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Controlled fermentation in artisanal bioreactors to produce specialty coffees

Fermentação controlada em biorreatores artesanais para a produção de cafés especiais

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ABSTRACT

A model of an artisanal bioreactor was tested for fermentations in specialty coffee production, monitoring parameters such as Brix, temperature, pH, electrical conductivity, and total dissolved solids. The results demonstrated that the bioreactor effectively controlled these parameters, ensuring consistent processes and reproducibility, as well as uniformity in the final product. Both washed coffees, one with lactic acid bacteria (LAB) inoculation and one without, were produced and evaluated using the Cup of Excellence (COE) format. The coffee without inoculation scored 84 points, while the coffee with LAB scored 85.9 points. This highlights a significant positive correlation between LAB treatment and the sensory quality of the coffee produced.

Index terms: Lactic acid bacteria; sensory profile; inoculation.

RESUMO

Um modelo de biorreator artesanal foi testado para fermentações na produção de café especial, monitorando parâmetros como Brix, temperatura, pH, condutividade elétrica e sólidos totais dissolvidos. Os resultados demonstraram que o biorreator controlou efetivamente esses parâmetros, garantindo processos consistentes e reprodutibilidade, bem como uniformidade no produto final. Ambos os cafés lavados, um com inoculação de bactérias lácticas (LAB) e outro sem, foram produzidos e avaliados usando o formato Cup of Excellence (COE). O café sem inoculação obteve 84 pontos, enquanto o café com LAB obteve 85,9 pontos. Isso destaca uma correlação positiva significativa entre o tratamento com LAB e a qualidade sensorial do café produzido.

Termos para indexação: Bactérias de ácido lático; perfil sensorial; inoculação.

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Introduction

Coffee is one of the few food products worldwide where fermentation is mostly carried out spontaneously (De Carvalho et al., 2020). However, to meet the demand of markets that seek coffees with different sensory profiles, producers have been developing new post-harvest practices focused on fermentation (De Carvalho et al., 2018; Da Mota et al., 2020; Pereira et al., 2020). Their goal has been to enhance the sensory quality of the coffee and improve its final score, aiming to achieve a better market position and higher product valuation.

This has encouraged the use of various tanks for coffee fermentation, as post-harvest processes have evolved, revealing the need to conduct the fermentation stage in a controlled manner (Bressani et al., 2021; Brioschi Junior et al., 2021). By primarily using starter cultures, this stage has been identified as promising for flavor development, the creation of volatile compounds, and product uniformity (Elhalis, Cox, & Zhao 2020; Pereira et al., 2020).

Although fermentation is related to the improvement of food safety by inhibiting the growth of pathogens and spores, different studies have reported the diversity of filamentous fungi found in coffee beans (Batista et al., 2009; Rezende et al., 2013; Santiago, 2020). A key variable is the initial microbial load (Bourdichon et al., 2012; Mokoena, 2017; Xiang et al., 2019). Thus, to obtain the desirable properties of fermented plant products, it is necessary to control both the fermentation conditions and the initial microbial load (Rodríguez et al., 2009; Parra, 2010; Di Cagno et al., 2014).

A bioreactor is a device that provides a controlled environment during the cultivation process, either in a solid or liquid state (Ruíz et al., 2007), so its use ensures optimal conditions of nutrient and oxygen transfer through stirring, aeration, temperature, and pH controls (Singh, Kaushik, & Biswas, 2014). Stainless steel bioreactors are often used in industry for controlled fermentation (De Carvalho et al., 2020); however, they are usually expensive, which means that the cost of processing coffee with them is up to 3.5 times higher than with a tank for spontaneous fermentation (De Carvalho et al., 2020).

This study aimed to test an artisanal bioreactor model for producing specialty coffees. It assessed the bioreactor's performance in controlling fermentation process parameters and its impact on the quality of the coffee produced.

Material and Methods

The experiment was carried out in San Cristobal de las Casas, in the facilities of Cafeología.

Design and operation of bioreactors

The study used 30 L high-density polyethylene barrels with lids (Figure 1). Polyethylene is a recyclable material approved by both the Food and Drug Administration (FDA) and the European Union (Oliveros et al., 2018). Although some studies report the formation of biofilms with repeated contact with food, these biofilms are indicative of spoilage, can lead to secondary fermentation, and result in additional over-oxidation (Yun, Kim, & Lee, 2019). These containers were chosen because they are the most used by producers. A high-pressure 12-volt diaphragm pump with a pressure switch of 4.5 L/Min 1.2 GPM 110 PSI was used for agitation, and an external circuit was used for mixing and circulating fluids.

Gas output was controlled by a pressure gauge and a brass pressure relief valve. *The temperature was controlled using a thermic well connected to the reactor vessel* that was kept in contact with the coffee mass. In turn, this was connected to a 1000 W 30–150 °C insulated silicone belt heater with a thermostat. *A 1/2"* PVC ball faucet connected to the vessel was installed for sampling collection.

Isolation and culture of microorganisms

To isolate lactic acid bacteria (LAB), coffee cherries were cultured in selective Man, Rogosa, and Sharpe (MRS) agar and were incubated in anoxia for 48 h at 32 ˚C. Then, three consecutive isolates of bacterial colonies were performed, maintaining the same incubation parameters and using MRS. LAB purity and identification were evaluated according to Walstra et al. (2001). Although several genera of lactic bacteria grew in the MRS medium, only three isolates identified as *Lactobacillus* were selected.

Biochemical identification of LAB according to the type of fermentation produced

The three selected isolates considered positive indicators of CO_2 production were transferred to a liquid MRS culture medium with a Durham tube and incubated at 30 °C for 48 h to evaluate bubble formation. Biochemical identification was carried out using an API 50 CH (BioMerieux) system. For application, LABs were cultivated at 30 ˚C in MRS broth until reaching a concentration of 10^9 cells·mL^{-1}. After growth, the bacterial suspension was centrifuged (4000 rpm for 10 min) and then resuspended in 500 mL of distilled water. This solution was inoculated in coffee (Figure 2), reaching a final concentration of approximately 10^7 cells·mL^{-1} according to the methodology by Cassimiro et al. (2022); at the end of the fermentation, 370x10^5 CFU/ml were reported.

Coffee

Bioreactor fermentation was evaluated using a mixture of coffee beans (varieties Typica 30%, Bourbon 35%, and Caturra 35%) grown at 1500 m. According to Lopez-Garcia et al. (2016), these are the main varieties cultivated in Mexico and involved in the international coffee trade. To discard damaged and rotten cherries, a hydraulic classification of coffee cherries was performed; to this end, clean, non-recirculated water was used (1.6 L/kg cherries) as proposed by Puerta (2015).

Coffee fermentation

The controlled fermentations of coffee cherries were discontinuous (in batches) and immersed in a closed system; this technique is used in processes in which water-soluble materials are used for microbial growth and biomass multiplication (Subramaniyam, & Vimala, 2012). Closed bioreactors were used that contained 18 kg of whole coffee cherries and 5 L of water. Coffee cherries were disinfected, and the compound was previously diluted in water using serial dilutions of 1/10.

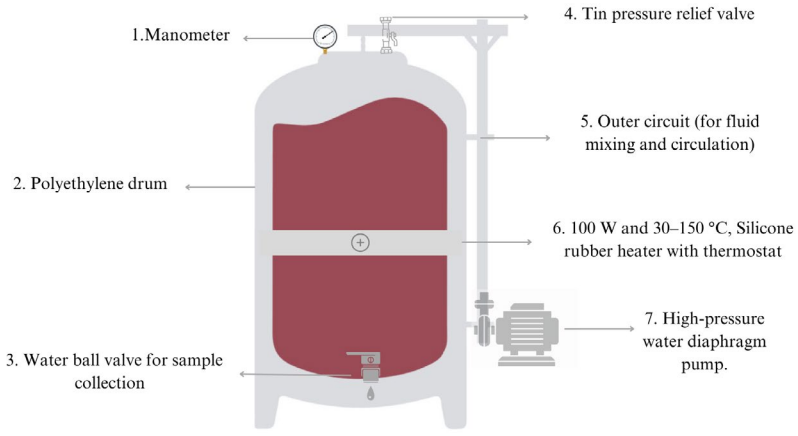

Figure 1: Artisanal bioreactor components.

From this solution, 0.001% was used for 15 minutes. The fermentation processes were carried out with inoculation of lactic acid bacteria (LAB) and without inoculation (Control), all in quadruplicate. The first fermentation phase was carried out with whole coffee cherries and stopped when pH reached 3.5 of the liquid fractions. Then, coffee beans were depulped and returned to the tanks for the second fermentation phase until reaching a pH of 3.5.

Fermentation monitoring

Fifteen minutes after fermentation started, the following process parameters were assessed: inner medium pressure, pH, electrical conductivity (EC), total dissolved solids (TDS), percent sugar (degrees Brix), and temperature (°C). The solution was mixed, for 20 minutes every 4 hours (5 L min^{-1}). The stirring system provided homogenization to maintain uniform conditions of temperature and distribution of the microbial concentration (Rodríguez, Cabrer, & Valencia, 2003). The inner pressure was maintained at 3 psi. Samples were collected every 8 hours during the fermentation phases for a total of 18 samples per reactor, a total of 144 hours of fermentation.

Sensory analysis

After drying, the samples were sent for tasting by panels of 5 expert tasters. The following attributes were evaluated: clean cup, sweetness, aroma, mouthfeel, taste, aftertaste, balance, and general impression. Attributes were scored on a 0-to-8 scale. Subsequently, each taster obtained the raw score (8 attributes, with 8 being the maximum score for each attribute), to which 36 points were added for a total score of 100 according to the Cup of Excellence protocols (Cup Of Excellence - COE, 2020). Depending on the final score, as per COE (2020) protocols, coffee is classified as Quality Award (final score of at least 90 points), Specialty Coffee (final score between 84 and 89 points), and Standard Coffee (score between 82 and 84 points).

Variables evaluated

The variables evaluated during the fermentation process were the following: V1: initial electrical conductivity; V2: initial TDS; V3: initial pH; V4: degrees Brix; V5: duration of fermentation; V6: fermentation temperature; and variables related to physical and sensory testing: V7: % Humidity; V8: WA (water activity); V9: clean cup; V10: sweetness; V11: acidity; V12: mouthfeel; V13: taste; V14: aftertaste; V15: balance; V16: taste score.

Statistical analysis

We used a completely randomized experimental design with the same number of observations (n = 8**),** two treatments: control (spontaneous fermentation) and LAB inoculation, and four replicates. Differences in the mean total score between treatments were evaluated using an analysis of variance (ANOVA). Also, a principal component analysis (PCA) was performed for 14 variables (variables 5 and 6 were excluded from this analysis because their values were constant during fermentation). These variables have different scales, so the components were defined based on a correlation matrix. The Bartlett test was used to evaluate the suitability of the proposed model, indicating that the data are adequate for a PCA (χ^2 = 384.87; df = 91; $P < 0.0001$).

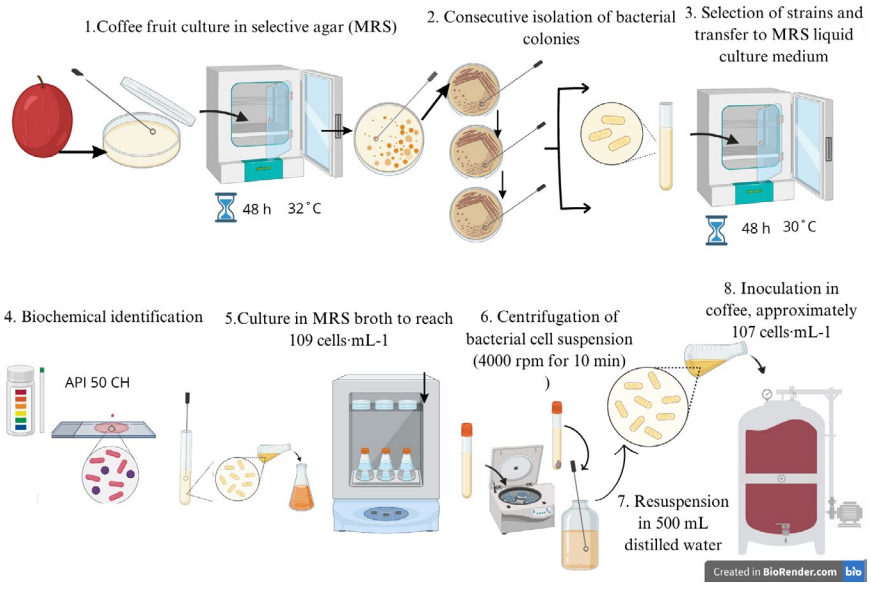

Figure 2: Isolation of lactic acid bacteria and inoculation in coffee fruits.

A linear mixed model (LMM) was performed for CE and TDS. Treatments were the fixed factor, and time was nested in the replicate (1 replicate/time) to consider multiple evaluations over time and avoid pseudo-replicates in the model. The model assumptions were evaluated by introducing all the proposed variables in the different contexts and testing the significance of the fixed effects with Wald's Chi-square test (type II). Generalized linear mixed models (GLMM) were constructed for pH and degrees Brix. The model structure was similar to that used in the LMM, except for the error distribution family, Gamma, in this case (a distribution suitable for continuous data where variance increases with the square of the mean).

All analyses were performed in R (4.3.0) and RStudio (version 2023.06.0+421) (R Core Team, 2023) using the packages lme4 (Bates et al., 2015), car, Dharma (Hartig, 2021) and ggplot2 (Wickham, 2016) for graphic design.

Results and Discussion

Analysis of coffee cup quality

The results of the sensory evaluation show a homogeneous differentiation between the treatment with lactic acid bacteria (LAB) and the Control in all attributes (Figure 3). This is mainly due to the adequate control of the main variables in the fermentation process with the bioreactor.

The analysis of variance (Table 1) determined no statistically significant differences between treatments.

However, the sensory evaluation showed differences of almost two points between the Control and the LAB treatment. The control group yielded a standard deviation of 0.55 of the mean for the cupping score, along with the following notes: ripe fruit, dry fruits, chocolate, molasses, hazelnut, butter, honey, seed fruits, brown sugar, and apple; in addition, the sample taste was markedly acidic. For the LAB treatment, the standard deviation was 1.25 in the tasting score from the four replicates evaluated, finding the following taste notes: caramel, chocolate, hazelnut, milk caramel (*dulce de leche*), vanilla, apple, syrup, peach, brown sugar, honey, lychee, and molasses; some samples in this treatment were slightly astringent.

In a lactic acid fermentation study in a stirring tank bioreactor developed by De Carvalho et al. (2018), the authors found caramel notes, an intense 'citrus' perception, and fruity notes, which coincide with the taste notes found in this controlled fermentations trial. Similarly, Pereira et al. (2016) found that lactic bacteria such as *Lactobacillus plantarum* significantly increase the formation of volatile aromatic compounds during fermentation, producing beverages with distinctive sensory notes and a marked increase in quality. In addition, studies about the metabolism of lactic acid bacteria to form esters of ethyl acetate, isoamyl acetate, propyl acetate, ethyl hexanoate, and n-butyl acetate indicated that the production of esters contributes to exotic sensory notes in coffee beverages (Evangelista, 2014a; Evangelista et al., 2014b; Pereira et al., 2015).

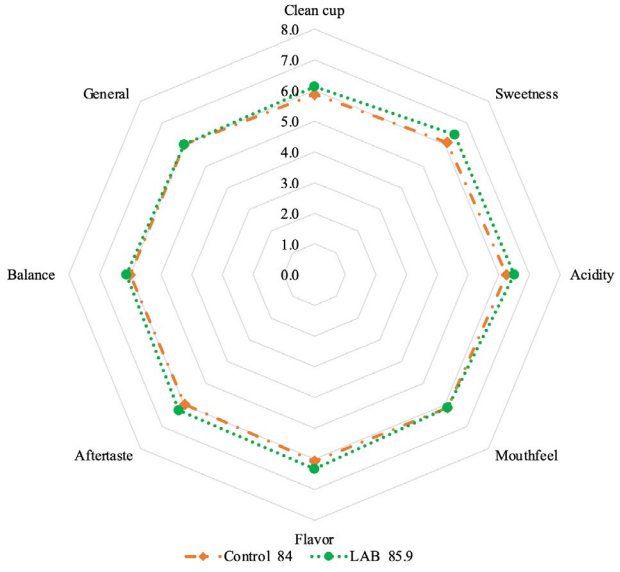

Figure 3: Sensory evaluation of fermentations controlled in the bioreactor, with attributes scored on the 0-to-8 scale.

Regarding the control of the key parameters in the fermentation process and the microbial load, this study confirmed that the use of bioreactors helps to standardize some notes detected in all of them. The replicates of each treatment showed a similar behavior of the variables. This is attributed to the control achieved with the bioreactor, which helped reproduce the fermentation processes in the replicates.

The degrees Brix and pH showed a slight increase at the beginning of the fermentation, followed by a marked decrease 72 hours later due to the completion of the first fruit fermentation phase when coffee was depulped. The subsequent samples showed a slight increase in pH and degrees Brix followed by a decrease in the latter.

Table 1: Analysis of variance of controlled fermentation treatments.

Source of the variations	Degrees of freedom	Sum of squares	Mean squares	Calculated *F*	Pr (>*F*)
Between groups	1	6.0378125	6.0378125	4.981573601	0.067086276
Within groups	6	7.272175	1.212029167		

Significance codes: 0 *** 0.001 ** 0.01 * 0.05 " 0.1 ' ' 1

Confidence intervals were used to determine the ranges of differences and whether they were significant from a practical perspective. In the case of the degrees Brix, the LAB and Control treatments showed no statistically significant differences (Figure 4) in 144 hours the fermentation. This implied a similar mucilage breakdown, since approximately 60% of sugars are used as a carbon source for microbial growth during the fermentation process (Elhalis, Cox, & Zhao, 2020; 2023). The reduction of sugars during fermentation is accompanied by the accumulation of acids, such as lactic, acetic, and succinic acids, as reported in different studies (De Carvalho et al., 2017; De Carvalho et al., 2018; Elhalis, Cox, & Zhao, 2020). Therefore, pH is an important variable that helps to certify the acidification process.

Regarding the pH, differences were found between the LAB and the Control treatments. The LAB treatment showed an initial pH of 4, which was lower than the control, which had a pH of 4.5. This is because the LAB fermentation mechanism of action involves different factors: the obstruction of the maintenance of the cell membrane potential, which inhibits the active transport, reducing intracellular pH and different metabolic functions and producing an unfavorable environment for other microorganisms, since lactic acid bacteria can survive and grow at a relatively low pH, unlike other microbial groups (Vásquez, Suárez, & Montoya, 2009). Another benefit of lactic fermentation is improved food safety by inhibiting pathogens and spore growth (Bourdichon et al., 2012; Sabo et al., 2014; Zapaśnik, Sokołowska, & Bryła, 2022). Likewise, a study by De Carvalho et al. (2020) recorded a decrease in pH throughout the fermentation process: the pH decreased along with a gradual increase in lactic acid concentration. On the other hand, Jackels and Jackels (2005) state that the acidification process promotes pectin breakdown (the main carbohydrate polymer in coffee mucilage), contributing to removing the mucilage layer of the fruit and the drying of grains (Germane et al., 2015; Kim et al., 2016; Pereira et al., 2017).

Electrical conductivity and total dissolved solids (TDS) were monitored throughout the fermentation process to check the functioning and repeatability associated with the use of the bioreactor. A higher electrical conductivity and TDS concentration were observed in the first phase, followed by a sharp decrease at 72 h due to the completion of the first fermentation phase; then, a slight increase in parameter values was recorded again during the second fermentation phase.

The LAB treatment showed a higher mean electric conductivity than the Control (Figure 5a). This rise in electric conductivity during fermentation confirms that it is largely due to the rupture of the cell walls of fermented fruits, as reported by Zambrano et al. (2010). Also, Ouyang et al. (2002) and Fessel et al. (2006) pointed out that, in immersion fermentations, water absorption by seeds during imbibition occurs along with a rapid release of solutes such as sugars, organic acids, ions, amino acids, and proteins to the surrounding environment. In this sense, the amount of ions that leach is inversely proportional to the integrity of the gran cell membranes (Aramendiz, Cardona, & Alzate, 2017).

In coffee, the mucilage soluble solids mainly contain sucrose, glucose, and fructose; malic, lactic, acetic, succinic, oxalic, formic, phosphoric, and galacturonic acids; ethanol and other alcohols; esters, polysaccharides, proteins, and ashes (Puerta, 2012). TDS are one of the most relevant parameters in anaerobic digestion due to their movement, ease of dissolution, transport of nutrients, and bacterial growth (Sadaka & Engler, 2003). The results of this study showed differences between the LAB and Control treatments (Figure 5b), with an increase in TDS concentration due to the dilution of compounds and consumption of soluble substrates by microorganisms (Soria et al., 2001).

Monitoring variables such as pH, degrees Brix, electrical conductivity, and TDS provided a better understanding of fermentation processes in coffee beans.

Figure 6 shows that the Control treatment component was not correlated to any variables, while the LAB treatment component is correlated to physical and sensory variables (V15; V7; V9; V14; V16; V10; V8; V13; V11): balance, % humidity, clean cup, aftertaste, tasting score, sweetness, WA (water activity), taste, and acidity. Similarly, a strong and positive correlation was found between these variables. Likewise, a strong and positive

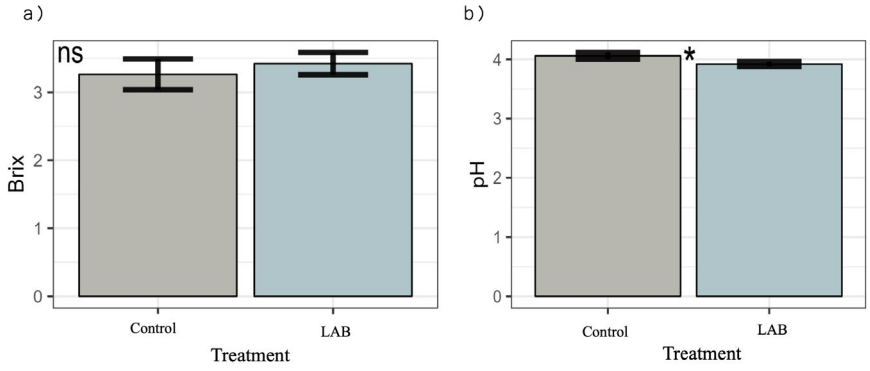

Figure 4: A) Degrees Brix (mean ± standard error) according to the Control and LAB treatments (GLMM: $\chi^2 = 1.03$; df = 1; $P = 0.30$). ns = not significant. b) pH (mean ± standard error) according to Control and lactic acid bacteria treatments (GLMM: $\chi^2 = 7.85$; df = 1; $P = 0{,}005$). * Indicates differences between treatments.

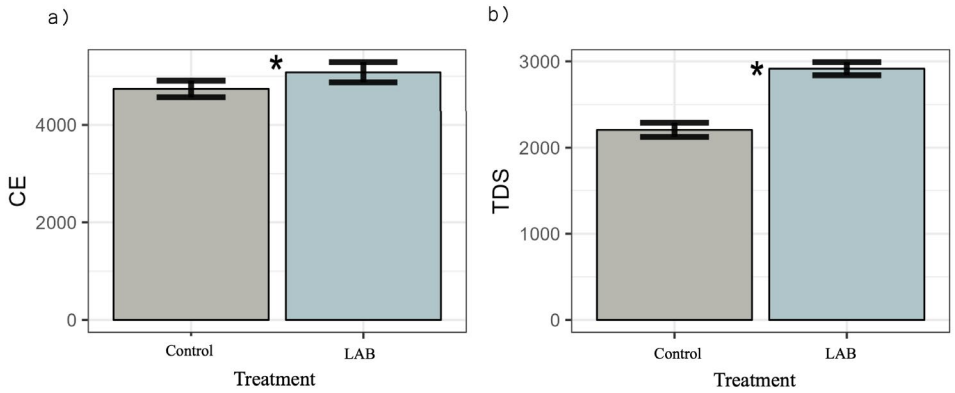

Figure 5: A) CE (mean ± standard error) according to the Control and LAB treatments (MML: χ^2 = 4.02; df = 1; *P* = 0,045). * Indicates differences between treatments. (b) TDS (mean ± standard error), according to the Control and LAB treatments (MML: χ^2 = 68.05; df = 1; *P* < 0.0001). * Indicates differences between treatments.

correlation was observed between the variables evaluated during fermentation (V1; V2; V3; V4): initial electrical conductivity, initial TDS, initial pH, and degrees Brix, although these variables were unrelated to any treatment. An inverse correlation was also observed between the variables V1, V2, V3, V4 and V8, V13, V11: electrical conductivity, TDS, pH, degrees Brix increase as WA (water activity), taste, and acidity decrease.

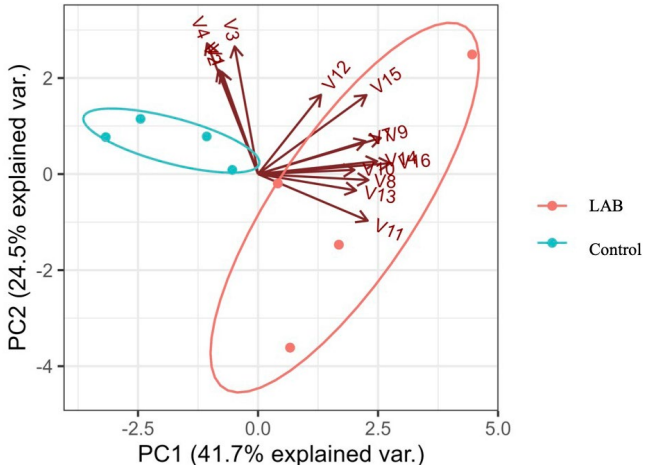

Figure 6: Ordination diagram of the principal component analysis categorized according to the LAB and Control treatments, N = 8 (n = 4 per treatment). The percentage values shown on the axis labels indicate the proportion of the total variance explained by each component. Variables evaluated during fermentation: V1= initial electrical conductivity; V2 = initial TDS; V3 = initial pH; V4 = degrees Brix; variables related to physical and sensory analyses: V7 = % humidity; V8 = WA (water activity); V9 = clean cup; V10 = sweetness; V11 = acidity; V12 = mouthfeel; V13 = taste; V14 = aftertaste; V15 = balance; V16 = tasting score.

These results confirm the statements in Wintgens (2004) Bhumiratana, Adhikari and Chambers (2011) and Sunarharum, Williams and Smyth, (2014), that the chemical composition of green coffee beans and the chemical changes during post-harvest processing, especially during fermentation, can directly impact final product quality and value. While it is true that the use and implementation of bioreactors at the farm level can facilitate standardizing processes and replicating certain profiles, it requires proper training of processors and producers, as well as the collaboration of research institutions that assist in transferring this technology to producers and farms.

Conclusions

The use of an artisanal bioreactor in specialty coffee production has proven to be a useful tool. It allows for precise control during fermentation processes, ensuring consistent treatment, essential process standardization, and replicability. The results obtained from evaluations using the Cup of Excellence (COE) format showed that coffees treated with lactic acid bacteria scored higher (85.9) compared to coffees without inoculation (84), improving the sensory quality of the coffee.

Authors Contributions

Conceptual idea: Morales, E.; Methodology design: Morales, E., Bolaños, M.; Artisanal bioreactor design: Cucuñame, J.; Data collection: Morales, E., Salazar, J.; Data analysis and interpretation: Morales, E., Salazar, J., and Writing and editing: Morales, E., Bolaños, M.

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