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Characterization of Fermentations with Controlled Temperature with Three Varieties of Coffee (*Coffea arabica* L.)

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Abstract: Temperature control is the starting point for the development of controlled fermentation and improving coffee quality. The characteristics of coffee varieties can influence fermentation behavior. To evaluate the effect of the coffee variety on the behavior of controlled fermentation and on coffee quality, a completely randomized design was used with three varieties (Castillo, Cenicafé1 and Tabi) and two control temperatures (15 and 30 °C). Spontaneous fermentation was the control for each controlled process. The fermentation time, pH, glucose and lactic acid contents, as well as, the count of mesophiles, yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB), were assessed. The sensory quality of the coffee was classified as very good and excellent based on the variety, with averages above 82 Specialty Coffee Association (SCA) points. The highest values were for the Cenicafé1 variety. Fermentation behaviors were similar among varieties but not based on the given condition. Compared with spontaneous fermentation, the treatment at 15 °C prolonged the degradation of mucilage in more than 24 h; additionally, there were differences in the final pH values, less than 3.5 and close to 4.0, respectively. Quality was not significantly different between the controlled fermentation and the spontaneous fermentation (Wilcoxon test $p > 0.05$) or between fermentation temperatures (Kruskal–Wallis test $p > 0.05$).

Keywords: coffee varieties; temperature-controlled fermentation; quality coffee; fermentation time

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1. Introduction

The coffee beverage from Arabica species is highly consumed worldwide and valued due to its flavor, smoothness, acidity and, in general, balanced attributes [1–4]. These attributes are obtained through the interaction of factors, such as varieties, good agronomic practices and postharvest transformation methods. Regarding the latter, the wet method produces so-called mild coffees, with highlighted quality characteristics [2,5–7]. The wet process refers to the transformation of coffee fruits into dry parchment coffee (dpc), removing parts, such as the pulp and mucilage, and in turn decreasing the water content, mainly through the stages of pulping, fermentation and drying [8]. During fermentation, mucilage is the appropriate culture medium for the activation of the microorganisms present to transform pectic substances and simple carbohydrates into water-soluble compounds [5,9,10]. This process traditionally occurs spontaneously, and there is no control of the variables involved, which also generates variable results that affect product consistency [3,5,11]. During fermentation, there are changes related to the temperature, time, acidity and beverage quality. The evolution of fermentation from a spontaneous, traditional process to a controlled process is necessary to reduce quality variability and take advantage of biochemical transformations that can modify the chemical composition of the grain, thus modulating and highlighting the attributes. Due to the exothermic nature of fermentation [12,13], the starting point should be temperature control. Knowing the behavior of fermentation carried out at controlled temperatures will help to develop strategies to modify fermentation processes and generate recommendations for the management of fermentation so that desired

flavor characteristics are generated [5,10]. One way to guarantee temperature control in coffee fermentation and to understand fermentation behaviors under controlled conditions is by using a stirred tank bioreactor. This equipment allows for the control of variables associated with the process, with the aim of improving and optimizing fermentation to obtain consistency in the results. The use of this equipment has yielded quality advantages when used for coffee of a single variety of *Coffea arabica* L. produced at different altitude ranges [14]. In that study, controlled fermentation at a temperature below the ambient temperature produced higher grades for coffee produced at a lower elevation range. However, coffee varieties of the same species have differences that are reflected in the physical and sensory quality, which could indicate different fermentation behaviors, which are necessary to consider for the development of controlled processes.

Colombian coffee is distinguished in the world market for its quality, which is due, among other factors, to the grown varieties [15]. In total, 85% of the productive area of the country is resistant to coffee rust (*Hemileia vastatrix*) [16], the main disease that affects this crop. These varieties, which include Castillo, Tabi and Cenicafé1, maintain, in their genetic diversity, the same sensory quality characteristics of traditional varieties, such as Típica, Bourbon and Caturra [16,17]. Characteristics related to variety must be considered in the development of controlled fermentation processes to ensure the availability of high-quality coffee beans.

Taking into account the described background, the objective of this study was to evaluate the effect of carrying out the fermentation in a controlled-temperature stirred-tank bioreactor on the quality of three varieties of coffee produced in the department of Cesar, Colombia. In this study, the behavior of the variables associated with fermentation was analyzed considering controlled temperatures. The results obtained provide information on the behavior of coffee varieties under different fermentation conditions and indicate a focus of future research work to further contribute to the development of controlled processes to reduce variability and improve beverage quality, taking into account the characteristics of intrinsic varieties.

2. Materials and Methods

2.1. Coffee Samples and Processing

Fermentation was carried out in the northern coffee zone of Colombia at the Pueblo Bello Cenicafé Experimental Station, located in the Department of Cesar at an 1134 m elevation, with an average annual temperature of 21.1 °C and 78.8% relative humidity (latitude 10°25′ N and 73°34′ W) [18]. Ripe fruits collected manually were used. For each batch of 100 kg of coffee collected, dense fruits were obtained through hydraulic classification pulped in a Gaviota Ref.300 machine (Ingesec, Bogotá, Colombia) and size classified. The pulped coffee obtained was divided into two equal parts (each part of about 30 kg) to conduct fermentation with temperature control (treatment) and spontaneous fermentation (control). No water was added in the process.

2.2. Experimental Design

This study was conducted through a one-way completely randomized design consisting of six treatments corresponding to the combination of two fermentation temperatures (15 °C and 30 °C) for three varieties of coffee, Castillo, Cenicafé1 and Tabi, from the species *Coffea arabica* L. For each treatment, spontaneous fermentation was the control treatment. Five repetitions of each treatment were carried out.

2.3. Controlled Fermentation Processes

To obtain a controlled fermentation temperature, the pulped coffee mass was deposited in the tank of a water-jacket bioreactor of 30 L, which had a water recirculation system at the selected temperature; mechanical agitation was performed by means of a flat-paddle stirrer at 3 rpm for 2 min every 6 h, as described in previous studies [19]. Spontaneous

fermentation (control treatment) was carried out in an open plastic container with a 30 L capacity, without agitation.

To identify the evolution of fermentation in each treatment and in the spontaneous fermentation (control), the temperature and pH of the mass were assessed at the beginning of and during the process. For this, a Hanna brand pH meter (HI 10532/Halo®) with automatic temperature compensation was used; the pH meter was calibrated before each use with pH 7.0 and pH 4.0 buffer solutions (HI 7004 L/C; HI 7007 L/C; HANNA®).

The fermentation time was considered the time to reach mucilage degradation greater than 95%. The mucilage degradation was measured by using a pectinolytic enzyme [14]; meanwhile, for spontaneous fermentation, the Fermaestro® method was used [14]. Additionally, the time needed for the coffee mass in the controlled fermentation to reach the control temperature, called the equilibrium temperature, was recorded, for which the difference between the two was considered to be less than 1.0 °C.

2.4. Determination of Glucose and Lactic Acid Contents

The glucose and lactic acid concentrations in the mucilage were determined in situ using a reflectometric method, i.e., the Reflectoquant RQflex®20 test (Merck SA, Darmstadt, Germany), with the test strips corresponding to these compounds [20]. The measurement of glucose is based on its conversion to gluconic acid lactone, in which the reaction of hydrogen peroxide with an organic redox indicator forms a blue–green tint. This measurement was carried out at the beginning and at the end of fermentation. The measurement of lactic acid is based on the oxidation of lactate to pyruvate, in which the production of NADH reduces tetrazolium salt [21,22]. Due to the low concentrations of this compound at the beginning of fermentation, this measurement was made only at the end of fermentation. Each sample was analyzed in triplicate.

2.5. Counting of Microbial Groups

Samples of coffee with mucilage (50 g) obtained at the end of fermentation were introduced into a sterile Whirl-Pack™ bag (B00992 WA). Serial dilutions up to 10^{-6} were prepared in peptone water, and 100 µL of the 10^{-3} to 10^{-6} dilutions was directly seeded in plate count agar (PCA), Man-Rogosa-Sharpe agar (MRS) supplemented with 0.1% sorbic acid (*w*/*v*) and acetobacter agar supplemented with absolute ethanol, glacial acetic acid and 10 mg/L cycloheximide, for determining the counts of aerobic mesophilic bacteria (AMB), lactic acid bacteria (LAB) and acetic acid bacteria (AAB), respectively; yeast-glucose-chloramphenicol extract (YGC) was used for determining the yeast count [14].

2.6. Physical and Sensorial Analysis

Dry parchment coffee samples with moisture between 10 and 12% w.b. were obtained by drying coffee beans with forced air at 40 °C following the recommendations to maintain coffee quality during drying [10]. The moisture content was determined using International Organization for Standardization (ISO) 6673, 2003 [23]. To obtain green coffee beans to determine the physical and sensory quality of the samples, the parchment was eliminated using a machine (C-250, Kaffemat, Bogotá, Colombia). Granulometry and physical defect analyses were carried out in accordance with the procedure established by Specialty Coffee of America—SCA [24].

To determine the sensory quality, the Cupping Specialty Coffee—SCA protocol was used [25], which defines the procedures for roasting, grinding and preparing the beverage and for rating the 10 attributes (fragrance/aroma, uniformity, cleanliness, sweetness, flavor, acidity, body, aftertaste, balance and overall impression) that compose coffee quality on a scale of up to 10 points. The overall quality of each sample was the sum of the values for the attributes, and higher scores indicated a better quality beverage. This procedure was carried out by a panel of three experts accredited in coffee cupping [26].

2.7. Statistical Analysis

The average, deviation, median, minimum and maximum values of the sensory variable scores were obtained for the attributes (fragrance/aroma, flavor, aftertaste, acidity, body, balance score), the total score for each variety and fermentation temperature and for the process variables (fermentation time, time to equilibrium temperature, glucose and lactic acid concentrations and microbial group counts by variety). Average values, standard deviations and 95% confidence intervals are presented.

For the total score, for each variety and temperature, the Wilcoxon test was performed [27] for related groups to evaluate the differences between the samples fermented under a controlled temperature and their respective control (spontaneous fermentation). An analysis of variance was performed to evaluate the effect of temperature on the cup quality. Because the data did not present a normal distribution but the variances were homogeneous, a nonparametric test was used [27]. The analyses were performed using SAS/STAT version 9.4 [28].

3. Results

3.1. Initial Characteristics of the Coffee Varieties

For the ripeness of the harvest, more than 85% of samples from each variety were concentrated within colors 4 to 7 of the method (Cromacafé®), which is a color chart to recognize, by eye comparison, eight different coffee maturation stages, as referenced by [14]. During the course of the fermentation, the ambient temperature averaged 26 °C, ranging from 23 °C to 31 °C, for which the initial fermentation temperature for the three varieties was close to the ambient average (Table 1). No differences were observed among the varieties in the initial characteristics of the masses.

Table 1. Averages and 95% confidence intervals for the general characteristics of the three varieties of coffee at the beginning of fermentation.

Variety	Ripeness (%)	Mucilage Content (%)	pH	Mass Temperature (°C)	Glucose Concentration (g/L)
Castillo	88.3 ± 1.6 a	27.4 ± 0.9 a	5.61 ± 0.11 a	23.8 ± 0.9 a	21.3 ± 3.3 a
Cenicafé1	89.5 ± 3.0 a	26.0 ± 0.8 a	5.67 ± 0.09 a	23.2 ± 1.4 a	21.3 ± 2.8 a
Tabi	87.7 ± 3.0 a	26.1 ± 0.7 a	5.53 ± 0.12 a	23.8 ± 0.7 a	23.8 ± 1.5 a

For each column, mean values with different lowercase letters are significant at $p < 0.05$ based on the *t*-test.

3.2. Fermentation Time

The time to achieve mucilage degradation greater than 95% was different for the given fermentation conditions, being shorter for spontaneous fermentation and significantly longer when the temperature was controlled at 15 °C (Table 2). Relative to spontaneous fermentation, temperature control prolonged the fermentation time. There were no differences among the varieties, except for fermentation at 30 °C, being significantly longer for the Castillo variety than for the Tabi variety.

Table 2. Averages and 95% confidence intervals for the fermentation time (hours) under different process conditions for three varieties of coffee.

Variety	Temperature Control		Spontaneous Fermentation
	15 °C	30 °C	
Castillo	42.1 ± 1.5 aA	20.0 ± 1.2 aB	17.7 ± 0.7 aB
Cenicafé1	41.7 ± 2.2 aA	18.8 ± 0.9 abB	17.3 ± 0.5 aB
Tabi	41.3 ± 3.0 aA	17.5 ± 0.6 bB	16.8 ± 0.5 aB

For each column, mean values with different lowercase letters are significant at $p < 0.05$ based on the *t*-test. For each row, mean values with different capital letters are significant at $p < 0.05$ based on the *t*-test.

3.3. Temperature and pH Behavior during Fermentations

In the spontaneous fermentation (control), the temperature of the coffee mass exhibited an upward trend, reaching close to 26 °C at the end of the process, without differences among varieties. There was a temperature delta (ΔT) between 2.8 and 3.4 °C. The temperature of the bioreactor water-jacket neither exceed 29.5 °C when it was controlled at 30 °C, nor was it lower than 16 °C when it was controlled at 15 °C. Therefore, the equilibrium temperature was maintained for an average of 14.7 h for 30 °C and 20.9 h for 15 °C, respectively. These variables increased or decreased depending on the control conditions, with temperature differentials between −5.97 and −7.36 °C for 15 °C and between 6.23 and 6.38 °C for 30 °C (Figure 1).

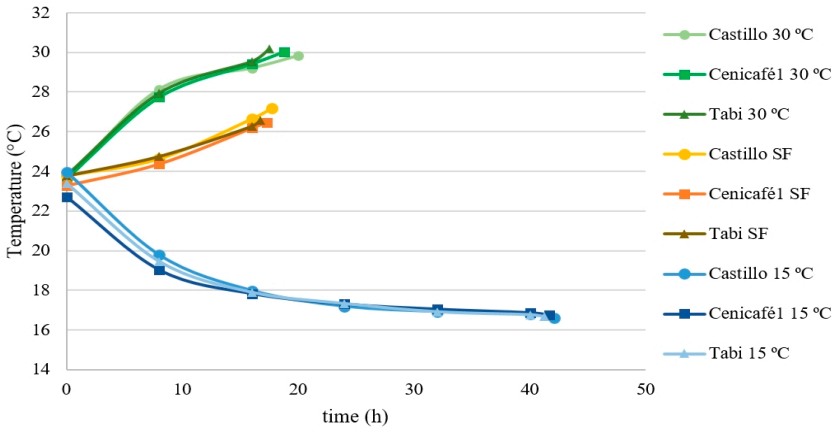


Figure 1. Behavior of the temperature of the coffee mass of three varieties of coffee under spontaneous fermentation and under temperature-controlled fermentation at 15 °C and 30 °C.

During the first 16 h of fermentation, there was a marked decrease in pH in the three varieties based on the fermentation condition (Figure 2). For the three varieties, the final pH of the fermentations presented the following pattern: the highest final pH for fermentation was at 15 °C, followed by fermentation at 30 °C and spontaneous fermentation, for which the values were below 3.50. Fermentation at 15 °C exhibited asymptotic behavior, maintaining values close to 4.0 for more than 20 h until the end of the process. The Cenicafé1 variety had the lowest pH values in all fermentation conditions.

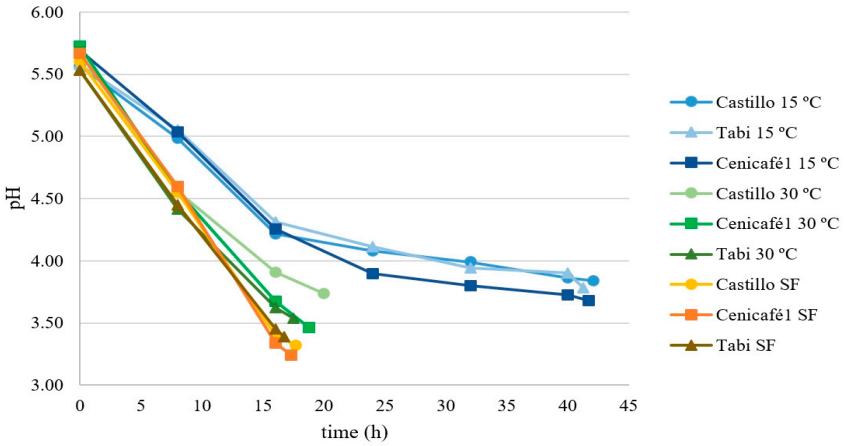
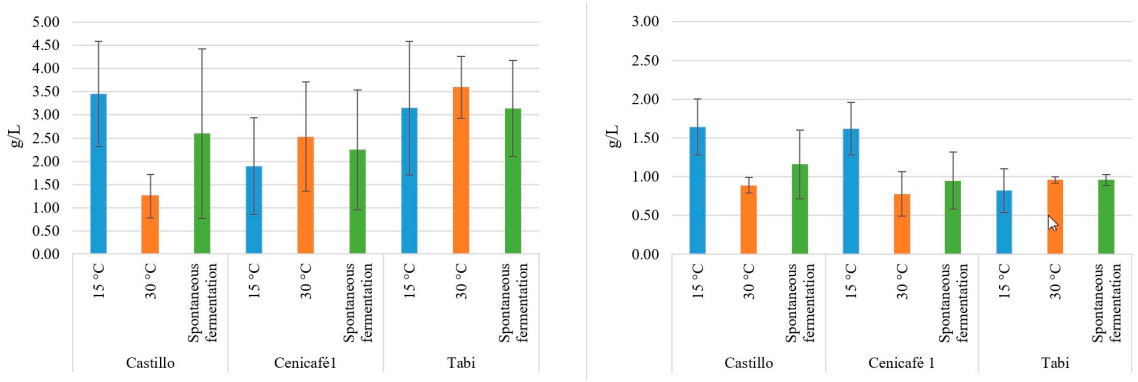


Figure 2. Behavior of the pH of the coffee mass of three varieties of coffee under spontaneous fermentation and under temperature-controlled fermentation at 15 °C and 30 °C.

3.4. Final Characteristics

As expected, there was a decrease in the glucose concentration in mucilage at the end of fermentation. The fermentation temperature affected the behavior of this variable for the

Castillo variety; the glucose concentration was significantly higher at 15 °C than at 30 °C. Additionally, under fermentation at 30 °C, there was a significant difference in the glucose concentration between the Castillo and the Tabi varieties (Figure 3a).



(**a**) (**b**)

Figure 3. Averages and 95% confidence intervals for the final concentrations of glucose (**a**) and lactic acid (**b**) in the coffee mucilage of three varieties of coffee under different fermentation conditions.

The mean lactic acid concentrations ranged from 0.78 to 1.64 g/L (Figure 3b). The lowest values were obtained for the three fermentations carried out with the Tabi variety. At 15 °C, the lactic acid concentration for the Tabi variety exhibited a significant difference from those for the Castillo and Cenicafé1 varieties. For the control treatments, the lactic acid concentrations ranged from 0.96 to 1.16 g/L, with no significant differences among varieties.

The counts of the microbial groups (Table 3) revealed that AAB was least represented. The Tabi variety had the greatest populations of mesophilic microorganisms and LAB. For spontaneous fermentation and temperature-controlled fermentation, LAB populations were highest at 30 °C and lowest at 15 °C. Likewise, there were more yeast populations in the Cenicafé1 and Tabi coffee varieties, fermented at 15 °C.

Table 3. Averages and standard deviations of population counts (log CFU·mL^{-1}) of bacteria and yeast under different process conditions for three varieties of coffee.

Variety	Temp. (°C)	Mesophile	Yeast	LAB	AAB
Castillo	15	7.33 ± 0.52	7.27 ± 0.16	7.04 ± 0.81	6.30 ± 2.91
	30	7.99 ± 0.40	7.11 ± 0.82	7.59 ± 0.50	6.11 ± 1.27
	SF	7.48 ± 1.23	7.56 ± 0.15	7.48 ± 0.43	6.91 ± 1.35
Cenicafé1	15	8.28 ± 0.24	7.99 ± 0.13	7.37 ± 0.32	6.72 ± 0.19
	30	7.72 ± 0.41	6.83 ± 0.14	7.48 ± 0.48	7.40 ± 0.50
	SF	8.46 ± 0.60	7.11 ± 0.23	8.23 ± 0.60	6.53 ± 0.96
Tabi	15	8.18 ± 0.05	7.67 ± 0.07	7.94 ± 0.30	6.75 ± 0.62
	30	8.44 ± 0.01	7.05 ± 0.67	8.19 ± 0.16	6.66 ± 0.76
	SF	8.21 ± 0.06	7.60 ± 0.03	8.21 ± 0.16	6.50 ± 1.74

3.5. Physical and Sensorial Analysis

For the dpc samples, the average moisture was 11.3% w.b., ranging from 11.0 to 11.7% w.b. The beans presented good physical quality because more than 90% were defect-free green coffee beans, with those of the Tabi variety having the lowest values (Table 4). Regarding the beans with physical defects, no immature grain was obtained due to the high concentration of ripe fruits and good quality of the harvest. Beans perforated by the coffee borer (brocade beans) accounted for 0.9 to 3.9% of the total beans; these values did not exceed the economic damage threshold of 5% [29]. The proportion of sour beans was below 0.9%. This type of bean is classified within category I, in which a maximum of one (1)

completely sour bean is accepted within a 350 g sample [24]. Within this category are also black beans and those affected by fungi, which were not found in the samples.

Table 4. Average values and standard error for the main physical characteristics of the coffee samples of the three varieties under different fermentation conditions.

Variety	Temp. °C	Moisture % wb	Defect-Free Green Beans (%)	Brocade Grains (%)	Sour Grains (%)	Mesh 17 and 18 (%)	Mesh 15 and 16 (%)
Castillo	15	11.3 ± 0.1	93.0 ± 1.1	2.9 ± 0.9	0.47 ± 0.3	69.7 ± 4.2	27.6 ± 3.9
	30	11.5 ± 0.2	93.7 ± 1.3	2.2 ± 0.7	0.69 ± 0.4	65.7 ± 5.9	30.7 ± 5.6
	SF	11.3 ± 0.2	91.8 ± 1.3	2.8 ± 1.0	0.89 ± 0.4	65.1 ± 5.1	32.6 ± 4.7
Cenicafé1	15	11.7 ± 0.2	93.8 ± 1.0	0.9 ± 0.3	0.72 ± 0.4	80.7 ± 0.9	17.3 ± 0.9
	30	11.5 ± 0.2	92.2 ± 1.0	0.9 ± 0.7	0.45 ± 0.4	81.4 ± 1.6	17.2 ± 1.3
	SF	11.3 ± 0.2	91.1 ± 1.1	1.9 ± 0.8	0.78 ± 0.4	79.6 ± 1.1	18.6 ± 1.0
Tabi	15	11.3 ± 0.2	91.7 ± 1.2	3.4 ± 0.8	0.92 ± 0.5	81.6 ± 1.5	15.8 ± 1.5
	30	11.1 ± 0.3	90.8 ± 2.3	3.9 ± 1.7	0.14 ± 0.3	83.7 ± 2.2	14.8 ± 2.0
	SF	11.0 ± 0.3	90.1 ± 1.8	3.9 ± 1.5	0.76 ± 0.4	82.2 ± 1.9	16.0 ± 1.8

Regarding size, more than 96% of all samples were larger than the 15 mesh (6.00 ± 0.08 mm); for the Cenicafé1 and Tabi varieties, 80% were larger than the 17 mesh (6.70 ± 0.08 mm), classified as "supreme", with more than 40% classified as "Premium" because they were larger than the 18 mesh (7.10 ± 0.09 mm). For Castillo, 30% of beans were considered "Premium".

The total score for the three varieties of coffee under the different fermentation conditions indicated that the coffee was very good (greater than 80 SCA points) and excellent (greater than 84 SCA points) (Table 5). The Wilcoxon test results indicated that there were no significant differences between the controlled-temperature fermentation and spontaneous fermentation ($p > 0.05$), indicating that temperature control did not affect the quality of the beverage despite the time differences for the degradation of mucilage. Similarly, there were no significant differences between fermentation temperatures (Kruskal–Wallis test $p > 0.05$), despite a 15 °C difference between treatments. However, for the Castillo variety, compared with the SCA scores for the control, those for fermentation at 15 °C and 30 °C were 1 point and 3 points higher. The Castillo variety had the lowest variability between the first and third quartiles. The Cenicafé1 variety had the highest means and medians.

Table 5. Descriptive statistics for the total score (SCA points) of sensory analyses of three coffee varieties under spontaneous fermentation and temperature-controlled fermentation.

Variety	Temp. °C	Average		Standard Deviation		Median		Minimum		Maximum		Interquartile Range	
		SF	Treat.	SF	Treat.	SF	Treat.	SF	Treat.	SF	Treat.	SF	Treat.
Castillo	15	82.5	83.1	1.3	1.4	82.3	83.0	80.8	81.3	84.3	85.3	1.4	0.1
	30	79.7	82.7	6.4	0.8	82.5	82.3	68.3	81.9	83.0	84.0	0.8	0.8
Cenicafé1	15	83.4	83.5	0.7	1.1	83.0	83.5	83.0	82.0	84.6	84.8	0.3	1.5
	30	83.8	83.6	0.8	0.9	84.0	83.8	82.8	82.5	84.6	84.6	1.0	1.1
Tabi	15	82.3	82.7	1.1	0.5	82.8	82.5	81.0	82.3	83.5	83.5	1.8	0.3
	30	83.7	83.8	1.1	1.4	84.0	84.5	82.3	81.8	84.8	85.0	1.5	1.6

For the analysis of the quality attributes, uniformity, clean cup and sweetness had the highest rating (10 points), indicating adequate characteristics suggestive of consistency, without negative impressions and with sweet notes characteristic of soft washed coffee of good quality. For the other attributes, the scores ranged from 7.0 to 7.9, with the fragrance/aroma and flavor of the Cenicafé1 and Tabi varieties, respectively, receiving the highest scores for fermentation at 30 °C (Figure 4). For the Castillo variety, fermentation at

15 °C resulted in the highest scores for flavor, body and balance; spontaneous fermentation resulted in the lowest scores for the six attributes.

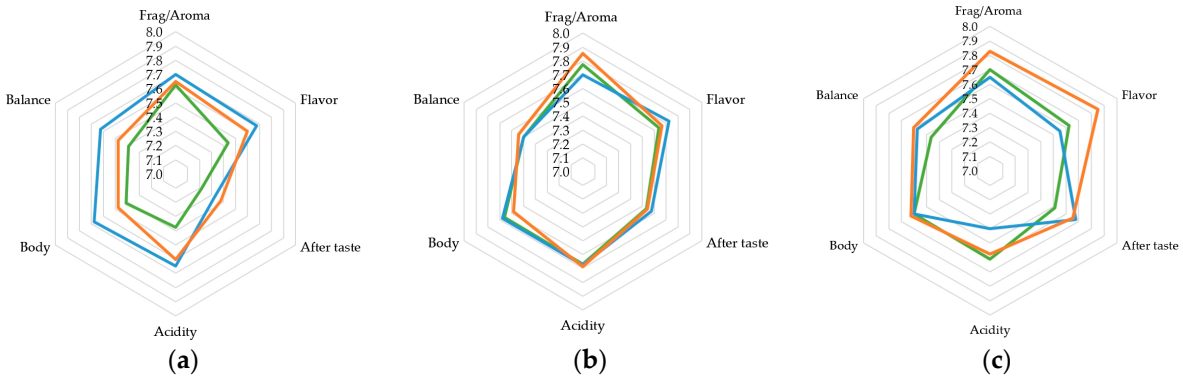


Figure 4. Average attribute scores for the Castillo (**a**), Cenicafé1 (**b**) and Tabi (**c**) varieties under spontaneous fermentation (green line) and temperature-controlled fermentation 15 °C (blue line) and 30° (orange line).

Regarding the sensory descriptors related to flavor, 21 descriptors were obtained; these descriptors were mentioned a total of 159 times (Table 6), of which fruit trees, citrus fruits, red fruits and other fruits, such as apple, pineapple and tropical fruits, occurred at the highest frequency (38.4%), followed by sweets, caramel, "panela" (brown sugar), honey and vanilla (29.6%). The preparations that received the highest number of descriptors (darker color) were those corresponding to the spontaneous fermentations, with one the most frequent descriptors being related to citrus fruits. "Other" attributes included astringent, dry herbal, cereal notes, which in general are attributes that are not desired in the sensory profile; these descriptors occurred in greater numbers for the control treatments and for the Castillo and Tabi varieties.

Table 6. Sensory descriptors (count) for coffee samples of three varieties under spontaneous fermentation and temperature-controlled fermentation.

Variety	Temperature	Caramel	"Panela"	Honey	Vanilla	Chocolate	Walnuts	Citrus Fruits	Red Berries	Other Fruits	Floral	Spices	Other
Castillo	15 °C	3	2	1		3		4	1	1			
	30 °C	1	1	2		2		2	1	1	1	2	2
	SF	4	3		2	3	1	4	1	2			5
Cenicafé1	15 °C	1			1	2		2	3		1		3
	30 °C	1			1	2	1	3	3	4			2
	SF	2	4	4	1	2	1	3	5	4	2		1
Tabi	15 °C		1		1			1	1			1	
	30 °C	3	1	1		1		3	1	2	1	2	
	SF	2	2	1	1	1	2	6		3	3	1	3

4. Discussion

Because the quality of coffee is influenced by several factors [6,8], to avoid confounding factors and to determine the effect of the variety on fermentation processes, this study was conducted at a single production site to guarantee the same environmental conditions and agronomic management of the varieties. Additionally, the drying process was controlled to avoid alterations in the quality of the beans during this stage [30]. The varieties of the

Arabica species evaluated in this study are crosses of the Caturra variety, with different sources of resistance of the natural Timor hybrid (*C. arabica* × *C. canephora*), to obtain genes with resistance to rust and to maintain good quality features [17,31]. This strategy also results in similar characteristics among the Castillo, Cenicafé1 and Tabi varieties (Table 1), for which there were no differences in the effects of the fermentation treatments or in the final quality of the coffee beverage. Therefore, the impact on the quality of varieties of the Arabica species has been determined considering the process method, dry, semidry and wet [32], and considering changes in the production environment [33].

In general, for the three varieties, temperature control at 15 °C yielded the greatest changes because both the process time and the pH were different from those after spontaneous fermentation and those after fermentation at 30 °C (Table 2 and Figure 1). Temperature control affected the pH. The asymptotic behavior of the pH in the fermentation at 15 °C coincided with the behavior of the temperature, which reached equilibrium at approximately 20 h (Figure 3), after which the pH remained close to 4.0. Several authors agree with the hypothesis that this pH value is an indicator of the completion of fermentation and can be used to avoid unwanted fermentation that affects quality [3,5,20,32] or even to improve coffee quality. However, the fermentation conditions evaluated generated different final pH values, but the coffee varieties had comparable sensory quality. The above implies that this variable reflects the evolution of fermentation but is not related to the final quality. The results also indicated the possibility of modifying the final pH through temperature control, showing a change in the behavior of microorganisms. The inverse relationship between the populations of AAB, specifically of the genus *Gluconobacter*, with the pH at the end of fermentation [11] could explain the lower values obtained for spontaneous fermentation and fermentation at 30 °C because these fermentation conditions led to the highest counts of this microbial group.

Through various investigations that have explored fermentation behaviors in coffee, it is known that the temperature evolves depending on several factors, such as the initial quality of the coffee, the temperature of the environment and the amount of coffee, among other factors [34–37]. In controlled processes, agitation is necessary to achieve temperature homogenization in the coffee mass, which is a challenge considering the changing physical properties of coffee beans suspended in the hydrolyzed mucilage [19]. In some types of fermentation, the final time is obtained when the temperature stabilizes [38]. However, under the conditions evaluated here, the stabilization of the temperature was achieved during the course of fermentation, with incomplete mucilage degradation (Figure 1). In this study, the end of the fermentation was determined objectively as mucilage degradation greater than 95% [14]. In each evaluated condition, differences in the completion time were identified, which must be taken into consideration for the development of controlled processes in which different sensory profiles are to be obtained. The differences in time did not generate differences in the coffee beverage quality (Table 5). This behavior reflects a modification of the activity of the microorganisms involved in the process at low temperatures, the growth and reproduction of the microorganisms slowing down and the fermentation speed [39]. Microbial communities from fermentation at 15 °C, compared with other fermentation conditions, showed higher activity toward carbohydrates and organic acids among different groups of carbon sources [14]. Although the fermentation time at 15 °C was more than double the time of spontaneous fermentations and fermentation at 30 °C, pH values and glucose and lactic acid concentrations suggest that this type of fermentation could be extended while maintaining the process conditions (Figure 3), with values higher than those found by other authors using this same method [20]. At the end of the fermentation of the Castillo variety at 15 °C, the concentration of glucose was higher, different from that at the end of fermentation at 30 °C and different from those for the Cenicafé1 and Tabi varieties at the same temperature. The initial glucose concentration was the same for the three varieties, and glucose consumption differed depending on the fermentation conditions and variety. The final glucose concentration is related to differences in the metabolic activity of the microorganisms involved in reducing sugars [32]. Additionally,

glucose is the primary substrate used to produce lactic acid [40]. The type of process has a greater influence than the variety on the glucose content, a behavior also identified in varieties of the Arabica species in Brazil [32]. The Tabi variety had the shortest fermentation times, a high final glucose concentration and high populations of mesophiles and LAB (Table 3).

The numbers of microbial groups are consistent with those reported in other investigations [14,41], indicating the dominant characteristic of mesophilic and lactic acid bacteria. In addition to the high diversity in coffee after processing using the wet method, the diversity varies between farms and regions [41,42]; however, characteristics did not vary among varieties maybe because they were produced in one place. The largest populations of mesophilic microorganisms require an optimal temperature to ensure and accelerate fermentation, which would be the case of those carried out at 30 °C. On the contrary, fermentations at low temperatures indicate that the processes can develop without problems, maintaining stable and reliable control [39], as in fermentations at 15 °C. The Castillo and Cenicafé1 varieties have different sizes, densities and thermal properties related to the humidity of the coffee [43], which could also affect the fermentation behavior. Some of these differences were observed with the Cenicafé1 and Tabi varieties, which had more beans of a larger size than did the Castillo variety (Table 4), a finding that could be related to the fact that this variety had the greatest descriptive changes in coffee drink scores. Given the diffusion of compounds that occurs from the mucilage to the endosperm, this is facilitated by the water content of the coffee in this state and the size of the bean [44]. The Castillo variety has a lower surface area and bulk and real density values than those for the Cenicafé1 variety [43]. Despite the fact that fermentation under the same conditions ended at similar times and that there was no significant difference in quality, differences in attributes were perceived, being more noticeable for Castillo. Additionally, the descriptors used indicated the possibility of controlling the sensory profile of the varieties through fermentation, for which temperature is one of the options to consider in the development of controlled processes.

5. Conclusions

The behavior of temperature-controlled fermentation at 15 °C and 30 °C was assessed with the Castillo, Cenicafé1 and Tabi varieties. Fermentation behavior differed based on the condition but not the variety, representing an advantage for the development of controlled processes that include recommendations for most of the coffee produced in Colombia.

Differences of more than 24 h to obtain mucilage degradation greater than 95% were observed between fermentation at 15 °C and spontaneous fermentation, influencing the pH, glucose and lactic acid contents. The pH dynamics showed lower acidification rates at lower temperatures and lower final pH values at higher temperatures. Given the independence of the variety from the fermentation behavior, the possibility of controlling pH dynamics and the pH value that marks the end of mucilage degradation through temperature control, they become a contribution to the controlled fermentation of coffee.

The intrinsic quality characteristics of each variety were expressed both in spontaneous fermentation and in controlled fermentation. The latter showed a tendency to improve the characteristics of the coffee, especially for the Castillo variety. The varieties of Arabica coffee evaluated in this research have the quality characteristics desired by the market and can be improved through controlled-temperature fermentation processes. The best sensory quality qualifications were for the Cenicafé1 variety, since it obtained the greatest means and Medias in all treatments.

Because higher grades were consistently identified for larger beans based on the variety, size could influence the diffusion of compounds into the bean during the fermentation process. Bean size and mass transport through different structures of the bean should be investigated in more detail.

Knowing the behavior of different varieties during this type of fermentation allows for the development of strategies to improve controlled fermentation processes and generate

recommendations for temperature management so that the desired quality characteristics are modulated.

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