxidant, reaching up to 113.39 ± 5.99 mEq Trolox/g. This result compared to the unfermented sample represents an improvement of up to 1.8 times in FRAP activity.

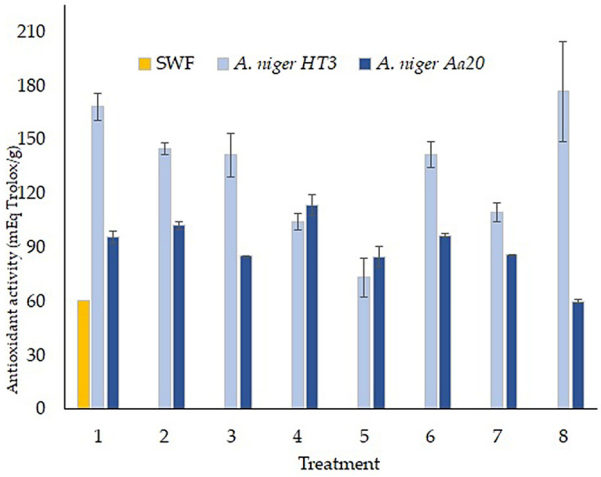


Figure 7. Antioxidant activity of pineapple peel waste of fermentation extracts for FRAP.

4. Discussion

4.1. Physicochemical Characterization

The results of WAI were similar to those reported by Polania-Rivera et al. [21] in pineapple peel (5.61 g/g dry peel). There exist similar works; for example, grape pomace achieved values of 3.38 g/g dry material [22] and mango seeds 3.4–4 g/g dry material [31]. In the case of CMP, the value was like that reported by Buenrostro-Figueroa et al. [30] in fig by-products (4.63%); however, it was lower than that reported by Polania-Rivera et al. [21] (14.37%). With these results, pineapple peel waste is a promising substrate for SSF.

The moisture content was higher than that reported by Selani et al. [32] (3.77%) in pineapple pomace. The values reported by Morais et al. [33] (8.8%) in oven-dried peel

were similar. However, the values reported by Huang et al. [34] (10.47–16.1%) in pineapple peel in different cultivars were higher. The ash content was similar to that reported by Sánchez-Prado et al. [35] of 1.5% for pineapple peel waste. However, Selani et al. [32] reported a higher ash content of 2.24% in freeze-dried pineapple pomace. The values reported by Aparecida-Damasceno et al. [36] of 4.57% in pineapple peel flour were higher. On the other hand, some authors mention that these results can be attributed to the fact that they are different varieties of pineapple and are grown in different areas of the world; in addition, they may depend on the type of soil and the ripening of the fruit [37]. The fat content reported in this work is within that reported by different authors; for example, Huang et al. [34] reported values of 2.8% fat in pineapple peel—the smooth cayenne variety, while Zakaria et al. [38] reported values of 1.8% fat in pineapple peel.

The total sugar content is within the range reported in different studies. Díaz-Vela et al. [39] reported values of 22.59% in pineapple peel flour. On the other hand, Huang et al. [34] reported values of 69.5% in pineapple peels—the smooth cayenne variety. The results obtained for reducing sugars were lower than those reported by Huang et al. [34] of 38% in pineapple peels—the smooth cayenne variety. Pineapple agro-industrial residues have been found to contain several types of sugars. According to reports by Sepulveda et al. [16] and Polania-Rivera et al. [21], the sugars found are mostly glucose, fructose, and sucrose.

The protein content is within the ranges reported by different authors; for example, Díaz-Vela et al. [39] reported values of 0.32% in pineapple peel flour. Selani et al. [32] reported values of 4.71% in pineapple pomace. The fiber content is within the ranges previously reported by different authors. Huang et al. [34] reported values between 7–8% in pineapple peel—the Tainung variety. Morais et al. [33] reported values between 14–16% in pineapple peels with different treatments, while Apareciada-Damasceno [36] reported values of 4.92% in pineapple peel flour.

4.2. Adaptation of Aspergillus Strains in Fermentation Kinetics

Andrade-Damián et al. [25] mentioned that *Aspergillus* strains can adapt to complex substrates such as *Curcuma longa* L. On the other hand, Buenrostro-Figueroa et al. [30] mentioned that filamentous fungi are the most widely used in SSF, as a result of their ability to adapt to the substrate that presents similarities with the medium in which they grow, in addition to having a great potential for the release of bioactive compounds. Another study demonstrated the ability of the fungus *A. niger* to develop and degrade compounds present in pineapple agro-industrial residues [40].

4.3. Release of Tannins by Solid-State Fermentation

Polania-Rivera et al. [21] mentioned that they performed an SSF process by *Rhizopus oryzae* on pineapple peel as the substrate; the results obtained were 83.77 mg GAE/g of HT and 66.5 mg QE/g of CT. Some authors mentioned that the low polyphenolic content during the fermentation of pineapple residues combined with soybean meal and *Rhizopus oligosporus* could be related to the fact that most of the compounds are bound to the inner membrane [41]. Another study mentioned that *Aspergillus* strains using the SSF help to release polyphenols and naturally produce enzymes that degrade the cell wall. Torres-León et al. [31] used SSF mango seeds with the *A. niger* GH1, where there was an increase of 3.3 times in the total phenolic content. On the other hand, Buenrostro-Figueroa et al. [30] used fig residues in SSF with *A. niger* HT4, and the results demonstrated an increase in total polyphenol content of 10.19 $\pm$ 0.04 mg GAE/g. In another research work, total phenolics, and flavonoids of jambolan fruit pulp after SSF treatment at different times using *Aspergillus niger* and *Aspergillus flavus* were studied. The results reveal that *A. flavus* fermented fruit pulp showed increased total phenolics (685.88 mg GAE/100 g) and flavonoids (388 mg QE/100 g) [42]. The results in this study indicate that SSF is a promising biotechnological process for obtaining bioactive compounds. Pineapple peels were a suitable substrate to be used as a source of carbon and energy for the filamentous fungus.

4.4. Evaluation of Antioxidant Activity in Fermentation Extracts

By autohydrolysis, pineapple waste extracts can have up to 80% and 94% by a DPPH and ABTS antioxidant assay, respectively [16]. Polania et al. [43] used SSF by *Rhizopus oryzae* on pineapple waste, and, as a result, the obtained fermented extracts exhibited 61.46 and 77.39% on the DPPH and ABTS antioxidant assays, respectively. Moreover, peach pomace from SSF using *A. niger* and *R. oligosporus* were evaluated and the extracts demonstrated the antioxidant potential determined by the DPPH radical scavenging assay increased significantly (>18%) throughout fungal growth. Furthermore, Torres-Leon et al. [31] found a correlation between polyphenols obtained from mango seed from SSF by *A. niger* GH1 and their antioxidant activity, which was attributed to the fermentation process, which, apparently, increases the antioxidant activity by improving the bioavailability of the bioactive molecules. Paz-Artega et al. [40] evaluated a solid bioprocess by *A. niger* GH1 on pineapple core and peels, and the results indicate a positive correlation with the release of polyphenols and antioxidant activity by DPPH by observing an increase of 25%.

On the other hand, Chiet et al. [44] evaluated the bioactive compound content and antioxidant capacity of Josapine, Morris, and Sarawak pineapple (*Ananas comosus*). The results demonstrated that the FRAP scavenging activity in Josapine samples showed the highest antioxidant capacity, followed by Morris, and then Sarawak having the lowest value. Brito et al. [45] obtained methanol and ethanol extracts by maceration from pineapple crowns, where the extracts showed a positive antioxidant effect using the FRAP method. Larios-Cruz et al. [46] mentioned that the valorization of grapefruit by-products can be carried out by the fermentation process because of the release of antioxidant compounds. The authors observed that the water content had an important effect on SSF; an increase in this parameter promoted the bioactive molecule release. Therefore, SSF is useful for releasing a large number of bioactive compounds and improving antioxidant activity; this was attributed to microbial enzymatic hydrolysis [47].

4.5. Identification of Polyphenolic Compounds by HPLC-MS

According to Banerjee et al. [48], different bioactive compounds were found in pineapple waste, such as phenolic acids, flavonoids, catechin, gallic acid, and ferulic acid. Other authors mentioned that, through the use of SSF with *Rhizopus oryzae* on pineapple waste, some compounds such as gallic acid, chlorogenic acid, catechin, caffeic acid, epicatech, epicatech, cumaric acid, scopoletin, and quercetin were identified [21]. On the other hand, Paz-Arteaga et al. [40], using SSF with *A. niger* GH1, identified 15 compounds, for example, caffeoyl hexoside, 5-caffeoylquinic acid, spinacetin 3-*O*-glucosyl-(1->6)-[apiosyl(1->2)]-glucoside, *p*-coumaric acid ethy ester, banzoic acid, *p*-coumaroyl tyrosine, *p*-coumaryl alcohol hexoside, 4-vinylphenol, *p*-coumaroyl glycolic acid, feruloyl aldarate, psoralen, caffeic acid 4-*O*-glucpside, *p*-coumaroyl hexoside, (+)-gallocatechin, and gallagyl-hexoside.

5. Conclusions

The results obtained in the present study show that solid-state fermentation using *Aspergillus niger* spp. strains and pineapple peels as the substrate are effective for obtaining a greater release of bioactive compounds and, thus, favorable antioxidant activity. The *A. niger* HT3 strain assisted in the release of hydrolyzable tannins, while the *A. niger* Aa20 strain assisted in the release of condensed tannins. In an HPLC analysis, up to 33 compounds were identified, of which three were the main ones, 3-feruloylquinic acid, caffeic acid, lariciresinol, and 3-hydroxyphloretin 2′-O-xylosyl-glucoside. The SSF process with pineapple peel as a substrate is an alternative for the release of molecules with potential application in the pharmaceutical and food industry.

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