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Fungal biotransformation of chlorogenic acid to caffeic acid from coffee pulp

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ABSTRACT

The present study involved using the coffee pulp which is considered a residue of the coffee bean roasting process known as shell. This by-product was used in a biotransformation process to obtain high value-added compounds with industrial applications such as chlorogenic acid and caffeic acid. The biotransformation of chlorogenic acid to caffeic acid by means of *Aspergillus niger* was evaluated, the amount of chlorogenic acid present in the coffee pulp was measured, as well as its ability to function as a substrate in a culture medium for the same strain. The amount of chlorogenic acid present in the coffee pulp before and after the biotransformation process was evaluated by high-performance liquid chromatography (HPLC) analysis. The results obtained show concentrations of the caffeic acid product from 0.21 to 0.71 mg/mL to 120 h fermentation, which demonstrates the feasibility of *A. niger* biotransformation on chlorogenic acid.

INTRODUCTION

Coffee is the most popular and important food product in the world, and it ranks second after crude oil out of all traded goods (Lashermes et al., 2008). Coffee cultivation is economically important at global level, about 60 countries with tropical and subtropical regions produce coffee as the main agricultural export for some of them (Moreira da Silva et al., 2008).

In 2014, 8.5 million tons of coffee were produced (ICO, 2016). The main sub-products obtained from the coffee industry are the husk and pulp; it is estimated that more than 3 million tons per year are generated (Sagarpa, 2017). The generation of waste derived from the coffee industry causes an increase in environmental pollution in the growing areas that influence public health (Cortes Rico and Ladino Soto, 2016). New strategies have been adopted to existing ones for the management and control of these solid wastes (Farah et al., 2005), because of its high content of antiphysiological compounds (Pushpa and Naidu, 2012a). Hydroxycinnamic acids have been reported in large quantities in various agricultural by-products such as coffee pulp and apple bagasse (Asther et al., 2005).

The phenolic compounds in coffee pulp were reported by Esquivel and Jiménez (2012), Peña-Maravilla et al. (2017) and Orozco et al. (2008). Hydroxycinnamic acids can be extracted by ultrasonic bathing technology or by liquid chromatography with fractional collection. These technologies influence the extraction performance of the compounds of interest (Azuola and Vargas-Aguilar, 2007). The extraction of bioactive compounds has focused on measuring the bioavailability of them, as a precursor in the manufacture of anticarcinogenic drugs, antioxidants and immunomodulators that are of great importance to health (Muñoz-Neira et al., 2018).

Several researches mention that coffee pulp contains a considerable amount of nutrients and bioactive

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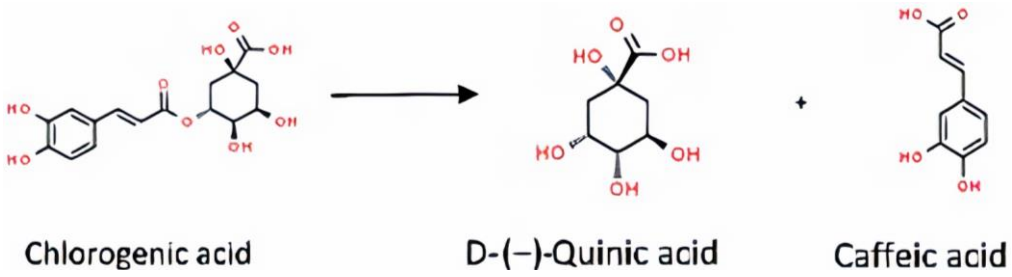

Figure 1. Chlorogenic acid [3-(3,4-Dihydroxycinnamoyl) quinic acid] biotransformation to caffeic acid [3-(3,4-dihydroxyphenyl) acrylic acid] and D-(−)-Quinic acid.

compounds (Ulloa Rojas et al., 2002; Janissen and Huynh, 2018; Rivera et al., 2013). different authors have given reports on the chemical composition of coffee pulp as is dry matter (259 ± 1.5), protein (80 ± 1.5), crude fat (29 ± 1.5), crude ash (89 ± 1.5), cellulose (286 ± 1.5), total phenols (20 ± 1.5), tannins (7.4 ± 1.5), caffeine (18 ± 1.5), values expressed in g/kg (Manals-Cutiño et al., 2018). Approximately 3095 mg/kg of chlorogenic acid, 1985 mg/kg of caffeic acid, 113 mg/kg of ferulic acid and 81 mg/kg of p-cumaric acid (Torres-Mancera et al., 2011 ;Noguera and Posada, 2017). Microbial biotransformation is an important biotechnolo-gical tool that allows high value-added products to be obtained from any residue containing the nutrients needed to enable the development of microorganisms (Leresche and Meyer, 2006). Biotransformations of hydroxycinnamic acids have been reported but at low concentrations due to their toxicity, by the formation of protein complexes that decrease the enzymatic activity of the microorganism (Mohammed et al., 2014).

In this work, we evaluate the ability of the *Aspergillus niger* strain to biotransform chlorogenic acid [3-(3,4-Dihydroxycinnamoyl) quinic acid] to coffee acid [3-(3,4-dihydroxyphenyl) acrylic acid] using coffee pulp as the only source of carbon (Figure 1). Coffee pulp contains approximately 3095 mg/kg chlorogenic acid, 1985 mg/kg of caffeic acid, 113 mg/kg of ferulic acid and 81 mg/kg of p-cumaric acid (Torres-Mancera et al., 2013), which makes this residue optimal for the production of antioxidant compounds and precursors of essences.

MATERIALS AND METHODS

The coffee pulp used in this study was obtained from the Variety *Coffea arabica* L. seeds provided by the company "Café Colibri" around 1 kg of coffee pulp was obtained after the depulping process, was frozen and stored at -20°C in order to preserve. 100 g of pulp were ground using a Brand Waring Commercial Heavy Dutty industrial blender, and sifted at particle size from 0.7 mm to 0.9 mm and preserved frozen.

The *A. niger* strain used belongs to the Center for Biotechnology Applicate Research (CIBA-IPN) collection. The freeze-dried strain was reactivated using 5 mL of sterile peptone water at 120°C. The rehydrated strain was taken together with a bacteriological loop and sown in Petri dishes that contain 30 mL of potato dextrose agar (PDA) were incubated for 5 days at a temperature of 30°C per triplicate.

Extraction of hydroxycinnamic acids

The extraction of bioactive compounds in coffee pulp have been reported by Esquivel and Jiménez (2012) previous studies, showing that the extraction yield is favored by an increase in the contact surface between the liquid phase and the solid phase (Peña-Maravilla et al., 2017). Authors performed a comparison between two extraction methods to recover coffee acid from coffee pulp. Pushpa and Naidu (2012b) took 10 g of sample and underwent ultrasound extraction and soxhlet where 1.02 g and 0.34 g of caffeic acid were obtained respectively using the same proportion of solvents (50% water, 50% methanol v/v).

Sifted coffee pulp was dried at 60°C for 24 h, 8 g were placed in an Erlenmeyer flask with 50 mL of HPLC grade methanol to extract the intern compounds. The extraction process is carried out with the support of an SB-3200 DTDN ultrasonic bath with a capacity of 8 L, with a frequency of 40 kHz and 130 W of power at 45°C, taking three extraction times of 2, 4 and 6 h per duplicate. Samples were filtered with Whatman 41 paper under vacuum, the recovered liquid was stored at 4°C for HPLC analysis.

Culture medium coffee pulp (agar plates)

The A. niger strain adapts to the coffee pulp medium, for 7 days at 30°C. The culture medium was prepared from coffee pulp previously tamed and dry with 4.5 g of dried pulp, 2 g of agar and 70 mL of distilled water, finally sterilized at 120°C for 15 min. The culture medium was

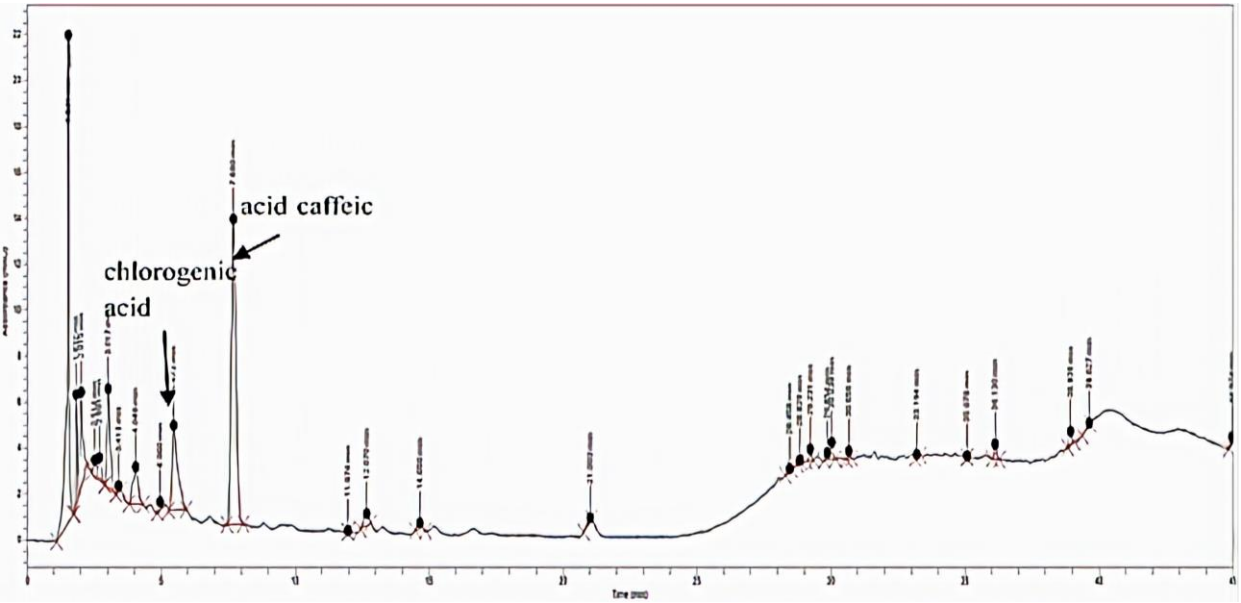

Figure 2. HPLC chromatogram of chlorogenic acid and caffeic acid in sample extraction of coffee pulp with ultrasonic bath.

poured into 3 Petri dishes which were inoculated by the *A. niger* strain and incubated for 7 d at 30°C.

Similarly, the coffee pulp agar medium was prepared in 250 mL Erlenmeyer flasks incubated for 5 d at 30°C to obtain spores in the submerged culture. The liquid fermentation test was performed in duplicate on 250 mL flasks with 97 mL of coffee pulp medium and the inoculum concentration was adjusted to 10^7 spores/mL flasks were incubated at 30°C with constant agitation at 100 rpm, samples were taken every 24 h for 7 days for further analysis.

Treatment of samples

Under sterile conditions, the samples were added 0.5 mL of HPLC grade methanol and placed in an ultrasonic bath for 1 h to 45°C, for the extraction of caffeic and chlorogenic acids, the biomass was separated by centrifugation in a thermoscientific equipment HERAEUS to 3000 rpm for 5 min for quantification.

Analysis by High-performance liquid chromatography

A Perkin Elmer Flexar HPLC instrument was used, equipped with a column ZORBAX Eclipse Plus C18, 4.6 mm × 250 mm, 5 µm, with autosampler. Analysis method was performed in reverse phase with acid solution 1% phosphoric (A) and acetonitrile (B) in gradient mode: 0 min – 10% B, 20 min – 20% B, 25 min – 30% B, 35 min – 40% B, 40 min – 40% B, with an injection volume of 10 µL and a total flow of 1 mL/min a diode array detector was used for acquisition via Chromera software. For the quantification of chlorogenic and caffeic acid of the biotransformation process a calibration curve was prepared from a standard solution of each compound with concentrations of 1, 0.5, 0.25, 0.125, 0.0625 and 0.03125 in mg/mL by serial dilution. Retention times (RT) of 5.01 min and 7.66 min for chlorogenic acid and caffeic acid respectively were identified (Figure 2).

Design and statistical analysis

The experimental design was factorial with three levels of 2, 4 and 6 h of extraction times. The variables evaluated were the extraction of caffeic and chlorogenic acids from coffee pulp and liquid fermentation that were carried out in duplicate. The results are presented as means of the variables and their standard deviation. The results were analyzed using ANOVA and were considered significant when $p \leq 0.05$. All analysis were conducted with Minitab.

RESULTS AND DISCUSSION

Adaptation of *Aspergillus niger* in culture medium coffee pulp

The first step was to develop the *A. niger* strain in the medium of culture of PDA and make a growth comparison between a PDA culture medium and coffee pulp-based culture medium (Figure 3), the performance of the

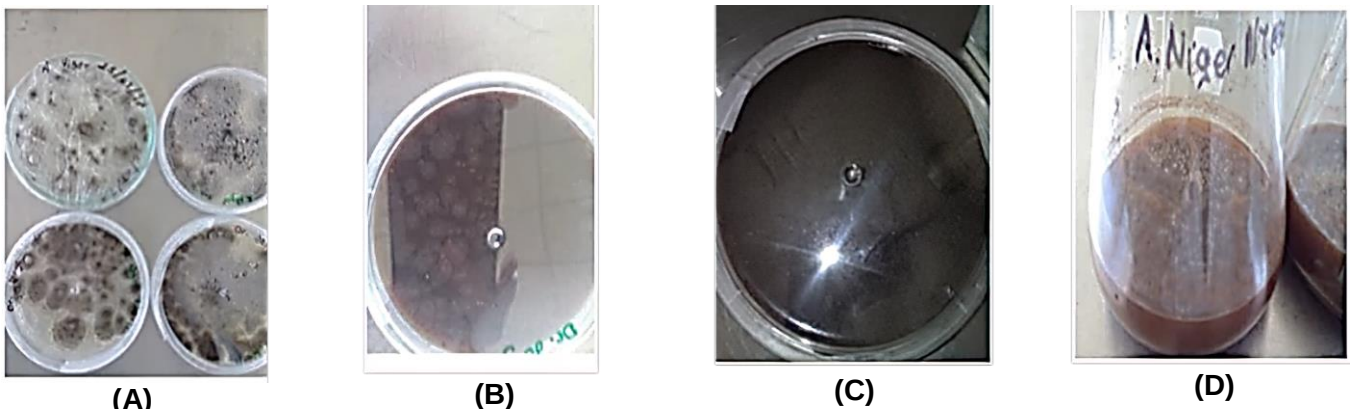

Figure 3. A, *A. nige*r harvested on PDA medium at 30°C for 5 days; **B,** first reseeding of *A. niger* in culture medium of coffee pulp under the same conditions in PDA medium; **C,** the third reseeding in culture medium of coffee pulp with *A. niger* adapted; **D,** agar plates fermentation for harvesting spores for subsequent fermentation liquid biotransformation process.

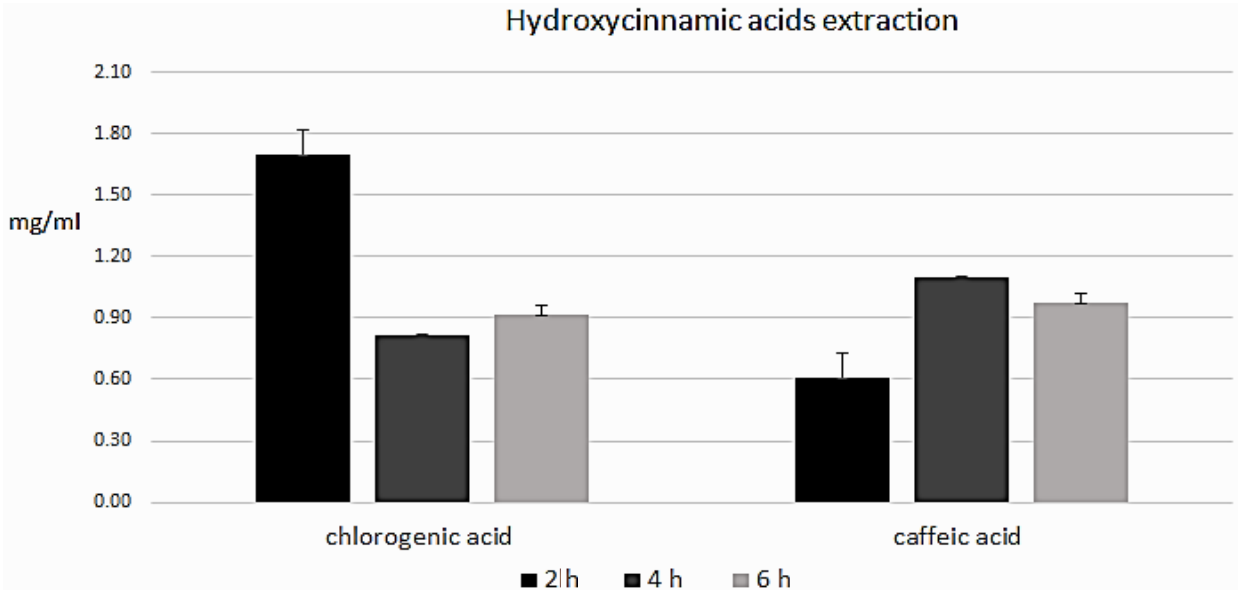

Figure 4. Chlorogenic acid and caffeic acid extraction yield at different times in an ultrasonic bath using methanol as solvent.

extraction of chlorogenic acid and caffeic acid at 2, 4 and 6 h in ultrasonic bath with methanol were analyzed by HPLC in Figure 4, the result shows a higher concentration of chlorogenic acid compared to caffeic acid at 4 and 6 h of extraction, there is an increase in concentration in caffeic acid extracts after of 20 h. The result explains that due to the thermal instability of chlorogenic acid during the roasting process of coffee beans can degrade to phenol derivatives, and some of the chlorogenic acid is isomerized and some is transformed into quinolactones due to dehydration with the formation of an intramolecular bond (Clifford, 2000), the hydrolyzed part degrades into low molecular weight compounds such as caffeic acid and ferulic acid (Farah and Donangelo, 2006).

A similar study examined the extraction of phenolic compounds from strawberries, with ultrasonic extraction causing less degradation of phenolic compounds and the process is faster compared with other extraction techniques, including the microwave assisted method (Herrera and Luque de Castro, 2005).

The results show higher chlorogenic acid extraction yield at 2 h of 0.364 ± 0.025 mg/g and caffeic acid were obtained 0.120 ± 0.025 mg/g as extraction time increases the extraction performance of caffeic acid increases and chlorogenic acid extraction decreases. Several authors have studied the extraction of antioxidant compounds from

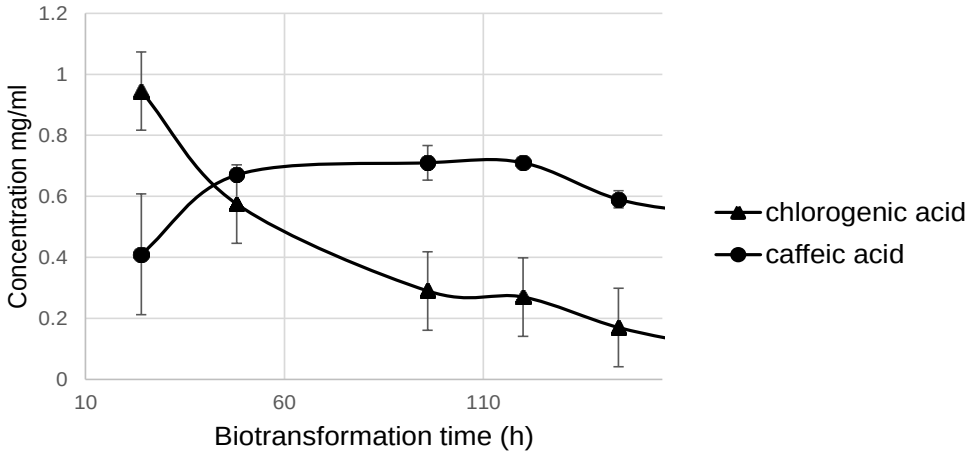

Figure 5. Biotransformation kinetics from chlorogenic acid to caffeic acid with *A. niger* in submerged culture.

coffee pulp similar results have been reported by Torres-Mancera et al. (2011) described extractions yield of 1627 mg/kg and 715 mg/kg of chlorogenic acid and caffeic acid respectively. Torres-Mancera et al. (2013) carried out the extraction of phenolic compounds including hydroxycinnamic acids in coffee pulp using three different solvents (water, methanol and ethanol) report phenolic compounds extraction yields of 5276 mg/kg with ultrasonic assisted extraction using a solvent ratio of 50% water, 50% methanol, results show that using water and ethanol as solvents increase extraction yields of these compounds, due to their polarity and affinity with the solvents. Hydroxycinnamic acids concentration depends on many biotic and abiotic factors such as the state of the raw material, variety and region where coffee seed is grown, storage time, handling and roasting process (Samuel de A. S et al., 2011).

Moreover, Nayive et al. (2012) explains when the particle size of coffee pulp increase, the contact area between the solvent and coffee pulp decrease, which makes the process low in extraction (Norbey de la Cruz et al., 2015). On the other hand, if particle size in too thin, it can do an overextraction process and the phenomenon produces a cohesive force that would hinder further analysis; therefore particle size has a marked influence on the extraction of phenolic compounds.

In the first instance some of the caffeic acid present in the coffee pulp can be removed before being used as a culture medium and after biotransformation, the caffeic acid produced can be extracted. Obtaining two stages of extraction.

Analysis of the biotransformation process

Chlorogenic acid is an ester and the hydrolysis of this compound produces quinic acid and caffeic acid, the reaction is catalyzed by the extracellular enzyme chlorogenate hydrolase (Torres-Mancera et al., 2011; Pushpa and Naidu, 2012b). The analysis of fermentation extracts was carried out by sampling every 24 h for 7 days, for HPLC analysis, the results obtained are shown in Figure 5.

Result shown a caffeic acid production of 0.70 ± 0.20 mg/mL at 24 and 48 h of fermentation. 55% chlorogenic acid consumption was obtained after 24 h. At 96 h of fermentation consumption increases to 80% and is maintained until 144 h, which decays to 0.16 mg/mL after of 120 h of fermentation *A. niger* incorporates the caffeic acid as a source of carbon after 168 h these results are similar to the reported by Torres-Mancera et al. (2013) 10% of chlorogenic acid is biotransformed in caffeic acid between 36 and 72 h, in this work accelerate the biotransformation due to the previous adaptation of the strain to the coffee pulp culture medium. This step allowed for conversions similar to those reported but in less time biotransformation.

Conclusions

Coffee pulp is considered an agro-industrial residue. *A. niger* is able to use the chlorogenic acid contained and use it as a source of carbon to biotransform it into coffee acid. It was shown that *A. niger* is able to grow in a coffee pulp culture medium without adding any external compound. After 48 h of fermentation the highest percentage of biotransformation was obtained. After this time *A. niger* begins to consume the caffeic acid with the possibility of obtain aroma precursors such as ferulic acid, vanillic acid and vanillin.

The evaluation of *A. niger* in the biotransformation

process shows that high value-added compounds can be obtained from a residue from the coffee industry without significant treatment. Adapting the strain to the coffee pulp culture medium improved the biotransformation process by reducing the time by 48 h compared to previous reports.

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