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Operating conditions of a bioreactor for coffee fermentation *

Condiciones de operación de un biorreactor para la fermentación en café

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ABSTRACT

Fermentation at a controlled temperature is proposed as a processing alternative to obtain benefits in terms of coffee quality. The changing physical characteristics in the coffee mass during fermentation become a challenge for the stirring system to achieve homogeneous temperature conditions. A fine-tuning process was carried out to determine the operating conditions of a bioreactor for fermentation in coffee with variations in rotation speed, stirring time, and the period between stirrings. Subsequently, the behavior of the fermentations was determined in a temperature range between 10 and 30 °C, at intervals of 5 °C. As a comparison for each process, spontaneous fermentation was carried out. The time to reach mucilage degradation greater than 95 % and the time to reach equilibrium between the temperatures of the coffee mass and the equipment's water jacket were determined. The final pH was also registered. Descriptive differences observed between the processes with controlled temperature, the spontaneous fermentation in the time to reach mucilage degradation, and the final pH and temperature values. The controlled processes required between 2 and 13 hours longer fermentations than the corresponding spontaneous process. Through a flat vane impeller, the best operating point was identified when the process was performed at 3 r•min^{-1} for 2 min every 6 hours. Low-speed agitation is required to reduce mechanical removal of mucilage.

KEY WORDS:
Agitation conditions; Bioreactor for coffee; Coffee mass; Controlled fermentation; Mucilage removal; pH; Temperature control; Temperature differential; Time fermentation; water jacket.

RESUMEN

Las fermentaciones con temperatura controlada se proponen como alternativa de procesamiento para obtener beneficios en la calidad del café. Las características físicas cambiantes en la masa de café, durante la fermentación, se convierten en un desafío para que el sistema de agitación logre condiciones homogéneas de temperatura. Con el objetivo de determinar las condiciones de operación de un biorreactor para fermentaciones en café, se realizó un proceso de puesta a punto, en el que se realizaron fermentaciones con variación en la velocidad de rotación, tiempo de agitación y periodo entre agitaciones. Posteriormente se determinó el comportamiento de las fermentaciones en un rango de temperatura entre 10 y 30 °C, con intervalos de 5 °C. Como testigo de cada proceso se realizó una fermentación espontánea. Se determinaron los tiempos de degradación de mucílago mayor a 95 % y de equilibrio de temperatura entre la masa y la chaqueta del equipo. Se observaron diferencias en el tiempo de fermentación y los valores finales de pH y temperatura de la masa, respecto al testigo y a la temperatura de control. Los procesos controlados tomaron entre 2 y 13 horas más de fermentación que el testigo correspondiente. Mediante un impulsor de paletas planas, se identificó el mejor punto de operación cuando el proceso se realizó a 3 r•m^{-1}, por 2 min, cada 6 horas. Para lograr una temperatura de fermentación en la masa de café, de acuerdo con sus características, se requiere una agitación a baja velocidad para no generar remoción mecánica de mucílago.

PALABRAS CLAVE:
Biorreactor para café; Chaqueta del biorreactor; Condiciones de agitación; Control de temperatura; Diferencial de temperatura; Fermentación controlada; Masa de café; pH; Remoción de mucílago; Tiempo de fermentación.

INTRODUCTION

Coffee fermentation is a process carried out in batches, in which the microorganisms available in the environment use the mucilage composition as a substrate for their metabolic processes. Several factors affect fermentation evolution and determine the activity of microorganisms with regard to the transformation of compounds therefore the final quality of the coffee, for example, the maturity of the harvested fruits, the environmental conditions in which the process is carried out and the use of water, among others (Peñuela-Martínez *et al*., 2013; Bastian *et al*., 2021). Traditionally, in Colombia, this process is carried out in containers that include buckets, cans, and tanks of different capacities and built-in materials such as masonry, plastic, and stainless steel, the latter driven by the development of technology for using a minimal amount of water when washing coffee in the fermentation process. As coffee is a product for human consumption, it is necessary to maintain aseptic conditions, which is why stainless steel is an appropriate material for its handling (Martínez *et al*., 2021). In addition, stainless steel allows a greater heat transfer with the environment, especially when much of this process occurs at night and the temperature is lower. Regardless of the type of tank or material, these containers allow fermentation to take place because a suitable environment is generated for microbial activity. The above mentioned scenario occurs spontaneously, without the control of the main variables that affect it. Faced with this variation, the result is unpredictable. When fermentation occurs spontaneously on mucilage, there is an increase in temperature typical of the type of process, which in turn is an indicator of microorganism activity (Kumar *et al*., 2021).

To create favorable environmental conditions and to present a desired type of reaction or, on the contrary, not to produce undesirable compounds, it is necessary to control variables such as temperature, pH, and stirring speed, among others. Temperature control in fermentation allows the induction of microorganism activity using a predefined temperature range with the potential to generate compounds that modify the chemical composition of the grain, effects that are later perceived in the beverage (Ruta and Farcasanu, 2021). It has also been suggested that there are economic advantages of the use of this type of technology to obtain sensory profiles different from conventional coffee that are valued in the market in such a way that the investment is rewarded (Magalhães Júnior *et al*., 2021). The first step to determine if temperature control in fermentation in coffee is a viable strategy to highlight sensory attributes is to generate information from investigations that evaluate fermentation in coffee with parameters controlled by a bioreactor. This equipment comprises a container that provides a controlled environment to generate the activity of desirable microorganisms and that protects the process from the uncontrolled external environment (Bossio, 2021). Likewise, it allows control, improvement and optimization of the process to facilitate greater reproducibility of fermentations (Batista da Mota *et al*., 2022).

The investigations carried out using bioreactors for coffee fermentation have occurred at the laboratory level, with low capacity equipment used to inoculate lactic bacteria (De Carvalho Neto *et al*., 2018) or yeasts (De Carvalho Neto *et al*., 2020) and evaluate the growth parameters of these microorganisms and their effect on the chemical and sensory characteristics. Bioreactors with capacities between 30 and 50 L have also been used, with temperature control at levels lower than those of the environment; the behavior of the microbial communities were affected, conferring benefits with regard to the quality of the coffee (Peñuela-Martínez *et al*., 2018; Vera Pacheco *et al*., 2018). Coffee with differentiated quality was obtained with fermentations in 300 L tanks using fruits and pulped coffee, decreasing the temperature of the mass through agitation (Martínez *et al*., 2021), and monitoring process variables in fermentations with water in 150 L tanks (Carbajal-Guerreros *et al*., 2022).

However, this technology is nascent, and the operating conditions of the equipment are unknown with regard to controlling the fermentation parameters in pulped coffee as well as the behavior of associated variables under these conditions, such as changes in the temperature and pH of the coffee mass, the need for stirring and the completion time. The objective of this research as an exploratory approach was to identify the operating conditions of the bioreactor with respect to agitation and temperature; the information generated will used to carry out fermentations with temperature control with different varieties of coffee and environmental conditions of production.

METHODS

This research was carried out at the Cenicafé Experimental Mill, located in Chinchiná Caldas, Colombia, at an altitude of 1.310 m, with an average annual temperature of 21,6 °C and relative humidity of 80,3 % (Centro Nacional de Investigaciones de Café, CENICAFÉ, 2021). Coffee fruit (*Coffea arabica* L.) varieties Castillo® and Cenicafé 1® were used in the process.

The fermentations were carried out in a bioreactor designed and built through a collaboration with Industrias Centricol® (Medellín, Colombia). The equipment consists of a vertical cylindrical water jacket tank in 316 stainless steel (30 L volume and 20 L effective capacity), an agitation system composed of a motor and a helical belt agitator, and a water circulation system through the water jacket, generated by water cooler, to evaluate different control temperatures. The bioreactor also had pH and temperature sensors (PT-100) and a digital control panel to select the speed, time and period of agitation and monitored variables. It had a data capture system (Unitronics DataXport software) that recorded the information regarding the work cycles supplied by the controller. The setup consisted of adjusting the stirring system to facilitate the handling of the coffee.

Subsequently, to define the control temperature, the equipment was operated at 10, 15, 20, 25 and 30 °C ± 1 (cases). For each case, was used 20 kg of pulped coffee obtained from approximately 40 kg of ripe berries. To obtain a homogeneous and good quality pulped coffee mass, were followed seven key practices to obtain quality coffee in the postharvest period (Peñuela and Sanz, 2021).

Simultaneously with the fermentations in the bioreactor, fermentations were carried out without temperature control (control treatment), and the fermentation time was determined using the Fermaestro® method (Peñuela *et al*., 2013). For the bioreactor, the fermentation time of the coffee mass was recorded when mucilage degradation greater than 95 % was obtained, as determined using an enzymatic method (Peñuela *et al*., 2010), and the temperature of the mass was balanced with the temperature of the jacket.

To define the agitation conditions, different speeds (r·min^{-1}), times (min) and periods (h) were evaluated to keep the temperature of the fermentation mass close to the temperature selected in the bioreactor. Green fruits of a similar size to the pulped beans were added, and the movement of the mass was assessed, checking that all the beans came in contact with the jacket. Mechanical damage to the beans caused by agitation was determined, taking a sample of 100 g of coffee after each fermentation to identify the amount of beans threshed (%) and/or bitten (%). Additionally, the temperature and pH of the mass were monitored during fermentation using a pH and temperature sensor (Halo FC 2022). In total, 20 fermentations were carried out.

Information analysis

The following condition were defined as criteria to obtain the stirring conditions of the equipment:

- There was no mechanical removal of mucilage or mechanical damage to the grains.
- The temperature difference (ΔT) [°C] considers the selected temperature, that of the bioreactor jacket and that of the mass, with the following equations:

$$\Delta T_1 = \left[T_s - T_{wj}\right] \quad (Eq.1)$$

$$\Delta T_2 = \left[T_s - T_m\right] \quad (Eq.2)$$

$$\Delta T_3 = \left[T_{wj} - T_m\right] \quad (Eq.3)$$

Where:
Ts is the operation set point temperature for the bioreactor (°C), T_{wj} is the bioreactor water jacket temperature (°C), and Tm is the temperature of the coffee mass during fermentation (°C).

RESULTS

Regarding the coffee used for the tests, 90,4 % were ripe fruits (ripe stage 4, 5 and 6) and less than 3 % were unripe fruits (ripe stage 1 and 2), according to Cromacafé® methodology, resulting in a homogeneous mass with less variability in the final results (Peñuela and Sanz, 2021).

During the tuning process of the bioreactor, were made the following adjustments:

- The feeding funnel of the pulped coffee was changed to facilitate the filling operation;
- The control panel programming was updated to avoid limiting the stirring speed;
- An emergency stop button was installed on the controller; and
- Micro-SD ports were installed for data logging.

Regarding the agitation system, the motor was replaced by a gear motor (OUKEDA OKD86-AG20) that reduces the rotation speed, generates more power and improves torque (Bossio, 2021). The gear motor has a reduction ratio of 1:20, with a range of 0 to 100 r·min^{-1}.

The helical belt impeller generated block movement of the coffee mass; therefore, it was replaced by an agitator with flat paddle, which was installed on top of the other agitator on the same axis, given the height of the tank. Silicone attachments were placed on the upper blades, acting as cleaners to detach the grains that adhered to the inner surface of the water jacket. The mixing of the grains with this type of agitator was effectively verified by the movement of the green fruits added one by one to the mass, which were displaced as the paddles moved. The agitator was designed based on the relationship between the dimensions of the bioreactor and the agitator (Table 1) for the mixture of particulate matter (Bossio, 2021).

Figure 1. Design coffee stirred tank bioreactor equipment.

Physical properties were also considered because mucilage has high viscosity and is classified as a non-Newtonian fluid with pseudoplastic behavior, i.e., the apparent viscosity decreases with the increase in the shear rate (Oliveros and Gunasekaran, 1994). The changes that occur in the mucilage during fermentation, such as detachment, generate a sticky and viscous mixture that requires high power to move the mass.

Table 1. Bioreactor and agitator dimensions.

Description:	Value (cm)	Relationship
H: Height of the bioreactor tank	45	---
Dt: Internal diameter of the bioreactor	32	---
Di: Impeller diameter	30	Dt/Di=1,06
c: Free distance between paddle and bioreactor	2	c/Di=0,07
Hi: Impeller height	45	Hi/Di=1,5
Wi: Pallet width	4	Wi/Di=0,14

The first stirring tests were carried out at 20 °C, close to room temperature. The rotating speed variated at 5, 7 and 10 $r \cdot min^{-1}$ for 3 min between 2 and 3 hours. Under these conditions, there was mechanical removal of the mucilage from the beginning of the process, which hindered the movement of the coffee mass. For the following six tests, the temperature was lowered to 15 °C to prolong the fermentation, and mechanical removal was maintained, with the consequent loss of the rheological properties, making agitation difficult. At this same temperature, the speed was decreased to 3 $r \cdot min^{-1}$ for 2 min, and the period was increased every 6 hours. These conditions were identified as the best operating point because the coffee mass temperature was maintained with a difference of less than 1,0 °C with respect to the control point. Additionally, there was no mechanical damage to the beans due to the movement of the coffee mass with the stirrer throughout the fermentations; the amount of cut and threshed beans was 0 % after the evaluations.

With the defined stirring conditions, the working temperature was varied according to the experimental methodology. The average temperature of the water jacket was above the selected temperature in most cases, except when it was higher than the ambient temperature. In the same way, the average temperature of the mass was higher, between 1,5 and 4,0 °C, even when it was operated at 20 °C, a value from which it was between 0,2 and 1,9 °C higher than that of the equipment (Table 2). This occurs because heat is released as part of the metabolic activity of microorganisms (Elhalis *et al*., 2021; Kumar *et al*., 2021; Batista da Mota *et al*., 2022). The equilibrium time of the process was when there was less than a 1,0 °C difference between the temperature of the jacket and that of the mass. This equilibrium point was more easily determined when the temperatures of the equipment were 15 °C and 30 °C.

Table 2. Average values and standard deviations of temperatures and temperature differentials during fermentations under different process conditions

Temperature Selected (°C)	Twj (°C)	Tm (°C)	ΔT1 (°C)	ΔT 2 (°C)	ΔT3 (°C)
10	12,2±0,57	13,8±0,28	-2,20±0,57	-3,8±0,28	-1,6±0,28
15	16,0±0,49	16,9±0,49	-0,95±0,49	-1,9±0,49	-0,9±0,00
20	20,6±0,78	21,6±0,00	-0,55±0,78	-1,6±0,00	-1,1±0,78
25	24,3±0,14	24,1±0,99	0,70±0,14	0,9±0,99	0,2±0,85
30	28,6±0,42	28,3±0,21	1,40±0,42	1,8±0,21	0,4±0,64

Fermentation time

The removal of mucilage is an objective variable to identify the advancement of degradation in fermentation. Previous studies have determined that when this removal is greater than 95%, washing can begin because the water-insoluble compounds have been degraded, and therefore, fermentation has ended, as indicated by the Fermaestro® method. Time has been shown to be a determining factor related to the quality of the beverage but varies depending on the given condition (Ruta and Farcasanu, 2021; Mahingsapun *et al*., 2022). Additionally, Vaz *et al*. (2022) identified a relationship between higher cup scores and sugar content under longer fermentation conditions and lower temperatures and vice versa, but not under intermediate conditions.

In this research, with an average mucilage content of 28,2 %, ranging from 26,0 to 31,8 %, the fermentations carried out as controls for the controlled processes ranged from 19,0 to 21,5 hours, and those carried out with temperature control required between 2 and 13 hours longer than the controls; this difference was greater at a lower control temperature (Figure 2).

In the control fermentations, there was an increase in the temperature of the coffee mass (approximately 3,0 °C), and the final temperature for those that occurred in the bioreactor was close to that selected for the equipment, being up to 1,9 °C higher when working from 15 °C to 20 °C and lower by the same measure (1,8 °C) when working at 25 and 30 °C (Figure 3a). For the control at 10 °C, the average difference from the final temperature was 3,8 °C because the temperature of the water jacket did not reach the control point and heat was generated from the process. For pH, the change was less when the processes were controlled with respect to the control, which presented greater acidity at the end of the fermentations (Figure 3b), indicating the possibility of controlling this variable using temperature.

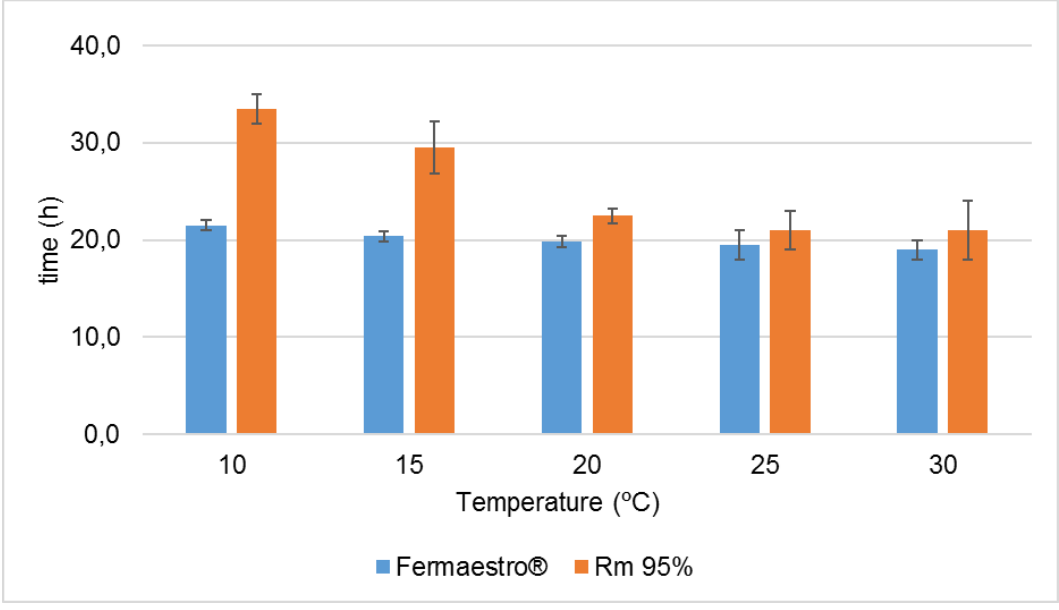

Figure 2. Average and variation in the processing time with degradation of mucilage greater than 95% in fermentations with temperature control in the bioreactor and the control.

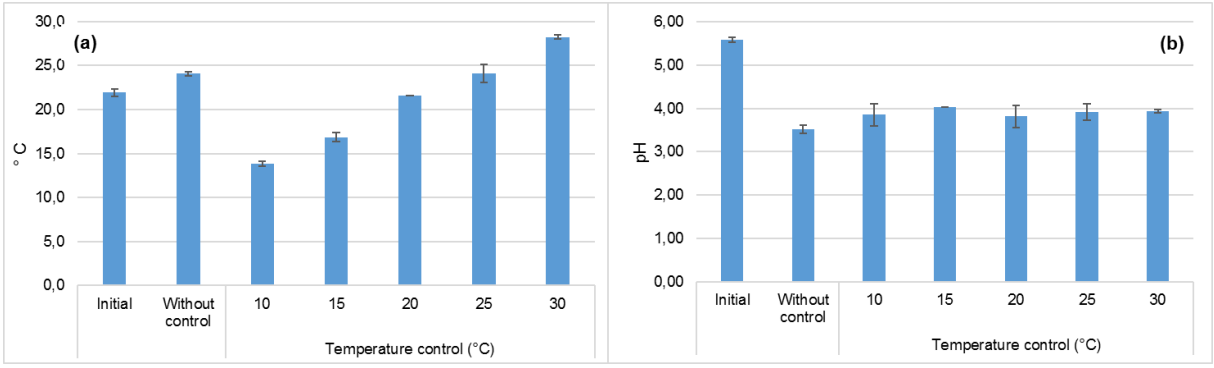

Figure 3. Initial and final mass temperature (a) and pH (b) values for coffee fermentations with and without temperature control.

The temperatures used to control the fermentation generated different behaviors, which affected the pH and the process time. The evolution of fermentations is determined by several factors, among which temperature and pH are considered the most important; for example, small changes in pH generate large differences in the metabolism and growth of microorganisms (Kumar *et al*., 2021). Elhalis *et al.* (2020) identified differences in the final pH values related to the metabolism of active microbial communities. Later, this author determined that changes in pH during fermentation affected the pH of the beans, influencing the formation of compounds perceived later in the aroma and flavor of coffee (Elhalis *et al*., 2021). The above result coincides with that observed by Mahingsapun *et al.* (2022) in fermentations with different inoculated yeasts; the final pH values were different and depended on the metabolic activity of the dominant microorganisms.

The temperatures of 15 and 30 °C were selected to control subsequent fermentations; these temperatures are related to the weather of the Colombian coffee zone, which temperature varies between 14,6 °C and 30,2 °C (CENICAFÉ, 2021). Under these conditions, fermentations were carried out without stirring to identify the time of equilibrium temperature (Figure 4). The ambient temperature was close to 25 °C most of the time, with values close to 30 °C in the warmest hours of the day. The water that circulated through the jacket quickly reached the levels of the working temperature. The equilibrium time was reached at 23 and 11 hours for 15 °C and 30 °C, respectively. This difference is explained by a lower rate of change with respect to time ($dT \cdot dt^{-1}$), which was a maximum of -0,5 °C · h^{-1} for the colder and 0,8 °C · h^{-1} for the warmer. Considering the exothermic process that occurs, at 15 °C, there was heat flow from the mass toward the bioreactor jacket, with temperature differentials between -0,3 and -4,5 °C. Contrary to the process conducted at 30 °C, heat flow was generated from the jacket to the coffee mass, with differentials that were between 0,2 and 4,7 °C.

The elimination of heat is important for an efficient fermentation process in accordance with the expected result. Due to the physical nature of the coffee mass and the metabolic activity generated during fermentation, agitation is necessary to maintain the temperature as close as possible to the conditions selected for the process or to avoid sources of heat accumulation. The use of a double-stirred bioreactor by Martínez *et al.* (2021) avoided the formation of hot spots and allowed the transfer of heat to the environment. In contrast, Batista da Mota *et al.* (2022) reported a fermentation mass temperature of 30 °C after 87 hours of processing in spontaneous fermentations using closed plastic tanks.

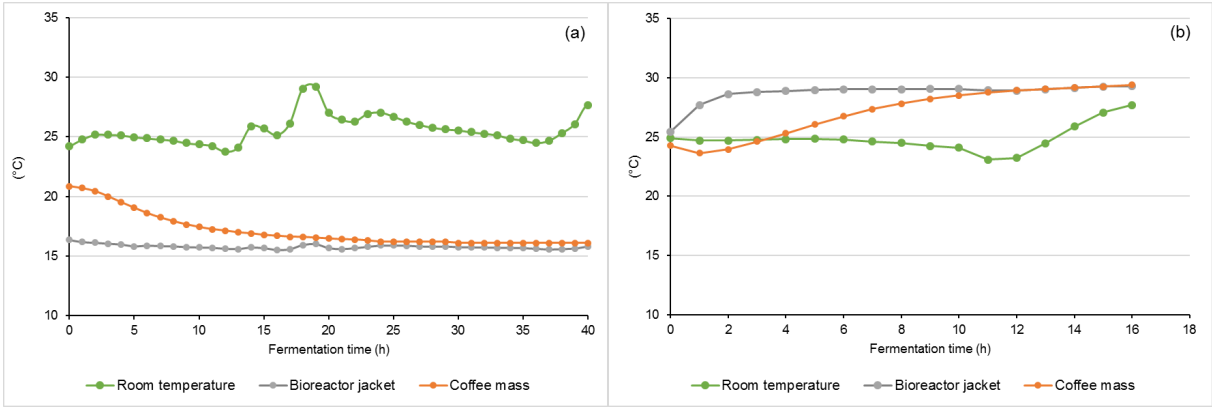

Figure 4. Behavior of the temperatures of the bioreactor jacket, of the coffee mass and of the environment during fermentations with temperature control, (a) 15 °C and (b) 30 °C, with respect to the fermentation time.

CONCLUSION

The best operating point of the bioreactor identified with respect to agitation and temperature, 3 r•min^{-1} for 2 min every 6 hours, and temperature of 15 °C and 30 °C; these conditions can be used to carry out fermentations with variations in production conditions and coffee varieties and determine their effect on the quality of the beverage. Agitation during fermentation is necessary to achieve the control temperature in the coffee mass; however, even at low rotation speeds, mechanical removal of mucilage generated, leading to alterations in the process. To define the fermentation time of a controlled process, it is necessary to establish criteria such as the times to achieve equilibrium temperature and mucilage degradation greater than 95 %.

Fermenting coffee is described as a pasty, highly viscous mass that is difficult to move by stirring. In addition, long times are required to achieve the control temperature, which is influenced by the exothermic nature of the process, and thermal properties, such as specific heat and thermal conductivity, make heat transfer slow. However, it is advisable to continue this work by determining these properties as the mucilage degrades to establish scaling models for bioreactors with larger quantities of coffee mass.

The control of temperature in coffee fermentation is projected as one of the first steps for improving a stage that has traditionally been used as a spontaneous process, which produces a wide variability of results, influencing the commercialization of this product. The industry can use the results to develop equipment that improves coffee farm production.

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