



THE POTENTIAL OF COFFEE (*COFFEA ARABICA* L.) PULP AND CACAO (*THEOBROMA CACAO* L.) HUSK AS A SOURCE OF PREBIOTICS, ANTIOXIDANTS, AND ANTIMICROBIAL COMPOUNDS

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ABSTRACT

The objective of this work was to determine the viability of the most important agro-industrial coproducts, such as cacao husk and coffee pulp, as sources of bioactive compounds, as prebiotic fiber, and polyphenols as antioxidants and antimicrobials. Main carbohydrates and prebiotic activity score were determined, as well as the characterization of main polyphenols, antimicrobial and antioxidant capability. The flours presented a higher dietary fiber content (64 % for coffee pulp and 70 % for cacao husk), with a higher glucose content in cacao husk flour. This explained the higher prebiotic activity score obtained, as compared to coffee pulp. During lactic acid fermentation, the SCFA (short chain fatty acids) was adequate, with a higher production of lactate and acetate when coffee pulp was employed as a carbon source. Total polyphenols content was higher in cacao husk, and although TEAC (trolox equivalent antioxidant capacity) was higher with this flour, DPPH, and total antioxidant activity were higher for coffee pulp. Cacao husk flour or coffee pulp can be employed as functional ingredients to improve intestinal health promoting the selective growth of probiotics, as well as inhibiting pathogens microorganisms due to their antimicrobial activity. Both ingredients can be employed as natural antioxidants as well.

1. Introduction

The Agro-industrial co-products are valuable ingredients and employed as a source of important biomolecules that represent an opportunity to impulse circular economics looking for the sustainability in the food industry chains. A circular economy implies systems that reuse and recycle resources in other materials that can be use in the same process or different ones, resulting in cycles to optimize resources and diminish residue production (Sauvé et al., 2016). There have a essential to keep up a cross-sectorial visualization to link the gaps between the co-products management and business opportunities, regarding four main

challenges according to Gontard et al. (2018): first, environmental and economic management strategies especially the lack of guidance to manage residues; second, the use of the actual residue converting technologies; third, the energy consumption during co-products recovery and conversion to biomass or bioproducts; and fourth, the integration of these residues in a circular economy context. In this respect, there are six types of circular business models proposed by Donner et al. (2020) related to: 1) biogas production by anaerobic digestion process, 2) environmental refineries to produce biofuels mainly, 3) production of high-value materials by upcycling entrepreneurship, 4)

spatial cluster of companies in an industrial symbiosis in a circular bio-based system, or agro parks, 5) agricultural cooperative as the association of persons involved during all the productive chain, and 6) support structure to coordinate, networking, and enlace different companies to take advantage of technological and logistics infrastructures.

These co-products from fruits and vegetables are fiber-rich ingredients that, after dehydration and milling, present a great physiological functionality due to the dietary fiber content, antioxidants, and many nutrients for example lipids, proteins, and glucosides (Garcia-Amezquita et al., 2018). Since the fiber amount is a major concern in processed food today, these agro-industrial co-products can be employed as an inexpensive ingredient to improve nutritional profile of processed foods. Most recent definition imply that the prebiotic must be metabolized by the native intestinal microbiota, must be able to withstand gastric conditions, mammalian enzymes before being absorbed in gastrointestinal tract, also can be used by the intestinal microbiota, stimulate selectively the activity of bacteria with propitious effect to the host (Lordan et al., 2020). Additionally, dietary fiber include compounds as: non digestible oligosaccharide, polysaccharide, and starch, and non-glucosides compounds or associated substances; since early definitions of fiber referred plant constituents (lignin, cellulose, hemicellulose), expanded later to the carbohydrate chains with the or more carbons, undigestible and non-absorbed in human intestine (Rezende et al., 2021). These ingredients, besides the digestive effect, can also present prebiotic properties, in addition to the naturally occurring polyphenol compounds, with antioxidant and antimicrobial properties.

Coffee and cacao are two economically important productive chains in developing countries, that also generate an important number of co-products. On one hand, coffee processing implies the transformation of the harvested fresh coffee cherries to low moisture (around 12%) green coffee beans, involving pulping, fermentation, and washing, where the

cherries are left with parchment and mucilage before drying process. From this wet processing method, coffee pulp and pulp can be recovered in conjunction fraction, named coffee pulp, representing around 40 % of the fresh coffee cherries (Esquivel and Jiménez, 2012). Coffee by-products comprise carbohydrates, proteins, fibers and other compounds, represent a good source of phytonutrients, in the pharmaceutical and food industry containing of polyphenols, hydroxycinnamic acids, flavanols and condensed tannins (Esquivel and Jiménez, 2012). Otherwise, cacao pod husk is a economic source of co-product as pectin, antioxidants, dietary fiber and minerals, being an fountain of organic constituents with nutraceutical characteristics, besides aroma compounds or food texturizing agents (Campos-Vega et al., 2018). Cacao husks present a larger quantity of phenolic compounds, proving to be an inexpensive source of bio-compounds, as soluble phenols, and tannins, with antioxidant activity, besides a considerable amount of soluble dietary fiber (Okiyama et al., 2017).

This work has the objective to determinate the capability of coffee pulp and cacao husk even as a fountain of functional compounds, suchlike as antioxidants, antimicrobial compounds, and prebiotics, to promote the circular economy of these co-products.

2. Materials and methods

2.1. Raw materials

Dry coffee pulp (*Coffea arabica* L.) be collected during the harvest time from January to March 2022. Dry cacao husks (*Theobroma cacao* L.) were collected during October and November 2022 as well. Both co-products were obtained at Angel Albino Corzo locality, at Chiapas, México (15°46'00" N, 92°41'00" W, 365 m.a.s.l.). Both co-products were dried in a Weston 74-1001-W oven as to 65 °C by 12 h, to homogenize moisture content (10-12%). Dry materials were pulverized in a food processor and sieved in a mesh #100 to obtain homogeneous dust named flour and stored in hermetic plastic containers until use.

2.2. Fiber, glucose, reducing sugars and prebiotic activity score.

The percent of fiber was determined by the enzymatic method, AOAC Official Method 991.43 (AOAC, 1996). The carbohydrates content was calculated by difference. Glucose was determined by HPLC, using an isocratic method with sulfuric acid 5 mM as eluant, flow 0.6 mL/min at 60 °C and retention time of 15 min. Samples were analyzed in a Shimadzu Prominence (Shimadzu Corp, Kyoto) with a hydrogen column HPX-87H (300×7.8 mm) (Sluiter et al., 2011).

For prebiotic activity, a set of flours coffee pulp and cacao husk was evaluated based on a report by Huebner et al. (2007). The different probiotic strains (*Lactobacillus* (L.) *rhamnosus* Rosell, *L. rhamnosus* GG, *L. casei* Shirota, and *L. acidophilus*) and the pathogen *Escherichia coli* were grown in a culture medium employing coffee pulp flour or cacao husk flour (1%) as carbon source (casein peptone 0.5 % along with yeast extract 0.3 %) and glucose was the control (1.0 %). After 18 h of incubation, the biomass production (calculate as optical density at $\lambda=600$ nm), the prebiotic activity score was calculated as:

$$\text{Prebiotic activity score} = \text{LAB} \frac{\frac{\Delta N \text{ Flour}}{\Delta N \text{ glucose}}}{\frac{\Delta N \text{ Flour}}{\Delta N \text{ glucose}}} - \text{Enteric} \quad (1)$$

Where: ΔN is the subtraction of the CFU/mL in the final time and CFU/mL at the initial time for the coffee pulp flour or cacao husk flour and the growing with glucose difference, the value of the pathogen (*E. coli*) from the employed probiotic lactic acid bacteria (LAB).

2.3. Short chain fatty acids (SCFA)

In the quantification of SCFA, acetic, propionic, butyric fatty acids, and lactic acid were determined using the probiotic strains *L. acidophilus*, *L. casei* Shirota, *L. rhamnosus* Rosell and *L. rhamnosus* GG, using gas chromatography on a GC HP5890, with a flame ionizer, AT-1000 column (10 m × 0.25 mm)

with a temperature gradient of 90 to 120 °C, increasing 5 °C/min, N₂ carrier gas, at a flow of 1 mL/min. The standards were prepared at a concentration of 25-700 ppm and were expressed in mg/mL.

2.4. Polyphenols content, polyphenols composition, and antioxidant properties.

An ethanolic extract (90:10 ethanol:water) from both flours (5% w/v) were obtained by maceration at 60 °C during 30 min subsequently was percolate, and polyphenols content was determined by Folin-Ciocalteu reagent (Singleton et al., 1999). Flavonoids were analyzed based on what was reported by Vega et al. (2017). Condensed tannins (pro anthocyanidins), as insoluble, water soluble, organic solvent soluble, were determined (Shay et al. 2017). And hydroxycinnamic acids were determined by HPLC (Rodriguez-Duran et al., 2014).

Antioxidant properties were determined as Trolox equivalent antioxidant capacity (TEAC), based on what was reported by Re et al., (1999). Antiradical power was determined as inhibition of DPPH in percent, as specified by Randhir and Shetty (2007). And the total antioxidant activity, based on the inhibition of linoleic acid peroxidation by compounds present in extracts (Starzynska-Janiszewska et al., 2008).

2.5. Antimicrobial activity of ethanolic extracts

The antimicrobial activity of coffee pulp and cocoa husk was determined using the methodology of (Bauer et al., 1966). As enteric strains (*Escherichia coli*, *Salmonella* (*S.*) *typhimurium*., *Pseudomonas* (*P.*) *fragilis*, *P. fluorescens*, *P. putida*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus subtilis* and *S. sp*), they were plated on Mueller-Hinton agar (2X10⁸ CFU/mL) and filter paper discs (1.0 cm diameter) containing commercial antibiotics such as erythromycin (NeoPharma, Mexico), chloramphenicol (Pharmacos Exakta, Mexico) and tetracycline (Bioresearch, Mexico) at a concentration of 5 mg/mL were used. The ethanolic extract of coffee pulp flour and cocoa

shell (ethanol:water 90:10) was diluted to 5 mg/mL of polyphenols and finally the inhibition diameters were quantified, reported as the mean of three reproducible assays.

2.6. Experimental design and statistical analysis

The influence of the flour's coffee pulp and cacao husk on antimicrobial activity was determined according to the model:

$$y_{ij} = \mu + \alpha_i + \beta_j + \epsilon \quad (2)$$

Where y_{ij} is the antimicrobial activity for the i -th type of antimicrobial compound and the j -th of the strain. The μ is the overall mean, α_i is the effect of the type of antimicrobial, and β_j is the

effect of the pathogen; and ϵ is the error terms of a presumed normal distribution, $N(\mu, \sigma^2)$ (Der and Everitt, 2001).

3. Results and discussion

3.1. Fiber, glucose, reducing sugars and prebiotic activity score.

The fiber content for both flours was around 60-70% (Table 1). The glucose content in coffee pulp flour was 26 %, and the reported range was from 17 to 33 % (Mawaddah et al., 2022). Cacao husk flour presents higher glucose content (58%). Total sugars content was close to 48% in coffee pulp flour (Pérez-Calvo et al. 2023), in cacao husk the content was higher (75%) (Table 1).

Table 1. Carbohydrates composition and prebiotic activity score for the coffee pulp flour or cacao husk flour

Parameter	Coffee pulp flour	Cacao husk flour
Main carbohydrates		
Dietary fiber (%)	63.93 ± 0.70	70.60 ± 0.30
Glucose (mg/g sample)	26 ± 7.8	58 ± 6.54
Total reducing sugars (%)	47.50 ± 1.12	74.50 ± 1.82
Prebiotic activity score		
<i>L. rhamnosus</i> GG	0.12	0.25
<i>L. rhamnosus</i> Rosell	0.08	0.25
<i>L. casei</i> Shirota	0.14	0.29
<i>L. acidophilus</i>	0.13	0.26

The results show that the coffee pulp flour and the cacao husk flour are suitable ingredients that employed to increase the quality of processed nourishment, as well as to carbon source for different fermentation process. By-products can be employed to produce relevant platform chemicals (single cells oil, specific C2-C6 metabolites) and biopolymers (as bacterial cellulose), where bioconversion processes are the most important techno-economic aspect (Koutinas et al., 2014). The coffee by-products singular organic composition made it an ideal substrate for microbial processes to earn betterment products. Likewise, manner, the interesting composition of cacao husk represents a source as well as the production of compounds

such as lignocellulosic biomass (Porto de Souza Vandenberghé et al., 2022).

The prebiotic activity score with *E. coli* as pathogen indicator microorganism for the different probiotic strains are show in Table 1, where coffee pulp flour presented higher scores than cacao husk flour. *L. casei* Shirota presented the higher score employing cacao husk as a carbon source, due to the higher sugars content. Inulin, as the prebiotic by antonomasia, presented scores higher than one (Huebner et al., 2007) since inulin composition is higher in soluble fiber (98%). Prebiotic potential is an important characteristic for dietary fiber, since only oligosaccharides, fructooligosaccharides, and inulin are classified as such, so for other

fibers to be prebiotic, demonstrating such an effect is important. (Rezende et al., 2021). The insoluble dietary fiber of cacao fiber contains in glucose, whereby that the most part of polysaccharides is cellulose and pectin, and the insoluble dietary fiber fraction in cacao fiber presents a considerable amount of mannose and galactose, suggesting the presence of galactomannans as monosaccharides part of the pectic substances, as major components of both soluble dietary fiber and insoluble cell wall polysaccharides. For its part, coffee pulp has xylans, and polysaccharide whit cellulose, hemicellulose, and lignin (Machado et al., 2023). Pectin in coffee skin contains uronic acid with different degrees of methyl esterification (Esquivel and Jiménez, 2012). The coffee coproducts could exert certain antimicrobial activity in the course the fermentation process in the prebiotic activity determination due to the melanoidins content (Jiménez-Zamora et al., 2015).

The fermentation rate of the prebiotic dietary fibers pivot on solubility and size inters other. The soluble fibers are fermented faster

than the insoluble ones, and oligosaccharides than the polysaccharides, so that the fermentation the fiber varied (like the obtained from agro-industrial coproducts, pulp or husks) will be different from the fermentation of individual fibers or a single carbon source (Rezende et al., 2021). For this reason, the determination of the prebiotic activity score is important in these kind of fiber resources, such as coffee pulp and cacao husk, although the inherent and concomitant differences in composition depending on seasonal changes and geographic factors, carbohydrate composition, as soluble or insoluble fiber, represent a nice fountain of prebiotic compounds.

3.2.Short chain fatty acids

For the results, the SCFA, were different depending on the strain employed. The highest concentration of lactic acid for both flours was produced by *L. acidophilus*, as well as highest amount of probiotic. Nonetheless, together butyric, and propionic acids results be similar among probiotic strains for both flours (Table 2).

Table 2. Short-chain fatty acids produced by different probiotic strains with coffee pulp flour or cacao husk flour as carbon sources.

Compound	Probiotic strain			
	<i>L. acidophilus</i>	<i>L. casei</i> Shirota	<i>L. rhamnosus</i> Rosell	<i>L. rhamnosus</i> GG
Coffee pulp flour				
Lactic acid	121.5 ± 0.04	84.3 ± 0.04	69.4 ± 0.07	68.6 ± 0.05
Acetic acid	25.7 ± 0.006	18.8 ± 0.01	12.7 ± 0.02	21.0 ± 0.06
Propionic acid	Nd	Nd	2 ± 0.01	Nd
Butyric acid	2.0 ± 0.04	2.1 ± 0.01	2.1 ± 0.01	2.0 ± 0.02
Cacao husk flour				
Lactic acid	129.7 ± 0.05	61.8 ± 0.04	60.7 ± 0.02	70.1 ± 0.06
Acetic acid	41.4 ± 0.03	10.6 ± 0.02	11.9 ± 0.03	17.2 ± 0.02
Propionic acid	1.6 ± 0.01	1.7 ± 0.01	2.5 ± 0.01	1.8 ± 0.01
Butyric acid	2.1 ± 0.01	2.1 ± 0.01	3.2 ± 0.02	2.0 ± 0.01

Short chain fatty acids (less than 6 carbons) are produced due to the non-digestible carbohydrates and are related to an indirect way to measure prebiotic capacity (Markowiak-Kopéc and Śliżewska, 2020). This phenomenon has been related to host health affecting the

integrity of the gastrointestinal epithelium, and glucose homeostasis (Ashaolu et al., 2021). The intensive metabolic activity of probiotic fermentation of available substrates decreased pH value and increase the production of probiotic fermentation of available substrates

decreased the pH value and increased the yield of organic acids (Nsor-Atindana et al., 2020), besides SCFA is acetate, propionate and butyrate (Gibson and Roberfroid, 1995). In addition, the fermentation of prebiotic fibers improves the growth of other non-probiotic microorganisms, which employ by-products generated by probiotics. Then, the cross-feeding situation is a dietary regulation of the microbiota results to prebiotic consumption where the nutrients competition and metabolites production with the concomitant pH reduction help to impede the proliferation of other microorganisms, considering the host genetic disposition as well, consumption of required dietary fiber, and/or the constitution of the individual microbiota (Ashaolu et al., 2021).

3.3. Polyphenols content, polyphenols composition, and antioxidant properties.

Total polyphenols content was higher in cacao husk flour, although the flavonoids content was similar for both flours. Condensed tannins content was similar in coffee pulp flour and cacao husk flour, with a higher quantity of insoluble tannins in cacao husk flour. Chlorogenic and caffeic acid were detected in coffee pulp flour, and vanillic acid was detected in cacao husk flour. Ferulic acid was detected in both samples (Table 3). The bio-accessibility and bioavailability of polyphenols are important, since although phenolic compounds decreased during digestion, hydroxycinnamic acids can reach colon and be metabolized by bifidobacteria and lactic acid bacteria (Cañas et al., 2022).

Table 3. Total polyphenol substances contain, polyphenols composition and antioxidant properties of coffee pulp flour or cacao husk flour ethanolic extracts.

Parameter	Coffee peel flour	Cacao husk flour
Total polyphenols content (Eq. gallic acid mg/g)	3.84±0.21	4.12±0.23
Flavonoids (Eq. catechin mg/g)	1.23±0.05	1.30±0.33
Condensed tannins		
Soluble in organic solvents (mg/g)	0.81±0.09	1.20±0.10
Soluble in water (mg/g)	1.00±0.01	0.85±0.01
Insoluble (mg/g)	0.23±0.01	0.63±0.02
Hydrocinnamic acids		
Chlorogenic acid (mg/g)	0.40±0.02	Nd
Caffeic acid (mg/g)	0.03±0.01	Nd
Ferulic acid (mg/g)	0.03±0.003	0.03±0.001
Vanillic acid (mg/g)		0.07±0.001
Antioxidant properties		
TEAC	13.79±0.90	44.83±0.10
Antiradical power by DPPH	81.67±0.50	72.30±0.67
Total antioxidant activity	80.50±0.60	68.40±0.10

For the Trolox equivalent antioxidant capacity (TEAC), cacao husk flour presented higher values as compared to coffee pulp flour. The antiradical power determined by DPPH was higher for the coffee pulp flour, as well as the total antioxidant activity (Table 3). Antioxidant activity pivot on the amount and the type of polyphenols presents, although the

relations among polyphenols content, the antioxidant activity is not directly related since there are several reactive molecules with different response as antioxidant (Alcalde et al., 2019). The different techniques employed measure different reactions and depend on factors such as the concentration of antioxidant-related compounds, extraction method, etcetera

(Yusof et al., 2019). Antioxidant capacity determination the of bio-accessible and the no bio-accessible (or insoluble) fractions of foods after a physiological-resembling digestion (Jiménez-Zamora et al., 2015). The coffee pulp has phenolic content in exerts an important antiradical-reduction effect, and the antioxidant activity could be attributed to the soluble melanoidins fraction (Jiménez-Zamora et al., 2015).

Some polyphenols, such as highly polymerized condensed tannins and phenols associated with proteins and polysaccharides from cell wall are associated with dietary fiber, and can resist digestive enzymes hydrolysis and although the congregation of the soluble phenolic made-up in cacao fiber was low than the expected, is important to notice that polyphenols present showed antioxidant capacity and free radical scavenging capacities, higher than the reported in other dietary fiber sources, like wheat bran. Minor chemical constituents in coffee pulp have (2-10% of weight) are caffeine, chlorogenic acids, and polyphenols, including flavonoids, being a valuable source of dietary antioxidants supplements, and a source of fiber as well. Coffee growth at different conditions has different antioxidants profile, but there are a useful and cheap source of molecules with biological activity to add to food products (Dorsey and Jones, 2017).

3.4. Antimicrobial activity of ethanolic extracts

A significantly higher antimicrobial activity was observed with erythromycin, followed by tetracycline and coffee pulp flour extract ($P > 0.05$). The lower antimicrobial activity was observed in chloramphenicol and cacao husk flour extract. In the same manner, *P. fragilis* was the significant ($P > 0.05$) most sensitive strain to the employed antimicrobials, followed by *L. monocytogenes* and *B. subtilis*, and *Salmonella*

sp. The rest of the strains were less sensitive to these antimicrobials (*E. coli*, *P. fluorescens*, *P. putida*, *S. aureus*, and *S. typhimurium*) (Table 4). These results indicate that the coffee pulp flour ethanolic extract presented an antimicrobial activity as good as tetracycline, and cacao husk flour ethanolic extract has the inhibitory pathogens capacity like chloramphenicol.

This is important since extracts from these agro-industrial co-products can be employed as antimicrobials, or in conjunction with drugs to reduce the risk of the antimicrobial resistance. A specific extraction of compounds of interest could be made to increase the antimicrobial capacity. Melanoidins present in coffee by-products present antimicrobial activity related to their chelating capacity (Jiménez-Zamora et al., 2015). The cacao pod husk extracts, Kayaputri et al. (2020) ensure the caffeine, as the leading alkaloid identified in chloroformic fraction, presented a bacteriostatic effect on Gram negative *P. aeruginosa*, and although theobromine has been reported as the main alkaloid in cacao, the synergic effects of different alkaloids are responsible of the antimicrobial effect. Gram-positive bacteria are most receptive to polyphenols and tannins, as hydrophobic compounds, since the cell membrane of Gram-negative bacteria, made-up of phospholipids, hydrophobic compounds are not absorbed. Polyphenols present antimicrobial activity because of the disruption of the perviousness of the cell membrane (Duangjai et al., 2016), and by a metal chelating effect of melanoidins (Rufián-Henares and de la Cueva, 2009). Changes in cell wall integrity due interactions with phenolic compounds resulted in the alteration of various intracellular functions, by hydrogen bonding enzymes, provoking an irreversible damage in cell cytoplasmic membrane, with the concomitantly cease of intracellular enzymes activity, and death (Khochapong et al., 2021).

Table 4. Antibacterial activity by disk diffusion method of ethanolic extract of coffee pulp flour or cacao husk flour as compared to commercial antibiotics against the different pathogen strains.

Pathogen	Diameter of inhibition zones (cm)				
	Chloramphenicol	Tetracycline	Erythromycin	Coffee pulp flour	Cacao husk flour
<i>E. coli</i>	0.45±0.07 C, d	0.63±0.14 B, d	1.00±0.07 A, d	0.67±0.05 B, d	0.48±0.04 C, d
<i>Salmonella sp.</i>	0.53±0.07 C, c	1.43±0.07 B, c	0.75±0.06 A, c	0.39±0.07 B, c	0.43±0.05 C, c
<i>P. fragilis</i>	1.43±0.56 C, a	0.48±0.15 B, a	0.58±0.04 A, a	0.26±0.05 B, a	0.35±0.03 C, a
<i>P. fluorescens</i>	0.48±0.07 C, d	0.50±0.14 B, d	0.95±0.07 A, d	0.34±0.02 B, d	0.38±0.02 C, d
<i>P. putida</i>	0.45±0.07 C, d	0.40±0.07 B, d	0.75±0.02 A, d	0.30±0.07 B, a	0.52±0.04 C, d
<i>L. monocytogenes</i>	0.60±0.06 C, b	0.50±0.06 B, b	1.03±0.14 A, b	0.39±0.05 B, b	0.45±0.05 C, b
<i>S. aureus</i>	0.55±0.05 C, d	0.90±0.14 B, d	0.80±0.07 A, d	0.30±0.03 B, d	0.47±0.06 C, d
<i>B. subtilis</i>	0.60±0.05 C, b	1.63±0.07 B, b	0.65±0.28 A, b	0.27±0.08 B, b	0.45±0.02 C, b
<i>S. typhimurium</i>	0.43±0.07 C, d	0.38±0.07 B, d	0.88±0.14 A, d	0.33±0.05 B, d	0.45±0.07 C, d

A, B - Means with the same letter in the same row are not significantly ($P < 0.05$) different for antimicrobial.

a, b - Means with the same letter in the same column is not significantly ($P < 0.05$) different for pathogen microorganisms.

4. Conclusion

Coffee pulp flour and cacao husk flour are a sources of functional ingredients, such as fiber and polyphenols, where the relatively high concentration of fiber present in these two by-products make them a functional ingredient that could improve intestinal health promoting the selective growth of probiotics, as well to inhibit pathogens microorganisms due to their antimicrobial activity.

The demonstrated ability to serve as a prebiotic carbon source and antimicrobial, in addition to the antioxidant capability of the polyphenols present in both cacao husk flour and coffee pulp flour, stands out the importance of these byproducts as a fountain of important bioactive substances, so that be employed as meal ingredients by enhance the nutritional value of processed foods. Cacao and coffee are commodities with important economic importance in several countries, implying as well small factories that can use these

ingredients in a circular economy, looking for a sustainable agricultural chain.

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