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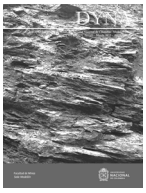

UNIVERSIDAD NACIONAL DE COLOMBIA

Effect of solid fermentation with *Rhizopus oryzae* on coffee pulp to obtain a food product

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Abstract
Coffee pulp is one of the most voluminous by-products in the wet processing of coffee beans. To maximize the use of this by-product, we conducted a solid-state fermentation (SSF) process with *Rhizopus oryzae* (MUCL 28168). In the SSF process, the effect of pH and carbon source on the content of nutritional compounds and condensed tannins was evaluated. Applying a factorial design revealed a significant ($p < 0.05$) interaction of factors affecting protein content, sugars, and condensed tannins, while phenol content and antioxidant capacity were only impacted by pH. The optimum conditions for the fermentation process were pH 6, 75% carbon source, temperature of 32 °C, and 28 h of processing. A coffee pulp infusion with sweet, honey, and woody notes was obtained. The SSF process reduced condensed tannins in coffee pulp by up to 60%, indicating its effectiveness in releasing functional compounds and reducing anti-nutritional factors.

Keywords: by-product utilization; solid state fermentation; coffee pulp; *Rhizopus oryzae*.

Efecto de la fermentación sólida con *Rhizopus oryzae* en pulpa de café para obtención de un producto alimentario

Resumen
La pulpa de café es uno de los subproductos más voluminoso en el tratamiento húmedo del grano. Con el propósito de dar un mejor aprovechamiento de este subproducto, se le realizó un proceso de fermentación en estado sólido (FES) con *Rhizopus oryzae* (MUCL 28168). En el proceso de FES se evaluó el efecto del pH y la fuente de carbono sobre el contenido de compuestos nutricionales y taninos condensados. Aplicando un diseño factorial, se encontró que la interacción de los factores tuvo un efecto significativo ($p < 0,05$) sobre el contenido de proteínas, azúcares y taninos condensados, mientras que el contenido de fenoles y la capacidad antioxidante se vio afectada únicamente por el pH. Las condiciones óptimas para el proceso de fermentación fueron pH 6, fuente de carbono del 75%, temperatura de 32 °C y 28 h de proceso. Se logró obtener una infusión de pulpa de café con notas dulces, a miel y madera. El proceso SSF redujo los taninos condensados en la pulpa de café hasta en un 60%, lo que indica su eficacia en la liberación de compuestos funcionales y la reducción de factores antinutricionales.

Palabras clave: Aprovechamiento de subproductos; Fermentación en estado sólido; Pulpa de café; *Rhizopus oryzae*.

1 Introducción

Coffee is one of Colombia's main export products, making it the third largest producer in the world with an annual production of 11.1 million 60 kg bags of green coffee in 2022 [1]. Despite increasing demand, the coffee sector faces significant challenges because only 5% of the harvested coffee cherries are used to produce the beverage [2]. The remaining 95% results in by-products such as pulp, mucilage, cisco, pasilla, borage, and coffee stalks [2].

Coffee pulp, a major by-product of wet coffee processing, accounts for 42% of the fruit's weight on a wet basis, as noted by Acevedo & Penaloza [3]. Traditional disposal methods that involve water lead to severe environmental issues,

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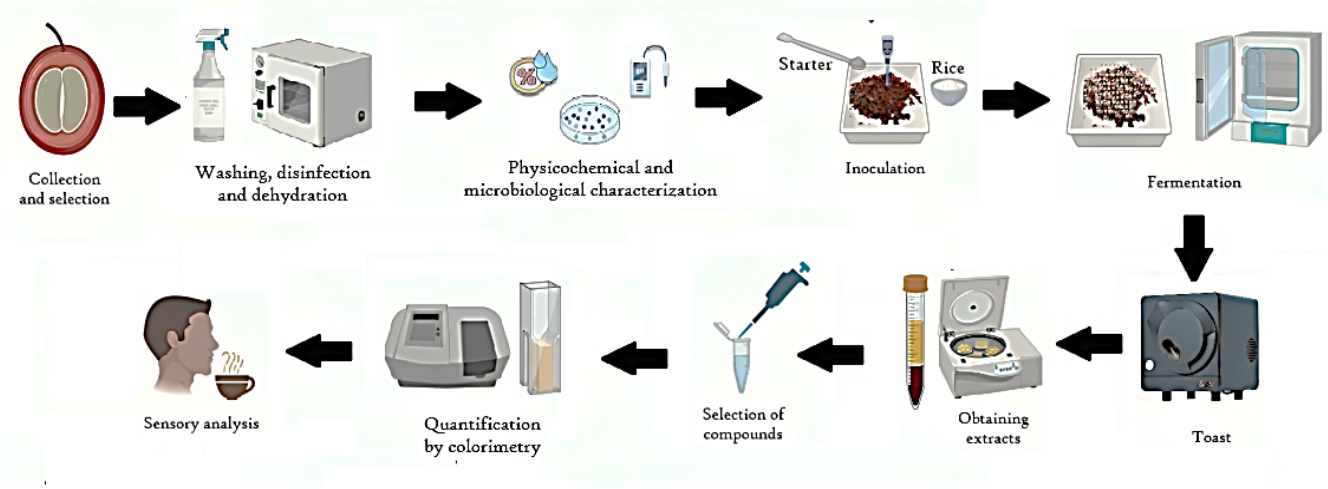

Figure 1. Graphic Summary.
Source: Author made

including contamination of nearby water sources, ecosystem imbalances, and adverse impacts on human health, as well as on local flora and fauna [4,5]. To mitigate these effects, covered pits have been implemented for pulp disposal; however, their effectiveness is limited due to the large volume of pulp produced.

Developing countries often lack the necessary technology and expertise to effectively utilize coffee residues. Consequently, various alternatives have been explored to valorize coffee residues, including their use in composting, animal feed, mushroom substrates, and the production of specialized beverages and infusions. These practices have somewhat increased the market value of coffee residues [6]. However, their application in the food industry is limited by the presence of anti-nutritional compounds such as condensed tannins [7]. Biotechnological processes, such as solid fermentation, are recommended to reduce these compounds to acceptable levels and to release high-value nutritional compounds.

In this context, the fungus *Rhizopus oryzae*, which is recognized as safe (GRAS), has been chosen for its tannase enzyme that hydrolyzes condensed tannins from coffee pulp, thereby improving the bioavailability of nutritional compounds [8,11]. Therefore, this research aims to develop a food product using fermented coffee residues from Coffea arabica var. Castillo with *Rhizopus oryzae* (MUCL 28168) (Fig. 1). This approach offers an environmentally sustainable solution and creates new economic opportunities for coffee farmers.

2 Materials and methods

2.1 Raw material

Coffee pulp from Coffea arabica var. Castillo, sourced from the north of Valle del Cauca, was utilized in this research. The samples were collected immediately after the pulping process and transported under controlled conditions to the Microbiology and Applied Biotechnology Laboratory (MIBIA) at Universidad del Valle. At the laboratory, the pulp was cleaned, sorted, and disinfected with 2% chlorinated water for 30 seconds. The samples were then dehydrated at 60°C until a moisture content of 12% was achieved, to minimize physicochemical and microbiological deterioration.

2.2 Physical and chemical characterization of coffee pulp

2.2.1 Determination of the initial moisture content

The initial moisture content of the coffee pulp was determined by applying the NTC 2558:2000 standard [12], which involves measuring the weight difference as described in eq. (1).

$$\%\,\mathbf{Moisture} = \left(\frac{\mathbf{Mass\ of\ water}}{\boldsymbol{Total\ mass}}\right) \cdot \mathbf{100} \quad (1)$$

2.2.2 Water Activity (Aw) and pH determination

Water activity was measured using an AQUALAB SERIES 4TE device with 1 gram of the dry sample. For pH determination, 1 gram of the product was weighed, suspended in 10 mL of distilled water for 30 minutes, and the pH was then measured using a sensitive pH-meter electrode (Thermo Scientific) until a stable reading was achieved [13].

2.2.3 Determination of Wáter Absorption Capacity (WAI)

The methodology employed was that developed by Londono [8], where 1.25 g of the sample was suspended in 15 mL of distilled water and agitated continuously for 10 minutes. Afterward, the samples were centrifuged at 3000 rpm for 10 minutes; the supernatant was decanted, and the weight of the resulting gel was recorded. Eq. (2) was used to calculate the water retention capacity:

$$WAI = \left(\frac{\text{Weight of the gel}}{\text{Weight of the dry sample}}\right) \quad (2)$$

2.2.4 *Microbiological characterization of unfermented coffee pulps*

Microbiological analyses were conducted on the coffee pulp following collection, sanitization, and dehydration processes. These analyses adhered to the methodologies outlined in standards NTC 4491-1 [14], 5034 [15], 4516 [16], 5733 [17], and 5698-1 [18], covering material preparation, and counts of lactic acid bacteria, total coliforms, enterobacteria, and yeasts.

2.3 Solid State Fermentation (SSF)

2.3.1 *Reactivation and Verification of the Purity of the Filamentous Fungus Rhizopus Oryzae*

Dried spores from the MIBIA group's culture collection were used to reactivate *Rhizopus oryzae* (MUCL 28168). These spores were centrally placed on Petri dishes containing Potato Dextrose Agar (PDA) as the stock culture medium. The dishes were incubated at 28°C until complete sporulation was observed. The purity of the cultures was verified using a stereoscope, and their morphological characteristics were examined under a microscope at 40X magnification.

2.3.2 *Preparation of inoculum*

The inoculum was prepared using the Tane-Koji method as described by Londoño [19]. This method involved inoculating cooked brown rice with a spore suspension of the fungus *Rhizopus oryzae,* extracted from test tubes previously seeded with the strain. The rice-filled containers were incubated for 75 hours to allow complete sporulation of the microorganism. Afterward, the rice was dried at 45°C for 24 hours until it reached a moisture content of 12%. The dried product was then ground using a blade mill, stored in sterile containers at 28°C, and spore counts were conducted using a Neubauer chamber.

2.3.3 *Experimental design*

A 2x3 factorial design was employed to determine the ideal process conditions, assessing the impact of pH and the percentage of carbon source (CS) on various response variables (Table 1). A total of 27 treatments were evaluated. Data analysis was performed using ANOVA and Tukey's test via Minitab 19.

Table 1.
Experimental design.

Independent Variables	Levels			Response Variable
pH	4	5	6	Content of tannins, total phenols, and caffeine present in coffee pulp
Carbon source (%)	25	50	75	

Source: Author made

2.3.4 *Evaluation of fermentation conditions*

Solid-state fermentation was conducted in sterile aluminum boxes at 32°C for 40 hours, maintaining substrate humidity at 65% and an inoculum density of 1 x 10^7 spores per gram. The initial pH of the substrate was adjusted using buffered solutions of NaOH and H2SO4. After fermentation, the samples were dehydrated at 60°C for 24 hours, sieved, and then roasted at 140°C for 1 minute and 20 seconds.

2.4 Quantification of Compounds of interest present in coffee pulp

The quantification of the study compounds was performed using aqueous solutions of coffee pulp samples across three stages: unfermented and unroasted (PST), unfermented with roasting (PCT), and fermented with roasting. Water was used for analyzing nutritional compounds and 80% methanol for condensed tannins. One gram of pulp was placed in centrifuge tubes with 10 mL of the respective solvent and subjected to ultrasonic extraction for 45 minutes at 40°C. The samples were then centrifuged at 3000 rpm for 15 minutes, the extract was filtered and stored at 4°C until analysis.

Soluble proteins were quantified using the modified Lowry method [20]. Total sugars were quantified using the Antrona method [21], and the content of reducing sugars was determined by the DNS method [22]. Total phenols were quantified following the methodology described by Londono [8]. Antioxidant capacity was evaluated using the DPPH (1,1-diphenyl-2-picryl-hydrazyl) technique as described by Medina et al. [23]. The quantification of condensed tannins followed the method established in NTC 602 [24].

2.5 Fermented product selection and design

Product selection and design were conducted using the "Response Optimization" feature in the statistical software Minitab 19. The objective was to minimize the levels of tannins and sugars while increasing the protein content, phenols, and antioxidant capacity. The qualitative sensory profile was evaluated by a panel of experts from Agricola Himalaya S.A., who assessed the aroma, flavor, and appearance in aqueous solutions of both the control sample (PCT) and the selected product.

3 Results and discussion

3.1. Physicochemical characterization of unfermented dry pulp

Following the methodologies outlined earlier in this document, the physicochemical analysis of the unfermented dry pulp was performed, and the results are recorded in Table 2.

Table 2.
Physicochemical characterization of coffee pulp.

Analysis	Methodology	Results
Initial Moisture Content	NTC 2558:2000	88.5±0.9
Final moisture content	NTC 2558:2000	11.5±1.5
WIA	Londoño (2015)	8.3±0.2
pH	Londoño (2015)	4.3±0.2
Aw	-	0.49±0.03

Source: Author made

Table 3.
Microbiological characterization of fresh and dehydrated pulp

Parameter	Fresh Pulp	Dry Pulp
Mesophilic Aerobes - PCA (UFC/g)	$4.9*10^6$	<3
Fungi and yeasts - PDA (UFC/g)	$1.2*10^7$	<3
Enterobacteriaceae count - VRBA (UFC/g)	$1.8*10^6$	<36
Lactic Bacteria's -MRS (UFC/g)	$3.7*10^6$	<30
Total Coliforms (NMP/g)	1000	0

Source: Author made

Coffee pulp is a solid by-product with a high moisture content, making it susceptible to microbial growth as well as changes in size, color, and odor [25]. After the drying process, the coffee pulp was found to have an average moisture content of 88.5%. Although several studies indicate that the moisture content of coffee pulp varies between 74% and 88% [25-27], the results obtained in this study are slightly higher. This increase could be attributed to the exudate released during the thawing process, which increased the free water content of the sample, facilitated by the concavity of the pulp.

The water activity of the material after drying was measured at 0.49 ± 0.003, very similar to those found by other researchers [8,28]. This confirms the microbiological and enzymatic stability of the coffee pulp after dehydration, given that the water activity of fresh premium material was reported to be 0.6 [29]. The water absorption capacity of the coffee pulp was recorded at 8.3, significantly exceeding the values reported by Londoño [8]. This indicates a higher hydration capacity of the dry coffee pulp, ensuring effective retention of water added during rehydration and achieving an optimal moisture level (60-70%) for microbial growth. Table 2 shows that the pH of the coffee pulp is 4.3 ± 0.2. Previous studies have shown that the pH of coffee pulp can vary between 3.92 and 5.63 [8,26,30]. This variability is attributed to factors such as geographical location, growing conditions, coffee variety, altitude, degree of fruit ripening, and production methods [8,31].

3.2. Microbiological characterization of the unfermented coffee pulp

Table 3 presents the results of the microbiological analyses performed on both the fresh and dehydrated coffee pulp.

As shown in Table 3, the high load of microorganisms in the fresh pulp was attributed to processing, product handling, and machinery. This is evident in the mesophilic microorganism count, as a higher presence of these microorganisms indicates deficiencies in sanitation practices and possible contamination during production [32]. Puerta et al. [32] reported that reducing two stages of the wet coffee processing method—sorting and sieving—resulted in a significant decrease in the count of mesophilic microorganisms, directly associated with product handling.

Fungal and yeast counts also showed elevated amounts, although they were within the range reported by several authors (1.4 × 10^5 to 3.1 × 10^8 CFU/g) [27,32]. At 72 hours, however, yeasts showed an absolute predominance, aligning with the findings of Blandon et al. [27] and Puerta et al. [32], who identified yeasts of the *genera Candida spp., Saccharomyces cerevisiae, Rhodotorula spp., and Cryptococcus terreu*s. In contrast, Martinez et al. [34] observed that fungi of the genera *Aspergillus, Penicillium, Fusarium, and Trichoderma* are more predominant in coffee fruit. The variability in the count and predominance of fungi and yeasts is influenced by factors such as coffee variety, altitude, and growing conditions.

Regarding lactic acid bacteria, a higher concentration was observed compared to that reported by Puerta et al. [32], who found that 33% of the initial bacterial population belonged to the genus *Lactobacillus.* However, this finding contrasts with Martinez (2022), who established a relationship between the quantity of lactic acid bacteria and the altitude at which the coffee is grown. Based on the results of this study, it was concluded that altitude did not have a significant effect on the lactic acid bacteria content of the samples analyzed.

Similarly, the fresh pulp showed a high concentration of coliforms, coinciding with the findings of Torres et al. [29]. These authors associated the presence of coliforms of the genera *Enterobacter, Klebsiella, Serratia, and Citrobacter* with the quality of the water used during the fruit washing process. Deficient water supply, storage, and distribution systems often harbor these bacteria, which negatively affects the microbiological quality of the product.

The high microbial load in the fruit was influenced by the moisture content of the substrate and the soluble carbohydrate content of the pulp. To prevent spoilage caused by microbiological agents, sanitation and drying processes were implemented. These measures significantly reduced the microbial count, as detailed in Table 3, thereby extending the shelf life of the product and eliminating microbial activity that could compete with the fungus during the solid-state fermentation process.

3.3. Solid state fermentation

Physicochemical analysis revealed that coffee pulp is a suitable substrate for the growth of Rhizopus oryzae, enhancing its survival, colonization, and enzyme production [34]. Under the established conditions (temperature of 32°C, substrate humidity of 65%, and relative humidity of 77%), optimal growth of the microorganism on the substrate was observed. At pH 4, maximum growth was achieved in 38-40 hours; at pH 5, it took 32-36 hours; and at pH 6, the time was reduced to 28 hours, indicating that pH significantly

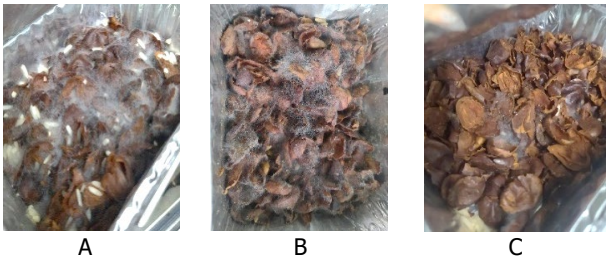

Figure 2. Comparison of the growth of Rhizopus oryzae at different concentrations of carbon source A) with 75% rice, B) with 50% rice, and C) with 25% rice.
Source: Author made

Table 4.
Results of the experimental runs for each of the parameters evaluated after the solid fermentation process.

pH	%CS	%PT	%AzT	%AzRT	Phenols	DPPH	%CT<
PST		17.32±0.36	56.95±0.96	44.26±1.51	80.00±0.03	9.84±1.30	0.300±0.006
PCT		6.40±0.70	24.93±1.05	16.56±0.80	110.24±8.50	11.74±0.51	0.305±0.015
4	25	5.28 ± 0.58^{F}	32.60 ± 2.08^{A}	18.54 ± 1.69^{A}	118.74 ± 2.04^{B}	11.21 ± 0.66^{B}	0.174 ± 0.021^{BC}
4	50	12.67 ± 0.83^{CDE}	21.89 ± 0.36^{C}	14.80 ± 0.29^{B}	139.45 ± 7.06^{B}	11.72 ± 0.12^{B}	0.191 ± 0.035^{AB}
4	75	21.17 ± 2.03^{A}	15.73 ± 0.62^{D}	10.50 ± 0.51^{D}	137.1 ± 12.03^{B}	11.69 ± 0.73^{B}	0.196 ± 0.017^{BC}
5	25	6.78 ± 0.68^{EF}	23.08 ± 0.80^{BC}	15.46 ± 0.65^{B}	170.23 ± 13.53^{B}	10.37 ± 0.71^{B}	0.178 ± 0.016^{BC}
5	50	14.56 ± 0.72^{BCD}	20.35 ± 1.65^{C}	14.71 ± 1.35^{B}	129.59 ± 2.30^{B}	12.87 ± 0.27^{B}	0.262 ± 0.017^{A}
5	75	8.17 ± 0.75^{DEF}	13.41 ± 1.09^{DE}	12.10 ± 0.89^{C}	201.80 ± 14.28^{B}	14.04 ± 0.40^{B}	0.153 ± 0.014^{CD}
6	25	9.17 ± 0.13^{DEF}	24.73 ± 0.87^{B}	19.61 ± 2.72^{A}	284.51 ± 16.25^{A}	17.09 ± 0.20^{A}	0.124 ± 0.015^{D}
6	50	16.72 ± 1.14^{ABC}	11.27 ± 1.52^{E}	11.05 ± 1.24^{CD}	337.14 ± 10.86^{A}	14.21 ± 0.80^{A}	0.175 ± 0.015^{BC}
6	75	19.85 ± 1.66^{AB}	7.21 ± 0.44^{F}	6.83 ± 0.44^{E}	332.63 ± 11.20^{A}	17.16 ± 0.06^{A}	0.165 ± 0.008^{CD}

Note: Results expressed in percentage are equivalent to the reagent used in the calibration curve/g sample on a dry basis. Phenols (mg Gallic Acid Equivalents GAE/g d.m) DPPH (µmol Trolox Equivalents TE/g). CS: Carbon source; AzT: Total sugars; AzRT: Reducing sugars; CT: Condensed tannins; PST: Pulp without roasting; PCT: Pulp with roasting. Values with the same letter within the same evaluated parameter do not show statistically significant differences.
Source: Author made

influences the mycelial growth rate. Additionally, it was demonstrated that incorporating uncooked white rice as a carbon source (CS) had a positive impact on mycelial growth. Treatments with 75% rice exhibited the highest growth rate (Fig. 2A), followed by those with 50% (Fig. 2B) and, finally, those with only 25% (Fig. 2C).

3.4. Quantification of compounds of interest present in coffee

Quantification of the compounds of interest was performed on unfermented and unroasted (PST), unfermented and roasted (PCT) coffee pulp samples, as well as for each of the treatment groups. Table 4 presents the obtained results.

Proteins are the main structural and functional components of cells [35]. However, plant proteins obtained from the solid-state fermentation process must be free of any contaminants or anti-nutritional factors [35,36].

Table 4 shows that after the roasting process, the control sample exhibited a 6% decrease in protein content. This decrease is attributed to chemical reactions caused by heating, primarily between amino acids and reducing sugars, commonly known as the Maillard reaction. These reactions lead to the creation of molecules with distinct odors and flavors, thus reducing the protein content [37]. Researchers such as Londoño et al. [38], Encalada et al. [39], and Fierro et al. [40] reported soluble protein contents of 14.83%, 12.56%, and 10.63%, respectively, which are slightly lower than those found in the PST sample. These differences are attributed to variations in the growing area, soil composition, and conditions, as well as treatments applied to the crop, factors that can influence the nutritional composition of the fruit [41].

According to the ANOVA and post-ANOVA analyses ($P<0.05$), it was observed that the treatments at pH 4 and pH 6 with a CF (carbon source) of 75% presented protein amounts of 21.17% and 19.85%, respectively. Ceballos et al. [42] attribute this increase to the fact that during the first 24 hours of fermentation, the microorganism *Rhizopus oryzae* accelerates its metabolic activity and degrades compounds such as tannins through the action of enzymes like polyphenol oxidase, which breaks down the tanninprotein structure. In contrast, at pH 4 and 5 with a CF of 25%, the protein content is low, possibly due to insufficient growth of the microorganism throughout the matrix, as the mycelium did not completely invade the substrate.

According to Navarro & Roa [43], any raw material intended for animal feed is considered to have good capacity if its protein content exceeds 11%. Therefore, the fermented coffee pulp is classified as a premium raw material based on its protein content. However, it should be noted that in this research, the product is intended for human consumption, although its use in animal feed is not ruled out.

3.4.1. Total sugars content of coffee pulp

The sugar content of the substrate significantly influences the growth, invasion, and metabolic activity of the fungus. In the PST sample, a total sugar content of 56% was observed, which is similar to that reported by Londoño et al. [26] for coffee pulp of the same variety. However, this amount decreased considerably to 24.9% after the roasting process due to chemical and biochemical reactions involved [44].

ANOVA analysis showed statistically significant differences in the interaction between the two factors analyzed on the total sugar content, and Tukey's test revealed that the greatest decrease occurred at pH 6 with CS (carbon source) of 75% and 50%. This is because the fungus was in an optimal growth environment, which allowed it to completely invade the substrate and perform transformations on it.

A study by Ibarruri & Hernandez [45] mentions that *Rhizopus oryzae* grows better on fruit residues than on other

by-products, due to the abundant presence of sugars in these substrates. Their research observed a 33% decrease in carbohydrate content after 3 days of fermentation. In contrast, a 71% decrease in carbohydrates (at pH 6 and CS of 75%) was achieved in a maximum time of 40 hours compared to the PCT sample. This was accomplished by optimizing fermentation conditions and adding a CS, which allowed higher growth of the microorganism and, therefore, a higher depletion of carbohydrates.

Thus, there was a higher consumption of carbohydrates from the substrate. It should also be noted that the decrease in this parameter is additionally due to the roasting process.

3.4.2. Reducing sugars content in coffee pulp

In terms of reducing sugars, coffee pulp, unlike coffee beans, is characterized by the presence of these compounds, especially glucose and xylose [45]. The PST samples contained 44% reducing sugars (Table 4), similar to levels reported by Gurram, Al-Shannag & Knapp [46] and Fierro et al. [40] for the same coffee variety. However, the PCT samples showed a reduction to 16.56%, representing a decrease of 62.4% in these compounds. ANOVA analysis revealed that the interaction of the two factors significantly influenced this response variable. Tukey's test indicated that a CS (carbon source) of 75% contributed to the decrease in reducing sugars in the coffee pulp samples, regardless of the pH level. This behavior can be attributed to the fact that, at a CS of 75%, there is a greater availability of reducing sugars, which enhances the growth and metabolic activity of the microorganism. This allows it to degrade compounds such as condensed tannins and increase the presence of proteins and phenolic compounds [36].

3.4.3. Content of total phenolics

Phenols are compounds known for their antioxidant, anti-inflammatory, and antimicrobial capacities [8, 47]. In the PST samples, the content of total phenolics was 80 mg GAE/g. When the pulp underwent the roasting process, a 37.5% increase in phenol content was observed (Table 3). This effect has also been reported by Diaz et al. [66] for roasted coffee, where the phenol content increased with roasting time, concluding that medium-grade roasting is favorable to obtain coffee with a high concentration of total phenols.

ANOVA analysis indicated that the interaction between factors did not significantly affect phenol content. However, post-ANOVA analysis revealed that pH did have an influence, with the highest phenol content observed at pH 6, averaging 318.1 mg GAE/g sample. The fermentation process increased the phenolic content by 189%, 52%, and 19.7% in the PCT sample at pH 6, 5, and 4, respectively. According to Londono [8], this increase is attributed to the crucial role of pH during the growth and metabolism of the microorganism. At pH between 5 and 6, the microorganism can secrete enzymes such as xylanases, α-amylases, and β-glucosidases, which perform the necessary hydrolysis to release phenols bound to other molecules and increase the content of soluble phenols.

According to Londono [8], after solid-state fermentation of coffee pulp with *Rhizopus oryzae*, approximately 46 phenolic compounds were identified, including ferulic acid, trigonelline, caffeine, and chlorogenic acid, along with 18 compounds found exclusively in coffee pulp. Meanwhile, Palomino et al. [49] reported that after fermentation of coffee pulp with *Penicillium purpurogenum,* the most abundant phenolic compound was chlorogenic acid, followed by caffeic acid and rutin.

Researchers have found significant variability in the phenolic content of coffee pulp. Authors such as Melendez [50], Cruzalegui et al. [51], and Myo & Khat-udomkiri [52] have determined that the polyphenol content can range from 46.31 to 891 mg GAE/100 grams of coffee pulp. These differences are attributed to several factors, including coffee variety, drying methods, geographical location of the crop, extraction techniques and solvents, as well as the degree of roasting. According to Diaz et al. [48], during roasting, chlorogenic and hydroxycinnamic acids and some other phenolic compounds are degraded, which increases the concentration of quinic acid. Meanwhile, Shanmagam [53] noted that trigonelline does not survive the roasting of coffee beans, being transformed into nicotinic acid, pyridine, and other volatile compounds.

When comparing the results with those of the cited authors, it is evident that fermentation and roasting significantly influenced the phenolic content of the coffee pulp. This led to an increase in its amount, showing figures similar to those recorded in the literature. However, many of these compounds were not extracted due to the methodology employed and/or were degraded during the roasting process.

3.4.4. Antioxidant capacity

The antioxidant capacity provides information on the ability of compounds present in coffee pulp to inhibit or reduce oxidative damage induced by oxidizing agents, in this case, the DPPH radical [54]. Table 4 shows a 19% increase in this capacity in the PCT samples compared to the PST samples. This increase is associated with the synthesis of secondary products such as melanoidins or brown compounds, which have antioxidant activity and are responsible for color, flavor, and reducing properties. These compounds are produced from the Maillard reaction caused by thermal processing [48]. In the ANOVA and post-ANOVA analysis, it was observed that this variable was influenced only by pH. It was found that pH 6 exhibited the highest average antioxidant capacity (64.12 µMol of TE/g), followed by pH 5 (12.42 µMol of TE/g) and then pH 4 (11.5 µMol of TE/g). The amounts obtained in this study are higher than those reported by authors such as Myo & Khat-udomkiri [52] and Thy Minh et al. [55], who found antioxidant capacities of 8.19 mg TE/g and 2.24 mg TE/g, respectively. Therefore, it can be concluded that the application of solid-state fermentation and roasting enhances the antioxidant capacity of the samples.

However, at pH 5 and 6 with a CF (carbon source) of 50%, it was found that there was no direct correlation with the phenolic content. A similar effect was reported by Lopez et al. [56], who, after fermenting coffee pulp with different

strains of lactic acid bacteria, observed that an increase in phenolic content did not clearly correlate with an increase in antioxidant capacity, as measured by the DPPH and ABTS methods. They attributed this effect to the presence of reductones, which could react with free radicals to stabilize and terminate the propagation of antioxidant chain reactions.

3.4.5. Condensed tannins content

The presence of condensed tannins impacts the utilization of coffee pulp as a premium material [57]. In the PST (Pre-Solid State Fermentation) and PCT (Post-Solid State Fermentation) samples, the condensed tannin content was recorded at 0.3%, and by solid fermentation, this compound was significantly reduced (see Table 4). Analysis of variance revealed statistically significant differences in the effect of the factors on condensed tannin content. Tukey's post-ANOVA analysis results indicated that the lowest tannin content, at 0.12%, was obtained at pH 6 and a carbon source (CF) concentration of 25%, representing a decrease of 60%. This finding contrasts with that reported by Londono et al. [26], who recorded a 52% reduction in the same compound. This behavior is attributed to the optimization of the substrate, which was enriched with carbon, nitrogen, phosphorus, and sulfur sources, under conditions favorable for the metabolism of the microorganism. This allowed for sufficient production of the enzyme tannase, responsible for degrading these compounds [36].

Authors such as Noriega, Silva, & Garcia [58] and Samayoa et al. [59] report that the tannin content in the Arabica coffee variety varies between 0.14% and 8.56%, which is in agreement with the results obtained for the control samples. However, this variability is attributed to factors such as the region of origin of the samples, genetics, plant species, soil fertility, and climatic conditions, as all these variables influence the quantity and type of tannins that the plant can synthesize [60].

On the other hand, Diaz et al. [48] found that in roasted coffee, tannin content increases with roasting time. Meanwhile, Thy Minh et al. [55] observed that in coffee pulp, drying with hot air at 70°C resulted in the lowest level of condensed tannins (2.34 mg ET/g DW in dry sample) compared to vacuum drying at 90°C and 110°C. This is because condensed tannins are sensitive to light and oxygen; during vacuum drying, reduced exposure to oxygen minimizes the degradation of these compounds. Therefore, both the drying and roasting processes significantly influence the tannin content present in the samples

3.5. Fermented product selection and design

In order to add value to this by-product and considering the characteristics of the premium material, it was decided to produce an infusion-type beverage. This type of beverage has traditionally been popular in countries such as Yemen, Sumatra, Ethiopia, Jamaica, and Sudan, where this by-product has been utilized for many years. In other regions, its popularity has been increasing over time, and companies such as SUTRACAFE, Cafe La Manchuria, Indio Ramirez, and Casa Eguia are already marketing the dried pulp for the preparation of this infusion.

Table 5.
Comparison of the nutritional composition of the infusion samples control and best experimental run

Component	PST	PCT	BET
Protein (%)	17.32±0.36	6.40±0.70	19.85± 1.66
Total sugars (%)	56.95±0.96	56.95±0.96	7.21±0.44
Reducing sugars (%)	44.26±1.51	16.56±0.80	6.83±0.44
Antioxidant capacity (mg/g)	9.84±1.30	11.74±0.51	17.16± 0.06
Phenols (mg/g)	80.00±0.03	110.24±8.50	332.63±11.20
Tannins (%)	0.300±0.006	0.305±0.015	0.165±0.008

Source: Author made
Note: Results expressed in percent are equivalent to the reagent used in the calibration curve/g sample on a dry basis. PST: Pulp without roasting; PCT: Pulp with roasting, BET: Best Experimental treatment.

Table 6.
Sensory evaluation of the infusion obtained versus the control sample

Organoleptic characteristics	PCT	BET
Aroma	Wood Fresh Vegetal Toasted	Wood Toasted Fermented
Taste	Acids Mild sweetness Wood with predominance over the other notes Slight sensation of astringency	Sweet Woody notes Slight sensation of astringency
Appearance in aqueous solution	Light yellow Translucent	Light yellow Slight turbidity

Note: PCT: Pulp with roasting, BET: Best Experimental treatment
Source: Author made

In the Minitab software, the best option was evaluated based on the increase of nutritional compounds and the decrease of condensed tannins. It was found that the fermentation at pH 6 with a carbon source (CS) of 75% (see Table 5) meets these requirements with a composite desirability index of 0.73.

To determine the sensory profile and the characteristic sensory notes of the product, panelists from Agricola Himalaya S.A., specializing in the collection of tea and aromatic infusions, were consulted. The results are presented in the Table 6.

The panelists identified sensory notes in the coffee pulp that align with the findings of other researchers. Amorocho & Cortes [82] noted herbal and sweet notes, as well as fruity aromas, in infusions made from dried and unfermented coffee pulp. Similarly, Prado [62] detected aromatic notes of raisins, while flavors of tamarind, red fruits, and coffee beans were also reported. These variations can be attributed to several factors, including the variety of coffee used. Additionally, Serna et al. [63] determined that the optimal conditions for extracting antioxidant compounds from a coffee pulp infusion were at a temperature of 90°C for 4.5 minutes, using 3.3 grams of dry coffee pulp immersed in 250 mL of drinking water.

4 Conclusiones

Physicochemical analysis of coffee pulp has revealed that this by-product possesses significant potential for use in

various industries, thanks to its content of bioactive compounds with functional properties. Additionally, it was determined that coffee pulp provides ideal conditions for undergoing solid-state fermentation processes, thereby maximizing its valorization as a premium material.

The optimum conditions for the fermentation process were identified as pH 6, a carbon source concentration of 75%, a temperature of 32°C, and a fermentation time of 28 hours. These conditions favor the metabolic activity of the microorganism, resulting in a significant increase in the content of proteins and total phenols. This is accompanied by a notable reduction in condensed tannins, thus enhancing the nutritional value of the final product.

The solid-state fermentation (SSF) process with Rhizopus oryzae proved effective in transforming coffee pulp, achieving up to a 60% reduction in condensed tannin content. This reduction is attributed to the fungus's ability to alter the chemistry of the substrate, highlighting its potential in the production of functional foods.

The infusion obtained from fermented coffee pulp exhibited attractive sensory profiles, characterized by sweet and woody notes. These attributes, along with its improved nutritional profile, position this product as an innovative alternative in the functional food and beverage market. This underscores the value of coffee pulp as a by-product with high potential to be transformed into a fermentation-enriched functional food, thus contributing to reducing the environmental impact associated with coffee production and generating new economic opportunities for farmers by diversifying their product offerings.

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