Evaluación de la aplicación de microorganismos comerciales en el proceso de fermentación para la obtención de cafés especiales

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Karen Dayana Calderón

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NOMENCLATURA

ARF: agua Residual de Fermentación

BAL: bacterias Ácido-Lácticas

CR: crecimiento Microbiano Relativo

DSD: diseño de Cribado Definitivo

PC: pulpa de Café

S: puntaje sensorial

SCA: Specialty Coffee Association

SS: sólidos Solubles

UFC: unidades Formadoras de Colonias

1 RESUMEN GENERAL

La fermentación del café, en la mayoría de los casos, se realiza sin control y ocurre de manera espontánea, generando una alta variabilidad en el perfil sensorial de la bebida final. Por tanto, el presente estudio se estructuró en dos fases complementarias con el objetivo de optimizar la fermentación semi-controlada del café mediante la aplicación de bacterias ácido-lácticas (BAL) y levaduras comerciales, integrando el desarrollo de inóculos a partir de subproductos y la optimización del proceso fermentativo.

En la primera fase, se desarrollaron inóculos microbianos empleando subproductos del procesamiento de café como fuentes de carbono, con el fin de proponer un sistema sostenible y de bajo costo para pequeños productores. La viabilidad de los inóculos se evaluó mediante un diseño de mezclas que variaron proporciones de pulpa de café (PC) y agua residual de fermentación (ARF), considerando como variable de proceso el tiempo de fermentación (36–48 h para BAL y 12–36 h para levaduras). La variable de respuesta principal fue el crecimiento relativo microbiano (CR). Los modelos estadísticos ajustados fueron de tipo cuadrático, con adecuados indicadores de ajuste (R^2 = 0.84 y 0.88; R^2 ajustado = 0.77 y 0.83, para BAL y levaduras, respectivamente). Los resultados mostraron que la combinación óptima para BAL correspondió a 100% PC con 48 h de fermentación, alcanzando 7.8×10^7 UFC/mL, mientras que para levaduras la formulación óptima fue 100% ARF durante 36 h, con 8.3×10^8 UFC/mL. Los inóculos optimizados se aplicaron posteriormente en fermentaciones de café, de manera individual y combinada, para evaluar su efecto en el perfil sensorial.

En la segunda fase, se implementó un enfoque experimental secuencial para optimizar el proceso fermentativo con los inóculos obtenidos. Esta fase incluyó un diseño de cribado definitivo (DSD) para identificar los factores críticos, seguido de un diseño factorial. Se evaluaron cinco factores: tiempo de fermentación (X_1), concentración del inóculo (X_2), contenido de agua (X_3), tipo de microorganismo (X_4) y método de preparación del inóculo (X_5). Se consideró CR como covariable y el Puntaje Sensorial (S) como variable de respuesta principal. Los resultados del DSD mostraron que X_4, seguido de X_1 y X_5, tuvieron mayor influencia sobre S. El modelo de optimización predijo que el valor máximo de S (85.14) se obtenía con un inóculo optimizado de levadura y 48 h de fermentación, en combinación con niveles bajos de X_2 (3%) y X_3 (50%).

Los hallazgos evidencian que los inóculos derivados de subproductos no solo incrementan la viabilidad microbiana, sino que también mejoran la calidad sensorial del café. La integración de

inóculos optimizados con estrategias experimentales secuenciales de optimización permite predecir condiciones de fermentación que incrementan significativamente S y mejoran la calidad del café fermentado. Esta aproximación constituye una alternativa viable, escalable y sostenible para pequeños productores que buscan diferenciarse en el mercado de cafés especiales.

Palabras clave: *subproductos del café, bacterias ácido-lácticas, levadura (Saccharomyces cerevisiae), optimización de procesos y perfil sensorial.*

Abstract

Coffee fermentation, in most cases, occurs without control and proceeds spontaneously, which generates high variability in the sensory profile of the final beverage. Therefore, the present study was structured in two complementary phases with the objective of optimizing semi-controlled coffee fermentation through the application of lactic acid bacteria (LAB) and commercial yeasts, integrating both the development of inocula from coffee by-products and the optimization of the fermentative process.

In the first phase, microbial inocula were developed using coffee-processing by-products as carbon sources, aiming to propose a sustainable and low-cost system for smallholder producers. The viability of the inocula was evaluated through a mixture design that varied the proportions of coffee pulp (CP) and coffee wastewater (CWW), considering fermentation time as a process variable (36–48 h for LAB and 12–36 h for yeasts). The main response variable was microbial relative growth (CR). The fitted statistical models were quadratic, with adequate goodness-of-fit indicators ($R^2 = 0.84$ and 0.88; adjusted $R^2 = 0.77$ and 0.83 for LAB and yeasts, respectively). Results showed that the optimal condition for LAB corresponded to 100% CP with 48 h of fermentation, reaching 7.8×10^7 CFU/mL, while the optimal formulation for yeasts was 100% CWW for 36 h, yielding 8.3×10^8 CFU/mL. The optimized inoculum were subsequently applied in coffee fermentations, both individually and in combination, to assess their effect on the sensory profile.

In the second phase, a sequential experimental approach was implemented to optimize the fermentation process using the developed inoculum. This phase included a Definitive Screening Design (DSD) to identify the critical factors, followed by a factorial design. Five factors were evaluated: fermentation time (X_1), inoculum concentration (X_2), water content (X_3), type of microorganism (X_4), and inoculum preparation method (X_5). CR was considered as a covariate, while the main response variable was the sensory score (S). DSD results showed that X_4, followed by X_1 and X_5, had the greatest influence on S. The optimization model predicted that the maximum S value (85.14) was obtained with an optimized yeast inoculum and 48 h of fermentation, combined with low levels of X_2 (3%) and X_3 (50%).

Overall, the findings demonstrate that inoculum derived from coffee by-products not only increase microbial viability but also enhance the sensory quality of coffee. The integration of optimized inoculum with sequential experimental optimization strategies allows the prediction of fermentation conditions that significantly increase S and improve the consistency of the final product.

This approach represents a viable, scalable, and sustainable alternative for smallholder producers seeking to differentiate themselves in the specialty coffee market.

Keywords: *coffee by-products, lactic acid bacteria, yeast (Saccharomyces cerevisiae), process optimization, and sensory profile.*

2 INTRODUCCIÓN

El café como producto agrícola es clave en los mercados globales y un pilar económico para muchas naciones, con una demanda creciente a nivel mundial (Vegro & de Almeida, 2020). Las características sensoriales del café, especialmente el sabor y el aroma, son de los factores más importantes en la elección del consumidor final. Por tanto, en los últimos años ha aumentado el interés por los cafés especiales, que tienen aromas y sabores únicos. La Asociación de Cafés Especiales (SCA por sus siglas en inglés) definió metodologías estandarizadas para evaluar la calidad sensorial de los granos de café, que consiste en un análisis de cata realizado por catadores especializados certificados como Q-graders, utilizando muestras de café tostado. En esta prueba se evalúan los granos de café con diez atributos: fragancia, sabor, regusto, acidez, cuerpo, uniformidad, equilibrio, dulzor, limpieza y puntuación sensorial general (SCA, 2024). Para la SCA, la calidad de los cafés especiales está dada por no tener ningún defecto en la bebida, alcanzar mínimo 80 puntos en la escala de calificación y presentar una calidad diferenciada en sabor y aroma (Vaz et al., 2023).

La calidad de los cafés especiales es bastante amplia y depende de la composición química de los granos, así como de la cosecha, el procesamiento y la preparación. Los diferentes métodos de procesamiento del café (seco, húmedo o semiseco) generan reacciones bioquímicas que transforman los azúcares y compuestos del mucílago en metabolitos, lo que puede afectar la composición química de los granos de café y, en consecuencia, su calidad de cata (De Bruyn et al., 2017). Este criterio de calidad en cata se ha consolidado en los mercados que buscan cafés con perfiles sensoriales diferentes y, por tanto, se han desarrollado nuevas prácticas durante el beneficio del café con el objetivo de aumentar la calidad del grano y la diferenciación sensorial (Pimenta et al., 2018; Raimondo, 2022).

Durante el beneficio y en especial en la etapa de fermentación del café, una amplia gama de microorganismos autóctonos, entre ellos levaduras, bacterias y, en menor medida, hongos filamentosos, realizan actividades metabólicas en los granos, las cuales tienen un impacto en el desarrollo de sabores y aromas característicos del café (de Sousa et al., 2023). La fermentación del café ocurre de forma natural, independientemente del método de procesamiento. y es fundamental para eliminar el mucílago del café pergamino (Carvalho Ferreira et al., 2023). En esta los microorganismos presentes desarrollan un proceso metabólico que utiliza los azúcares, ya sea en ausencia (anaeróbica) o presencia (aeróbica) de oxígeno.

Teniendo en cuenta lo anterior, se ha generado la necesidad de llevar a cabo la etapa de fermentación de manera controlada, principalmente ligada al uso de cultivos iniciadores (Carvalho Ferreira et al., 2023; Silva et al., 2013). Este control microbiano permite dirigir la fermentación hacia la producción de compuestos deseados, como ácidos orgánicos y metabolitos aromáticos, que

contribuyen a desarrollar sabores y aromas más complejos y agradables (de Sousa et al., 2023). Como resultado, los cafés sometidos a fermentación controlada con cultivos iniciadores suelen obtener puntajes de cata superiores en comparación con aquellos fermentados de manera espontánea (Cassimiro et al., 2022). Esto los posiciona en la categoría de cafés de especialidad, donde las características sensoriales distintivas, como notas frutales, cítricas o florales, son más pronunciadas y apreciadas.

Como parte de las innovaciones en las operaciones de postcosecha, se han comenzado a introducir microorganismos específicos, como levaduras y bacterias ácido-lácticas (BAL) al procesamiento del café (Bressani, Martinez, Batista, et al., 2021; da Mota et al., 2020). Estos microorganismos pueden obtenerse externamente o aislarse del mismo proceso de fermentación. Su adaptación generalmente se realiza en medios enriquecidos que requieren conocimientos técnicos y son de alto costo (de Melo Pereira et al., 2014; Zhao et al., 2023). La inoculación intencionada con estos microorganismos se realiza para modificar y controlar la fermentación del café que de manera natural es espontanea, lo que conduce a tiempos de fermentación más cortos y a resultados más predecibles y deseables. Actualmente, múltiples investigaciones validan que la inoculación durante la fermentación del café puede mejorar las cualidades sensoriales de la bebida (Carvalho Ferreira et al., 2023; de Sousa et al., 2023). Por tanto, este proyecto tiene como objetivo evaluar el uso de levadura (*Saccharomyces cerevisiae*) y BAL (*Lactobacillus delbrueckii subsp. bulgaricus* y *Streptococcus Thermophilus*) como alternativa sencilla y accesible para mejorar la fermentación del grano de café, orientada a la producción de cafés especiales.

El desarrollo de los objetivos de este trabajo se estructura en las siguientes partes: parte 1: desarrollo de inóculos optimizados usando levadura y BAL comerciales a partir de subproductos del café y parte 2: determinar las condiciones óptimas del proceso de fermentación semi-controlado usando inóculos de microorganismos comerciales.

3 PLANTEAMIENTO DEL PROBLEMA

En Colombia el cultivo de café se realiza mayormente en fincas pequeñas, las plantaciones promedio de los caficultores se encuentran entre 1 y 2 hectáreas (Vegro & de Almeida, 2020). Estos cultivos reciben una alta dosis de cuidado y atención por parte de las familias residentes en sus parcelas (Navarro Muñoz et al., 2024), lo cual se ajusta a la tendencia actual del mercado de cafés especiales. Este mercado favorece los lotes pequeños debido a la capacidad de los agricultores para identificar y clasificar productos de alta calidad, aspecto esencial para este tipo de cafés. Sin embargo, la falta de acceso a tecnologías modernas asequibles y la importancia de estandarizar los procesos, así como la carencia de prácticas sostenibles en la producción, limitan la capacidad de producir cafés especiales y competir en este mercado de creciente demanda global.

El café en Colombia ha adquirido un valor agregado a lo largo de los años gracias al proceso de beneficio, en el cual la cereza de café se transforma en café pergamino seco mediante el método tradicional de vía húmeda (Maya, 2023; Rodríguez et al., 2015). Dentro de este proceso, la fermentación del café se produce principalmente durante la degradación del mucílago, etapa determinante para la calidad final de la bebida. En este entorno, las levaduras y bacterias autóctonas presentes en los granos generan enzimas que transforman carbohidratos, lípidos, proteínas y ácidos en metabolitos como alcoholes, ácidos, ésteres y cetonas. Estos compuestos son responsables de definir el perfil sensorial del café, aportando atributos como notas dulces, cítricas, frutales o tostadas (Carvalho Ferreira et al., 2023). Sin embargo, en la mayoría de los casos la fermentación ocurre de manera espontánea y sin ningún tipo de control, lo que genera una alta variabilidad en los perfiles de taza y con frecuencia resultados inconsistentes que afectan la percepción de calidad. Para minimizar este problema y evitar la formación de características indeseables en los granos, resulta necesario implementar sistemas de fermentación que regulen factores críticos como la temperatura, el tiempo de fermentación, el sustrato y el uso racional del agua, entre otros factores (Carvalho Ferreira et al., 2023; Puerta & Echeverry, 2015). Dada la importancia de estos procesos bioquímicos en la calidad final y las características sensoriales del café, es esencial que los caficultores cuenten con alternativas prácticas y efectivas para llevar a cabo la fermentación de manera adecuada, y que les permita obtener perfiles sensoriales característicos y distintivos.

Actualmente, diversas investigaciones han explorado el uso de microorganismos iniciadores para mejorar el control de los procesos bioquímicos durante la fermentación de café. Estos microorganismos, generalmente autóctonos del café, se utilizan para preparar inóculos en sustratos enriquecidos bajo condiciones de laboratorio y en ambientes asépticos. La aplicación de estos inóculos ha permitido obtener perfiles sensoriales diferenciados y de mejor calidad en comparación

con la fermentación espontánea (Cassimiro et al., 2022; de Melo Pereira et al., 2014; Ribeiro et al., 2017). Sin embargo, se identifican pocas alternativas en el proceso de fermentación del café que los pequeños productores puedan implementar fácilmente para la producción de cafés especiales.

En este contexto, la problemática de este proyecto se centra en la escasez de métodos de fermentación que sean prácticos, asequibles y adaptados a las condiciones de los pequeños caficultores, los cuales permitan llevar a cabo fermentaciones controladas orientadas a mejorar la calidad sensorial del café. Una alternativa prometedora es el uso de microorganismos comerciales de fácil acceso, que podrían favorecer la obtención de cafés especiales con mayor calidad y consistencia. Esto teniendo en cuenta la importancia de controlar y evaluar el efecto de los principales factores durante el proceso de fermentación en la obtención de cafés especiales.

Pregunta de investigación

¿Cuál es el efecto del uso de microorganismos comerciales (levadura y BAL) en la obtención de café especiales, como una alternativa asequible para pequeños productores?

4 JUSTIFICACIÓN

La producción mundial de café pergamino se estimó en 171.4 millones de sacos de 60 kg para el año 2023-2024, y en Colombia alcanzó los 11.5 millones de sacos, ocupando el tercer puesto a nivel mundial (USDA, 2024). En consecuencia, el café ocupa un lugar estratégico en la economía y en la cultura de Colombia, no solo como uno de los principales productos de exportación, sino también como un sector que sostiene a miles de familias caficultoras (Navarro Muñoz et al., 2024). Actualmente, generar un mayor valor agregado en el café es una estrategia adoptada por diversas instituciones y productores de la industria cafetera, tanto colombiana como mundial. La diferenciación en la producción del café desempeña un papel central en esta estrategia, al resaltar características como el origen, la calidad y otros atributos específicos, para así lograr precios superiores a los de los cafés estándar en los mercados internacionales (Raimondo, 2022).

Una de las principales barreras para los productores de pequeña escala es la ausencia de métodos de fermentación que sean simples, reproducibles y al mismo tiempo efectivos para mejorar los atributos de taza. Diversos estudios han demostrado que un proceso de fermentación bien controlado y estandarizado es esencial para garantizar el desarrollo de microorganismos que mejoren la calidad y el aroma del café (Elhalis et al., 2020; Wang et al., 2020b). En este sentido, una fermentación mediante el uso de cultivos iniciadores (inóculos) puede generar una calidad estandarizada y reducir las pérdidas económicas del productor, como ha ocurrido con alimentos y bebidas fermentados, como el queso, el yogur, el pan, la cerveza y el vino (Silva et al., 2013; Wang et al., 2020b). Sin embargo, la aplicación de estas tecnologías puede ser compleja para los pequeños productores, ya que se requieren recursos económicos y conocimientos específicos sobre los parámetros involucrados en los procesos.

A pesar de estas limitaciones, el interés por los cafés especiales ha crecido significativamente en los últimos años, debido a sus perfiles sensoriales diferenciados y a su alta aceptación en el mercado, lo que les permite alcanzar precios más elevados (Raimondo, 2022). Una alternativa prometedora para generar valor adicional y que sea aplicable a escala de producción real, es la biotransformación de los granos de café mediante un proceso de fermentación semi-controlado con la adición de microorganismos específicos comerciales. Este enfoque no solo mejora la calidad sensorial del café, sino que también ofrece oportunidades innovadoras para los pequeños productores con la obtención de cafés especiales.

Un aspecto clave en el mercado del café colombiano es la búsqueda constante de alternativas que permitan diferenciar los productos. En este sentido, el uso de inóculos de microorganismos

comerciales constituye una técnica con el potencial de optimizar la fermentación y resaltar atributos de sabor, aroma y calidad del grano. En el marco del Plan Nacional de Desarrollo 2022-2026, que promueve la innovación y el fortalecimiento del sector agroindustrial, este tipo de proyectos contribuye a la modernización y diferenciación de la caficultura nacional, beneficiando tanto a pequeños productores como a grandes exportadores. Al desarrollar métodos de fermentación asequibles y adaptados a la realidad del caficultor, se busca promover una producción de café de alta calidad que incremente la competitividad internacional, incentive prácticas sostenibles y genere valor agregado en las regiones productoras, alineándose con los objetivos de desarrollo económico y social del país.

En este contexto, un proceso de fermentación semi-controlado optimizado utilizando inóculos de microorganismos comerciales podría contribuir significativamente a una alternativa viable para pequeños caficultores, al ofrecerles un mecanismo práctico para estandarizar la fermentación, mejorar el perfil sensorial del café y aumentar su competitividad en el mercado de cafés especiales.

5 HIPÓTESIS

H_0: El uso de inóculos de microorganismos comerciales en un proceso de fermentación semi-controlado optimizado no produce diferencias significativas en la calidad sensorial del café en comparación con la fermentación espontánea tradicional.

H_1: El uso de inóculos de microorganismos comerciales en un proceso de fermentación semi-controlado optimizado mejora significativamente la calidad sensorial del café en comparación con la fermentación espontánea tradicional.

6 OBJETIVOS

Objetivo general: Optimizar el uso de levadura (*Saccharomyces cerevisiae*) y bacterias ácido-lácticas (*Lactobacillus bulgaricus* y *Streptococcus Thermophilus*) como alternativa asequible para mejorar la fermentación del grano de café, orientada a la obtención de cafés especiales de alta calidad.

Objetivo específicos

1. Evaluar el uso de los subproductos del café (*Coffea arabica*) en el desarrollo de inóculos para la fermentación semi-controlada de café con levadura (*Saccharomyces cerevisiae*) y bacterias ácido-lácticas (*Lactobacillus bulgaricus* y *Streptococcus Thermophilus*) comerciales.
2. Determinar las condiciones óptimas del proceso de fermentación semi-controlado de café que mejore los perfiles sensoriales y la calidad del café en taza.

7 ANTECEDENTES

El uso de inóculos con microorganismos iniciadores en el proceso de fermentación es una alternativa eficaz para obtener un mayor control en comparación con la fermentación espontánea (Schwan et al., 2012). Diversos estudios han explorado la preparación de estos inóculos utilizando microorganismos seleccionados, cultivados en condiciones específicas que favorecen su desarrollo (Cassimiro et al., 2022; Ribeiro, Ribeiro, et al., 2017). Estos estudios se han enfocado en microorganismos provenientes de colecciones de cultivos, los cuales han sido previamente aislados del grano de café o son usados en la fermentación de otros productos alimentarios (Kim et al., 2022). Los cultivos de colección requieren una activación previa y la inoculación en medios específicos para garantizar un crecimiento óptimo. Posteriormente, estos cultivos se aplican al proceso de fermentación, ya sea de forma directa o mediante una resuspensión en solución, como se muestra en la Tabla 1.

Tabla 1. *Inóculos usados en la fermentación de café.*

Microorganismos iniciadores	Origen	Sustrato	Aplicación	Referencia
Levaduras: *S. cerevisiae* y *Pichia kluyveri* BAL: *Lactococcus lactis subsp. Cremoris*	Colección de cultivos liofilizados	Levaduras: Caldo nutritivo de levaduras. BAL: Medio con glucosa (GM17).	En solución con NaCl	(Wang et al., 2020a)
Levaduras: *Meyerozyma. caribbica*, *S. cerevisiae* y *Torulaspora delbrueckii*.	Colección de cultivos	Medio YEPG	En solución de agua de peptona	(Bressani, Martinez, Sarmento, et al., 2021)
Levaduras: *S. cerevisiae*, *Candida parapsilosis* y *Pichia guilliermondii* BAL: *Leuconostoc mesenteroides*, *Lactiplantibacillus plantarum* y *Pediococcus pentosaceus*	Colección de cultivos	Levaduras: Caldo de malta de levadura. BAL: Caldo MRS.	Directa al café	(Kim et al., 2022)
Levadura: *P. kluyveri*, *Wickerhamomyces anomalus* BAL: *L. lactis*	Aislados del proceso de fermentación espontánea del café	Levaduras: Caldo de malta de levadura. BAL: Caldo MRS.	Directa al café	(Mahingsapun et al., 2022)
Levaduras: *S. cerevisiae* y *Torulaspora delbrueckii*	Colección de cultivos aislados del café	Medio líquido con melaza, cloruro de calcio, urea y polisorbato	Microencapsulados	(Mynsen Machado Martins et al., 2023)

Rhodotorula mucilaginosa y *W. anomalus*	Aislados del proceso de fermentación espontánea del café	Pulpa seca y agua	Directa al café	(Zhao et al., 2023)
Levadura: *S. cerevisiae* BAL: *L. plantarum*	Colección de cultivos liofilizados	-	Directa al café	(Rabelo et al., 2024)

BAL: bacterias ácido-lácticas

Los sustratos de los inóculos influyen significativamente en la adaptación de los microorganismos iniciadores a las condiciones de la fermentación de café. Zhao et al. (2023) utilizaron pulpa de café como sustrato para el cultivo iniciador de microorganismos aislados del grano de café, lo que proporcionó una adaptación preliminar al medio fermentativo, que permitió el desarrollo de compuestos aromáticos característicos que pueden mejorar la calidad sensorial del café. Estos resultados evidenciaron que el tipo de microorganismo empleado y el sustrato de adaptación, son determinantes en la composición química del grano de café.

En general, la fermentación controlada mediante la inoculación de microorganismos, como levaduras y bacterias lácticas (BAL), ha demostrado mejoras significativas en las características organolépticas del café, reflejándose en puntuaciones más altas según los criterios de la Asociación de café de especialidad (SCA, por sus siglas en inglés). Frente a la fermentación espontánea, la aplicación de inóculos contribuye a reducir la variabilidad de los perfiles en taza y a estandarizar atributos sensoriales específicos.

Mahingsapun et al. (2022) reportaron que el co-inóculo de levaduras (*Pichia kluyveri* y *Wickerhamomyces anomalus*) alcanzó un Puntaje Global Sensorial (PGS) de 82.4, frente a 75.6 en fermentación espontánea. Resultados similares fueron observados por Cassimiro et al. (2023), quienes mostraron incrementos en el PGS al emplear co-inóculos de levaduras y BAL, llegando hasta 81.3, en comparación con 75.3 en fermentación espontánea. De igual manera, Pereira et al. (2016) documentaron un incremento notable (88.3 frente a 80) al utilizar *Lactobacillus plantarum*, mientras que Jiménez et al. (2023) mostraron un aumento de 84.25 a 86.5 con *Torulaspora delbrueckii*.

Estos hallazgos, resumidos en la Tabla 2, confirman que el uso de inóculos en la fermentación de café no solo mejora de manera consistente el puntaje en taza, respecto a la fermentación espontanea, sino que también permite alcanzar los estándares requeridos para clasificar como cafés de especialidad de acuerdo con los criterios de la SCA.

Tabla 2. *Fermentación vía húmeda de café con inóculos.*

Inóculo	Tipo de fermentación	Puntaje sensorial	Referencia
Levaduras: *P. kluyveri* y *W. anomalus.*	Húmeda	- 82.4 co-inóculo de levaduras. - 81.1 co-inóculo de *P. kluyveri* y *L. lactis*. - 75.6 fermentación espontánea.	(Mahingsapun et al., 2022)
BAL*: L. lactis.* Levaduras: *T. delbrueckii* y *S. cerevisiae*.	Húmeda	- 81.33 co-inóculo de *L. Mesenteroides* y *S. cerevisia*. - 81.00 con *L. Mesenteroides*. - 80.33 co-inóculo de *L. mesenteroide* y *L. plantarum*. - 75.33 fermentación espontánea.	(Cassimiro et al., 2023)
BAL: *L. mesenteroides* y *L. plantarum*.			
BAL: *L. plantarum*.	Húmeda	- 88.3 con inóculo - 80 fermentación espontánea.	(Pereira et al., 2016)
Levadura: *T. delbrueckii*	Húmeda	- 86.5 con inóculo - 84.25 fermentación espontánea.	(Jimenez et al., 2023)
Levaduras: *Hanseniaspora. uvarum* y *Pichia kudriavzevii*.	Húmeda	- 82.0 con co-inóculo de levaduras. - 81.5 con inóculo de *P. kudriavzevii.* - 80.3 fermentación espontánea.	(Elhalis et al., 2021)
BAL: *Bacillus licheniformis*	Húmeda	- 79.77 con inóculo de *B. licheniformis* - 78.73 control	(Bravim et al., 2023)

BAL: bacterias ácido-lácticas

8 MARCO TEÓRICO

8.1 Café

El cafeto es una planta perteneciente a la familia *Rubiaceae*. Dentro de esta familia, el género *Coffea* es el más producido comercialmente a nivel mundial, destacándose principalmente las especies: *Coffea arabica*, *Coffea canephora*, *Coffea liberica* y *Coffea excelsa* (da Costa et al., 2023). Entre ellas, *C, arabica* y *C. canephora* son las más importantes en términos de producción global.

En Colombia predomina el cultivo de la especie *C. arabica* (Flórez et al., 2021), especie altamente valorada por su suavidad, acidez balanceada y perfiles sensoriales diferenciados. Este posicionamiento se explica en gran medida por las condiciones agroecológicas del país, caracterizadas por suelos de origen volcánico, altitudes entre 1.200 y 1.800 m s. n. m., temperaturas promedio de 18–22 °C y elevada pluviosidad, que en conjunto favorecen la obtención de granos de alta calidad (Navarro Muñoz et al., 2024). Debido a estos atributos el café colombiano ha logrado reconocimiento internacional tanto en mercados tradicionales como en la creciente categoría de cafés especiales, respaldado por denominaciones de origen que certifican su diferenciación (Maya, 2023). Actualmente, la producción nacional se extiende en más de 500 municipios y 23 departamentos, con una base social de alrededor de 540.000 familias caficultoras que dependen directamente de este cultivo como principal fuente de ingresos y sustento (Navarro Muñoz et al., 2024).

8.1.1 Estructura de la cereza de café

El fruto del cafeto, conocido como cereza, es una drupa de forma globular u ovoide, con un peciolo. Su longitud varía entre 10 y 15 mm, mientras que su diámetro oscila entre 11 y 14 mm (Flórez et al., 2013). La cereza de café presenta una estructura formada por varias capas (Figura 1). Su piel exterior, conocida como exocarpio, es de color rojo brillante y constituye la capa más externa del fruto (Hall et al., 2022). En las etapas iniciales de desarrollo, esta capa es rica en contenido de clorofilas, lo que le confiere un color verde. A medida que el fruto madura, la clorofila disminuye y aumenta el contenido de antocianinas responsable del color rojo característico (Flórez et al., 2013).

Debajo del exocarpio se encuentra el mesocarpio, también conocido como mucílago. Esta capa intermedia es pastosa y rica en carbohidratos, como glucosa y fructosa, además contiene péctina, proteínas, minerales, polifenoles y cafeína (Elhalis et al., 2023). A medida que el fruto alcanza su madurez, el mesocarpio sufre transformaciones químicas, incluyendo un aumento en el contenido de agua y carbohidratos solubles lo que lo convierte en el principal sustrato para el metabolismo microbiano durante la fermentación (Arya et al., 2022).

Seguidamente del mesocarpio está el endocarpio, que es una fina capa que, en la etapa final de desarrollo, forma el pergamino que recubre la semilla, también conocido como cascarilla. Este pergamino es blanco, flexible y resistente, con un espesor de aproximadamente 0.1 mm, que al retirarse del grano el café se denomina café verde (Flórez et al., 2013). Finalmente, la semilla del fruto de café (endospermo) o grano está recubierto por una capa delgada llamada cascarilla plateada. El grano está compuesto de proteínas, azúcares, ácidos grasos, minerales, ácidos orgánicos y compuestos bioactivos (Hall et al., 2022). En la mayoría de los casos, la cereza de café contiene dos granos, aunque en ocasiones puede presentarse un solo grano redondeado conocido como caracolillo o peaberry. Esta variación estructural, junto con la composición química del grano, contribuye a la diversidad en las características físicas y organolépticas del café.

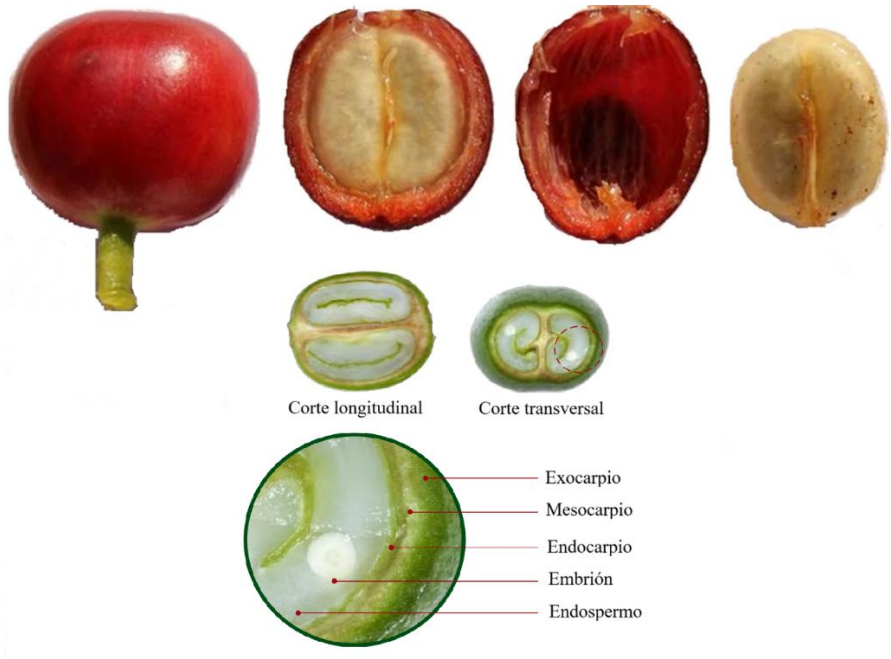

Figura 1. *Estructura de la cereza de café.*
Adaptado de Arya et al. (2022) y Flórez et al. (2013)

8.2 Procesamiento de la cereza de café

La transformación de la cereza de café en café verde se puede llevar a cabo mediante métodos húmedos, secos o semiseco (De Bruyn et al., 2017). La elección del método tiene un impacto significativo en la calidad del grano y en los controles necesarios durante el proceso. Esta etapa es fundamental en la producción de café de alta calidad, ya que influye directamente en el perfil sensorial final de la bebida. Independientemente del método utilizado, la cereza de café se cosecha

generalmente cuando alcanza una coloración roja, indicando su madurez (Flórez et al., 2013). A partir de esta cosecha, se implementan distintos procedimientos para obtener el café verde, los cuales se describen a continuación:

3. Método seco

El método seco, también conocido como natural, es el más sencillo. Después de la cosecha, las cerezas de café se secan directamente al sol en patios durante 1 o 2 semanas, o en secadores de aire, hasta reducir su contenido de humedad a un rango entre el 10% y el 12% (Das, 2021). Una vez secas, se retira la cáscara, formada por la pulpa seca (exocarpio y mesocarpio) y el pergamino, para obtener el grano verde. Este método requiere un control riguroso de la humedad y de la exposición a microorganismos para evitar defectos como moho o fermentaciones indeseadas. El producto final obtenido a través de este proceso se conoce como café verde natural.

4. Método húmedo

El método húmedo (también conocido como método lavado) es un proceso muy utilizado para obtener granos de café de alta calidad. Este proceso comienza con el despulpado mecánico de las cerezas de café, lo que elimina la piel exterior (Batista et al., 2015). Tras el despulpado, los granos, aún cubiertos por el mucílago, se colocan en tanques de fermentación durante un período de 12 a 36 horas (Rodríguez et al., 2015). La fermentación puede ser natural o controlada con la adición de microorganismos o enzimas, y su objetivo principal es descomponer el mucílago, lo cual es crucial en los perfiles sensoriales del producto final. Durante este proceso, aumenta la acidez y el pH puede disminuir hasta 4.5 (Das, 2021). Es fundamental controlar la fermentación para evitar sabores agrios indeseables.

Una vez completada la fermentación, los granos se lavan para eliminar los restos de mucílago. Este lavado garantiza un grano limpio y homogéneo, preservando sus cualidades intrínsecas. En esta etapa, el café pergamino húmedo tiene un contenido de humedad de aproximadamente 57%. Posteriormente, los granos se secan al sol o mediante secadores mecánicos hasta alcanzar un contenido de humedad entre 10% y 12% (Batista et al., 2015). Una vez secos los granos, conocidos como café pergamino, se almacenan en esta forma hasta la comercialización. Antes de venderse, el café pergamino se trilla para retirar la capa protectora, obteniendo así el café verde. Este proceso garantiza un producto homogéneo, con pocos defectos, lo que contribuye a una mayor calidad y valor en el mercado internacional.

Este método es ampliamente utilizado en regiones donde el café se cosecha manualmente, como Colombia, América Central y Asia, debido a su capacidad para resaltar el perfil sensorial del café. En Colombia, este proceso es conocido como beneficio húmedo, y se aplica especialmente a la especie *C. arabica*, considerada de alta calidad (Rodríguez et al., 2015).

5. *Método semiseco y semihúmedo*

En el método semiseco, los granos de café se separan de la pulpa mediante un proceso de despulpado, que elimina el exocarpio, y de una serie de lavados que elimina la mayor parte del mesocarpio. Sin embargo, se deja una parte del mucílago adherida al grano durante el secado, lo cual contribuye a desarrollar perfiles de sabor más dulces y complejos. Por otro lado, en el método semihúmedo se deja el mesocarpio adherido al grano después del despulpado. Los granos se secan al aire libre, generalmente durante 10 a 15 días, dependiendo de las condiciones meteorológicas. Al igual que los métodos anteriores, el secado se considera completo cuando los granos alcanzan un contenido de humedad entre el 10% y el 12%. Durante esta fase, se produce un proceso de fermentación en el que los microorganismos degradan los restos del mucílago adherido a los granos, por tanto, es esencial controlar cuidadosamente el secado para evitar la sobrefermentación y garantizar la calidad del grano. Luego del secado, a los granos se les retira el endocarpio para obtener el café verde. El producto final obtenido a través del método semihúmedo se conoce como café verde honey (Batista et al., 2015; Sanz-Uribe & Velásquez-Henao, 2022).

8.2.1 Subproductos del procesamiento del café por el método húmedo

La producción de granos de café verde comercializables genera varios subproductos según el método de procesamiento utilizado. El principal subproducto del método seco está compuesto por las tres primeras capas de la cereza de café (exocarpio, mucílago y pergamino), todos juntos en una sola fracción (cáscaras de café). En cambio, el beneficio húmedo permite una recuperación diferenciada: la pulpa (exocarpio) que constituye una primera fracción (43.2% p/p de la cereza entera), el mucílago conforma una segunda fracción cuando este se retira del grano (11.8% p/p), y el pergamino representa una tercera fracción (6.1% p/p) (Esquivel & Jiménez, 2012). Dado que más del 50% del fruto del café no se utiliza en la producción del café verde comercial y se descarta durante el procesamiento, es fundamental explorar aplicaciones potenciales para estos subproductos que son ricos en compuestos orgánicos e inorgánicos.

A continuación, se describe la composición de los principales subproductos del beneficio húmedo del café:

6. Residuos sólidos: pulpa de café

Entre los residuos sólidos, la pulpa es el primero en generarse. En el proceso de despulpado de la cereza de café se retira el exocarpio junto con parte del mesocarpio, dando como resultado la pulpa. Esta pulpa está compuesta principalmente por carbohidratos, proteína cruda, fibra cruda y ceniza (Campos et al., 2021), como se muestra en la Tabla 3. Además, tiene un contenido de ácidos fenólicos significativo. Entre los ácidos fenólicos de la pulpa de café, los flavan-3-oles, los ácidos hidroxicinámicos, los flavonoles y las antocianidinas son los más representativos (Ramirez-Coronel et al., 2004). En cuanto al contenido de carbohidratos, predominan los polisacáridos pécticos, así como algunos monosacáridos, como arabinosa, galactosa, glucosa, xilosa y manosa, con concentraciones de 5.8, 5.2, 20.2, 4.2 y 4.7% en materia seca, respectivamente (Bhandarkar et al., 2021). Estos compuestos desempeñan un papel importante, ya que las actividades microbianas los hidrolizan para generar ácidos orgánicos y compuestos aromáticos (Zhao et al., 2023), lo cual influye directamente en la composición química del grano de café y, por ende, en su perfil sensorial final.

Tabla 3. *Composición de la pulpa de café.*
Adaptado de Campos et al. (2021)

Componente	**Cantidad (%, bs)**
Carbohidratos	74.10
Fibra cruda	15.0
Proteína cruda	8.25
Cenizas	8.12
Potasio	3.17
Nitrógeno	1.32
Cafeína	0.75
Calcio	0.32
Fósforo	0.05
Ácidos fenólicos	1.1

bs: base seca.

7. Residuos líquidos

Los residuos líquidos generados durante el beneficio húmedo provienen principalmente del uso de agua, especialmente durante la fermentación y el lavado del mucílago residual. El agua residual de la fermentación de café (ARF), también conocida como “agua miel”, es un subproducto que contiene altos niveles de materia orgánica, azúcares, pectinas, cafeína, y compuestos fenólicos, derivados de la degradación del mucilago (Elhalis et al., 2023; Rodríguez et al., 2015). Durante la

fermentación, la hidrólisis de la pectina libera azúcares simples, como glucosa, ramnosa, L-arabinosa y D-galacturonato, que sirven como fuentes adicionales de carbono para el metabolismo microbiano. Además, los metabolitos primarios generados en esta etapa incluyen etanol, acetaldehído y ácido acético, a partir de los cuales se producen compuestos de bajo peso molecular, como ésteres, alcoholes superiores, aldehídos, cetonas y terpenos (De Bruyn et al., 2017). Entre los terpenos formados, se destacan β-citronelol, linalool, geraniol, α-terpineol y β-citronelol, los cuales provienen de precursores glucósidos liberados por la acción de la enzima β-glucosidasa de levaduras (de Melo Pereira et al., 2019). También durante la fermentación se producen compuestos ácidos, lo que resulta en un pH bajo que contribuye a la acidez de este residuo líquido (Campos et al., 2021). Este subproducto líquido, rico en metabolitos y productos de degradación de moléculas complejas, representa una fuente potencial de carbono para procesos microbiológicos.

Por otra parte, las aguas generadas durante el lavado del mucilago después de la fermentación tienen una concentración de nutrientes destacable, especialmente azúcares, ya que está relacionada con la remoción del mucílago adherido a los granos. Por tanto, su composición refleja la del propio mucílago, que está compuesto por agua (84.2%), proteínas (8.9%), azúcar (4.1%), sustancias pécticas (0.91%) y cenizas (0.7%). El análisis de la composición de los residuos insolubles mostró la presencia de sustancias pécticas (aprox. 30%), celulosa (aprox. 8%) y polisacáridos neutros no celulósicos (aprox. 18%). Además, las pectinas contenidas en el mucílago incluyen aproximadamente un 60% de ácidos urónicos, con un alto grado de metilesterificación y un grado moderado de acetilación (Esquivel & Jiménez, 2012).

8.3 Fermentación del grano de café

La fermentación es un proceso metabólico microbiano en el cual las moléculas complejas se descomponen en moléculas más simples y se generan productos líquidos y gases (compuestos volátiles). Este proceso puede ocurrir en condiciones anaeróbicas (sin oxígeno) o aeróbicas (con oxígeno), por medio de la acción de levaduras y bacterias autóctonas del café. El fruto del café se somete a uno de los tres métodos de procesamiento mencionados anteriormente (ver Sección 6.2) inmediatamente después de la cosecha, generando así la fermentación espontánea. Dependiendo del tipo de procesamiento, el tiempo necesario para la fermentación varía. El objetivo principal del proceso de fermentación en todos los métodos es eliminar la capa de mucílago y disminuir el contenido de agua de los granos de café. Sin embargo, cuando se maneja con cuidado, este proceso también mejora significativamente los atributos de calidad del café (Carvalho Ferreira et al., 2023; Haile & Kang, 2019b).

En el método húmedo después de despulpar los frutos del café, los granos se fermentan en tanques con agua. En este proceso el contenido de glucosa y fructosa se reduce, debido al metabolismo del azúcar (es decir, fermentación alcohólica o láctica debido a la degradación de compuestos en el procesado húmedo) (de Melo Pereira et al., 2019). Por el contrario, en el método seco la fermentación ocurre durante el secado de los frutos sin despulpar, lo que permite que los compuestos del mucílago se degraden naturalmente. El procesamiento semiseco es una combinación de ambos métodos, en el que se despulpan los frutos del café, pero el proceso de fermentación ocurre directamente bajo el sol en una plataforma, como el método seco (Febrianto & Zhu, 2023).

La fermentación espontánea del café está influida por una serie de factores, entre los que se incluyen el medio ambiente, el pH, la temperatura, la microbiota y el nivel de contaminación en el agua utilizada, las diferencias de variedad en las cerezas maduras utilizadas y el origen geográfico (Girma & Sualeh, 2022). Por tanto, el proceso de fermentación influye en la calidad final del café, ya que modula los perfiles sensoriales que se van a percibir en la bebida debido a la composición química del grano que se desarrolla durante este proceso.

8.3.1 Factores que influyen en la fermentación

La fermentación de café es un proceso crítico que define el perfil sensorial final del grano, a partir de las diversas reacciones metabólicas de los microorganismos presentes en el proceso. Por tanto, diversos factores influyen en este proceso:

8. *Composición del sustrato:* la materia prima del proceso de fermentación está compuesta por los granos de café, que pueden ser despulpados o enteros, y contienen el mucilago que será fermentado, cuya proporción y composición depende de la variedad del café y el estado de madurez del fruto, oscilando entre el 2% y el 30% del peso del grano. Además, la presencia de pulpa residual, insecticidas u otras sustancias en el grano puede influir en la producción de metabolitos secundarios durante la fermentación, afectando directamente los sabores y aromas del café final (Carvalho Ferreira et al., 2023; Puerta Quintero, 2012).
9. *Condiciones del medio:* las condiciones fisicoquímicas del medio como el pH, la acidez y los sólidos solubles son parámetros clave para monitorear las condiciones y las actividades durante la fermentación. El pH del grano de café despulpado fresco varía entre 5.3 y 6.0 cuando se sumerge en agua (Puerta Quintero, 2012). Durante la fermentación, el pH del sustrato disminuye debido a la producción de ácidos orgánicos como láctico, acético, cítrico, fórmico, málico, fosfórico y succínico, derivados de los procesos metabólicos de los microorganismos (Vaz et al., 2023). En contraste, la acidez del sustrato aumenta como

resultado de estos mismos ácidos generados. Los sólidos solubles indican el grado de madurez del grano despulpado y, por tanto, la composición del sustrato (Cassimiro et al., 2022). Un control adecuado del pH y del nivel de azúcares asegura que la fermentación no desarrolle sabores indeseados. Este monitoreo riguroso permite resaltar atributos específicos del café, mejorando la calidad sensorial y diferenciando los perfiles de sabor.

10. *Microorganismos:* los microorganismos presentes durante el procesamiento de la cereza de café provienen de diversas fuentes, como el suelo, el aire, el agua, las plantas, las personas, los animales, los insectos, los equipos y las instalaciones. De manera natural, en el café se encuentran levaduras, bacterias (como las lácticas) y algunos hongos (Haile & Kang, 2019b). Esta microbiota presente durante la fermentación juega un papel crucial en la composición química final del grano de café y, en consecuencia, impactan en los perfiles sensoriales (Puerta Quintero, 2012; Schwan et al., 2023).
11. *Temperatura:* debido a su composición química y microbiológica, el mucílago del café se fermenta de manera natural en las condiciones ambientales de las zonas cafeteras, donde las temperaturas del aire pueden variar entre 12 °C y 34 °C entre el día y la noche, dependiendo de la altitud (Puerta Quintero, 2012). Durante la fermentación, la temperatura de los granos de café experimenta variaciones debido a la actividad metabólica de los microorganismos presentes. La mayoría de estos microorganismos fermentadores crecen en un rango de temperatura que oscila entre 0 °C y 37 °C (da Mota et al., 2020).
12. *Tiempo:* este es un parámetro que se debe controlar durante el proceso de fermentación, ya que es durante el cual se van a desarrollar las actividades microbianas y se van a producir los diferentes compuestos que influirán en la calidad de café. Por lo general, en el método vía húmeda, el café se deja fermentando entre 12 a 36 horas, superior a esto requiere de un mayor control de las condiciones del medio para evitar aromas y sabores indeseables (Febrianto & Zhu, 2023; Rodríguez et al., 2015).

8.3.2 Microorganismos presentes en la fermentación de café

La microbiota del café es compleja, compuesta principalmente por bacterias, levaduras y hongos filamentosos. Durante la fermentación, se produce una intensa actividad microbiana y diversas reacciones bioquímicas dentro y fuera de los granos. Los microorganismos presentes de forma natural en el entorno productivo metabolizan los azúcares presentes en la pulpa y el mucílago, excretando ácidos orgánicos y otros metabolitos que influyen en las características sensoriales del café (Batista et al., 2015). Además, la actividad microbiana tiene otras funciones, como producir

enzimas pectinolíticas que degradan la pectina del mucílago, e inhibir el crecimiento de hongos toxigénicos (Schwan et al., 2023).

13. *Tipos de microorganismos*

Bacterias: dominan en las etapas iniciales de fermentación. Se han identificado especies como *Tatumella ptyseos*, *Pseudomonas putrefaciens*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Bacillus subtilis* y *Paenibacillus amylolyticus* (Schwan et al., 2012).

Las bacterias ácido-lácticas (BAL) (*Leuconostoc mesenteroides*, *Lactobacillus* spp., *Streptococcus faecalis*) están presentes principalmente en cerezas frescas y fermentaciones húmedas o semisecas. Durante la fermentación húmeda, las condiciones anaerobias producto de la mezcla de agua con los granos de café favorecen su desarrollo. Se han identificado especies como *Leuconostoc citreum*, *Leuconostoc pseudomesenteroides*, *Weissella cibaria* y *Weissella soli*. En fermentaciones secas, las poblaciones son muy bajas (Carvalho Ferreira et al., 2023; de Melo Pereira et al., 2017). Las BAL homofermentativas, como *Lactobacillus plantarum*, convierten los azúcares en ácido láctico, mientras que las heterofermentativas como *Leuconostoc mesenteroides* producen, además, etanol, ácido acético y otros metabolitos. Estos compuestos contribuyen significativamente a la acidez de los granos de café aunque, en exceso, pueden afectar la calidad sensorial final (Schwan et al., 2012, 2023).

Levaduras: estas son predominantes a medida que la actividad del agua y pH del medio disminuye. Las levaduras metabolizan sacarosa, glucosa, fructosa y ácido cítrico a valores de pH bajos para su crecimiento durante la fermentación, y son potenciales productores de enzimas pectinolíticas (Torrez et al., 2023). En el procesamiento natural, destacan géneros como *Pichia* (38.9%), *Candida* (22.3%), *Arxula* (18.9%) y *Saccharomycopsis* (9.7%) (Silva et al., 2008). Por otra parte, en el procesamiento despulpado, se identificaron especies como *Pichia anomala*, *Pichia caribbica*, *S. cerevisiae*, *Saccharomyces bayanus*, *Hanseniaspora uvarum* y varias especies de *Candida* spp. En Colombia, *Pichia. nakasei* es la especie dominante (Elhalis et al., 2020; Schwan et al., 2023).

Hongos: los hongos endófitos en las cerezas aparecen en las etapas finales, especialmente en condiciones de fermentación húmeda no controlada, y pueden ser los causantes de sabores indeseados en el café como sabores. Además, los principales hongos filamentosos que se han encontrado en el café con potencial para producir Ocratoxina A fueron *Aspergillus ochraceus*, *Aspergillus carbonarius* y *Aspergillus niger* (Haile & Kang, 2019b; Schwan et al., 2012).

8.3.3 Fermentación controlada de café

La fermentación controlada en el café es una técnica donde se regulan cuidadosamente las condiciones del proceso para optimizar la calidad y el perfil sensorial del grano, produciendo bebidas con aromas y sabores especiales, dulces, cítricos, frutales y tostados, que agregan valor al producto. Este método permite a los productores manipular factores clave como tiempo, temperatura, pH y tipo de microorganismos presentes, asegurando una fermentación uniforme (Puerta & Echeverry, 2015).

En la fermentación controlada es importante establecer sistemas que permitan monitorear y controlar las condiciones del café durante el proceso (como, tiempo y temperatura). Estos sistemas consideran el tipo de alimentación del sustrato, que puede ser discontinua, realizada en tanques cerrados o abiertos, o continua, donde se añaden sustratos y microorganismos en intervalos específicos (Jimenez et al., 2023; Puerta & Echeverry, 2015). En este contexto, la aireación y la agitación son factores cruciales. En la fermentación discontinua, generalmente no se añade ni se retira oxígeno, y en sistemas abiertos, el CO_2 se libera de manera natural. En cambio, los procesos continuos requieren un suministro controlado de oxígeno para mantener el crecimiento de levaduras y bacterias fermentadoras. Por tanto, esta técnica influye en la proporción del tipo de aromas y sabores que se desarrollan en el grano de café (Puerta & Echeverry, 2015; Wang et al., 2020b).

Por otra parte, la fermentación semi-controlada es un enfoque que combina elementos de las fermentaciones espontánea y controlada, permitiendo cierto grado de supervisión sobre factores clave, aunque sin intervenir de manera completa en todas las variables. Este método ofrece flexibilidad y es especialmente común en procesos artesanales o en regiones donde no se dispone de tecnología avanzada. En esta técnica, es fundamental establecer y controlar cuidadosamente las condiciones iniciales del proceso, como la aireación y la duración de la fermentación. A diferencia de la fermentación controlada, no es habitual la inoculación con cultivos específicos. En su lugar, se realizan verificaciones periódicas para evaluar el progreso y, si es necesario, ajustar ciertos parámetros. Esto previene la fermentación excesiva y asegura que el proceso se mantenga dentro de los estándares deseados, sin comprometer la calidad del producto final (Febrianto & Zhu, 2023; Ribeiro, et al., 2017b).

8.3.3.1 Microorganismos iniciadores

Una de las formas de realizar una fermentación semi-controlada es mediante la adición de inóculos de cultivos iniciadores o de partida, lo cual favorece al desarrollo de perfiles específicos y a aumentar el valor económico del café (da Mota et al., 2020; Haile & Kang, 2019a). Se le denomina inóculo a la concentración de microorganismos de un cultivo microbiano. En estos cultivos se tienen

en cuenta las múltiples interacciones entre bacterias-bacterias y bacterias-levaduras para la conversión del sustrato y mejorar el rendimiento del proceso fermentativo, como la producción de enzimas extracelulares, metabolitos volátiles y no volátiles y cambios de pH, que contribuyen a la formación de los precursores del sabor (Braga et al., 2016).

Un cultivo iniciador es un cultivo microbiológico que acelera el proceso de fermentación. Los microorganismos que se seleccionan para los cultivos iniciadores deben tener ciertas características, como ser no patógenos, no toxigénicos y adaptables a las materias primas y al proceso (Girma & Sualeh, 2022). Además de esas características, los cultivos iniciadores aplicados al café deben tener la capacidad enzimática de degradar los componentes del mucílago, la producción fermentativa de compuestos volátiles, ácidos deseables y generar notas sensoriales positivas en los cafés fermentados (Haile & Kang, 2019a). Como las especies de bacilos que producen una serie de enzimas extracelulares, que pueden contribuir a la descomposición de la celulosa y pectina presente en la pulpa y el mucílago de las cerezas del café (Girma & Sualeh, 2022). Otro ejemplo es la aplicación de inóculos de levaduras, las cuales han demostrado que facilitan el proceso de fermentación al acelerar la degradación de la capa de mucílago, generando ácidos orgánicos y compuestos aromáticos volátiles (Haile & Kang, 2019b). Igualmente, las BAL son una categoría importante de la microbiota, por tanto también son usadas como cultivo indicador en la fermentación sumergida de las cerezas de café despulpado en el proceso húmedo (Oktaviani et al., 2020; Wang et al., 2020b).

8.4 Calidad del café

La calidad del café depende de múltiples factores, tanto intrínsecos como extrínsecos, incluyendo la altitud, las características del suelo, la variedad de café, el origen geográfico y las condiciones estacionales. También influyen los métodos postcosecha (lavado, fermentación, secado), el almacenamiento, el transporte, así como los procesos de tostado y preparación (Bressani, Martinez, Batista, et al., 2021; Carvalho Ferreira et al., 2023; Pimenta et al., 2018). Las definiciones de calidad del café varían según el contexto, la organización o el país. Sin embargo, la SCA ha estandarizado métodos para garantizar consistencia en el análisis sensorial del café de calidad (Vaz et al., 2023). Los parámetros de calidad se clasifican en características físicas, composición bioquímica y propiedades organolépticas, que dependen de la especie (Torrez et al., 2023).

14. Calidad física del café: esta se evalúa en los granos verdes usando descriptores físicos como el tamaño del grano, peso del grano y el porcentaje de granos rechazados. Dentro del porcentaje de granos rechazados se incluyen los granos inmaduros, los granos marchitos, los

granos flotantes, los granos negros totales o parciales, los granos agrios totales o parciales, los granos dañados por hongos, los granos dañados por insectos y los granos rotos o astillados (Torrez et al., 2023).

15. Calidad de la composición bioquímica del café: esta se evalúa en el grano tostado, debido a los compuestos que se perciben en taza producto de las reacciones químicas simultáneas de la degradación de proteínas, polisacáridos, trigonelina y ácidos clorogénicos durante el tostado (Torrez et al., 2023).
16. Calidad organoléptica del café: esta se evalúa aplicando el método de catación, el cual se basa en la percepción sensorial de catadores certificados (Q-grader) después de tostar, moler y preparar el café siguiendo protocolos estandarizados de la SCA (SCA, 2024). Los catadores evalúan los siguientes atributos y la suma de su puntuación determina la clasificación del café y su calidad de acuerdo con la Tabla 4.

Tabla 4. *Escala de puntuación y atributos de captación.*
Adaptado de SCA (2024)

Atributos	Puntaje	Calificación	Clasificación
Fragancia Aroma Sabor Regusto Acidez Cuerpo Equilibrio Dulzura Taza Limpia Uniformidad Puntuación global Defectos	90.00 – 100.00	Destacado	Café de especialidad
	85.00 – 89.99	Excelente	
	80.00 – 84.99	Muy bueno	
	75.00 – 79.99		
	68.00 – 74.99	Calidad especial baja	No especialidad
	< 67.00		

9 MODULATING COFFEE FERMENTATION QUALITY USING MICROBIAL INOCULUMS FROM COFFEE BY-PRODUCTS FOR SUSTAINABLE PRACTICES IN SMALLHOLDER COFFEE PRODUCTION

En el marco de esta investigación, uno de los objetivos específicos fue evaluar el uso de los subproductos del café en el desarrollo de inóculos para la fermentación semi-controlada de café con levadura (*S. cerevisiae*) y bacterias ácido-lácticas (*L. bulgaricus* y *S. Thermophilus*) comerciales. Para ello, se optimizaron las proporciones de dichos subproductos como sustrato de cultivo mediante un diseño experimental que consideró variables críticas como el tiempo de fermentación y el crecimiento microbiano. El artículo que se presenta a continuación aborda este objetivo mediante la descripción detallada de la metodología empleada, los resultados obtenidos y su discusión en el contexto de la producción de cafés especiales. Las referencias, figuras y tablas de este capítulo mantienen la numeración del artículo original.

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9.1 Texto del artículo completo

Modulating Coffee Fermentation Quality Using Microbial Inoculums from Coffee By-Products for Sustainable Practices in Smallholder Coffee Production

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1. Introduction

Coffee, a globally consumed commodity, faces multifaceted challenges that impact its economic, social, and environmental sustainability, ranging from socioeconomic factors to environmental concerns and market volatility. In response, different strategies have been used to

reduce volatility, increase productivity, and adopt sustainable practices to improve quality and prices. Producing nations have invested in differentiating their products as 'specialty coffees' through origin, processing techniques, and sustainability practices. Colombian mild-washed coffee serves as an exemplary case, distinguished by its unique characteristics that meet growing consumer demands for high-quality, sustainably produced coffee. At the producer level, diversifying postharvest processes, such as fermentation reactions, is an essential strategy for enhancing coffee distinctiveness and shaping sensory attributes and overall quality [1]. The link between postharvest processes and sensory characteristics emphasizes the importance of exploring modifications to traditional fermentation techniques. This topic has garnered significant attention from industry and academic researchers in recent years [2].

In wet coffee processing, coffee cherries are de-pulped and fermented after harvesting to remove residual mucilage, a process that significantly influences flavor through the production of microbial metabolites [3]. This workflow involves key steps: de-pulping, washing, fermentation lasting 12–18 h, and drying beans to achieve a final moisture content of 10–12%. However, this process generates substantial environmental challenges. On average, processing one ton of dried coffee beans generates approximately 3.6 tons of fresh by-products [4]. Approximately 40–45 L of wastewater (CWW) are produced per kilogram of processed coffee, with a chemical oxygen demand (COD) of 3000–7000 mg/L [5]. Untreated CWW and by-products often lead to water contamination, microbial proliferation, and unpleasant odors in rural areas, where waste management infrastructure is limited.

Coffee pulp (CP), comprising 29–43% (*w*/*w*) of the fruit, and mucilage, which contains 84.2% water, 8.9% protein, and 4.1% sugar [6,7], are among the key by-products of wet processing. These materials are rich in carbohydrates, providing valuable carbon sources for microbial fermentation. Additionally, CWW is rich in sugars and bioactive compounds, including caffeine and phenolics, and has potential applications in food, pharmaceutical, and cosmetic industries [8].

Despite their environmental risks, these by-products present an opportunity for sustainable reuse. Their composition makes them ideal substrates for microbial fermentation, transforming waste into valuable resources and aligning with circular economic principles in coffee production. Such innovations can mitigate environmental impacts while contributing to the sustainable development of the coffee industry. Fermentation is a critical stage in coffee processing and shapes flavor and aroma profiles. Natural fermentation occurs regardless of the post-harvest method used to obtain parchment coffee [9,10]. In wet or submerged fermentation, the medium is excess water, while in dry or semi-dry methods, it is the mucilage itself. Innovations in post-harvest operations include the introduction of specific microorganisms, such as LAB and yeast, either alone or in combinations, sourced

externally or internally isolated from the same coffee process [11,12]. These microorganisms are intentionally added to initiate fermentation, leading to shorter fermentation times and desirable outcomes. These modifications aim to amplify desired flavor attributes while mitigating undesirable ones, resulting in coffee with consistent flavors. These innovations increase the marketability of specialty coffees, reinforcing the importance of fermentation in the pursuit of coffee quality and differentiation, and are being studied in different steps of the processes, as in the coffee processing per se [13,14], in the green coffee beans already processed [15,16], and in the development of new beverages of brewed coffee [17,18,19].

LAB and yeast are significant components of the indigenous microbiota associated with the submerged fermentation of de-pulped coffee cherries in wet processing [9,20]. During mucilage fermentation, LAB produces organic acids, such as lactic and acetic acid, and volatile compounds that significantly contribute to the flavor of the coffee profile [21]. These organic acids enhance the acidity and overall complexity of the coffee. Additionally, LAB contributes to developing desirable fruity and floral notes by producing esters and other volatile compounds. LAB also plays a crucial role in reducing undesirable compounds that may negatively affect coffee flavor by outcompeting spoilage microorganisms and lowering the pH, thus creating a favorable environment for beneficial flavors [22,23]. Conversely, yeast produces volatile metabolites, particularly aromatic esters, that can remain after roasting and contribute to the fruity and floral attributes of the roasted coffee [2,20,21,22,23,24,25]. The metabolic activities of yeast significantly influence the modulation of soluble carbohydrates, organic acids, alcohols, volatile compounds, and other substances in the coffee pulp and mucilage. This metabolic exchange alters flavor-related constituents in green coffee beans, modifying the final roasted coffee flavor [2].

Previous studies on coffee fermentation were conducted with monocultures of various commercial microorganisms, including fungi, yeasts, and LAB [12,26]. The favorable biomodifications of coffee aroma by the tested microorganisms during green coffee bean fermentation were mainly achieved through the direct production of fruity volatile compounds by yeasts [25] and the acidification of green coffee beans by sugar-supplemented LAB fermentation, which indirectly elevated the production of caramel-smelling furan derivatives during roasting [16,22,27]. Also, not only have individual microbial applications taken place, but the synergistic interactions between yeast and LAB such as *Pichia fermentans* and *Pediococcus acidilactici* have been reported to impact aroma complexity significantly [23].

However, some methodologies used in these studies are not feasible at the farm scale, as they often require sterilization of coffee or rely on advanced technology and specialized knowledge. While

scientifically valid for understanding the effects of each inoculum, this approach is impractical for smallholder coffee growers due to their particular characteristics [28]. Additionally, the preparation of inoculums using specific microbiological media presents a significant drawback. These media often demand controlled laboratory environments and precise formulations, which are not easily replicable outside specialized facilities. This further limits the applicability of these methods for farmers with limited resources or access to advanced equipment.

Despite operating on a small scale, smallholder coffee farmers are vital to the industry. They represent 95% of coffee farms, with 84% managing less than two hectares of land [28,29]. Developing accessible, low-cost, and sustainable fermentation methods that utilize coffee by-products can contribute to sustainability by empowering smallholder farmers, promoting rural development, ensuring equitable access to innovation, and encouraging environmentally conscious coffee production. This study aimed to optimize the use of coffee by-products in developing inoculum for semi-controlled fermentation with commercial LAB and yeast at a smallholder scale. By focusing on accessible and replicable methods, the study seeks to advance sustainable practices in coffee processing, reducing waste, improving resource efficiency, and enhancing the livelihoods of smallholder farmers. To achieve this, a three-step procedure was followed: (1) designing and optimizing mixture proportions of CP and CWW for microbial viability variation, (2) applying these optimized inoculums to coffee fermentation, and (3) evaluating the fermentation parameters and sensory qualities of the final product.

2. Materials and Methods

A research roadmap was designed to ensure a replicable approach toward advancing sustainable coffee processing practices. This structured approach is illustrated in Figure 1, providing a clear overview of the study's methodology.

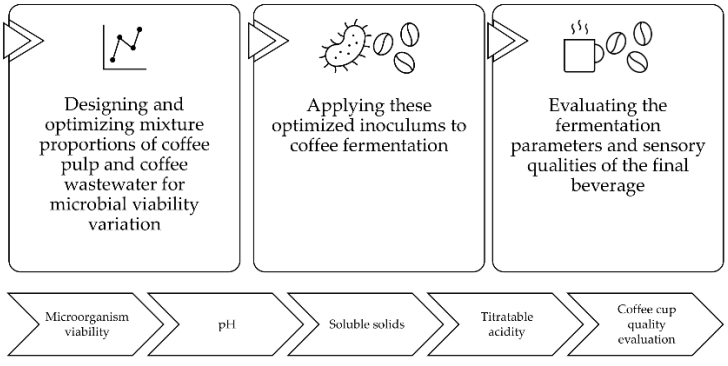

Figure 1. *Research roadmap.*

2.1. Coffee Beans

Freshly harvested coffee cherries (*Coffea arabica* var Colombia) were obtained at 1557 m above sea level in Morales, Cauca, Colombia. During harvest, cherries were selected based on health and maturity criteria; any green, overripe, or damaged cherries were discarded.

2.2. Coffee Pulp

The coffee pulp (CP) was collected immediately from the de-pulping machine during a traditional wet process. It was washed to remove debris, stored in plastic bags, and frozen until needed. The pulp was thawed, blended in a commercial blender with water at a 1:2 pulp-to-water ratio, and boiled for 5 min to stop spontaneous fermentation.

2.3. Coffee Wastewater

The coffee wastewater (CWW) was produced during the traditional wet fermentation process, in which de-pulped coffee cherries were submerged in water at a 1:1 ratio and allowed to ferment spontaneously for 24 h. Following fermentation, the coffee mass was removed, and the CWW was immediately boiled for 5 min, cooled, and then frozen.

2.4. Microorganisms

Fresh commercial yeast *Saccharomyces cerevisiae* (Levapan, Cali, Colombia) and lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* commercial yogurt (Colacteos, Pasto, Colombia) were used for fermentation.

2.5. Analytical Methods

2.5.1.Microorganism Viability

Viable aerobic bacterial counts were obtained by enumerating LAB on de Man Rogosa Sharpe agar and culturing yeast on potato dextrose agar (Scharlab, Barcelona, Spain). For microbiological analysis, 1 mL samples from each CWW were diluted in a 10-fold series with peptone water (Scharlab, Barcelona, Spain), ranging from 10^1 to 10^6. Microbial counts were determined using the standard spread plate method, with agar plates incubated at 37 °C for 48 h for bacteria and 24 h for yeasts. Total viable bacterial counts were obtained by counting the colony-forming units (CFU). The reported population data represents the means of triplicate analyses.

2.5.2. pH

The pH of the inoculum and the coffee fermentation liquid was monitored by taking samples from each at every sampling point according to AOAC method 981.12 [30]. The pH of the brewed coffee was determined using a technique described by Mazzafera (1999), where 2.25 g of ground coffee was mixed with 50 mL of deionized water heated to 80 °C, then cooled to room temperature before measuring the pH. The reported data represents the mean values from triplicate analyses [31].

2.5.3.Soluble Solids

The total soluble solids (SS) of the inoculum and the coffee fermentation liquid were monitored by taking samples from each at every sampling point using a digital and portable refractometer (Atago, Tokyo, Japan), according to AOAC Official Method 932.12 [30].

2.5.4. Titratable Acidity

Titratable acidity (TA) was determined by mixing 1 mL of liquid media from the inoculum, fermentation, and brewed coffee with distilled water. Acidity was titrated using 0.1 N sodium hydroxide with phenolphthalein as the indicator. The results reported are the means of triplicate analyses with standard deviation, according to AOAC Official Method 942.15 [30], using lactic acid as the standard acid with a specified equivalence of 0.009.

2.6. Coffee Cup Quality Evaluation

Cup quality was evaluated by an external coffee taster certified as a Q-Arabica grader. The evaluation was performed in triplicate as a blind assessment, with each sample assessed three times by the same certified grader to ensure consistency. The coffee samples were prepared following Specialty Coffee Association (SCA) protocols [32], roasted at 115.6 °C for 9–10 min until reaching a roast degree of 48–63 Agtron, and ground according to SCA cupping protocols. For brewing, 150 mL of hot water (92–94 °C) was poured over 8.25 ± 0.25 g of ground coffee, and the mixture steeped for 4 min. Sensory assessments commenced at 65 °C for olfactory evaluation and 43 °C for gustatory evaluation. Attributes assessed included aroma, taste, acidity, body, balance, aftertaste, and overall quality, each rated on a scale from 0 to 10 in 0.25 increments. A descriptive analysis and a total score, with a maximum of 100 points, were assigned, covering fragrance/aroma, flavor, acidity, body, uniformity, balance, sweetness, clean cup, and overall quality. To ensure an anonymous review, samples were masked, and the grader was not informed of the experimental treatments of each sample.

2.7. Experimental Methods

2.7.1.Bacteria and Yeast Inoculums Optimized Production

The experiments utilized a mixture design, part of the response surface methodology class. Mixtures were defined by varying the percentages of coffee pulp (CP) and coffee wastewater (CWW) in experimental units of 100 mL with 5% (*v*/*v*) inoculation of LAB and 1% (*w*/*v*) yeast. The optimization of the mixtures was based on the relative microbial growth (RG) of CFU/mL from the initial inoculation to the maximum CFU/mL during the 48 h fermentation, with measurements taken at 0, 12, 18, 24, 36, and 48 h. SS, TA, and pH were also monitored during the 48 h inoculum fermentation. An analysis of variance (ANOVA) was performed to determine the effect of these parameters on inoculum generation during the process variable levels of each microorganism. Statistical analyses were performed with MINITAB version 19.

Relative microbial growth (RG) was quantified as the maximum increase in microbial viability and the time required to achieve it, as shown in Equation (1):

$$\mathrm{RG} = \frac{(CFU_{ti} - CFU_{t0})}{CFU_{t0}} \quad (1)$$

where CFU_{ti} represents the highest viable count reached by each microorganism during the experiment at time *ti*, and CFU_{t0} is the viable count at the initial time point.

The experiments were conducted in a randomized order to reduce systematic errors. As indicated in Equation (2), the sum of the proportions equals one.

$$\sum_{i=1}^{k} Xi = 1 \;\; 0 \leq Xi \leq 1 \; i = 1 \ldots k \quad (2)$$

Where Xi stands for the proportions of the i^{th} component of the mixture represents the total number of components.

This study employed a quadratic regression model, as shown in Equation 3, to analyze the relationship between the dependent variable $\hat{y}$ and the independent variables Xi and Xj:

$$\hat{y} = \sum_{i=1}^{k} \beta i Xi + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta ij Xi Xj \quad (3)$$

Where $\hat{y}$ is the predicted dependent variable, Xi (CP) and Xj (CWW) represent independent variables; βi and βij denote the linear and quadratic coefficients, respectively. The first term captures the linear effects of the variables, while the second term accounts for the interactions between variable pairs, capturing quadratic effects.

Table 1 presents the proportions of components, the experimental conditions of the mixture design, the response, and the process variables. The experiments consisted of 18 runs, including cubic, central, and axial points. The proportions of the components were evaluated for both yeast and LAB. Additionally, different growth times were assessed for each microorganism: 36 and 48 h for LAB and 12 and 36 h for yeast.

***Table 1**. Component and factor levels used in mixture design of experiment.*

Run	Components (%)		Process variable Time (h)		Response variable
	CP	CWW	LAB	Yeast	
1	25	75	36	12	Relative Microbial Growth (RG)
2	25	75	48	36	
3	50	50	36	12	
4	50	50	48	36	
5	75	25	48	36	
6	50	50	36	12	
7	100	0	48	36	
8	50	50	48	36	
9	50	50	48	36	
10	50	50	36	12	
11	0	100	48	36	
12	0	100	36	12	
13	75	25	36	12	
14	100	0	36	12	
15	50	50	48	36	
16	50	50	36	12	
17	50	50	48	36	
18	50	50	36	12	

An ANOVA was performed to assess the adequacy and significance of the model. The statistical significance of the model and its equation terms were evaluated based on the *p*-value ($p < 0.05$). Statistical analyses were carried out using MINITAB version 19. The quality of the model was evaluated by computing the coefficient of determination (R^2) to determine the proportion of variance

in the dependent variable explained by the independent variables, as shown in Equation (4). The adjusted R^2 to measure the variance between model predictions ($\hat{y}$) and experimental data (y) as described in Equation (5). Values close to one indicate strong agreement between experimental and simulated data. The root mean square error (RMSE) was calculated to evaluate the predictive accuracy of the model using Equation (6), where lower RMSE values suggest higher model precision [33].

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(yi - \hat{y}i)^2}{\sum_{i=1}^{n}(yi - \bar{y})^2} \quad (4)$$

$$Adjusted\ R^2 = 1 - \frac{(1 - R^2)(n - 1)}{n - P - 1} \quad (5)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(yi - \hat{y}i)^2}{n}} \quad (6)$$

where yi represents the experimental data, $\hat{y}i$ is the model prediction, $\bar{y}$ the experimental data, n is the number of observations, and P is the number of predictors. SS, TA, and pH were also monitored during the 48 h inoculum fermentation. An ANOVA was performed to determine the effect of these parameters on inoculum generation during the process variable levels of each microorganism. Statistical analyses were performed with MINITAB version 19.

2.7.2.Optimization

A response optimizer was used to find the optimal mixture using the desirability compound, aiming to maximize the RG as the response variable. All variables were equally weighted. The selection was based on response variable models explaining over 70% of behavior (R^2 adj. > 0.7).

2.7.3.*Experimental Validation*

Validation experiments were performed by reproducing inoculum production under optimal conditions. The observed responses were compared to the optimal predictions from the optimization, using relative error (*RE*) as shown in Equation (7):

$$RE = \frac{y - \hat{y}}{y} * 100\% \quad (7)$$

The optimized procedure and control fermentation were performed three times, with three samples per replicate.

2.8. Coffee Processing with Optimized Inoculums

The coffee processing was conducted using the wet method. Freshly harvested Coffea arabica cherries were de-pulped using a DH-2 depulper (Penagos, Colombia), and the resulting beans were mixed with potable water to ferment and remove the mucilage. The laboratory-scale setup consisted of 1 L flasks containing 800 g of de-pulped beans and 400 mL of water.

LAB and yeast inoculums were prepared according to the optimized conditions obtained in the Section 2.7.2. prior to coffee processing. The following six fermentation treatments were applied with 6% (*v*/*w*) of the respective inoculum: (1) Direct LAB inoculation sourced from commercial yogurt without prior optimization; (2) Optimized LAB inoculum prepared under optimized growth conditions; (3) Fresh yeast inoculation without prior optimization; (4) Optimized yeast inoculum prepared under optimized growth conditions; (5) Mixture of optimized LAB and yeast inoculum; (6) Control treatment without microbial inoculation.

All coffee processing was conducted at room temperature without sterilization of the materials to mimic real-world coffee farm conditions. The fermentation vessels were left undisturbed for the process, allowing for the natural degradation of mucilage. After fermentation, the coffee beans were removed from the vessels, washed with potable water, and dried at 40 °C for 24 h until the beans reached a moisture content of 10–12%.

A principal component analysis (PCA) was used to evaluate both physicochemical and sensory attributes across the different coffee samples. The sensory data included fragrance/aroma, body, flavor, uniformity, and overall score, while the physicochemical parameters were assessed both at the initial (I) and end (E) stages.

3. Results

The results are structured into four sections. Section 3.1 presents the monitoring of key fermentation parameters such as pH, SS, TA, and RG during the inoculum fermentation process. Section 3.2 focuses on the statistical analysis of the development of the inoculum, providing results on RG, statistical models, and further analysis of the interactions between coffee by-products and RG. Section 3.3 addresses the optimization of the results using the desirability compound and the chosen model to maximize RG, along with the validation of the optimized inoculum, comparing the predicted and observed outcomes to evaluate the accuracy of the optimization process. Finally, Section 3.4 evaluates the performance of the optimized inoculum during coffee fermentation, emphasizing fermentation parameters and the sensory evaluation of the final product.

3.1. Dynamic Monitoring of Inoculum Fermentation Parameters

The mixture design of experiments (DOE) for both microorganisms included eighteen experimental runs in five formulations, each varying by 25%. The initial physicochemical parameters of CP and CWW used as carbon sources in the inoculum were as follows: pH 4.24 and 3.81 ± 0.06, TA 0.46 ± 0.02 and 0.65 ± 0.02, and SS 3.20 ± 0.33 °Brix and 4.91 ± 0.33 °Brix, respectively. Figure 2 shows the growth of LAB and yeast across the different formulations. The initial microbial viability among formulations varied between values of 1.4 ± 0.1 × 10^6 CFU/mL for LAB and 5.8 ± 0.4 × 10^7 CFU/mL for yeast.

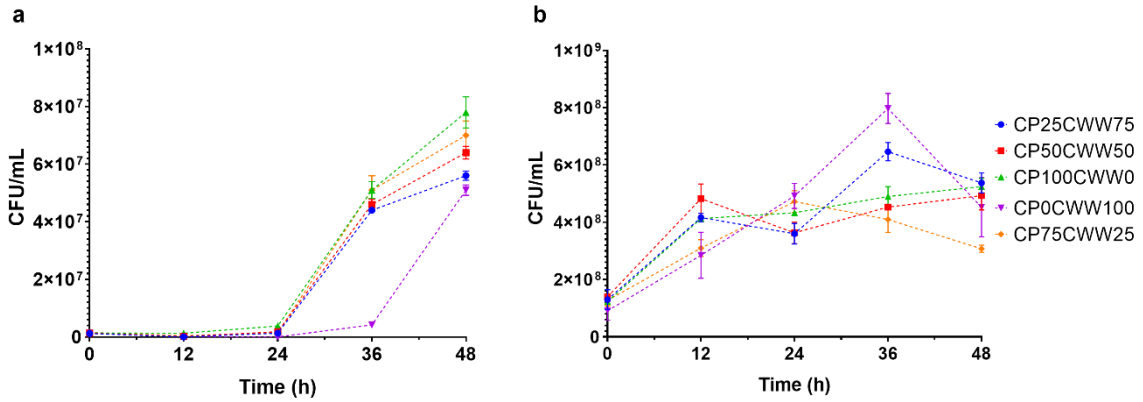

Figure 2. *Lactic acid bacteria (**a**) and yeast (**b**) growth in different formulations of inoculum. Labels represent the percentage of each component in the total inoculum: CP (coffee pulp) and CWW (coffee wastewater). Data are reported as mean ± SD from measurement replicates, with experimental replicates based on the DOE.*

The growth of each microorganism exhibited opposite trends. LAB viability was primarily influenced by the proportion of CP in the formulations, reaching 7.8 × 10^7 CFU/mL after 48 h of incubation in CP100CWW0. In contrast, yeast viability was driven by the percentage of CWW, peaking at 8.3 × 10^8 CFU/mL after 36 h of incubation in CP0CWW100.

Figure 3 illustrates the fermentative parameters SS, pH, and TA for the different formulations, highlighting the dynamic changes observed during fermentation for both inoculums. A consistent decline in SS was observed throughout the 48 h fermentation period in all formulations. Initial SS values ranged from 2.2 to 4.6 °Brix, influenced by the proportions of CP and CWW in each formulation. As fermentation progressed, SS levels gradually decreased, with the most significant drop observed in the yeast-inoculated blends after 12 h, indicating sustained sugar consumption by the yeast formulations CP0CWW100 with yeast and CP100CWW0 with LAB exhibited the sharpest

declines, suggesting higher sugar availability and consumption by the inoculated microorganisms than initially measured.

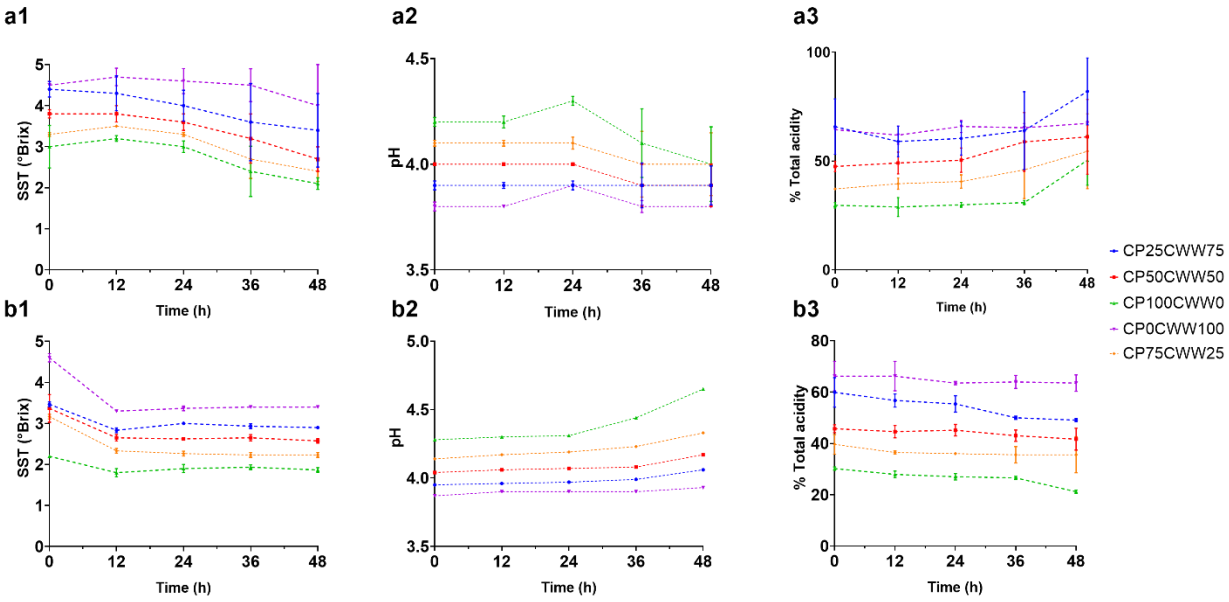

Figure 3. *Fermentative parameters of lactic acid bacteria (**a1–a3**) and yeast (**b1–b3**) in different inoculum formulations: total soluble solids (SST) (**1**), pH (**2**), and titratable acidity (TA) (**3**). Labels represent the percentage of each component in the total inoculum: CP (coffee pulp) and CWW (coffee wastewater). Data are reported as mean ± SD from measurement replicates, with experimental replicates based on the DOE.*

The pH levels exhibited slight fluctuations during fermentation. In LAB-inoculated formulations, particularly CP100CWW0 and CP75CWW25, pH values dropped after 24 h, which is consistent with the production of organic acids as LAB metabolizes sugars and growth. In contrast, yeast-inoculated formulations, such as CP0CWW100 and CP25CWW75, maintained stable pH levels or showed slight increases over time, indicating that yeast metabolism results in less acid production compared to LAB.

TA increased significantly in LAB-inoculated formulations over the 48 h fermentation period, especially in CP100CWW0 and CP75CWW25. The sharp increase in TA between 36 and 48 h aligns with active acid production by LAB, as seen in the corresponding decrease in pH. On the other hand, yeast-inoculated formulations exhibited minimal or stable changes in TA, with CP0CWW100 even showing a slight reduction. This further supports the observation that yeast fermentation produces lower amounts of acid compared to LAB.

3.2. Statistical Analysis

The independent and dependent variables were fitted to the various models proposed in the experimental design. Table 2 summarizes the observed and predicted responses for both microorganism inoculums based on the results of the mixture design experiment. The linear predictive models for LAB and yeast are shown in Equations (8) and (9), respectively, while the quadratic predictive models for these microorganisms are presented in Equations (10) and (11).

Table 2. *Experimental results of the maximum increment in microbial viability of lactic acid bacteria (LAB) and yeast across different inoculum formulations, along with the model quality results for both microorganisms.*

Exp. Number (n)	Components		Response variable Relative growth (RG)							
			Process variable	LAB			Process variable	Yeast		
	X1	X2	X3				X3			
	Pulp (%)	Wastewater (%)	Time (h)	Observed	Linear model	Quadratic model	Time (h)	Observed	Linear model	Quadratic model
1	25	75	36	34.33	24.23	24.68	12	5.32	5.46	5.50
2	25	75	48	44.30	42.22	42.33	36	11.52	10.19	10.12
3	50	50	36	34.06	30.65	35.16	12	6.88	5.89	6.31
4	50	50	48	51.12	46.13	47.15	36	9.18	8.49	7.80
5	75	25	48	45.87	50.03	50.14	36	6.87	6.80	6.73
6	50	50	36	28.40	30.65	35.16	12	6.38	5.89	6.31
7	100	0	48	53.14	53.94	51.29	36	7.03	5.10	6.91
8	50	50	48	38.06	46.13	47.15	36	7.17	8.49	7.80
9	50	50	48	42.71	46.13	47.15	36	8.30	8.49	7.80
10	50	50	36	35.38	30.65	35.16	12	6.97	5.89	6.31
11	0	100	48	34.40	38.32	35.67	36	13.19	11.89	13.70
12	0	100	36	1.92	17.80	6.06	12	3.85	5.03	3.93
13	75	25	36	33.34	37.07	37.52	12	5.16	6.32	6.36
14	100	0	36	34.53	43.49	31.76	12	6.07	6.74	5.64
15	50	50	48	53.09	46.13	47.15	36	6.44	8.49	7.80
16	50	50	36	37.80	30.65	35.16	12	6.71	5.89	6.31
17	50	50	48	52.48	46.13	47.15	36	6.75	8.49	7.80
18	50	50	36	36.07	30.65	35.16	12	6.05	5.89	6.31
R2					0.6736	0.8364			0.7321	0.8797
Adj-R2					0.6036	0.7682			0.6747	0.8295
c					6.6516	4.7093			1.1311	0.7598

R^2: Coefficient of determination; Adj-R^2: adjusted R^2 to measure the variance between model predictions and experimental data; RMSE: root mean square error.

The quadratic model demonstrated a better fit for the LAB and yeast inoculum, as evidenced by statistical values. For the LAB inoculum, the quadratic model resulted in an R^2 of 0.84, an adjusted R^2 of 0.77, and an RMSE of 4.71. Similarly, the quadratic model yielded an R^2 of 0.88, an adjusted R^2 of 0.83, and an RMSE of 0.76 for the yeast inoculum. These values indicate a reasonable fit between the predicted and observed data, especially for the yeast inoculum.

Linear model,

$$RG_{LAB} = 12.163\,X_1 - 43.744\,X_2 + 0.870\,X_3X_1 + 1.710\,X_3X_2 \quad (8)$$

$$RG_{yeast} = 7.563\,X_1 + 1.609\,X_2 - 0.068\,X_3X_1 + 0.285\,X_3X_2 \quad (9)$$

Quadratic model,

$$RG_{LAB} = -26.848\,X_1 - 82.755\,X_2 + 216.060\,X_1X_2 + 1.628\,X_3X_1 + 2.467\,X_3X_2 - 4.196\,X_1X_2X_3 \quad (10)$$

$$RG_{yeast} = 5.002\,X_1 - 0.952\,X_2 + 14.185\,X_1X_2 + 0.053\,X_3X_1 + 0.407\,X_3X_2 - 0.672\,X_1X_2X_3 \quad (11)$$

where RG represents relative microbial growth; X_1 and X_2 are independent variables CP and CWW, respectively; and X_3 is process variable.

The ANOVA of quadratic models revealed significant effects of the concentrations of CWW, CP, and time on the viability of the microorganisms. Both CWW and CP concentrations, along with incubation time, were statistically significant components and factors for both inoculums. LAB viability increased with higher time values and was significantly influenced by the proportion of CP in the mixture (p = 0.025). Similarly, yeast viability increased with higher time values but was primarily driven by the percentage of CWW (p = 0.00) (Supplementary Materials Table S1).

3.3. Optimization and Validation

The response variable was optimized using the desirability function to maximize microbial growth for both the LAB and yeast inoculums. According to the model, the highest RG for the LAB inoculum was predicted with 100% CP (CP100CWW0) and a fermentation time of 48 h, yielding a predicted RG of 51.3, equivalent to a theoretical count of 7.1×10^7 CFU/mL. For the yeast inoculum,

the highest RG was predicted with 100% CWW (CP0CWW100) and a fermentation time of 36 h, resulting in an RG of 13.7 and a theoretical count of 8.5×10^8 CFU/mL.

Validation experiments were performed in triplicate under these optimized conditions. At the end of the fermentation period, the experimental RG values for LAB were 45.98 ± 4.30 (equivalent to $6.4 \pm 0.6 \times 10^7$ CFU/mL), while for yeast, they were 12.14 ± 1.16 (equivalent to $7.6 \pm 0.7 \times 10^8$ CFU/mL). These results confirmed the accuracy of the model, as the experimental values closely matched the theoretical predictions. The RE was 10% for LAB and 11% for yeast, demonstrating the reliability of the optimization process, as shown in Table 3.

Table 3. *Validation model for LAB and yeast inoculum.*

Parameter	Experimental value	Predicted Value	RE (%)
RG_{lab}	45.98 ± 4.30	51.3	10.35
RG_{yeast}	12.14 ± 1.16	13.70	11.33

RE: relative error.

3.4. Performance Evaluation of Optimized Inoculum

The viability of LAB and yeast in coffee fermentation was evaluated using both direct inoculation from commercial sources and optimized inoculums. The initial LAB count from direct inoculation using commercial yogurt (fermentation 1) was $2.9 \pm 0.5 \times 10^6$ CFU/mL, while the optimized LAB inoculum (fermentation 2 and 5) had an initial count of $7.1 \pm 0.7 \times 10^7$ CFU/mL. For yeast, direct inoculation using fresh yeast (fermentation 3) showed an initial count of $3.5 \pm 0.4 \times 10^9$ CFU/mL, whereas the optimized yeast inoculum (fermentation 4 and 5) started with $8.2 \pm 0.8 \times 10^8$ CFU/mL. The SS, pH, and TA were also measured during fermentation, as detailed in Table 4.

Table 4. *Experimental results of lactic acid bacteria (LAB) fermentation parameters and yeast-inoculated coffee.*

Fermentation	SS (°Brix)		pH		TA (%)	
	Initial	End	Initial	End	Initial	End
1	5.3	5.9	5.04	3.84	13.21	65.76
2	5.1	3.1	4.88	3.78	12.31	54.95
3	2.3	3.3	4.58	4.77	31.83	33.03
4	4.3	3.1	4.48	4.20	20.72	33.03
5	4.4	3.0	4.61	3.90	16.81	46.24
6	5.0	6.1	5.38	3.86	7.51	62.43

SS: soluble solids; TA: titratable acidity. 1. Direct LAB inoculation sourced from commercial yogurt without prior optimization. 2. Optimized LAB inoculum prepared based on optimized growth conditions. 3. Fresh yeast inoculation without prior optimization. 4. Optimized yeast inoculum prepared based on optimized growth

conditions. 5. Mixture of optimized LAB and yeast inoculum. 6. Control treatment without any microbial inoculation.

Regarding pH, LAB-inoculated coffee (fermentations 1 and 2) showed a decrease, indicating increased acidity, which is typical of LAB-driven fermentations. This aligns with the expected production of organic acids by lactic acid bacteria. In contrast, yeast-fermented coffee (fermentations 3 and 4) maintained stable pH levels or exhibited only slight fluctuations, indicating lower acid production compared to LAB-fermented coffee. Regarding TA, coffee inoculated with LAB (fermentations 1 and 2) demonstrated a significant increase in acidity during fermentation, consistent with the observed pH decrease. Conversely, yeast-fermented samples (fermentations 3 and 4) exhibited more stable TA levels, indicating lower acid production. The control treatment (fermentation 6) followed a similar TA trend to yeast-fermented coffee. LAB-inoculated coffee (fermentations 1 and 2) showed a substantial increase in TA, correlating with the pronounced pH decrease, confirming that LAB fermentation was characterized by elevated acid production from the breakdown of carbohydrates into organic acids, driving the overall acidification of the coffee. In contrast, TA levels in yeast-fermented coffee (fermentations 3 and 4) showed a modest increase, indicating that yeast fermentation resulted in lower acid production. This aligns with the more stable pH values observed, suggesting that yeast activity primarily influenced other metabolic pathways, such as the production of flavor compounds, rather than significant acid generation.

The inoculation of LAB and yeast on the sensory properties is shown in Table 5. Regarding the sensory evaluation, variations in fragrance/aroma, flavor, aftertaste, and acidity scores were recorded across the fermentation setups. Treatment 5, which involved a mixture of optimized LAB and yeast inoculum, consistently scored highest in key sensory attributes, such as fragrance/aroma (8.25), flavor (8), and overall score (8.25), indicating a direct relationship between fermentation parameters and sensory outcome as the observed increase in titratable acidity in treatment 5, which showed the highest final TA and lowest pH.

Table 5. Sensory profile of lactic acid bacteria (LAB) fermentation parameters and yeast-inoculated coffee.

	Fermentation					
	1	2	3	4	5	6
Characteristic	Score					
Fragrance/aroma	7.75	8	8	7.75	8.25	8
Flavor	8	8	8	7.75	8	8
Aftertaste	7.75	7.75	7.75	7.75	7.75	7.75
Acidity	7.75	7.75	7.75	8	7.75	7.75
Body	7.75	7.75	7.75	7.5	7.75	7.75

Uniformity	10	10	10	10	10	10
Sweetness	10	10	10	10	10	10
Clean cup	10	10	10	10	10	10
Balance	7.75	7.75	7.75	7.5	7.75	7.75
Overall	7.75	8	8	7.75	8	8
Final score	84.5	85	85	84	85.25	85
Sensory notes	Fragrance of tangerine with walnut, aroma of almond and grapefruit, flavor of tangerine, hazelnut, and molasses, lingering honey-like aftertaste of sugarcane and walnut, creamy body, citrus honey-like acidity.	Fragrance of vanilla and maple syrup, aroma of red apple with honey, flavor of vanilla, butter, and orange, molasses aftertaste with tangerine, silky body, tangerine citrus acidity.	Fragrance of honey with hazelnut, aroma of orange and maple syrup, creamy flavor of grapefruit, tangerine, and butter, honey aftertaste with vanilla, creamy body, citrus honey-like acidity.	Fragrance of molasses and hazelnut, aroma of maple syrup with molasses, flavor of coriander seed, celery, and molasses, herbal and molasses aftertaste, citrus honey-like acidity, light and silky body.	Fragrance of delicate mango with toasted hazelnut, aroma of orange with honey, delicate flavor of vanilla, molasses, and tropical fruits, caramel aftertaste with a subtle hint of honey, silky body, bright citrus acidity.	Fragrance of honey and molasses, aroma of maple syrup with anise, flavor of lime, tangerine, and maple syrup, honey-like aftertaste, syrupy body, juicy citrus acidity.

1. Direct LAB inoculation sourced from commercial yogurt without prior optimization. 2. Optimized LAB inoculum prepared based on optimized growth conditions. 3. Fresh yeast inoculation without prior optimization. 4. Optimized yeast inoculum prepared based on optimized growth conditions. 5. Mixture of optimized LAB and yeast inoculum. 6. Control treatment without any microbial inoculation.

Aftertaste showed little variation across treatments, suggesting that despite differences in acidity, the aftertaste remained consistent. Similarly, body, or mouthfeel, exhibited slight variation, with scores consistently between 7.5 and 7.75, indicating that while LAB and yeast inoculum influenced acidity and flavor, their impact on body was minimal. Uniformity maintained a perfect score of 10 across all setups, highlighting that the fermentation processes were well-controlled and resulted in minimal sensory defects across samples, regardless of physicochemical changes. Clean cup, which indicates the absence of off-flavors or defects, remained constant at 10 for all fermentations. This stability implies that the inoculum types did not introduce undesirable flavors, even in treatments with higher acidity.

In contrast, balance and other key sensory characteristics such as fragrance/aroma and flavor showed more variation, particularly in treatment 5. Balance scored 7.75 across most treatments but increased to 8 in treatment 5, suggesting that the LAB and yeast inoculum mixture in this treatment created a more harmonized sensory profile. This improvement aligns with both physicochemical changes—especially higher acidity—and the enhanced aroma and flavor scores, indicating a more cohesive sensory experience. The highest fragrance/aroma score (8.25) was also observed in treatment 5, which had high TA and a notably lower pH. This suggests that volatile organic acids and other by-products may have contributed to enhanced aroma and flavor perception. Lower pH values in fermented products are often linked to more complex profiles, as organic acids and other metabolites contribute to sharper, more distinct tastes and aromas. The strong flavor profile of treatment 5 further supports this, indicating that the mixed inoculum enriched flavor complexity and balance. Acidity also correlated with physicochemical changes; treatment 5 had a high acidity score (7.75), corresponding to the higher TA (62.43%), suggesting that fermentation increased both acid content and perceived acidity, enhancing the sensory experience.

Across all fermentations, certain sensory attributes appeared consistently. Notably, citrus acidity with honey-like qualities was present in each sample. Fragrance and aroma notes featured nutty undertones, such as hazelnut, walnut, and maple syrup. The body of the coffee varied slightly from light and silky to syrupy, yet remained smooth across treatments, suggesting minimal impact of inoculum variations on mouthfeel.

PCA was performed to visually assess the relationships between physicochemical parameters and sensory attributes across the fermentation treatments (Figure 4). The PCA biplot reduces data dimensionality, enabling the identification of key contributors to sensory variation and facilitating the interpretation of correlations between physicochemical variables and sensory characteristics. The first two principal components (PC1 and PC2) accounted for 71.0% and 15.6% of the total variance, respectively, effectively capturing a significant portion of the variability in the data.

The positioning of the treatments in the PCA space reveals distinct profiles. PC1, which aligns with variables such as final titratable acidity (TA(E)), soluble solids (SS(E)), and overall score, is positively associated with higher sensory scores. This trend is particularly evident for treatments 1, 2, 3, 5, and 6, which cluster along PC1, showing strong correlations with sensory attributes such as fragrance/aroma, body, flavor, uniformity, and final score.

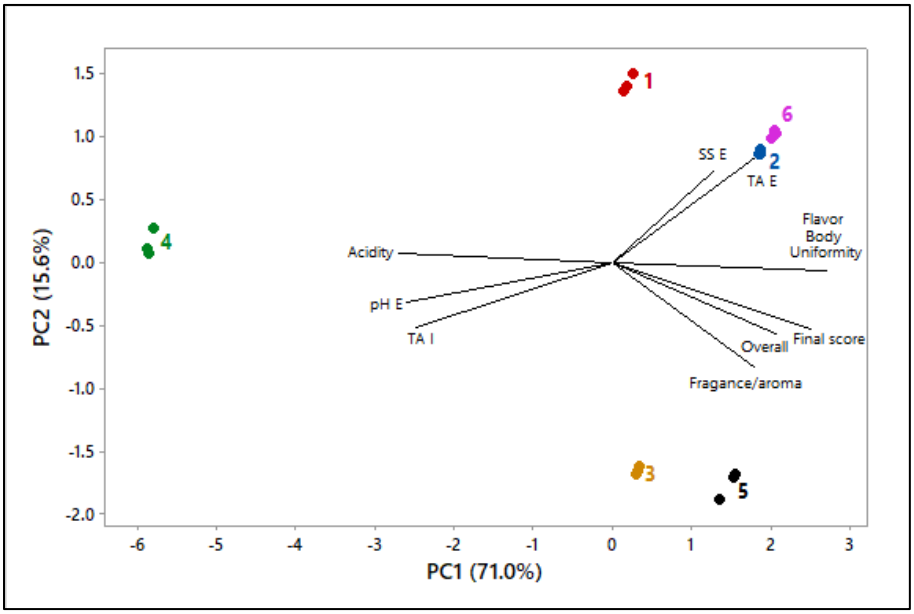

Figure 4. *Biplot of the principal component analysis (PCA) of physicochemical and sensory attributes across the different coffee samples. A total of 86.6% of the variance was described by the two principal components (PC). Dots correspond to individual replicates of 1. Direct LAB inoculation sourced from commercial yogurt without prior optimization. 2. Optimized LAB inoculum prepared based on optimized growth conditions. 3. Fresh yeast inoculation without prior optimization. 4. Optimized yeast inoculum prepared based on optimized growth conditions. 5. Mixture of optimized LAB and yeast inoculum. 6. Control treatment without any microbial inoculation.*

4. Discussion

This study focused on optimizing inoculum formulations by utilizing coffee processing by-products, specifically CP and CWW. Due to their large volumes, these by-products present significant environmental challenges for coffee farms. CP constitutes approximately 45% of the fresh coffee cherry, meaning that producing 1 ton of dried coffee beans generates 3.64 tons of fresh CP. Additionally, wet processing methods produce 40–45 L of CWW per kilogram of dried coffee, resulting in around 40,000–45,000 L of CWW for each ton of dried coffee beans [5]. Utilizing these by-products mitigates environmental impact, reduces waste, and creates value-added products, thereby improving the sustainability and efficiency of the coffee production system.

4.1. Impact of Coffee By-Products on LAB and Yeast Growth in Different Inoculum Formulations

The optimization of inoculum formulations demonstrated the critical role of coffee by-products, such as CP and CWW, in enhancing microbial viability. The results confirmed that LAB, by producing organic acids, lowered the pH of fermentation media, creating a favorable environment for

inhibiting spoilage microorganisms and enhancing desirable flavor attributes [10,19,31]. For example, the significant pH reduction observed in CP-rich formulations correlates with the higher viability of LAB, aligning with its known role in producing organic acids. Similarly, yeast-driven formulations, particularly those enriched with CWW, showed increased production of aromatic esters and modulated essential substances, including soluble carbohydrates and organic acids, as reflected in the enhanced sensory complexity of treatments involving yeast inoculum [34,35].

The predictive model that optimized inoculum formulations performed well in predicting microbial viability, though minor deviations were observed between theoretical predictions and experimental outcomes. These discrepancies could be attributed to unmodeled environmental factors, such as variations in initial microbial load or slight temperature fluctuations during fermentation. Nevertheless, the model proved robust, optimizing conditions for maximizing LAB and yeast viability.

The composition of coffee by-products significantly influences microbial growth during fermentation. A higher percentage of CP favored LAB viability, while higher concentrations of CWW enhanced yeast growth. Formulations with balanced ratios of CP and CWW, such as CP50CWW50 and CP25CWW75, supported moderate growth rates for LAB and yeast. However, after 36 h, yeast viability declined, which is associated with nutrient depletion as the SS decreased or the accumulation of metabolic by-products [18].

The concentration of SS reflects the availability of fermentable sugars, a critical factor for microbial growth. Yeasts, particularly *Saccharomyces cerevisiae* and *Pichia* species, thrive in CWW-rich environments due to their higher sugar content, leading to a more rapid decrease in SS than CP-dominant formulations. The ability of the yeast to quickly consume sugars supports the production of volatile compounds, which contribute to the fruity and floral aromas of the coffee. LAB, however, metabolizes sugars more slowly, particularly complex carbohydrates, resulting in a more gradual decline in SS. This difference in sugar utilization between yeast and LAB reflects their complementary roles in fermentation dynamics.

Changes in pH, SS, and TA during coffee fermentation are closely tied to the metabolic activity of LAB and yeast. The decision not to adjust initial physicochemical parameters, such as pH, SS, or TA, was made to replicate the natural fermentation conditions typically encountered on smallholder coffee farms, where such variations are not controlled. This allowed us to evaluate the performance of LAB and yeast in realistic scenarios, ensuring the findings are directly applicable to practical, field-scale operations. This approach is particularly relevant as smallholder farmers often lack the resources

or infrastructure to precisely adjust these parameters during fermentation. The fermentative metabolism of LAB and the respiratory metabolism of yeast, especially under aerobic conditions, lead to distinct patterns in these parameters across different formulations.

Differences in SS between the formulations reflected variations in sugar content, with CWW providing a more abundant carbon source for yeast due to its higher concentration of soluble sugars [6,36]. Changes in pH levels across different formulations correlated with carbohydrate availability. Microbial activity led to the hydrolysis of polysaccharides into monosaccharides, producing organic acids, which caused a decrease in pH [3,6,37], particularly in CWW-rich formulations [37]. After 36 h, pH levels in all formulations begin to rise, signaling reduced microbial activity due to nutrient depletion or the accumulation of inhibitory by-products. This shift indicates a decline in fermentation efficiency as LAB and yeast become less metabolically active.

TA, an indicator of organic acid production, shows a marked increase during the initial stages of fermentation, especially in yeast-inoculated formulations with higher CWW content. The rapid fermentation of sugars by yeast results in the accumulation of organic acids such as succinic and malic acid, contributing to the acidic profile of the coffee [38]. In LAB formulations with higher CP content, TA rises more gradually as LAB ferments complex carbohydrates over a more extended period [26]. This prolonged acidification enhances the sensory complexity, contributing to a well-rounded flavor and aroma.

4.2. Impact of LAB and Yeast Inoculum in Coffee Fermentation and Sensory Profile

Fermentation 1, involving direct LAB inoculation, increased SS instead of the expected decrease. This suggests that microbial activity or enzymatic breakdown of coffee mucilage components may have released more soluble solids into the fermentation leachate, contributing to the higher °Brix values. This result contrasts with typical fermentation patterns, where microorganisms consume sugars. Fermentation 2, which used an optimized LAB inoculum, showed a significant decrease in SS, indicating that this inoculum was more efficient at metabolizing sugars. This sharp reduction is consistent with the production of organic acids and fermentation by-products, which lead to a notable drop in soluble solids. The pre-adaptation of the LAB in the inoculum facilitated an active metabolism of the carbon sources typical of coffee fermentation.

Fermentation 3, using fresh yeast without prior optimization, showed an increase in SS similar to that of direct LAB fermentation, suggesting that the yeast did not efficiently consume sugars and released additional soluble solids from the coffee beans. In fermentation 4, the yeast inoculum resulted in a moderate decrease in SS, indicating active sugar consumption, albeit slower than LAB

fermentations. This slower utilization suggests that yeast ferments sugar into alcohol and other compounds more gradually. In fermentation 6 (the control), an unexpected increase in SS was observed due to passive release from the coffee beans in the absence of inoculated microorganisms. This implies that natural processes, such as leaching or slight spontaneous fermentation by native microorganisms, contributed to the accumulation of this soluble solid without a deliberate fermentation process.

The mixed inoculum fermentation (fermentation 5) balanced the two trends, showing a moderate increase in TA and a slight decrease in pH. This suggests that the combination of LAB and yeast resulted in more controlled acid production. The yeast's metabolic activities tempered LAB acidification, leading to a more balanced flavor profile. The significant SS decrease and the pH and TA increase in treatments with combined inoculations indicated an ecological interaction between these microbial groups. The interaction between LAB and yeasts also involves yeast autolysis, which releases essential nutrients such as amino acids and polysaccharides that support LAB growth. At the same time, LAB acidification creates an environment conducive to yeast fermentation [23,26]. The control fermentation (fermentation 6), which lacked microbial inoculation, exhibited minimal changes in pH and TA, reflecting limited natural fermentation activity. Despite the lack of deliberate inoculation, fermentation 6 demonstrated a significant increase in TA due to natural spontaneous fermentation by indigenous microorganisms. The elevated TA alongside relatively stable pH indicates that acid production occurred, potentially driven by native lactic acid bacteria or yeast in the environment.

LAB are known for enhancing acidity and flavor complexity in fermented foods, including coffee by-products [39,40]. In our study, the behavior of LAB microbial growth related to pH reduction and increased TA, particularly when CP was used as a carbon source, is similar to the findings of de Melo Pereira et al. (2015), where LAB fermentation also resulted in similar pH shifts [40]. Additionally, the fermentation process led to considerable sugar consumption, further supporting the active metabolism of LAB during the fermentation of CP as a carbon source, which increased bacterial biomass. LAB species such as *Leuconostoc* and *Lactococcus* produce volatile compounds such as diacetyl and acetoin, which enhance flavor [21]. This aligns with previous work highlighting LAB's role in producing aroma-enhancing metabolites [41], which likely contributed to the sensory improvements observed in our study. Although the metabolomic analysis of the resulting coffee was not within the scope of this research, the relationship between specific metabolites produced by LAB and the modulation of sensory profiles during coffee fermentation is consistent with our results and other studies [21,39,41,42,43].

Yeasts, particularly *Saccharomyces cerevisiae* and *Pichia* species, also play a critical role in the fermentation process by altering the carbohydrates, organic acid, and volatile compound content of the coffee beans. They produce vital flavor-active compounds, such as 2,3-butanedione (buttery flavor), acetaldehyde (fruity flavor), and hexanal (green bean flavor) [24,44]. Furthermore, *S. cerevisiae* contributes terpenes and esters derived from amino acids, adding sweet, caramel, fruity, and floral characteristics to the final coffee product [35,45]. Recent studies on mixed-culture fermentations involving LAB and yeasts have demonstrated improved fermentation efficiency and enhanced volatile compound production [23]. The combination of *Pichia fermentans* and *Pediococcus acidilactici*, for instance, significantly increased lactic acid and ethanol production in ripe coffee beans, highlighting the complementary roles of these microorganisms. Yeast activity, particularly in sugar consumption, was more efficient with *P. fermentans*, especially in fructose utilization, compared to LAB-only fermentations [23]. These observations are consistent with findings from other fermentations, where LAB acidification and yeast activity jointly modulate sensory outcomes. This ecological interaction leads to improved sensory attributes, such as increased concentrations of ethanol (alcoholic notes), isoamyl alcohol (banana and pear notes), and ethyl acetate (fruity notes), which were observed in spontaneous coffee fermentations [26,46]. The balance of these microbial activities contributes to the overall complexity and richness of the coffee's flavor profile.

The sensory profile variations across different fermentation setups appear to be directly influenced by physicochemical changes occurring during fermentation. Specifically, increased acidity and decreased pH in treatments, such as treatment 5. These findings support research showing that fermentation, especially with mixed LAB and yeast inoculum, significantly alters the sensory profile of coffee by producing organic acids and metabolites that enrich flavor complexity and aroma intensity [23,39,47]. Attributes such as body, aftertaste, and clean cup were consistently maintained across treatments, indicating that variations in fermentation conditions remained controlled and did not introduce negative sensory defects. Treatment 5's high TA and balanced acidity, paired with optimal aroma scores, underscore how mixed inoculum fermentation can enhance sensory outcomes by promoting beneficial by-products without compromising consistency [48,49].

Tailoring coffee flavor profiles through controlled fermentation offers substantial socio-economic and environmental benefits. By leveraging microbial activity, smallholder producers can create stable cup profiles that meet commercial quality standards while appealing to diverse consumer preferences. This adaptability supports long-term market viability and provides a competitive advantage by enabling product differentiation. Furthermore, this research integrates microbiology,

food science, and socio-economic considerations [50], contributing to a sustainable coffee production model that balances economic growth, environmental stewardship, and social resilience.

In addition to socio-economic gains, controlled fermentation promotes resource efficiency by utilizing local by-products such as CP and CWW. These practices reduce waste and align with sustainable agricultural methods, allowing producers to enhance coffee quality without compromising environmental responsibility. By refining fermentation techniques, small-scale farmers can produce high-quality coffee tailored to market demands while supporting environmental preservation and economic sustainability. This integration of innovation and resourcefulness underscores the potential of microbial fermentation as a transformative tool in coffee processing [51,52].

Although the overall final scores across treatments did not change significantly, this consistency is a positive outcome, as it indicates that quality remains uncompromised even as sensory profiles are modulated. The positive influence of LAB and yeast inoculum on both physicochemical and sensory attributes is further evident in treatments 4 and 5, which exhibited favorable parameters and scored highest in sensory qualities. These results align with previous studies indicating that optimal fermentation conditions, particularly through the synergistic effects of LAB and yeast, enhance desirable flavor and aroma compounds [48].

Additionally, PCA results illustrate relationships between physicochemical and sensory characteristics, showing how microbial activity shapes overall coffee quality [48,49,53,54]. Variables such as initial titratable acidity (TA(I)) and final pH (pH(E)) are more closely aligned with sensory attributes related to acidity, indicating their influence on this specific sensory dimension. Treatments positioned in the lower-right quadrant of the biplot (e.g., treatments 1, 2, and 6) exhibit higher overall sensory scores, suggesting a significant influence of the final-stage physicochemical parameters (SS(E) and TA(E)) on sensory outcomes. Interestingly, the contribution of PC2, while smaller, highlights variability not fully explained by the evaluated physicochemical parameters. This suggests that achieving higher final scores and enhanced sensory attributes (e.g., fragrance/aroma) may also depend on other variables not assessed in this study. For instance, treatments applying inoculum developed with yeast or mixtures of LAB and yeast show strong associations with improved sensory profiles, emphasizing the potential of these inoculums in enhancing coffee quality.

These findings raise questions about additional physicochemical or biochemical factors influencing sensory attributes, underscoring the need for further research to explore other contributors to coffee quality beyond the parameters evaluated in this study. Carefully controlled fermentation using LAB and yeast thus emerges as a promising method to achieve specific sensory qualities,

providing a foundation for future studies aimed at optimizing fermentation to improve product development.

5. *Conclusions*

The optimized inoculum formulations enable the repurposing of coffee by-products, such as coffee pulp (CP) and coffee wastewater (CWW), reducing waste while promoting microbial production. By adjusting these formulations, microbial viability and sensory enhancement were balanced—CWW concentrations boosted yeast growth, while CP concentrations supported LAB viability. These findings support large-scale applications in coffee processing facilities, reinforcing sustainability, and delivering both environmental and economic benefits. The optimized formulations balance the growth and viability of LAB and yeast, offering a scalable solution that enhances process control while maintaining the desired sensory quality of the final product. This research highlights the potential of optimized microbial inoculums to enhance sensory attributes and promote sustainable coffee fermentation practices, offering a scalable solution for smallholder farmers.

Supplementary Materials

The following supporting information can be downloaded at: **https://www.mdpi.com/article/10.3390/su17051781/s1**.

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10 SEMI-CONTROLLED FERMENTATION OF COFFEE BY THE ADDITION OF COMMERCIAL LACTIC ACID BACTERIA AND YEAST AT A SMALLHOLDER CA-PACITY

En esta sección de la investigación, se abordó el último objetivo específico que consistió en determinar las condiciones óptimas del proceso de fermentación semi-controlado del café, con el propósito de mejorar los perfiles sensoriales y la calidad final en taza. Para ello, se emplearon los resultados obtenidos en el desarrollo y optimización de inóculos de levadura (*S. cerevisiae*) y bacterias ácido-lácticas (*L. bulgaricus* y *S. thermophilus*), presentados en el artículo de la sección 9, los cuales sirvieron como base para el diseño experimental aplicado en este capítulo. El artículo que se presenta a continuación aborda este objetivo mediante la evaluación de unas variables críticas de fermentación y su influencia en los atributos sensoriales, aportando evidencia sobre cómo el uso de inóculos optimizados puede potenciar la diferenciación y calidad del café en taza. Este trabajo fue sometido para su consideración en una revista de MDPI (Multidisciplinary Digital Publishing Institute) dedicada a la sostenibilidad, dada su relevancia para la producción sostenible y de valor agregado en la caficultura. Las referencias, figuras y tablas de este capítulo mantienen la numeración del artículo original.

10.1 Texto del artículo completo

Semi-controlled fermentation of coffee by the addition of commercial lactic acid bacteria and yeast at a smallholder ca-pacity

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1.Introduction

Smallholder coffee farmers, who produce most of the global coffee supply, face increasing challenges in adapting to market demands for specialty coffees with distinctive flavor profiles and sustainable practices (Bermudez et al., 2022; Siles et al., 2022). Improving coffee quality through fermentation represents a viable strategy to enhance sensory characteristics and commercial value.

Fermentation plays a central role in defining the sensory profile of coffee. Traditional methods, although rooted in local practices, often produce inconsistent results due to environmental fluctuations and limited control over microbial dynamics. Recent advances in microbial fermentation, particularly the use of selected lactic acid bacteria (LAB) and yeast strains, have demonstrated potential to improve flavor consistency and complexity (Aswathi et al., 2024; de Sousa et al., 2023). However, these approaches remain largely inaccessible to smallholders because of limited resources, technical barriers, and the need for specialized equipment (de Melo Pereira et al., 2019; Vera, 2020).

Semi-controlled fermentation methods provide practical alternatives suitable for small-scale producers. These methods enable the management of key variables—such as microbial populations, temperature, and oxygen availability, resulting in more consistent outcomes. The use of commercially available LAB and yeast simplifies implementation, reducing the need for technical training and infrastructure while improving coffee quality (Calderon et al., 2023; de Melo Pereira et al., 2019; Elhalis et al., 2020).

LAB and yeast play an essential role in forming organic acids and volatile compounds that contribute to acidity, floral and fruity notes, and overall sensory complexity. Specific strains have been studied individually (Wang et al., 2020c, 2020a), in LAB or yeast consortia (Cassimiro et al., 2022), and in co-cultures combining both types of microorganisms (Borém et al., 2024; Cassimiro et al., 2022; Wang et al., 2020b). These controlled fermentations offer smallholders a viable alternative to natural fermentation processes, which often lack consistency due to environmental variability.

In wet-processing regions such as Colombia, semi-controlled fermentations integrate well with traditional practices while introducing manageable process control. The reduced need for sterilization and intensive monitoring makes these approaches feasible in low-resource contexts, offering scalable options to improve coffee quality (Cortés-Macías et al., 2022; Febrianto & Zhu, 2023; Rotta et al., 2021).

This study aims to design and implement a semi-controlled fermentation protocol adapted to smallholder conditions using commercial LAB and yeast. The objective is to enhance the sensory quality of coffee through accessible and economically viable techniques, contributing to sustainable production and strengthening the position of smallholders in the specialty market. The approach consisted of three stages: (1) identification and optimization of key variables through a definitive screening design (DSD), focusing on the relationship between sensory score and main effects; (2) refinement of significant factors through factorial design to improve microbial performance and

sensory attributes; and (3) validation and scale-up of the protocol under real farm conditions, evaluating its practicality, impact on sensory quality, and suitability for low-resource environments.

2. Materials and Methods

2.1 Coffee bean processing

Fresh coffee cherries (*Coffea arabica* var. Colombia) were harvested at 1557 meters above sea level in Morales, Cauca, Colombia. Only mature and healthy cherries were selected, while green, overripe, and damaged cherries were excluded to ensure sample uniformity. The cherries were de-pulped using a DH-2 depulper (Penagos Hermanos, Antioquia, Colombia), and the resulting beans were fermented using the wet processing method.

Fermentation was conducted in a laboratory setup using 1 L flasks containing 800 g of de-pulped beans and varying volumes of potable water (400–800 mL). Spontaneous fermentation, relying solely on native microorganisms present on the cherries, was used as the control. To replicate smallholder conditions, no sterilization was applied to fermentation materials.

After fermentation, the beans were washed with potable water and dried in a convective oven at 40 °C for approximately 24 hours until the moisture content reached 10–12%. Moisture content was determined using a Moisture Meter DH-200 (Quantik, Quindío, Colombia), based on the capacitance method, which compares the dielectric constant of the sample with that of water. The dried beans were hulled with a Lab Coffee Huller C-200 (Quantik, Colombia) to remove the parchment layer.

All fermentation runs within each experimental design were performed simultaneously, using coffee cherries harvested on the same day and from the same farm to avoid variability associated with collection time or origin.

2.2 Lactic acid bacteria and yeast inoculum

Inoculum formulations derived from coffee pulp and wastewater developed in previous studies were used for fermentation. Fresh commercial *Saccharomyces cerevisiae* yeast (Levapan, Colombia) and lactic acid bacteria from commercial yogurt containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Colacteos, Colombia) were employed (Duque-Buitrago et al., 2025)

2.3 Analytical methods

2.3.1Microorganism viability

The viability of lactic acid bacteria (LAB) was determined on de Man Rogosa Sharpe (MRS) agar, while yeast was cultured on potato dextrose agar (Scharlab, Spain). One milliliter of coffee wastewater (CWW) was serially diluted in ten-fold steps (10^1–10^6) using peptone water (Scharlab, Spain), and aliquots were plated by the spread plate method. LAB plates were incubated at 37 °C for 48 hours, whereas yeast plates were incubated for 24 hours. Colony-forming units (CFU) were then counted, and results were expressed as mean values from three independent replicates.

2.3.2 pH

The pH of the inoculum and fermentation liquid was measured at each sampling point following AOAC Method 981.12 12 (AOAC, 2023).

2.3.3 Soluble solids

Total soluble solids in the inoculum and fermentation liquid were determined at each sampling point using a digital portable refractometer (Atago, Japan), following AOAC Official Method 932.12 (AOAC, 2023).

2.3.4 Titratable acidity

Titratable acidity was measured by titrating 1 mL of inoculum or fermentation liquid with 0.1 N sodium hydroxide, using phenolphthalein as an endpoint indicator. Results were expressed as lactic acid equivalents (0.009) and reported as the means of triplicate analyses with standard deviations, based on AOAC Official Method 942.15 (AOAC, 2023).

2.4 Coffee cup quality evaluation

Cup quality was evaluated by a Q-Arabica certified grader following Specialty Coffee Association (SCA) protocols (SCA, 2024). Samples were roasted to 48–63 Agtron at 115.6 °C for 9–10 minutes, ground, and brewed with 8.25 ± 0.25 g of coffee in 150 mL of water at 92–94 °C, steeped for 4 minutes. Sensory evaluation was performed at 65 °C for olfactory attributes and 43 °C for gustatory attributes. Scores (0–10, in 0.25 increments) were assigned for fragrance/aroma, taste, acidity, body, balance, aftertaste, and overall quality, contributing to a maximum total score of 100. Samples were anonymized, and the grader was blinded to treatment conditions.

2.5 Experimental Design and Statistical Analysis

2.5.1 Experimental Strategy

This study was conducted in two sequential stages. First, a Definitive Screening Design (DSD) was applied to identify the process variables with the most significant impact on coffee fermentation. In the second stage, a factorial Design of Experiments (DOE) was implemented as an optimization step to develop a detailed predictive model using only the significant factors identified in the screening phase.

2.5.2 Screening with Definitive Screening Design (DSD)

The DSD methodology was selected for its efficiency in balancing model resolution with the number of required experiments (Erler et al., 2013; Jones & Nachtsheim, 2011). Based on the principle of effect sparsity, this design enables the identification of significant main effects, two-factor interactions, and quadratic effects with a minimal number of experimental runs (Goos & Jones, 2011).

The DSD evaluated five process parameters, as detailed in Table 1: fermentation time (h), water content (%), inoculum concentration (%), microorganism type (LAB, yeast, or a consortium of both), and inoculum preparation method (optimized vs. direct).

Table 1. *Independent variables and their levels in definitive screening design.*

Independent variable	Code	Levels		
		Minimum (−1)	Medium (0)	Maximum (+1)
Time (h)	X_1	24	36	48
Inoculum Concentration (%)	X_2	3	4.5	6
Water (%)	X_3	50	75	100
Microorganism Type	X_4	LAB	LAB and yeast	Yeast
Inoculum Preparation Method	X_5	Inoculum	-	Direct

The design included two central points to enable an initial assessment of curvature. A non-linear quadratic model generated by the DSD is presented in Equation 1:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i<j}^{k} \beta_{ij} x_i x_j + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \beta_c C + \varepsilon \qquad 1$$

where y denotes the measured response, β_0 is a constant, β_i are the coefficients of the linear effects of factors x_i, β_{ij} are the coefficients of the interactions between the factors x_i and x_j, β_{ii} are the coefficients of the quadratic effects of each factor x_i^2, β_c are the coefficients of co-variables C and ε is experimental error.

The response variable of the fermentations was the sensory score (S), determined by the certified Q-grader. The co-variable was the relative microbial growth (RG), quantified as the increase in microbial viability from the start to the end of fermentation in each experimental run, as shown in Equation 2:

$$\mathrm{RG} = \frac{(CFU_t - CFU_{t0})}{CFU_{t0}} \qquad 2$$

where CFU_t represents the highest viable count reached by each microorganism during the experiment at time *t* (X1), and CFU_{t0} is the viable count at the initial time point.

An ANOVA was conducted to evaluate the adequacy and significance of the model. The statistical significance of the model and its terms was determined using the p-value ($p < 0.05$). Statistical analyses were performed with MINITAB version 19. Model quality was determined using the coefficient of determination (R^2) and the adjusted R^2, as shown in Equations (3) and (4). Predictive accuracy was evaluated through the root mean square error (RMSE) (Equation 5), where lower RMSE values indicated greater model precision.

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(yi - \hat{y}i)^2}{\sum_{i=1}^{n}(yi - \bar{y})^2} \qquad 3$$

$$Adjusted\ R^2 = 1 - \frac{(1 - R^2)(n-1)}{n - P - 1} \qquad 4$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(yi - \hat{y}i)^2}{n}} \qquad 5$$

Where yi represents the experimental data, $\hat{y}i$ is the model prediction, $\bar{y}$ the experimental data, n is the number of observations, and P is the number of predictors.

Physicochemical parameters of the fermentation, including soluble solids, total titratable acidity, and pH, were monitored.

2.5.3 Optimization with factorial design

Following the screening stage, a factorial DOE was implemented to develop a detailed optimization model. This design focused exclusively on the independent variables identified as statistically significant in the DSD.

During the selection phase, the analysis prioritized the main effects with the greatest magnitude. The excluded factors were kept constant within controlled minimum operating ranges and were therefore omitted in the subsequent phase to optimize resources and concentrate the analysis on the most influential variables.

The primary response variable was the sensory score (S), while the co-variable was the relative microbial growth (RG), as in the DSD. The physicochemical parameters previously described above were also monitored.

2.5.4 Experimental validation

The final validated model was applied to determine the optimal fermentation conditions using the compound desirability function implemented in Minitab software (version 19). This multivariate tool enabled the simultaneous optimization of sensory and microbial responses, maximizing the overall quality of the final coffee. This optimum was experimentally validated at a pilot scale with 10 kg of pulped coffee. Experimental results were compared with model predictions by calculating the relative error (RE) (Equation 6).

$$RE = \frac{y - \hat{y}}{y} * 100\% \qquad 6$$

Both the optimized procedure and the control fermentation were conducted in triplicate (n = 3 biological replicates). From each biological replicate, three measurement replicates were collected for analysis.

2.5.5 Multivariate Analysis of Validation Samples

Principal Component Analysis (PCA) was applied to the dataset to explore relationships between sensory attributes (e.g., fragrance/aroma, body, flavor) and the physicochemical parameters measured at the initial and final stages of fermentation for all samples.

3. Results

3.1 Screening with definitive screening design (DSD)

A DSD was implemented to identify the most influential variables affecting the sensory quality of fermented coffee. Five independent variables were evaluated: fermentation time (X_1), inoculum concentration (X_2), water content (X_3), microorganism type (X_4), and inoculum preparation method (X_5). For each of the 14 experimental runs, relative microbial growth (CFU/mL) and physicochemical parameters (pH, TA, and SS) were monitored at the beginning (T_0) and end (Tf) of fermentation. The initial microbial concentrations of the inoculum were $7.00 \times 10^7 \pm 0.71$ CFU/mL for LAB and $8.25 \times 10^8 \pm 0.35$ CFU/mL for yeast, values comparable to those reported in previous work on inoculum optimization (Duque-Buitrago et al., 2025).

Table 2 presents the DSD matrix with the specific factor levels for each run, the physicochemical parameters (pH, TA, and SS) monitored at the start and end of fermentation, and the final observed and model-predicted values for the SC.

3.1.1 Physicochemical dynamics during fermentation

Analysis of the physicochemical and microbial data revealed consistent trends indicative of active fermentation across all 14 experimental runs. Treatments with inoculum showed a more pronounced reduction in soluble solids, from 5.50 ± 0.01 to 1.97 ± 0.06 °Brix, accompanied by an increase in titratable acidity from 8.10 ± 1.56 to 49.24 ± 2.27%. These changes reflected greater degradation of the coffee mucilage, confirming the direct relationship between microbial activity and substrate transformation during fermentation.

Fermentations carried out with the optimized inoculum showed a marked decrease in soluble solids and reached higher final TA values compared with those inoculated directly (X_5). This suggests that the preculture stage promoted the establishment of a microbial population with higher metabolic activity, leading to a faster and more complete substrate conversion. In contrast, no significant differences were observed between inoculations with LAB or yeast in terms of the trends of the evaluated physicochemical parameters.

Table 2. *Experimental components and factor levels of the definitive screening design (DSD) used to predict the Sensory Score (S).*

	Independent variables					Co-variable	Sensory Score		Initial fermentation time (T_0)			End of fermentation (Tf)		
Run	**X_1**	**X_2**	**X_3**	**X_4**	**X_5**	**RG**	**Observed**	**Predict**	**pH**	**SS (°Brix)**	**TA (%)**	**pH**	**SS (°Brix)**	**TA (%)**
1	24	6.0	75	LAB	D	40.39	83.25	83.19	4.75	4.30±0.01	10.81±2.38	3.86	4.56±0.06	52.85±1.88
2	48	6.0	50	LAB/Y	I	73.15	85.50	85.38	4.57	5.50±0.01	13.81±1.04	4.04	3.10±0.00	43.54±1.38
3	36	6.0	100	LAB	I	44.72	83.25	83.31	4.74	3.37±0.06	8.10±1.56	3.89	2.10±0.00	37.23±1.04

4	24	3.0	100	LAB/Y	D	15.61	83.50	83.62	4.33	2.60±0.01	16.51±2.75	4.49	1.80±0.00	18.02±0.90
5	24	4.5	100	Y	I	26.82	83.75	83.63	4.56	3.20±0.01	9.91±0.90	4.07	1.97±0.06	24.62±0.52
6	36	4.5	75	LAB/Y	I	46.63	84.50	84.57	4.63	4.30±0.01	12.01±0.52	4.00	2.50±0.00	36.63±2.75
7	48	3.0	100	LAB	D	76.00	83.00	82.91	4.89	3.40±0.01	8.71±0.52	3.64	3.37±0.06	66.65±0.90
8	48	6.0	100	Y	D	1.97	84.00	84.03	4.06	1.40±0.01	27.02±0.01	4.39	2.03±0.06	29.42±0.52
9	36	3.0	50	Y	D	1.09	84.75	84.69	4.12	3.53±0.06	35.73±7.66	4.37	3.03±0.06	39.63±3.25
10	36	4.5	75	LAB/ Y	D	19.11	83.25	83.19	4.31	2.77±0.06	29.73±5.91	4.24	2.20±0.00	30.62±0.90
11	24	3.0	50	LAB	I	36.77	83.00	82.97	4.72	5.40±0.01	10.51±1.04	3.92	3.80±0.00	49.24±2.27
12	48	4.5	50	LAB	D	95.05	84.00	84.12	4.73	5.70±0.01	15.91±1.38	3.74	5.50±0.00	81.07±1.80
13	48	3.0	75	Y	I	31.00	84.00	84.06	4.65	3.93±0.06	10.21±1.04	3.95	2.53±0.06	38.43±1.38
14	24	6.0	50	Y	I	31.15	83.50	83.59	4.38	5.00±0.01	20.12±1.88	4.15	3.03±0.06	32.12±2.60
				R^2			0.986							
				Adj-R^2			0.909							
				RMSE			0.007							

X_1: Fermentation time (h); X_2: Inoculum Concentration (%); X_3: Water (%); X_4: Microorganism type; X_5: Inoculum preparation method; RG: relative microbial growth; LAB: lactic acid bacteria; Y: yeast; D: direct inoculation (baker's yeast or commercial yogurt with LAB); I: optimized inoculum. R^2: coefficient of determination; Adj-R^2: adjusted R^2; RMSE: root mean square error.

3.1.2 Statistical modeling and identification of significant factors

A significance level of $\alpha = 0.1$ was adopted to identify the most influential variables from the screening experiment. The standardized effects of all factors, their interactions, and quadratic terms are shown in the Pareto chart (Figure 1). Any effect exceeding the statistical significance threshold ($t = 2.920$) was considered active. Eight of the fourteen evaluated terms were statistically significant. The most influential factor was microorganism type (X_4), which showed the largest standardized effect. Several interaction and quadratic terms also had a significant impact on the sensory score, including the interaction between time and inoculum concentration (X_1X_2), the quadratic effect of time (X_1X_1), and the interactions involving inoculum preparation method (X_1X_5 and X_2X_5). The main effects of inoculum preparation method (X_5) and fermentation time (X_1) were also significant. In contrast, the main linear effects of water content (X_3) and inoculum concentration (X_2) were not statistically significant at this stage. Complete statistical details, including the specific p-values for each term, are presented in Table 3.

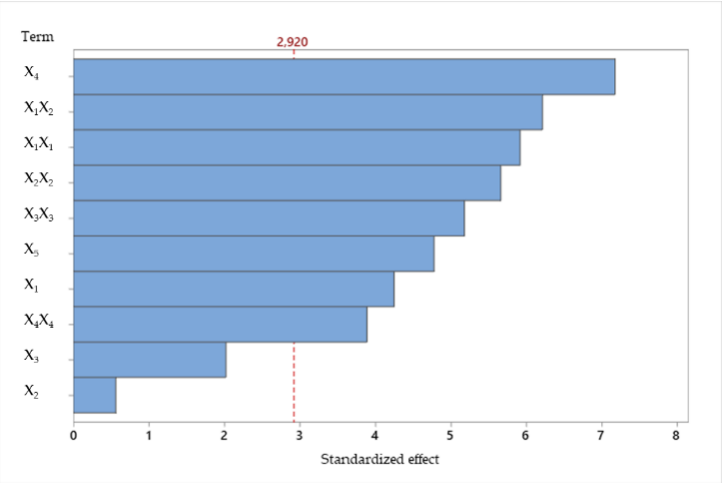


Figure 1. *Pareto chart of standardized effects for the Sensory Score (S), showing the relative influence of fermentation variables (α = 0.05).*

Table 3. *Model estimated by the DSD showing the relative influence of fermentation variables (α = 0.05).*

Term	Coeff.	Std. Error	p-value
Constant	80.872	0.532	0.000
RG	0.091	0.015	0.024
X_1	-0.916	0.215	0.051
X_2	-0.042	0.074	0.630
X_4	-2.302	0.321	0.019
X_5	-0.567	0.119	0.041
X_3	0.223	0.110	0.180
$X_1 * X_1$	-1.858	0.314	0.027
$X_2 * X_2$	1.743	0.308	0.030
$X_4 * X_4$	1.299	0.334	0.060
$X_3 * X_3$	-2.008	0.387	0.035
$X_1 * X_2$	1.694	0.273	0.025

Based on these results, and applying the principle of effect sparsity pragmatically, variables without a significant linear contribution, such as water content (X_3) (p = 0.180) and inoculum concentration (X_2), were excluded from the primary optimization model.

3.2 Optimization with factorial design

Following the screening phase, an optimization experiment was conducted using the three most influential factors. For this purpose, a factorial design was applied to systematically evaluate the interactions between variables and their effect on the response. The independent and dependent variables were fitted to the different models proposed in the experimental design.

Table 4 summarizes the observed and predicted responses for the sensory score (S) based on the DSD results. The linear predictive model did not provide an adequate fit, with $R^2 = 0.634$, adjusted $R^2 = 0.472$, and RMSE = 0.228. In contrast, the interactions model showed improved performance ($R^2 = 0.886$, adjusted $R^2 = 0.726$, and RMSE = 0.069) after excluding run 14, which corresponded to a central point that did not conform to the overall data trend.

Table 4. *Experimental components and factor levels of the factorial design were used to predict the Sensory Score (S).*

	Independent variables			co-variable	Sensory Score		
Run	**X_1**	**X_4**	**X_5**	**RG**	**Observed**	**Linear Model**	**Interactions Model**
1	36	LAB/Y	D	0.63	83.75	83.79	83.75
2	24	LAB	D	0.49	83.50	83.04	83.30
3	24	LAB	I	19.89	83.25	82.77	83.12
4	36	LAB/Y	I	19.58	83.75	83.55	83.72
5	36	LAB/Y	I	32.12	83.00	83.07	83.37
6	36	LAB/Y	D	1.20	83.25	83.77	83.74
7	48	Y	I	2.04	85.50	84.98	85.37
8	24	Y	D	0.30	82.50	82.87	82.21
9	48	LAB	I	22.48	83.75	84.37	83.71
10	48	LAB	D	28.18	84.25	83.67	83.96
11	36	LAB/Y	D	1.09	83.25	83.77	83.74
12	48	Y	D	0.04	85.00	84.58	84.80
13	24	Y	I	5.09	83.50	83.17	83.46
14	36	LAB/Y	I	18.02	82.75	83.61	-
R^2						0.634	0.886
Adj-R^2						0.472	0.726
RMSE						0.228	0.069

X1: Fermentation time (h); X4: Microorganism type; X5: Inoculum preparation method; RG: relative microbial growth; LAB: lactic acid bacteria; Y: yeast; D: direct inoculation (baker's yeast or commercial yogurt with LAB); I: optimized inoculum. R^2: coefficient of determination; Adj-R^2: adjusted R^2; RMSE: root mean square error. Factors X_2 and X_3 were excluded from the factorial design and were held constant at the minimum levels used in the initial DSD.

The predictive model for the sensory variable (S) was significant ($p = 0.039$). The ANOVA (Table 5) indicated that fermentation time was the only factor with a positive and highly significant effect on the response ($p = 0.004$). Neither X_4 nor X_5, nor their interactions, were statistically significant ($p > 0.05$), although inoculum preparation method (X_5) showed a positive trend. Similarly, the interactions among the factors were not statistically significant ($p > 0.05$), suggesting that their combined influence was limited and that the factors acted predominantly independently under the

evaluated conditions. These findings confirm that fermentation time was the main determinant of the response under the evaluated conditions.

Table 5. *Model estimated by the factorial design showing the relative influence of fermentation variables (α = 0.05).*

Term	Coeff.	Std. Error	p-value
Model	-	-	0.039
Constant	84.015	0.244	0.00
RG	-0.0278	0.199	0.222
X_1	0.813	0.164	0.004
X_4	0.002	0.218	0.992
X_5	0.246	0.170	0.208
$X_1 * X_4$	-0.289	0.171	0.152
$X_1 * X_5$	-0.191	0.165	0.300
$X_4 * X_5$	-0.257	0.151	0.149

Based on the obtained model, the response variable was optimized with the objective of maximizing the predicted outcome. The final model quantified the contribution of each factor and predicted the sensory response under controlled fermentation conditions. Optimization using the desirability function indicated that the highest predicted sensory score (S) was achieved with the optimized yeast inoculum and a fermentation time of 48 h, combined with the lower levels of the previously discarded factors (X_2 = 3% and X_3 = 50%). Under these conditions, the predicted score reached 85.14 with a relative microbial growth (RG) of 10.24.

3.2.1 Physicochemical dynamics during fermentation

Table 6 presents the physicochemical parameters monitored during coffee fermentation across the 14 runs of the factorial design. The variation in soluble solids (°Brix) was not pronounced between treatments with direct addition and those with inoculum, in contrast to the screening phase. This stage was carried out using the lowest levels of X_2 and X_3 (minimum percentages of water and inoculum). As a result, °Brix and pH values showed only minor changes. In contrast, titratable acidity exhibited more evident variations, consistent with previous trends, with greater increases in treatments with optimized inoculum compared to those with direct inoculation. It is important to note that a fixed inoculum concentration corresponding to the minimum level evaluated was used in this phase.

Table 6. *Experimental runs and physicochemical parameters monitored through the experiment factorial optimization.*

	Initial fermentation time (T_0)			End of fermentation (Tf)		
Run	pH	SS (°Brix)	TA (%)	pH	SS (°Brix)	TA (%)
1	4.11	3.00±0.15	38.73±2.55	4.07	2.80±0.00	50.14±2.60

2	4.04	3.70±0.06	41.14±2.75	4.08	3.07±0.06	46.24±1.38
3	3.93	3.20±0.00	26.72±0.52	3.88	3.80±0.00	62.76±0.52
4	3.84	3.40±0.00	27.92±0.90	3.93	3.20±.00	51.65±1.04
5	3.91	3.10±0.00	25.82±1.04	3.94	3.10±0.00	57.95±1.88
6	4.00	3.30±0.10	41.44±1.27	4.05	2.87±0.06	47.44±1.88
7	3.88	3.40±0.00	29.13±1.38	3.92	3.10±0.00	62.16±1.80
8	4.07	2.50±0.00	38.13±1.04	4.08	2.83±0.06	40.84±0.52
9	3.88	3.30±0.06	27.92±0.90	3.81	3.50±0.00	70.56±1.88
10	3.94	3.70±0.00	30.03±0.52	3.74	3.70±0.00	81.37±1.88
11	4.04	2.90±0.06	38.13±2.08	4.05	2.93±0.06	46.84±0.90
12	4.00	2.50±0.00	42.94±3.16	4.00	2.90±0.00	68.16±1.04
13	3.86	3.40±0.00	29.73±0.90	3.96	3.20±0.00	50.44±3.82
14	3.86	3.20±0.00	28.53±1.04	3.94	2.97±0.06	50.44±0.90

3.3 Experimental validation

Validation experiments were performed in triplicate under these optimized conditions with the yeast optimized inoculum concentration was 8.43 x 10^8 ± 0.25 CFU/mL. The average sensory score (S) of the samples was 84.83 ± 0.88, with an RG of 13.48 ± 1.30. These results confirmed the accuracy of the model, as the experimental values closely matched the theoretical predictions. The relative error (RE) was 0.36%, demonstrating the reliability of the optimization process.

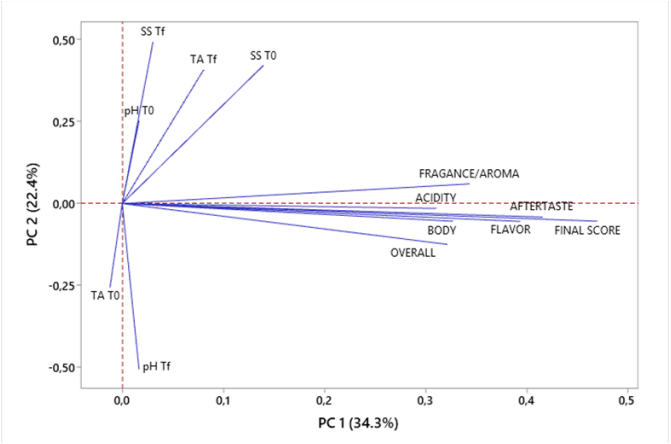
3.4 Principal Component Analysis

A Principal Component Analysis (PCA) was performed to explore the relationships between physicochemical parameters measured at the initial (T_0) and final (Tf) stages of fermentation and the sensory attributes of the final coffee. The first three principal components explained 74% of the total variance (PC1: 34.3%, PC2: 22.4% and PC3: 16.8%) and are presented in Figure 2. As shown in the figure, both component comparisons (PC1 vs PC2, Figure 2A, and PC1 vs PC3, Figure 2B) revealed clear trends in the distribution of variables. Sensory attributes tended to cluster along PC1, while physicochemical parameters were more strongly associated with the variance explained by PC2 and PC3. This suggests a differentiation between sensory and physicochemical contributions to the overall variability captured by the PCA.

PCA allowed the identification of clear patterns between physicochemical and sensory variables associated with the fermentation process. PC1 grouped the sensory descriptors: fragrance/aroma, flavor, aftertaste, acidity, body, final score, and overall. These attributes showed a positive association with initial soluble solids (SS T_0), whereas SS T_0 and initial titratable acidity (TA T_0) were oriented in the opposite direction, indicating a negative correlation with the sensory parameters. These results

suggest that higher soluble solids content and lower initial acidity favor the sensory quality of coffee. PC2 primarily explained the inverse relationship between initial pH (pH T_0) and final titratable acidity (TA Tf) versus TA T_0 and final pH (pH Tf), reflecting the acidification dynamics during fermentation. This process is characterized by a progressive decrease in pH and an increase in titratable acidity (TA) compared to their initial values, because of organic acid production by microbial metabolism. PC3 was more closely related to the distribution of physicochemical parameters, again showing an inverse relationship between pH T_0 and TA T_0, consistent with PC2. Altogether, the first three components explained 74% of the total variance, highlighting that PC1 mainly discriminates with initial conditions associated with sensory quality, while PC2 and PC3 capture metabolic changes occurring during fermentation.

A.



B.

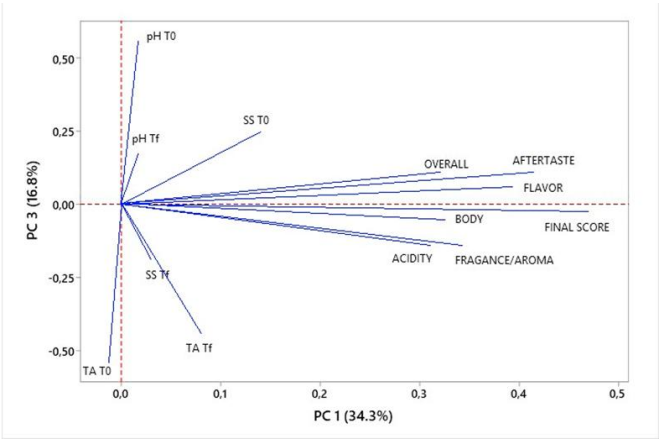


Figure 2. *Principal Component Analysis (PCA) biplot showing the relationship between physicochemical parameters and sensory attributes of previously evaluated fermented coffee samples.*

Comparison of Figures 2 and 3 shows that the clustering of sensory attributes in PC1 was primarily associated with fermentations using yeast inoculum and optimized consortia of yeasts and lactic acid bacteria, suggesting that this inoculum contributed to improving coffee sensory quality. In contrast, PC2 reflected the relationships between SS Tf and TA Tf, which in Figure 3 were oriented toward fermentations with LAB inoculum. This indicates that these microorganisms were more strongly associated with physicochemical modifications and, as previously observed, generated greater changes during fermentation compared with treatments with yeasts.

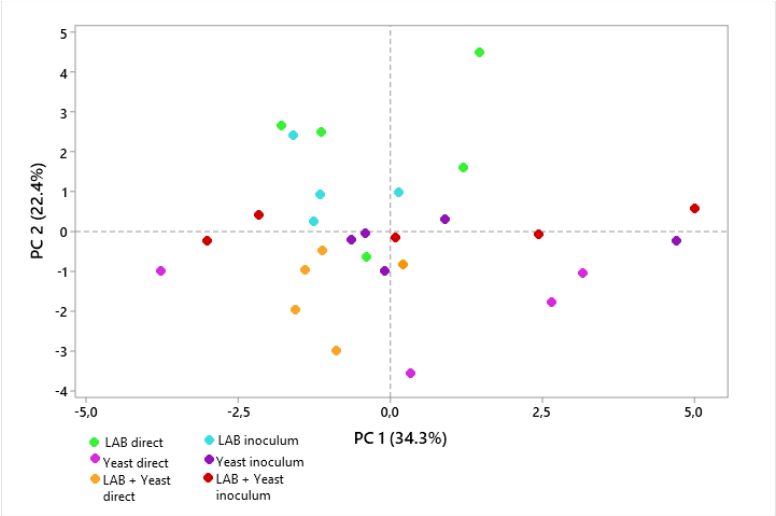


Figure 3. *Principal Component Analysis (PCA) biplot showing the distribution of fermented coffee samples differentiated by inoculum.*

4. Discussion

4.1 Critical Variables in Coffee Fermentation and Sensory Impact

The screening design identified the factors with the greatest influence on the sensory quality of coffee. Among the evaluated variables (fermentation time, inoculum concentration, water percentage, microorganism type, and inoculum application method), microorganism type and inoculation method had a statistically significant effect ($p < 0.05$) on the overall sensory score. These results confirm that the way fermentation is initiated directly influences the biochemical transformations of the system, modulated by the specific metabolic dynamics of each microbial group.

The screening confirmed that microorganism type (X_4) was the main determinant of sensory differentiation in the studied fermentation system. This finding is consistent with the literature, which indicates that the metabolic pathways of yeasts and lactic acid bacteria (LAB) differ substantially and drive the synthesis of organic acids, esters, and volatile compounds that define the final coffee profile (Mahingsapun et al., 2022; Osorio et al., 2023). Inoculation method also directly influenced fermentation dynamics, as it determined the speed at which microorganisms colonized the substrate and the homogeneity of their distribution. Efficient inoculation supports microbiota control, reduces process variability, and promotes the synthesis of desirable metabolites, improving the sensory quality of the final beverage (Kim et al., 2022; Wang et al., 2020a, 2020b).

The Pareto chart (Figure 1) revealed notable complexity, with significant interactions (e.g., X_1X_2) and nonlinear effects (e.g., X_1X_1, X_2X_2, and X_3X_3). This suggests that fermentation outcomes

are shaped not by isolated factors but by dynamic interactions among them. Nevertheless, to develop a practical protocol applicable under farm conditions, the most significant linear effects were prioritized. Based on this criterion, although inoculum concentration (X_2) was involved in significant interactions, its linear effect was not statistically relevant ($p > 0.05$) and was therefore considered a secondary factor in the optimization strategy. Similarly, although water percentage (X_3) exhibited significant nonlinear effects, its linear effect was not statistically significant ($p > 0.05$) and was excluded from subsequent analysis.

Experimental results, however, showed that water percentage, despite lacking a significant linear effect, indirectly influenced the process by modifying the concentration of available carbohydrates in the medium (Table 2). Lower water addition was associated with higher soluble solids (SS), which favored the metabolism of yeasts and LAB. This increased nutrient availability stimulated metabolic pathways such as glycolysis and lactic and alcoholic fermentations, enhancing the synthesis of secondary metabolites (organic acids, higher alcohols, esters, and volatile compounds) responsible for distinctive sensory attributes such as fruity, floral, and acidic notes. According to De Bruyn (De Bruyn et al., 2017), carbohydrate availability during fermentation not only regulates microbial activity but also determines the diversity of aromatic compounds released into the bean. In line with this behavior, fermentations with lower water percentages—and thus higher SS concentrations—achieved the highest sensory scores. For this reason, although not included in the statistical analysis, the lowest evaluated water level was selected as the reference condition for the optimization phase, as it supported a positive effect on final beverage quality.

In addition to microorganism type and inoculation method, fermentation time was identified as a variable with a trend toward influencing sensory quality ($p = 0.051$). The Pareto chart showed that this factor exceeded the significance threshold. Fermentation time is a critical factor in coffee processing, as it regulates the extent of microbial activity and the accumulation of metabolites such as organic acids, alcohols, and volatile compounds that define aroma and flavor. Controlled fermentation enables a balance in the production of these metabolites, favoring the development of more complex and appreciated sensory profiles, whereas excessive or insufficient times may result in defective or underdeveloped notes (Bressani et al., 2020; Zhang et al., 2019).

4.2 Influence of Microbial Inoculum on Fermentation Dynamics and Sensory Profile

This study demonstrated that a targeted inoculation strategy, optimized through a sequential experimental design, can improve both the sensory profile of washed coffee and the fermentation dynamics underpinning these changes. Based on the significant factors identified during the screening

phase, factorial optimization established conditions in which microbial inoculum led to higher sensory scores and a more predictable fermentation process.

Sensory analysis confirmed that inoculation with selected strains of S. cerevisiae and LAB produced differentiated and superior flavor profiles compared with uncontrolled spontaneous fermentations (Jimenez et al., 2023).This finding is consistent with previous studies highlighting the central role of microbial metabolism in generating aromatic and gustatory compounds (Bollen et al., 2025; Cassimiro et al., 2023; Mahingsapun et al., 2022). In particular, the homofermentative and heterofermentative pathways of LAB directly influence the production of organic acids and volatiles that define key beverage attributes, favoring the synthesis of lactic and acetic acids (Dias et al., 2025; Wang et al., 2020a). These compounds enhance the perception of bright acidity and freshness in the cup. Yeasts, such as S. cerevisiae, metabolize sugars and nitrogen compounds to generate higher alcohols, esters, and other volatiles that contribute fruity, floral, and sweet notes. The combination of both microbial groups enhanced fermentation dynamics and allowed a balance between acidity, aromatic complexity, and body, thereby improving the sensory quality of fermented coffee (Rabelo et al., 2024; Cassimiro et al, 2023; Haile & Kang, 2019).

In terms of fermentation dynamics, principal component analysis (PCA) established clear relationships between physicochemical variables and sensory attributes. The results mainly reflected the acidification dynamics of the fermentation medium, showing the inverse relationship between pH and titratable acidity over time. This behavior corresponds to microbial metabolic activity, characterized by the production of organic acids (lactic, acetic, malic), which progressively lower pH and increase final titratable acidity (Vaz et al., 2023; Zhang et al., 2019). The consistency of this pattern indicates that these variables are reliable markers of microbial activity during coffee fermentation.

Comparative analysis across all treatments by PCA highlighted differentiated performance among the inoculum: LAB were primarily associated with modifications in physicochemical parameters (titratable acidity and final soluble solids), whereas S. cerevisiae showed a more pronounced effect on improving sensory scores. Yeast inoculations achieved high-quality coffees both with selected cultures and with commercial baker's yeast, confirming the robustness and adaptability of this species (Bressani et al., 2020; Elhalis et al., 2023; Mahingsapun et al., 2022). In the case of LAB, although specialty-grade coffees were obtained, direct inoculation with yogurt produced slightly more favorable profiles than those obtained with isolated cultures or LAB–yeast

consortia. This suggests that the microbial composition of the source matrix or the presence of specific strains may play a key role in flavor modulation.

The fermentation conditions used in this study (covered containers to prevent contamination, without hermetic sealing or agitation) created a heterogeneous environment with oxygen gradients, which influenced metabolic succession. Initially, the surface layer of the coffee contained dissolved oxygen, allowing S. cerevisiae, as a facultative anaerobe, to perform respiration and promote rapid cell growth (Jimenez et al., 2023). As oxygen was depleted and CO_2 displaced air, conditions shifted toward a microaerophilic and later anaerobic environment, particularly in the lower layers, favoring the transition of S. cerevisiae to alcoholic fermentation. At this stage, yeast produced not only ethanol but also key secondary metabolites such as esters, higher alcohols, and glycerol, which contribute fruity notes, complexity, and body—attributes widely documented in the literature (Simmer et al., 2025; Aswathi et al., 2024; Bressani et al., 2021).

Similarly, in LAB, which are aerotolerant anaerobes, these conditions affected their metabolic output. Even low oxygen levels can modify the cellular redox balance and influence fermentative pathways, as in heterofermentative LAB, which generate lactic acid, acetic acid, ethanol, and CO_2. The absence of agitation likely favored the formation of microenvironments with high acidity, acting as selective pressure and determining the final proportion of organic acids—a key factor in the coffee's sensory profile (do Rosário et al., 2024; Wang et al., 2019; Tawali et al., 2018).

These findings demonstrate that microbial inoculum have a decisive impact on both the metabolic dynamics of fermentation and the resulting sensory profile of coffee. Selected strains modulate biochemical pathways and influence the production of organic acids, alcohols, and volatiles that define cup attributes. The evidence supports the development of directed fermentations in which microbial activity is managed strategically to achieve reproducible outcomes and distinctive sensory signatures. Greater control over fermentation trajectories reduces the variability inherent to spontaneous processes and enhances the potential to produce coffee with unique and high-value profiles. This approach strengthens the role of specialty coffee as a differentiated product, enabling smallholder producers to access premium markets, meet evolving consumer demands, and reinforce sustainability and competitiveness within the sector.

4.3 Implications for the production of specialty coffees for Smallholders

The findings of this study provide valuable insights for the specialty coffee industry, where consistency and sensory differentiation are critical for accessing high-value markets. A key

contribution of this research is the redefinition of "optimization" in this context. Although the use of a pre-cultured and optimized inoculum did not result in a statistically superior sensory score compared to direct inoculation as all optimized treatments already achieved high specialty coffee scores (>80) (SCA, 2024) it significantly improved fermentative performance. Greater consumption of soluble solids and more pronounced acidification indicate a more vigorous and efficient fermentation process, with practical implications beyond final cup quality.

This enhanced metabolic activity suggests the potential to reduce fermentation times without compromising quality, contributes to lower organic load in coffee wastewater, and provides a biological control mechanism by inhibiting undesirable microorganisms, particularly valuable in low-tech farm settings (Serna-Jiménez et al., 2022). Therefore, the value of the optimized inoculum is validated not by a marginal increase in cup score, but by its contribution to a more efficient, predictable, and sustainable process, providing producers with tools to improve process management and environmental performance, cornerstones of modern specialty coffee production (Sofía Torres-Valenzuela et al., 2020).

An additional contribution of this study lies in its potential to foster a circular economy within coffee processing. Optimization included not only fermentation conditions but also the substrate used to cultivate the inoculum, repurposing by-products such as coffee pulp and wastewater, which are typically considered environmental liabilities. This approach reduces reliance on external inputs and addresses waste management challenges, representing a practical and sustainable innovation for on-farm production (Serna-Jiménez et al., 2022; Sofía Torres-Valenzuela et al., 2020).

The experimental design employed significantly enhanced the feasibility of the approach. The use of a Definitive Screening Design (DSD) streamlined the optimization process, minimizing the number of experimental runs while providing statistically robust results. This methodological efficiency makes the approach more feasible, scalable, and transferable to producers seeking entry into specialty coffee markets.

In terms of applicability for small- and medium-sized producers, the robustness of Saccharomyces cerevisiae, both in selected cultures and commercial baker's yeast, demonstrates its adaptability and availability as a practical tool. Similarly, lactic acid bacteria (LAB) showed that accessible matrices such as yogurt can function as inoculum sources, producing cup profiles within the specialty coffee category. This democratizes access to directed fermentations, enabling quality improvement without relying exclusively on isolated commercial cultures.

Furthermore, the strategic combination of yeasts and LAB, adapted to farm-specific conditions, allows for fermentation designs oriented toward specific sensory outcomes, diversifying profiles and strengthening the competitiveness of Latin American coffees in international markets. The non-significance of water quantity as a factor reinforces that excellent sensory results can be achieved while minimizing water use, contributing to process sustainability.

It is also important to consider the socioeconomic context of producers. While the technical findings are promising, adoption of biotechnological innovations at origin requires attention to factors such as training, technical support, and infrastructure, which remain limited in many coffee-growing regions. Therefore, any proposed protocol must be cost-effective, simple to apply, and clearly linked to economic benefits. Considering controlled fermentation not only as a microbial technique but as a strategic tool for product differentiation and value generation could facilitate its adoption. Building producer awareness of how fermentation contributes to cup quality and traceability strengthens their position in the specialty coffee value chain, promoting more equitable and sustainable participation.

Sensory scores obtained in this study should also be contextualized. While all optimized fermentations produced high-quality coffees, current market trends for "competition-level" coffees often involve more complex protocols, such as sequential anaerobic-aerobic fermentations or honey processing (Freitas et al., 2024). This study intentionally focused on optimizing a single-stage, accessible washed fermentation protocol, so the results should not be considered the maximum achievable quality, but rather a robust and significantly improved baseline achievable with low-technology inputs. This opens opportunities for future research, where this optimized protocol could serve as the first stage in more complex fermentation chains.

4.4 Future Research Directions

This study also opens several avenues for future work. To enhance fermentation consistency, simple interventions such as pH monitoring and adjustment with food-grade buffers should be explored, as they have been shown to stabilize microbial activity (Bravim et al., 2023). Additionally, a broader spectrum of commercial and native microbial strains should be investigated to develop tailored fermentation "recipes" adapted to different coffee varieties, regions, and desired flavor profiles.

Nutrient supplementation represents another promising approach for sensory modulation. For instance, the targeted addition of simple sugars or nitrogen sources during fermentation could direct microbial metabolism toward the production of specific desirable compounds, such as esters (fruity

notes) or higher alcohols (complex aromas). These approaches offer a powerful means to elevate coffee quality and complexity, turning consistent production into a science-backed art.

Finally, this study should not be interpreted as a rigid prescription for fermentation, but rather as a scientifically validated framework designed to empower farmers. Through a clear, simplified protocol suitable for implementation with low-technology inputs, this work provides a foundational tool for smallholders to take control of their own productive processes. The goal is to encourage experimentation and adaptation, allowing producers to vary these methodologies based on their unique territorial capacities, available resources, and cultural preferences, thereby fostering innovation from the ground up and strengthening the entire specialty coffee value chain.

Collectively, these future directions provide a robust foundation for advancing applied research in coffee fermentation. They support efforts to increase process predictability, improve sensory outcomes, and reinforce the positioning of specialty coffee as a high-value, sustainable product within international markets.

5. Conclusions

This study demonstrates that optimizing microbial inoculation in coffee fermentation provides benefits that extend beyond achieving high sensory scores. The use of the commercial microbial inoculum evaluated in this work, combined with the sequential analysis of critical fermentation variables, enhanced fermentative performance and increased process predictability. The application of commercial microorganisms, such as baker's yeast and lactic acid bacteria from yogurt, enabled the production of specialty-grade coffees; although the best results in the optimization were achieved with the yeast inoculum, all strategies showed potential to produce high-quality coffee. Additionally, some process variables, such as water quantity and additional inoculum dose, did not have a statistically significant effect on sensory quality but influenced microbial metabolic development, modulating fermentation efficiency. These improvements provide producers with practical tools to obtain consistent, high-quality specialty coffees while minimizing technological and economic constraints, making this approach accessible to small- and medium-scale farms.

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11 SÍNTESIS

La presente investigación se enfocó en el diseño de un proceso de fermentación semi-controlado de café, desarrollado bajo un enfoque práctico y asequible para caficultores, con el propósito de mejorar el perfil sensorial y la calidad en taza a partir de recursos locales. La estrategia para el desarrollo de los inóculos consistió en emplear como sustrato subproductos del mismo beneficio del café: agua residual de fermentación (ARF) y pulpa de café (PC), aprovechando su disponibilidad en finca. Para el desarrollo de los inóculos se utilizaron cepas comerciales: levadura de panadería (*S. cerevisiae*, marca Lavapan) y bacterias ácido-lácticas (*Lactobacillus bulgaricus* y *Streptococcus thermophilus*) provenientes de un yogurt comercial.

Durante la etapa de optimización de los inóculos, se evaluaron diferentes proporciones de estos subproductos como fuentes de carbono y nutrientes mediante un diseño de mezclas. Los resultados demostraron que estos sustratos favorecieron un crecimiento microbiano exponencial, alcanzando concentraciones cercanas a 10^8 UFC/mL para *S. cerevisiae* y alrededor de 10^7 UFC/mL para las BAL. Además, se evidenciaron tendencias de cambios en los parámetros fisicoquímicos a lo largo del monitoreo, lo que confirma su actividad metabólica y su capacidad para generar condiciones fermentativas adecuadas.

Posteriormente, se evaluó el efecto del uso de los inóculos optimizados en el proceso fermentativo de café mediante un análisis secuencial de factores críticos, con el objetivo de identificar las variables con mayor impacto en la calidad sensorial en taza. En la etapa de diseño de cribado definitivo, se consideraron como factores: el tiempo de fermentación (24–48 h), la concentración de inóculo (3–6 %), la cantidad de agua de fermentación (50–100 % p/v), el tipo de microorganismos utilizados (levadura o BAL) y el método de aplicación del inóculo (directo u optimizado). Como variable de respuesta principal se consideró el puntaje sensorial global, incluyendo simultáneamente el crecimiento microbiano relativo (CR) como covariable para comprender mejor la dinámica microbiana durante la fermentación.

Los resultados mostraron que las variables con mayor influencia en la calidad sensorial fueron el tiempo de fermentación, el tipo de microorganismos y el método de aplicación del inóculo. En contraste, la cantidad de agua de fermentación y el porcentaje de inóculo tuvieron un efecto menor y fueron descartados como variables principales. No obstante, se mantuvieron en sus niveles inferiores, lo que permitió reducir el consumo de recursos, fortaleciendo el carácter eficiente y práctico del proceso.

A partir de los factores con mayor influencia, se determinaron las condiciones óptimas para el proceso de fermentación semi-controlado de café, con el objetivo de maximizar el puntaje sensorial, mediante un diseño factorial. Los resultados mostraron que los mejores puntajes se obtienen con el inóculo optimizado de levadura. En general, se evidenció que el uso de inóculos optimizados permite obtener perfiles sensoriales característicos: la levadura influyó principalmente sobre los descriptores sensoriales, mientras que las BAL mostraron efectos más marcados sobre los parámetros fisicoquímicos monitoreados, los cuales a su vez impactan el metabolismo y el desarrollo de compuestos que modulan el perfil sensorial del café. Esto demuestra que la optimización de la inoculación microbiana en la fermentación del café proporciona beneficios que van más allá de la obtención de altas puntuaciones sensoriales.

Por tanto, el análisis secuencial permitió realizar una estimación preliminar mediante los modelos generados, considerando los factores de mayor influencia. Esto facilita dirigir el proceso hacia perfiles sensoriales deseados, los cuales dependerán de los inóculos adicionados, constituyendo un proceso práctico y replicable para los caficultores.

12 CONCLUSIONES

Las formulaciones optimizadas de inóculo permiten la reutilización de subproductos del café, como la pulpa de café (PC) y las aguas residuales del café (ARC), reduciendo el desperdicio y promoviendo la producción microbiana. Al ajustar estas formulaciones, se logró un equilibrio entre la viabilidad microbiana y la mejora sensorial. Las concentraciones de 100% ARC con 36 h de fermentación impulsaron el crecimiento de la levadura (8.3×10^8 UFC/mL), mientras que las concentraciones de 100% PC con 48 h de fermentación favorecieron la viabilidad de las BAL (7.8×10^7 UFC/mL).

A partir de los inóculos optimizados, los resultados demuestran que la fermentación de café con inóculos microbianos comerciales mejora el perfil sensorial, consolidándose como una alternativa viable para la producción de cafés especiales a partir de recursos disponibles en la misma cadena. Además, el tipo de microorganismo empleado influye directamente en los parámetros fisicoquímicos del medio de fermentación, lo cual repercute en los descriptores sensoriales del café, reforzando la importancia de realizar una fermentación controlada.

Las condiciones optimizadas obtenidas para una fermentación semi-controlada son: tiempo de fermentación de 48 h, 50 % de agua y 3 % de inóculo optimizado de levadura, lo que permite alcanzar un puntaje sensorial global (PSG) de 85.14. Estas mejoras proporcionan a los productores herramientas prácticas para obtener cafés especiales de alta calidad y consistentes, al tiempo que minimizan las limitaciones tecnológicas y económicas, ofreciendo un proceso accesible, práctico y escalable para explotaciones agrícolas de pequeña y mediana escala.

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