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Valorization of red *Coffea arabica* var. Caturra pulp: Development of a kombucha beverage

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Abstract

Kombucha is a fermented beverage traditionally made from tea. This study investigates the use of coffee pulp —a polluting by-product of coffee processing— as an alternative to tea in kombucha development. The research aimed to compare kombucha made from dried and fresh coffee pulp with traditional black tea kombucha, focusing on ethanol production, sugar consumption, phenolic content, antioxidant activity, caffeine levels, and pH. The results indicated that dried coffee pulp kombucha produced more ethanol (1.53%) and consumed more sugar (3.80 g/100mL) than fresh pulp kombucha (0.64% ethanol, 0.61 g/100mL sugar). However, fresh pulp kombucha had higher total phenols content (71.24 mg eq GAE/330 mL) and antioxidant activity (4.03 mmol Trolox eq/mL) compared to dried pulp kombucha. Additionally, the fresh pulp version had a higher concentration of catechins, whereas the dried pulp kombucha had greater caffeine content. In terms of pH, fresh coffee pulp kombucha had the lowest value (2.67), followed by black tea kombucha (2.98), and dried pulp kombucha had the highest pH (3.21). Fresh pulp kombucha also presented the highest level of soluble solids (11.33 °Brix). The study concludes that fresh coffee pulp kombucha, with superior antioxidant properties and phenolic content, holds the most promise. This innovation could create new economic opportunities for coffee producers in Colombia and globally, as the kombucha market continues to grow.

Keywords: antioxidant capacity, by-products, phenolic compounds, SCOBY

Valorización de pulpa de *Coffea arabica* var. Caturra rojo: Desarrollo de una bebida kombucha

Resumen

La kombucha es una bebida fermentada tradicionalmente a base de té. Este estudio investiga el uso de la pulpa de café —un subproducto contaminante del procesado del café— como alternativa al té en la elaboración de kombucha. El objetivo de la investigación fue comparar la kombucha elaborada con pulpa de café seca y fresca con la kombucha tradicional de té negro, centrándose en la producción de etanol, el consumo de azúcar, el contenido fenólico, la actividad antioxidante, los niveles de cafeína y el pH. Los resultados indicaron que la kombucha de pulpa de café seca produce más etanol (1,53%) y consume más azúcar (3,80 g/100mL) que la kombucha de pulpa fresca (0,64% de etanol,

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0,61 g/100mL de azúcar). Sin embargo, la kombucha de pulpa fresca tuvo un mayor contenido de fenoles totales (71,24 mg eq GAE/330 mL) y actividad antioxidante (4,03 mmol Troloxeq/mL) en comparación con la kombucha de pulpa seca. Además, la versión de pulpa fresca tuvo una mayor concentración de catequinas, mientras que la kombucha de pulpa seca tuvo un mayor contenido de cafeína. En cuanto al pH, la kombucha de pulpa de café fresca presentó el valor más bajo (2,67), seguida de la kombucha de té negro (2,98), y la kombucha de pulpa seca tuvo el valor más alto (3,21). La kombucha de pulpa fresca también tuvo el nivel más alto de sólidos solubles (11,33 °Brix). El estudio concluye que la kombucha de pulpa de café fresca, con sus propiedades antioxidantes y su contenido fenólico superiores, es la más prometedora. Esta innovación podría crear nuevas oportunidades económicas para los productores de café de Colombia y del mundo, a medida que el mercado de la kombucha siga creciendo.

Palabras clave: capacidad antioxidante, subproductos, compuestos fenólicos, SCOBY

Valorização da polpa de *Coffea arabica* var. red Caturra: Desenvolvimento de uma bebida de kombucha

Resumo

Kombucha é uma bebida fermentada tradicionalmente preparada a partir de chá. Este estudo explorou o uso da polpa de café –um subproduto do processamento do café com potencial poluente– como alternativa ao chá na preparação da kombucha. O objetivo foi comparar a kombucha feita com polpa de café, tanto seca quanto fresca, à kombucha tradicional de chá preto, analisando a produção de etanol, o consumo de açúcar, o conteúdo fenólico, a atividade antioxidante, os níveis de cafeína e o pH. Os resultados mostraram que a kombucha preparada com polpa de café seca apresentou maior produção de etanol (1,53%) e maior consumo de açúcar (3,80 g/100 mL) em relação à versão com polpa fresca (0,64% de etanol e 0,61 g/100 mL de açúcar). Por outro lado, a kombucha de polpa fresca destacou-se pelo maior teor de fenóis totais (71,24 mg eq GAE/330 mL) e pela superior atividade antioxidante (4,03 mmol Troloxeq/mL). Essa versão também apresentou maior concentração de catequinas, enquanto a kombucha de polpa seca demonstrou um conteúdo mais elevado de cafeína. Quanto ao pH, a kombucha de polpa fresca registrou o valor mais baixo (2,67), seguida pela kombucha de chá preto (2,98), enquanto a de polpa seca teve o pH mais alto (3,21). Além disso, a kombucha de polpa fresca apresentou o maior nível de sólidos solúveis (11,33 °Brix). Concluiu-se que a kombucha de polpa de café fresca, com suas propriedades antioxidantes e elevado teor fenólico, é a alternativa mais promissora. Essa inovação pode gerar novas oportunidades econômicas para os produtores de café na Colômbia e em outros países, à medida que o mercado de kombucha continua a crescer.

Palavras-chave: capacidade antioxidante, subprodutos, compostos fenólicos, SCOBY

1. Introduction

Kombucha, a beverage discovered many years ago, is prepared from the fermentation of the sweetened infusion, typically from black or green tea leaves. This fermentation process is facilitated by a Symbiotic Culture of Bacteria and Yeasts (SCOBY)[1]. Studies have indicated that the beneficial properties of the tea infusion persist in the fermented beverage[1][2]. Consequently, kombucha provides antioxidant[3], anti-inflammatory[4], and antimicrobial[5] compounds, aiding in the prevention of cancer[2], hypertension, diabetes[6], and cardiovascular diseases[1].

During the production of kombucha, numerous reactions take place in which the enzymes of the microorganisms present in the SCOBY are able to break down the large molecules of the infusions of plant material into smaller molecules[7]. Sales and others[4] report that the esterases within the SCOBY are able to break down some glycosides, aglycones, flavonoids, and catechins, leading to an increase in the concentration of rutin, quercetin, gallic acid, among others over the course of fermentation[2][8][9]. In these cases, these reactions can either increase or decrease antioxidant activity, depending on the nature of the smaller molecules[4]. Addition-

ally, thanks to these reactions and those employed by the microorganisms for their most vital processes, the conditions of the medium are altered, resulting mainly in a decrease in pH and soluble solids[10].

Although kombucha is traditionally produced using tea leaves, alternative raw materials, such as coffee pulp, can also be utilized. The coffee production process generates significant volumes of by-products[11], some of which pose environmental challenges due to the presence of caffeine, tannins, and other compounds that can contaminate soil and water in coffee-growing regions[12]. Coffee pulp, which constitutes the largest proportion of coffee by-products by weight, has been extensively studied for its high content of bioactive compounds, including polyphenols, carotenoids, and micronutrients such as soluble fibers, ascorbic acid, and minerals[13][14][15][16][17][18]. However, fresh coffee pulp undergoes rapid browning and loses its organoleptic properties due to the action of microorganisms and enzymes. To address this issue, dehydration treatments can be applied, extending the shelf life of the pulp and preserving its valuable characteristics[19].

Finding alternatives for valorizing coffee pulp could have a positive impact on the nearly half a million coffee-growing families in Colombia. It would enable them to generate income from what was previously an economic and environmental burden due to its disposal. Likewise, this valorization effort allows progress to be made in at least 7 of the 17 Sustainable Development Goals proposed by Colombia to be achieved by 2030. These are briefly, the first: no poverty; the sixth: clean water and sanitation; the eighth: decent work and economic growth; the ninth: industry, innovation, and infrastructure; the twelfth: responsible consumption and production; the thirteenth: climate action, and the fifteenth: life on land[20].

Currently, there are some studies on kombucha brewed with coffee pulp[4][21][22]. These studies have assessed several aspects including the antioxidant activity of kombucha through in vitro experiments, some through oxidative stress experiments in cells, while others do so through chemical free radical scavenging methods. Studies agree that the antioxidant activity of coffee pulp kombucha is remarkable. Other studies that have analyzed the antioxidant activity of kombuchas in general show that this property varies over time. In some instances, the antioxidant activity shows a decrease[21][23] after 12-14 days, and in others, it shows an increase[24][25][26] after 12-15 days.

Thus, this research aims to explore an alternative valorization of coffee pulp by comparing kombucha brewed from fresh coffee pulp (FCP) and dried coffee pulp (DCP) and using black tea (BT) kombucha as a control. The comparison is made in terms of its kinetic of sugar consumption and alcohol production, total phenolic content, antioxidant activity, its phenolic compounds profile, and a microbiological analysis of the finished beverages. On the other hand, a study of the kombucha market is carried out and a product identification for a beverage such as the one in the study is proposed. The study stands out for its approach to the industrial sector of kombucha production with coffee pulp and establishes an alternative for obtaining resources for the production of kombucha with coffee pulp and for Colombian coffee families, changing the paradigm of coffee pulp as a waste product in the coffee processing process.

2. Materials and Methods

2.1 Reagents and Samples

In this study, coffee pulp samples were supplied by Tint Café S.A.S Company, Villeta, Colombia. For black tea, ready-to-use Hindú® tea bags were used (Colombia). The chemicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) (≥98%), 2,2'-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) (≥98%), monopotassium phosphate (≥ 98%), disodium phosphate (≥99%), potassium persulfate (≥99%), Trolox (97%), Folin-Ciocalteau reagent (2 N), gallic acid (≥97.5%), sodium carbonate (99.5%) were used from Sigma-

Aldrich. Methanol (≥99.8%), sulfuric acid (98%), absolute ethanol (≥99.5%) was obtained from Panreac (Gmbh). Dextrose was purchased throughout CIACOMEQ SAS (Bogotá, Colombia). SCOBY and a ready to drink kombucha (starter) were acquired from La Jaguara (St Agnes Brewing Co., Bogotá, Colombia).

The following standard compounds were used for the determination of the phenolic compound profile: (±)-catechin (C) (Sigma-Aldrich, Part N° C1788-500MG), (-)-epigallocatechin gallate (EGCG) (Sigma-Aldrich, Part N° E4143-50MG, Part N° E4143-50MG), (-)-epicatechin (EC) (Sigma-Aldrich, Part N° E1753-1G), (-)-epicatechin gallate (ECG) (Sigma-Aldrich, Part N° E3893-10MG), (-)-epigallocatechin (EGC) (Sigma-Aldrich, Part N° E3768-5MG), caffeic acid (Sigma-Aldrich, Part N° C0625), p-coumaric acid (Sigma-Aldrich, Part N° C9008), rosmarinic acid (Sigma-Aldrich, Part N° 536954-5G), quercetin (Sigma-Aldrich, Part N° Q4951-10G), naringenin (Sigma-Aldrich, Part N° N5893-1G), luteolin (Sigma-Aldrich, Part N° L9283-10MG), kaempferol (Sigma-Aldrich, Part N° K0133-50MG), pinocembrin (Sigma-Aldrich, Part N° P5239), apigenin (Sigma-Aldrich, Part N° A3145-25MG), cyanidin 3-glucoside (Sigma-Aldrich, Part N° G36428), pelargonidin 3-glucoside (Sigma-Aldrich, Part N° 53489); alkaloids: caffeine (Sigma-Aldrich, Part N° C8960-250G), theobromine (Sigma-Aldrich, Part N° T4500-25G), and theophylline (Sigma-Aldrich, Part N° T1633-25G).

2.2 Sample Preparation

The whole coffee cherry was disinfected with sodium hypochlorite (100 ppm), then manually pulped. On the other hand, for dried pulp kombucha, after disinfection and pulping, it was dried in a tray oven at 40 °C for 24 hours (UOP8 MKII, Armfield Limited, England). In the case of black tea, the bags —containing the ground leaves— were emptied until the required weight of leaves was gathered.

2.3 Kombucha Fermentation

Following the methodology of Sales and others[4], the infusions were made by adding water at 90 °C to the sample (3% w/v) and leaving them in contact for 15 minutes, after which the coffee pulp or tea leaves were removed using a cheesecloth previously disinfected. Dextrose (10% w/v) was added and manually agitated. When the sweetened infusion reached room temperature, 10% v/v of the ready to drink kombucha was added as starter for pH reduction and to facilitate the fermentation beginning. Finally, 2.5% w/v of SCOBY was added, and the top of the flask was covered with gauze and kept using a rubber band. The flasks were fermented at 23 °C in an incubator (Orbital Shaker HD 3000CT, Innovaciones Actum SAS, Colombia) without agitation for 10 days, and 8 or 9 samples were collected along the fermentation time.

2.4 Sugar Consumption and Ethanol Production Analysis

Determination of dextrose consumption and ethanol production was performed on a High-Performance Liquid Chromatographer (HPLC) coupled to a refractive index detector (RID) using a Aminex HPX-87H (300 × 7.8 mm) column (Bio-Rad Laboratories Inc, California, USA) at 50 °C. The eluent was a 5 mM sulfuric acid solution at a flow rate of 0.6 mL/min and injection volume of 20 µL of sample[27]. A calibration curve of ethanol (0.15625-10 mL/100 mL, seven data points with a R^2 = 0.9999) and dextrose (0.15625-10 g/100 mL, seven data points with a R^2 = 0.9972) was carried out for identification and quantification.

2.5 In Vitro Total Phenolic Content and Antioxidant Capacity Assays of Kombucha

The total phenolic concentration (TPC) was measured by reacting the sample with Folin-Ciocalteu's reagent and comparing its reducing capacity against a gallic acid standard[28]. For this purpose, dilutions of known concentration of the sample were made and reacted (1% v/v) with Folin-Ciocalteu's reagent (5% v/v) in the presence of 20% w/v sodium carbonate solution (15% v/v) in aqueous solution (79% v/v). The mixture was left to react for 2 hours isolated from light, and its absorbance was measured at 760 nm on a UV-Vis spectrophotometer (Thermo Scientific Genesys 20®, USA). The calibration curve was performed with the same proce-

dure changing the sample dilutions for dilutions of gallic acid. The absorbances of the samples were compared against those of gallic acid to find the concentration in units of mg of gallic acid equivalents (mg GAE)/330 mL kombucha (one serving).

Antioxidant capacity was determined by both the Trolox Equivalent Antioxidant Capacity (TEAC) assay and the EC50 method, both of which are based on the colorimetric measurement of the sample's ability to react with ABTS+ and DPPH+ free radicals. In the case of the TEAC test, ABTS radicals were formed by reacting to a dilution of 7mM ABTS (98.3% v/v) with a dilution of 139.8 mM potassium persulfate (1.7% v/v) for 16 hours at room temperature. Subsequently, the ABTS radical was diluted to a concentration of 0.1 mM with PBS (monopotassium phosphate, disodium phosphate) buffer (5 mM). Then, the sample was diluted to known concentrations and reacted (1% v/v) with the diluted ABTS+ radical (99% v/v) for 45 minutes and its absorbance at 734 nm was measured in the spectrophotometer. The calibration curve was performed by changing the sample with known concentrations of TROLOX. The absorbance of the samples was compared against those of TROLOX, and the results were presented in terms of mmol TROLOX equivalents/mL kombucha. On the other hand, in the case of EC50 determination, DPPH+ was diluted in methanol (6x10-5 M) and the sample was diluted to known concentrations. The sample reacted (2.5% v/v) with DPPH+ (97.5% v/v) for 4 hours isolated from light and its absorbance was measured at 516 nm in the spectrophotometer. The calibration curve was made by diluting the DPPH+ to known concentrations. The absorbance of the sample was compared against that of the calibration curve and the data were reported in terms of EC50 (mL needed to react with 50% concentration of DDPH+)[29][30].

2.6 Phenolic Compounds Profile and Caffeine, Theobromine and Theophylline Determination

An ultra-high performance liquid chromatograph (UHPLC), Dionex Ultimate 3000 (Thermo Scientific, Sunnyvale, CA, USA), equipped with a binary gradient pump (HP G3400RS), an automatic sample injector (WPS 300TRS) and a thermostatic column unit (TCC 3000), was used. The LC-MS interface technique was electrospray ionization (ESI), and the high-resolution mass spectrometer was equipped with an Orbitrap ion current detection system operated in positive mode with a capillary voltage of 3.5 kV. A Hypersil GOLD Aq Column (Thermo Scientific, Sunnyvale, CA, USA; 100 × 2.1 mm, 1.9 µm particle size) was used. The mobile phase was A- 0.1% v/v formic acid and 5 mM ammonium formate (water) and, B- 0.1% v/v formic acid and 5 mM ammonium formate (methanol). The initial gradient condition was 100% A, changing linearly to 100% B (8 min); it was maintained for 4 min and then returned to initial conditions in 1 min; the total run time was 13 min, with 7 min for post-run. Compound identification was performed using full scan acquisition mode and extraction of ionic currents (EIC) corresponding to protonated [M+H]+ molecules of compounds of interest, mass measurement with accuracy and precision of Δppm< 1, review of isotope ratios and fragmentation patterns. Quantification of the analytes of interest was based on calibration curves using certified reference materials. The study was carried out by the Chromatography and Mass Spectrometry Laboratory of the Universidad Industrial de Santander (Bucaramanga, Colombia).

2.7 Soluble Solids and pH

The concentration of total soluble solids for each day was determined with a refractometer (Pocket Refractometer Pal-1, ATAGO, Tokyo, Japan). Results were expressed in °Brix. Meanwhile, pH was measured using a multiparameter at 15 °C (SevenMulti S47, Mettler Toledo SA, Ciudad de México, México).

2.8 Microbiological Analysis

The corresponding cultures were performed to detect the presence of molds (ISO 21527-1:2008) and yeasts (ISO 21527-1:2008), *Escherichia coli* (NTC 4458 - 2018) and *Salmonella* spp. (ISO 6579-1:2017). According to the Colombian standard (Resolution 1407 of 2022 of the Ministry of Health and Social Protection)[31], there

should be up to a maximum of 1 × 10^2 CFU/mL of molds and yeasts, 10 CFU/mL of *Escherichia coli* and Absence/25mL for *Salmonella* spp. This analysis was carried out by Laboratorio Nulab S.A.S. (Bogotá, Colombia).

2.9 Market Analysis

Economic and financial information on the kombucha business was extracted from specialized portals such as Yahoo finance[32], a Skyquest[33] report, and Euromonitor International's Passport tool. The Google® search engine was used for the search; the search keywords were "kombucha sales worldwide", and the most convenient pages were chosen from the first 15 results. The information that we tried to collect came from reports of specialized companies. In addition, after a brief characterization of potential customers, a label is proposed using Colombian standards as a basis.

2.10 Statistical Analysis

The Minitab® software (version 21.4) was used and the data from days 0, 3, 7, and 10 were adjusted to perform an ANOVA and apply Tukey's test to compare the fresh and dry coffee pulp kombuchas at a confidence level of 95% and find significant differences between their properties. In the statistical tests, BT kombucha was not included because its superiority to coffee pulp kombuchas does not allow appreciation of the differences between these raw materials.

3. Results and Discussion

3.1 Sugar Consumption and Ethanol Production Analysis

Figure 1 shows the behavior of dextrose consumption and ethanol production over the days of fermentation for fresh coffee pulp (FCP) and dried coffee pulp (DCP) kombucha as well as black tea (BT). There is a different behavior of both dextrose consumption and ethanol production in each fermentation; in the case of FCP kombucha, it shows the least variation in dextrose concentration and the lowest ethanol production. In this fermentation, 10% of the initial dextrose is consumed and 0.64% ethanol is produced. Meanwhile, DCP kombucha shows an intermediate behavior between tea and fresh pulp; about 60% of the initial dextrose is consumed and up to 1.53% ethanol is produced. Finally, BT kombucha shows a higher consumption of dextrose, which is reduced by up to 74%, and about 2.15% of ethanol is produced.

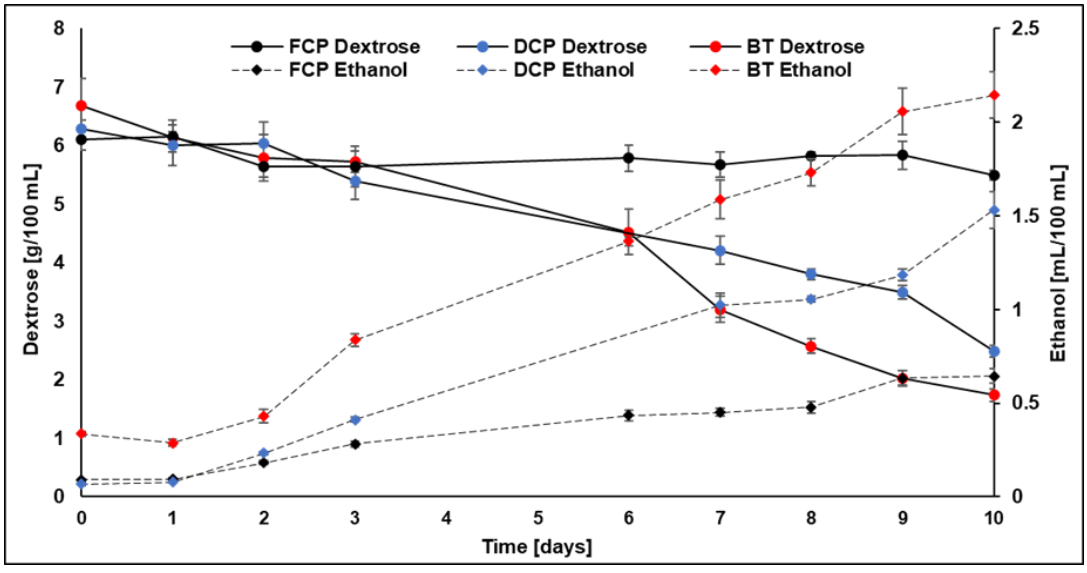

Figure 1. Dextrose consumption and ethanol production for Black Tea (BT), Fresh Coffee Pulp (FCP), and Dried Coffee Pulp (DCP) kombuchas over 10 days of fermentation

Indeed, while it is true that kombucha is not intended to have a high ethanol content, the low dextrose consumption for FCP kombucha indicates that there may be some compound in that medium that is inhibiting the development of SCOBY microorganisms. Some studies show that coffee pulp and mucilage contain compounds (mainly chlorogenic acids) that inhibit bacterial growth[34][35]. In addition, another study mentions that the tea drying process allows the fermentation of the leaves and thus the formation of simpler molecules, which would explain why in BT kombucha fermentation there was a higher consumption of dextrose[24]. Although drying is performed at a higher temperature than ambient and for a shorter time, a similar phenomenon could occur during the drying of coffee pulp, potentially resulting in higher ethanol production compared to fresh pulp. As a suggestion, the addition of enzymes to the infusion prepared with fresh pulp could replicate this effect and mitigate other consequences of drying, such as reduced antioxidant activity[36].

3.2 In vitro Antioxidant Capacity and Total Phenols Assays of Kombucha

Table 1 shows the results of the TPC, TEAC, and EC_{50} tests for FCP and DCP kombucha as well as BT kombucha. The comparison of FCP, DCP, and BT in terms of Total Phenolic Content (TPC), Trolox Equivalent Antioxidant Capacity (TEAC), and EC_{50} revealed significant differences in bioactive properties and their development during fermentation. Black tea consistently exhibited the highest TPC (>4400 mg GAE/330 mL) and TEAC (~63.48–70.07 mmol Trolox eq/mL), with minimal variation over time, reflecting its rich and stable phenolic profile. In contrast, FCP demonstrated the most substantial improvements during fermentation, with TPC increasing from 51.45 to 72.46 mg GAE/330 mL and TEAC rising from 2.89 to 4.03 mmol Trolox eq/mL, accompanied by a marked decrease in EC50 (0.95 to 0.73 mL), indicating enhanced antioxidant efficiency. DCP, while starting with slightly higher TPC (59.39 mg GAE/330 mL) than FCP, showed slower and less pronounced changes, with TEAC peaking at only 2.16 mmol Trolox eq/mL and EC_{50} fluctuating around 1.00 mL by Day 10. These findings suggest that fermentation enhances the phenolic content and antioxidant capacity of fresh coffee pulp, making it a promising substrate for developing functional kombucha beverages, whereas the drying process in DCP appears to limit phenolic bioavailability and antioxidant potential. Black tea, however, remains superior in absolute values, underscoring its intrinsic bioactive properties.

In the literature, several studies report the TCP of the kombuchas they produced, some with alternative raw materials and others with tea. Muzaifa and others[21] report that their coffee pulp kombucha reached on day 8 of fermentation with 3% (v/v) starter a value of 105.20 mg GAE/mL, meanwhile in this study 0.22 mg GAE/mL is reported in FCP kombucha. These differences could be linked to the region of origin of the coffee pulp (Indonesia vs. Colombia) and the coffee variety (Guayo Arabica vs. Caturra). On the other hand, studies such as that of Abuduaifibu & Tamer[37] or Ulusoy & Tamer[26] report values that are closer to those found in this study. In the first case, Abuduaifibu & Tamer[37] report 10.79 mg GAE/mL for black tea kombucha after a 48-hour fermentation at 28 °C, a value that is close compared with 15.27 mg GAE/mL found in this study for black tea after 10 days of fermentation at 23 °C. These variations may be linked to the difference in fermentation temperature or the origin of the black tea (Turkey vs. Colombia). In the second case, Ulusoy & Tamer[26] report that green tea kombuchas with black carrot and red raspberry extracts obtained values of 13 mg GAE/mL at 48 h of fermentation. These values are even closer to those obtained in this study for black tea.

For better identifying the specific differences between FCP and DCP kombuchas, an additional statistical analysis was conducted (based on **Table 1** data) excluding BT kombucha. This decision aligns with the primary focus of the study, which is to highlight the unique characteristics and performance of these alternative raw materials. **Figure 2** shows the TPC, TEAC and, EC_{50} test results for FCP and DCP kombuchas, for 0, 3, 7, and 10 days.

Table 1. Total Phenol Content, TEAC, and EC_{50} values of Black Tea, Fresh Coffee Pulp, and Dried Coffee Pulp kombucha over 10 days of fermentation

Day	TPC [mg GAE/330 mL]			TEAC [mmol Trolox eq/mL]			EC_{50} [mL to 50% inhibition]		
	FCP	DCP	BT	FCP	DCP	BT	FCP	DCP	BT
0	51.45 ± 3.70	59.39 ± 4.00	5038.86 ± 132.32	2.89 ± 0.07	2.06 ± 0.10	67.53 ± 2.62	0.95 ± 0.03	0.94 ± 0.03	0.014 ± 0.0008
1	52.62 ± 2.50	59.89 ± 0.71	4405.36 ± 251.73	2.91 ± 0.12	1.99 ± 0.11	63.91 ± 1.65	0.97 ± 0.04	0.85 ± 0.02	0.013 ± 0.0009
2	53.53 ± 4.07	57.64 ± 0.35	4388.69 ± 312.33	3.00 ± 0.08	1.87 ± 0.08	64.16 ± 2.96	0.95 ± 0.01	0.94 ± 0.03	0.013 ± 0.0003
3	54.79 ± 3.4	64.39 ± 0.71	4563.73 ± 176.82	3.15 ± 0.10	2.22 ± 0.11	67.13 ± 1.64	0.86 ± 0.05	0.98 ± 0.01	0.015 ± 0.0006
4	-	-	4772.12 ± 202.13	-	-	66.02 ± 4.26	-	-	0.017 ± 0.0007
6	56.58 ± 1.59	-	-	3.47 ± 0.20	-	-	0.81 ± 0.06	-	-
7	65.83 ± 0.88	63.89 ± 0.71	4788.79 ± 218.00	3.43 ± 0.02	2.09 ± 0.14	70.07 ± 2.95	0.77 ± 0.05	0.94 ± 0.03	0.014 ± 0.0010
8	69.46 ± 2.83	69.56 ± 3.88	4638.75 ± 141.46	3.54 ± 0.20	2.02 ± 0.02	68.56 ± 5.38	0.78 ± 0.02	1.03 ± 0.04	0.013 ± 0.0003
9	72.46 ± 1.41	69.39 ± 1.41	4588.74 ± 200.05	3.74 ± 0.32	2.12 ± 0.12	63.48 ± 4.04	0.74 ± 0.04	0.91 ± 0.06	0.012 ± 0.0006
10	71.54 ± 2.27	67.14 ± 3.18	4813.80 ± 247.55	4.03 ± 0.08	2.16 ± 0.09	67.39 ± 3.85	0.73 ± 0.01	1.00 ± 0.04	0.013 ± 0.0009

Values are presented as 3 values mean ± standard deviation

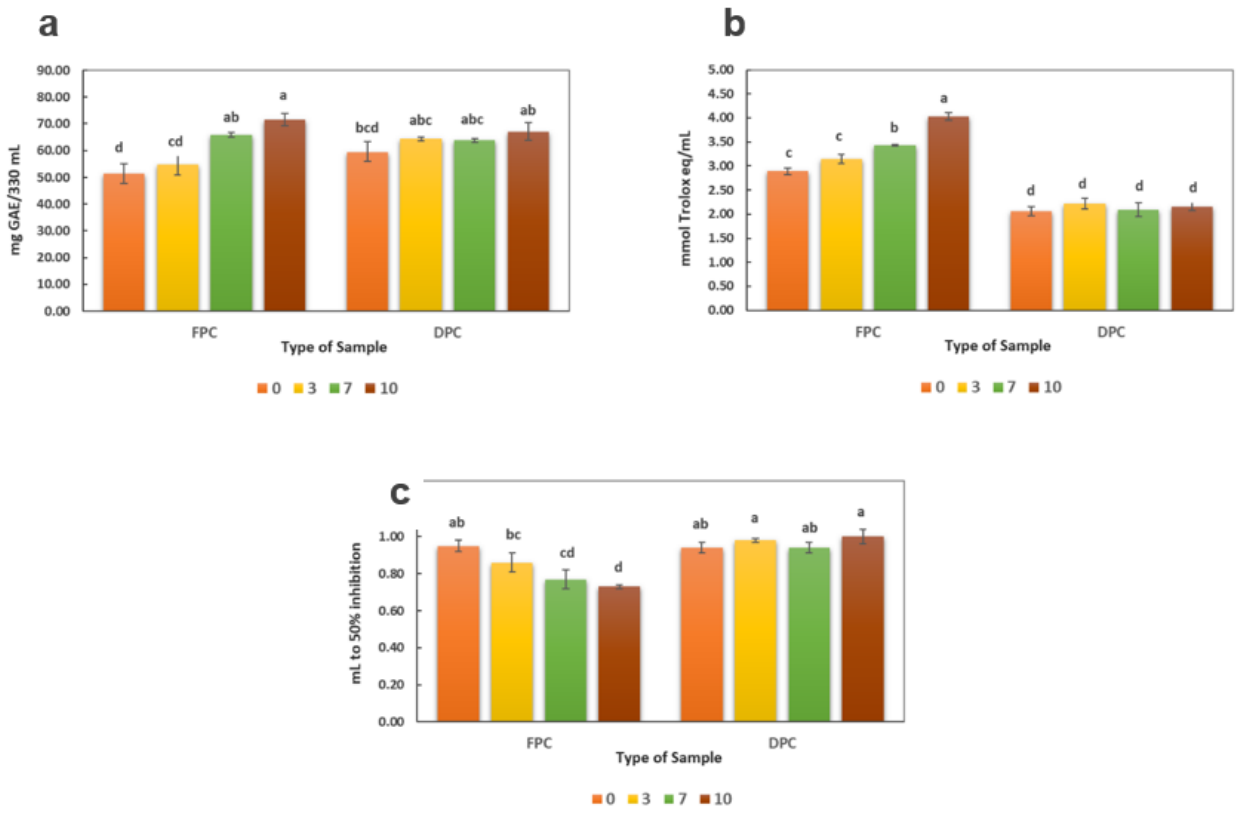

Figure 2. TPC (a), TEAC (b), EC_{50} (c) and values of Fresh Coffee Pulp and Dried Coffee Pulp kombucha over 0, 3, 7, and 10 days of fermentation. Values are presented as 3 values mean ± standard deviation. For the same property, values that do not share a letter show a significant difference ($p>0.05$)

According to **Figure 2**a, FCP showed a significant increase in TPC from 51.45 ± 3.70 mg GAE/330 mL on day 0 to a peak of 71.54 ± 2.27 mg GAE/330 mL on day 10, with particularly notable growth between days 3 (54.79 ± 3.40 mg GAE/330 mL) and 7 (65.83 ± 0.88 mg GAE/330 mL). In contrast, DCP started with a higher TPC (59.39 ± 4.00 mg GAE/330 mL) but increased at a slower rate, peaking at 67.14 ± 3.18 mg GAE/330 mL on day 10, indicating that drying may limit the release or transformation of phenolic compounds during fermentation. Despite this, the t-Student test indicates that there is no significant difference ($p > 0.05$) between the TPC values on day 10 of fermentation among those raw materials.

In terms of TEAC (**Figure 2**b), FCP demonstrated a significant and consistent increase in antioxidant capacity, starting at 2.89 ± 0.07 mmol Trolox eq/mL on day 0 and reaching 4.03 ± 0.08 mmol Trolox eq/mL on day 10. Notably, significant increments were observed between day 3 (3.15 ± 0.10 mmol Trolox eq/mL) and day 7 (3.43 ± 0.02 mmol Trolox eq/mL), resembling the behavior observed for TPC. This trend reflects a robust enhancement in antioxidant activity throughout the fermentation period. In contrast, DCP exhibited minimal improvement in TEAC, beginning at 2.06 ± 0.10 mmol Trolox eq/mL, fluctuating slightly by day 3 (2.22 ± 0.11 mmol Trolox eq/mL), and ending at 2.16 ± 0.09 mmol Trolox eq/mL on day 10. This indicates that the antioxidant potential of DCP is less responsive to fermentation, likely due to a reduced release or transformation of phenolic compounds.

A t-Student test revealed a significant difference ($p < 0.05$) between the mean TEAC values of FCP and DCP, with the TEAC of FCP being 62% higher at the 10-day mark. Heeger and others[13] reported TEAC values for their coffee cascara beverage of 3.02 ± 0.006 mmol Trolox eq/L, which are approximately three orders of magnitude lower than those reported in this study. This substantial variation may be attributed to the use of

dried cascara and the pasteurization process employed in their study (83 °C for 30 minutes), which likely caused a significant reduction in antioxidant activity due to the thermosensitive nature of the bio-compounds that influence this characteristic.

The EC_{50} values (**Figure 2**c) further underscore the differences in antioxidant efficiency between FCP and DCP. FCP exhibited a notable reduction in EC50, decreasing from 0.95 ± 0.03 mL on day 0 to 0.73 ± 0.01 mL on day 10, indicating a substantial improvement in antioxidant efficiency over time. Conversely, DCP displayed fluctuating EC_{50} values, starting at 0.94 ± 0.03 mL on day 0 and increasing to 1.00 ± 0.04 mL on day 10, which suggests a decline in antioxidant efficiency during fermentation. Muzaifa and others[21] reported a percentage inhibition of up to 51.69% for their sun-dried coffee pulp kombucha fermented for 8 days with a starter concentration of 7% (v/v) at full concentration. Despite its higher TPC concentration, their kombucha exhibited lower antioxidant activity compared to the results of the present study.

Overall, FCP outperformed DCP across all evaluated parameters, showing greater increases in TPC and TEAC as well as more efficient antioxidant activity, evidenced by its lower EC_{50} values. These findings highlight the potential of fresh coffee pulp as a more suitable substrate than dried coffee pulp for producing kombucha beverages with enhanced bioactive and antioxidant properties. This superiority becomes even more pronounced when the raw materials are compared on a wet weight basis. According to Rojas-Orduña and others[38], the dehydration process can reduce the weight of fresh coffee pulp by up to 80%. Consequently, for the same weight of coffee pulp on a wet basis, DCP exhibited TPC and antioxidant activity levels that were approximately five times lower.

However, this superiority of FCP is contrasted by the lower ethanol production observed in the fermentation kinetics analysis. This suggests that the dehydration process may alter the raw material in ways that facilitate fermentation but negatively impact the bioactive compounds in the pulp. These changes could be attributed to increased exposure of the pulp to oxygen during drying, as noted by Kieu Tran and others[39] and Wojdyło and others[40]. An alternative improvement might involve dehydrating the coffee pulp using a vacuum oven, as proposed by Kieu Tran and others[39], with careful consideration of the drying temperature to minimize the degradation of bioactive compounds.

3.3 Phenolic Compounds Profile and Caffeine, Theobromine, and Theophylline Determination

Table 2 presents the characterization of FCP, DCP, and BT kombuchas on day 10 of fermentation in terms of phenolic compounds and alkaloids like caffeine, theobromine, and theophylline. Five relevant catechins (Epigallocatechin, Catechin, Epigallocatechin gallate, Epicatechin, Epicatechin gallate) were detected in both coffee pulp and black tea pulp kombuchas. In all cases, the concentration found in the FCP kombucha sample was higher than that found in the DCP kombucha sample, which would indicate that the dehydration process generates this decrease in concentration due to temperature and greater contact with oxygen in the air.

Rutin was detected in all kombuchas, being higher in DCP kombucha (2.9 mg/kg) than in FCP (1.8 mg/kg) this difference has to be linked to the dehydration process as it is the difference with respect to the other raw material. These two values are well below the concentration found in BT kombucha (26.0 mg/kg); however, if compared to other studies that have quantified rutin in tea kombucha, the one presented in this study is even double. Specifically, Ivanišová and others[41] report a value of 13.35 ± 0.22 mg/L of rutin in a kombucha fermented at 22 °C for 7 days that was made with black tea of Indian origin. This difference in rutin values may be linked to the difference in the origin of the tea.

The concentration of all phenolic acids (p-Hydroxybenzoic acid, Caffeic acid, Vanillic acid, p-coumaric acid, Ferulic acid, Rosmarinic acid, Trans-cinnamic acid, Carnosic acid, Ursolic acid) was below the MQL for coffee pulp and BT kombuchas. Although phenolic acids have been reported in a low proportion in kombuchas[41][42],

Sales and others[4] reported an increase in the concentration of these compounds with the passage of time due to the degradation of chlorogenic acids among other compounds. It could have happened in this case as well, but on the final day of fermentation (day 10) it was not enough to be detected in the phenolic compound profile quantification assay.

Table 2. Retention times (t_R), minimum quantification level (MQL), and results of analysis of phenolic compounds in kombuchas from FCP, DCP, and BT, analyzed by UHPLC-ESI+/--ORBITRAP-HRMS

Compound	t_R[min]	MQL [mg/kg]	Concentration [mg/kg]		
			FCP	DCP	BT
Epigallocatechin (EGC)	3.4	< 0.1	3.0	1.3	4.1
Catechin (C)	3.5	< 0.1	1.1	0.5	2.2
Epigallocatechin gallate (EGCG)	3.8	< 0.2	6.7	1.0	22.2
Epicatechin (EC)	4.0	< 0.1	3.6	1.8	6.8
Epicatechin gallate (ECG)	4.4	< 0.1	2.2	0.2	21.7
Rutin	5.2	< 0.1	1.8	2.9	26.0
p-Hydroxybenzoic acid	3.5	< 0.4	< 0.1	< 0.1	< 0.1
Caffeic acid	3.9	< 0.1	< 0.1	< 0.1	< 0.1*
Vanillic acid	4.4	< 0.1	< 0.1	< 0.1	< 0.1
p-coumaric acid	4.5	< 0.1	< 0.1	< 0.1	< 0.1
Ferulic acid	5.2	< 0.1	< 0.1	< 0.1	< 0.1
Rosmarinic acid	5.2	< 2.5	< 2.5	< 2.5	< 2.5
Trans-cinnamic acid	5.9	< 0.4	< 0.4	< 0.4	< 0.4
Carnosic acid	8.1	< 0.4	< 0.4	< 0.4	< 0.4
Ursolic acid	9.3	< 0.1	< 0.1	< 0.1	< 0.1
Luteolin	6.2	< 0.1	< 0.1	< 0.1	< 0.1*
Quercetin	6.0	< 0.1	< 0.1*	< 0.1*	0.7
Pinocembrin	7.0	< 0.1	< 0.1	< 0.1	< 0.1
Naringenin	6.0	< 0.1	< 0.1	< 0.1	< 0.1
Apigenin	6.5	< 0.1	< 0.1	< 0.1	< 0.1
Pelargonidin 3-glucoside	4.0	< 0.1	< 0.1	< 0.1	< 0.1
Caffeine	3.8	< 0.1	75.0	80.4	920.8
Theobromine	2.9	< 0.1	1.2	0.4	47.7
Theophylline	3.4	< 0.1	< 0.1*	< 0.1	< 0.1

*Detected below the minimum level of quantification and above the minimum detection level (MDL) of the method used.

Other important flavonoids such as luteolin and quercetin presented values above the MDL and below the MQL. In the case of luteolin in FCP and DCP kombuchas, it was not possible to reach the MDL. However, in BT kombucha, the detection level was reached but not the quantification level. On the other hand, in the case of quercetin, coffee pulp kombuchas reached the detection level but not the quantification level, while BT kombucha presented a value of 0.7 mg/kg. This value agrees with those reported by Vázquez-Cabral and others[43] who compare kombuchas brewed from oak leaves against BT kombucha and report a concentration of 0.708 ± 0.05 mg/L in the latter mentioned beverage.

Finally, in the case of alkaloids determination, the concentration of caffeine was, in contrast, higher in the DCP kombucha (80.4 mg/kg) than in the FCP kombucha (75.0 mg/kg) and much higher in the BT kombucha (920.8 mg/kg). These results vary from those obtained by Sales and others[4], who found a similar concentra-

tion of caffeine in kombuchas made with coffee pulp and black tea (16.9 and 18.2 mg/100 mL, respectively), on the last day of fermentation (day 9), and may be linked to the origin of the tea. Theobromine concentration is also higher in BT kombucha (47.7 mg/kg) than in coffee pulp kombucha; however, it presents a higher concentration in FCP kombucha (1.2 mg/kg) than in DCP kombucha (0.4 mg/kg), which could be explained by its reactivity profile[44], indicating that it may be sensitive to light or to acidic or basic conditions. These conditions could have occurred during the dehydration process. The concentration of theophylline was in the case of all three kombuchas below the MQL, in the case of BT and DCP kombuchas, below the MDL. According to the above and considering the results of the determination of antioxidant activity, a relationship can be found between the antioxidant activity of the kombuchas and their concentration of phenolic compounds. Thus, when a kombucha has a high antioxidant activity, it can be expected that the concentration of its phenolic compounds is also high.

3.4 Soluble Solids and pH

Figure 3 shows the behavior of pH (A) and soluble solids concentration (B) over the days of kombucha fermentation. Data was also taken for the pH of the infusion of each raw material, the data are 4.92 ± 0.01, 4.58 ± 0.05, and 4.84 ± 0.01 for FCP, DCP, and BT infusions, respectively. The FCP kombucha shows a lower initial value (3.04 ± 0.01) than the other two beverages, then continues to decrease with time and at day 10 it presents a pH of 2.67 ± 0.02. The DCP kombucha presents an initial value of 3.45 ± 0.04 and is the one that varies the least with time, arriving at day 10 with a pH of 3.21 ± 0.08. In comparison, BT kombucha has the highest initial pH (3.80 ± 0.05) and also shows the most variation throughout fermentation, reaching a pH of 2.98 ± 0.01 on day 10. The pH values agree with those reported by Sales and others[4], who also made kombucha from coffee pulp. The pH values in their infusions were between 5.5 and 4.3 and immediately after adding the starter they dropped to 3.7 and 3.8. The study by Sales and others[4] also presents the phenomenon that the pH of BT kombucha has greater variation than the pH of coffee pulp kombuchas. This could be an indication that there is some affectation for SCOBY microorganisms produced by coffee pulp. Muzaifa and others[21] also reported pH values of kombucha made from sun-dried coffee pulp, with pH values between 3.1 and 2.8 for 7% v/v starter concentration and 8 and 12 days of fermentation, respectively. These values are similar to the final values determined in the present study. Low pH in kombuchas also plays an important role in maintaining conditions unsuitable for other types of undesirable microorganisms that can become pathogenic for humans[10].

The concentration of soluble solids, on the other hand, shows particular behaviors for each raw material. In the case of kombucha FCP, the trend over the days is decreasing even though the value of the final day is higher than the initial day (10.93 and 11.33 °Brix for the initial and final day, respectively). This indicates that sugar consumption is very slow, so the concentration of SCOBY microorganisms would be expected to be lower compared to the other kombuchas, therefore reaffirming the results of the kinetic analysis of sugar consumption and ethanol production. In the case of the DCP kombucha, this is the one that starts with the lowest value of soluble solids (8.97 °Brix) and until day 3 presents an increasing trend. After this day, it begins to decrease at a constant rate until it reaches a value of 7.10 °Brix on the final day of fermentation. BT kombucha starts with the highest soluble solids value (11.30 °Brix) and maintains a constant decrease throughout the fermentation days until reaching 5.30 °Brix on day 10. Sales and others[22] report values between 11.6 and 9.9 °Brix for coffee pulp kombuchas between the initial and final day, respectively, which corresponds to a slow decrease similar to that shown by FCP and DCP kombuchas. On the other hand, that study also reports a decrease in soluble solids for black tea kombucha from 10.4 to 9.3 °Brix between the initial and final day, respectively. The black tea kombucha in the study by Sales and others[22] also shows the lowest value of the three kombuchas on the last day. This could indicate that some properties of black tea kombucha allow sugar consumption reactions to develop better.

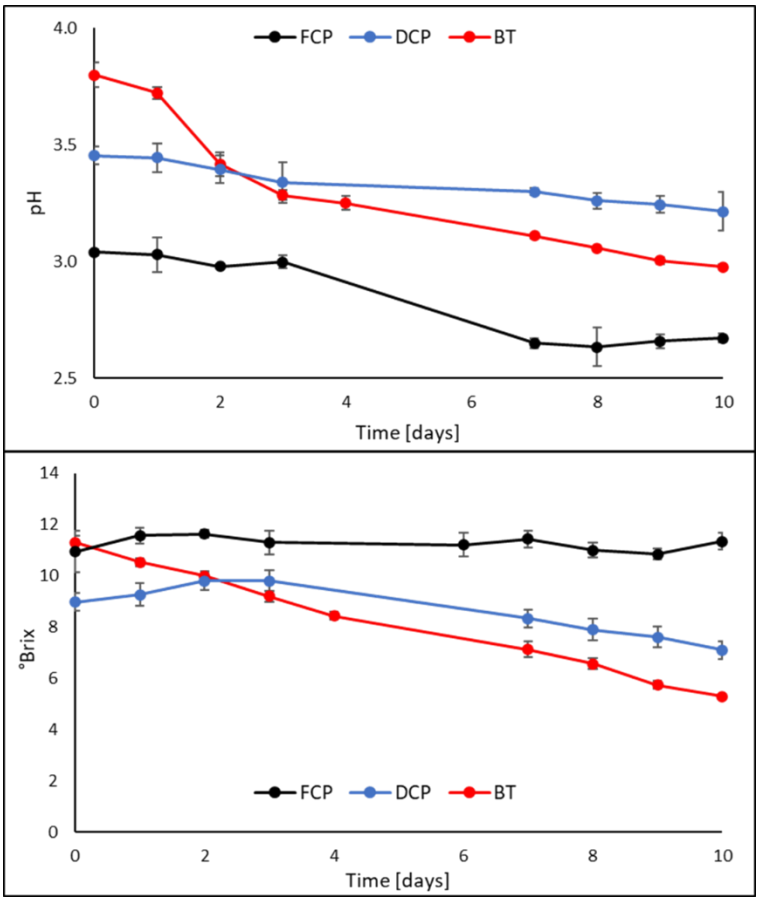

Figure 3. Variations in pH (A) and soluble solids content (°Brix) (B) of Fresh Coffee Pulp (FCP), Dried Coffee Pulp (DCP), and Black Tea (BT) kombuchas over 10 days of fermentation

3.5 Microbiological Analysis

Table 3 shows the results of the microbiological analysis. The kombucha samples in this study comply with the Colombian standard and do not show the presence of pathogenic microorganisms. The concentration of *E. coli* is less than 10 CFU/mL of kombucha; the concentration of yeasts was higher for FCP (1000 CFU/mL) and BT (1500 CFU/mL) kombuchas compared to DCP (<100 CFU/mL). This could be due to the fact that fresh pulp and tea have a higher concentration of phenolic compounds, some of which favor the growth of microorganisms such as yeasts, as mentioned by Zailani & Adnan[45], and Antolak and others[46]. Nevertheless, the three beverages are within the limits allowed by the standard; the concentration of molds, on the other hand, was less than 100 CFU/mL in the three beverages. Finally, the absence of *Salmonella* spp. is reported, so kombucha consumption does not represent a health hazard to consumers. In some cases, kombucha producers choose to pasteurize the beverage to ensure it is free of pathogenic microorganisms. However, Içen and others[5] report that kombucha has demonstrated significant antimicrobial capacity, not only thanks to the raw materials used in the preparation, but also to the bacteria and yeasts present in the SCOBY. According to these statements and the results of the microbiological analysis of this study, the kombucha obtained in this research can be offered to consumers without the need for pasteurization.

Table 3. Microbiological analysis results of Fresh Coffee Pulp (FCP) and Dried Coffee Pulp (DCP) kombucha as well as Black Tea (BT) kombucha at the 10th day of fermentation

Analysis	Result		
	FCP	DCP	BT
Salmonella spp.	Absent	Absent	Absent
Escherichia coli	< 10 CFU/mL	< 10 CFU/mL	< 10 CFU/mL
Yeast	1000 ± 2 CFU/mL	< 100 CFU/mL	1500 ± 2 CFU/mL
Molds	< 100 CFU/mL	< 100 CFU/mL	< 100 CFU/mL

3.6 Market Analysis

The mass-produced kombucha market is young, with about 24 years of existence. Euromonitor reports that in the United States the large-scale kombucha market starts in 1999 with GT's Kombucha[47]. However, the best documented market movements are from the second half of the 2010's onwards. Kombucha is a beverage that has been gaining a lot of popularity in this new century thanks to its beneficial health properties. Consumers have started to become more and more conscious of what they drink and to change their lifestyle to one in which products of natural origin are much more important[48]. It is thanks to this behavior that the kombucha market has grown to USD 3 billion by 2023 and is estimated to grow steadily (17.14% of Compound Annual Growth Rate) over the remainder of the decade to USD 11.2 billion worldwide, according to Vantage Market Research[32].

Currently, the North American market is the biggest in the world and is divided into GT's®, KeVitta® (Pepsi Company), Health-Ade (Coca Cola Company), and Brew Dr Kombucha® major distributors, but Pilot Kombucha®, Kombucha®, and Tonica Kombucha® also have an important space[49]. **Figure 4** shows the percentages of brand share in the North American market. Furthermore, in Europe the two predominant brands are Captain Kombucha® (Portugal) and KomVida® (Spain). Another large market is Oceania, Australia being the largest. The leading brands in this region are Good Brew® (Australia), Tea Gardens® (Australia), and Remedy Drinks® (Australia). Finally, in Asia, the trend is more inclined to small locals, who produce their kombucha on a small scale and sell it locally[33].

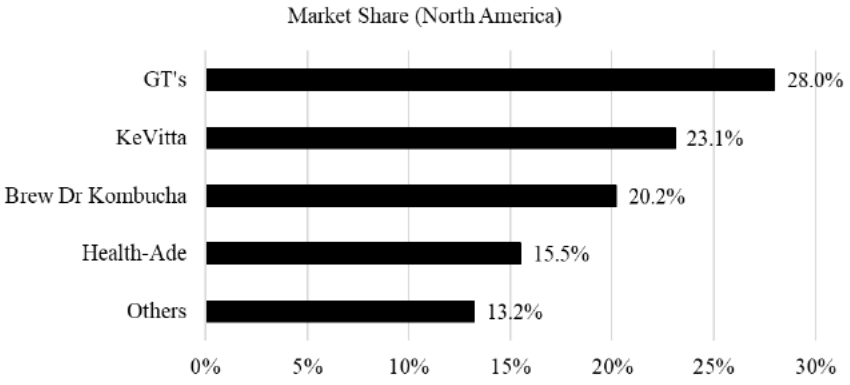

Figure 4. Market share of the largest kombucha brands in North America

The kombucha market is designed for a young type of customer, and most of the marketing is done to convince the consumer that it is a refreshing and healthy beverage. The kombucha market is also characterized by having a wide variety of flavors for the consumer to choose from, most of which are aromatic plants and fruits; there is not much preference for traditional flavors[48]. In the context of innovative product design, there are some important key concepts that should be considered in order to design a label that is eye-catching to

consumers. The use of appropriate colors and eye-catching typography are key factors when the consumer sees the product on the shelf[50]. This is why a label in **Figure 5** was designed with Colombian standards for a potential kombucha made with coffee pulp. The label is designed with colors such as yellow and orange that evoke freshness, acidity, and joy. The typography is calm, stylized, and youthful, as it is aimed at an audience with these characteristics. The flavoring was chosen due to the trend of having flavored kombucha in the market, and the passion fruit flavor was selected because this fruit is mainly acidic and connects quite well with the intention of being a refreshing drink. The label presents a possible nutritional table for the product and the health indications for being a beverage with an alcohol content of less than 2.5%.

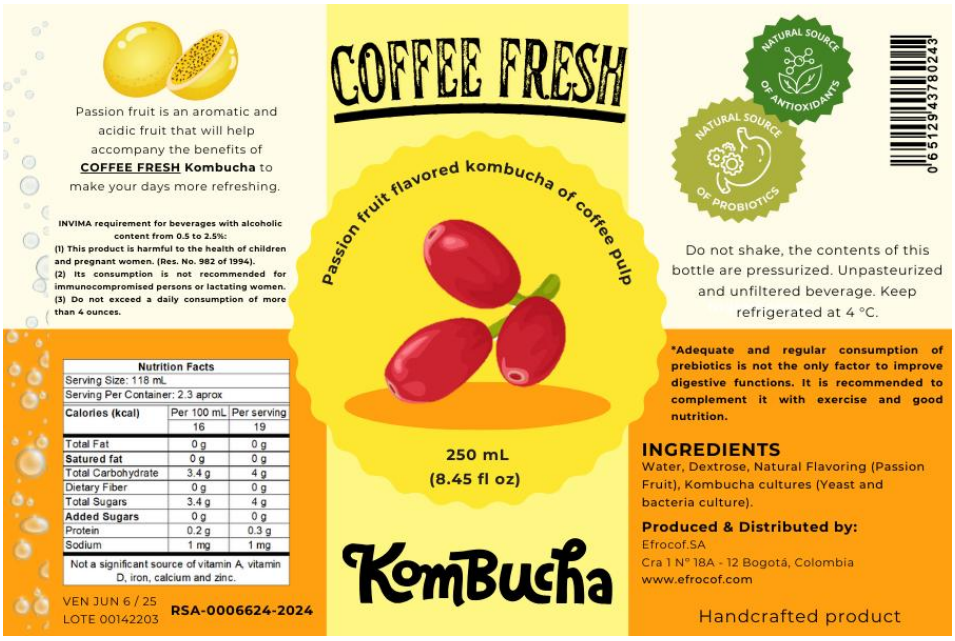

Figure 5. Label proposal for a potential kombucha type product produced with coffee pulp

The label was designed for a young-consuming public destined product, in the trend of flavored kombuchas.

4. Conclusions

Kombuchas made from fresh and dried coffee pulp were compared. The results showed that kombucha made from fresh coffee pulp had a higher antioxidant capacity (TEAC, 4.03 ± 0.08 mmol Trolox eq/mL; EC_{50}, 0.73 ± 0.01 mL for 50% inhibition) compared to that made from dried pulp (TEAC, 2.16 ± 0.09 mmol Trolox eq/mL; EC_{50}, 1.00 ± 0.04 mL for 50% inhibition) after 10 days of fermentation. This finding was corroborated by phenolic compound profiling, which revealed a higher concentration of catechins in the fresh coffee pulp kombucha than in the dried coffee pulp kombucha. Additionally, kombucha made from fresh pulp produced less ethanol and consumed less sugar than that made from dried pulp. This suggests that further investigation is needed into the effects of vacuum-drying coffee pulp at medium temperatures to potentially combine the strengths of both fresh and dried pulp.

The soluble solids content of fresh coffee pulp kombucha remained relatively stable, around 11.30 °Brix, on the last day of fermentation. In contrast, the dried coffee pulp kombucha exhibited a decrease in soluble solids from the third day of fermentation, reaching 7.10 °Brix by the tenth day. Black tea kombucha showed the most rapid and significant decrease, dropping to 5.30 °Brix on the last day of fermentation. Microbiological analysis

confirmed that all three beverages are safe for consumption without pasteurization, as *Salmonella spp*. was absent, and the concentrations of *E. coli*, yeasts, and molds were within the limits set by Colombian standards.

A market study indicated that the kombucha industry is promising, with market size expected to grow steadily until at least 2030. This research successfully developed a novel approach for valorizing coffee pulp, providing coffee growers in Colombia and globally with an environmentally friendly method to reduce waste and generate alternative income streams beyond coffee production.

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Transparency of data

Available data: The final results of this study were published in the article itself. Entire data is available if needed.

Author contribution statement

ERO: Conceptualization; Investigation; Writing – original draft

MHC: Validation; Formal Analysis; Writing – review & editing

ASC: Conceptualization; Project administration; Writing – review & editing, Resources

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