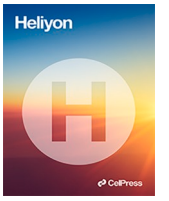
Heliyon 10 (2024) e33508

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Effects of cherries Sanitization methods and fermentation times on quality parameters of coffee beans

Andrea Milena Sánchez-Riaño, Carolina Vega-Oliveros, Wilmer Licerio Ladino-Garzón, Dayana Alejandra Orozco-Blanco, Andrés Felipe Bahamón-Monje, Nelson Gutiérrez-Guzmán, Claudia Milena Amorocho-Cruz *

Universidad Surcolombiana, Facultad de Ingeniería, Grupo de Investigación Agroindustria USCO, Centro Surcolombiano de Investigación en Café – CESURCAFÉ, Código Postal, 410001, Avenida Pastrana Borrero Carrera 1a, Neiva, Huila, Colombia

ARTICLE INFO

Keywords:
Coffea arabica
Coffee fermentation
Sanitization process
Fermentation times
Safety coffee
Coffee quality cup

ABSTRACT

For the first time, the dual effect of coffee cherry sanitization methods to control the microbial load in processing and the influence of fermentation time on coffee quality parameters was evaluated. Two assays were carried out by wet processing: I) Sanitization of the coffee cherry (ST1: Unclassified processed cherries; ST2: Classified and sanitized cherries with drinking water; ST3: Classified and sanitized cherries with a chemical agents and II) Fermentation times (FT1: 12 h; FT2: 24 h; FT3: 48 h; FT4: 72 h and FT5: 96 h). pH, temperature, and dissolved oxygen were monitored during fermentation. Counts of Lactic Acid Bacteria - LAB, mesophiles, and yeasts were carried out on the coffee mass before and after fermentation. Caffeine and chlorogenic acid contents were determined by HPLC-DAD and the sensory profile by methodology for specialty coffees (SCA). The main findings showed that: sanitization with Timsen® did not significantly influence the evolution of pH during fermentation ($p > 0.05$), but it can reduce to a small extent the action of LAB at the end of the process. It was observed that the temperature of the coffee mass tends to balance with the ambient temperature, with significant effects ($p < 0.05$) of sanitization (ST2 and ST3) on the stability of this variable during fermentation. Timsen® as a disinfectant affected microbial populations and improved the sensory profile in the cup. In prolonged coffee fermentations (FT3, FT4 and FT5), the pH of the coffee mass tended to stabilize after 36 h, regardless of the process time. Likewise, a correlation was evident between a higher microbial load correlated with better sensory profiles in FT4 and FT5. Neither the sanitization process nor the fermentation time significantly affected the caffeine and chlorogenic acid contents of the coffee, both in its green and roasted states. Consequently, the sanitization of cherry coffee with Timsen® and prolonged fermentation times favor the safety and coffee final quality in the cup.

Abbreviations: CFU, Colony-forming unit; FMC, Final Moisture Content of dry parchment coffee; GCaw, Green Coffee Water Activity; GRCaw, Ground Roasted Coffee Water Activity; IMC, Initial Moisture Content of washing coffee; LAB, Lactic acid bacteria; PCA, Plate Count Agar; PLS-DA, Partial Least Squares Discriminant Analysis; RCaw, Roasted Coffee Water Activity; RDO, Rugged Dissolved Oxygen; SCA, Specialty Coffee Association; TSS, Total Soluble Solids..

* Corresponding author.

E-mail addresses: andrea.sanchez@usco.edu.co (A.M. Sánchez-Riaño), carolina.vega@usco.edu.co (C. Vega-Oliveros), wilmer.ladino@usco.edu.co (W.L. Ladino-Garzón), dayana.orozco@usco.edu.co (D.A. Orozco-Blanco), andres.bahamon@usco.edu.co (A.F. Bahamón-Monje), ngutierrezg@usco.edu.co (N. Gutiérrez-Guzmán), claudiamilena.amorocho@usco.edu.co (C.M. Amorocho-Cruz).

https://doi.org/10.1016/j.heliyon.2024.e33508
Received 22 November 2023; Received in revised form 14 June 2024; Accepted 21 June 2024
Available online 25 June 2024

1. Introduction

Coffee is one of the most consumed drinks in the world. According to the International Coffee Organization-ICO [1], by 2023/24 coffee consumption is expected to grow by 2.2 %–177.0 million bags, while its total world production (Arabica and Robusta) is estimated around of the 178 million 60 kg bags, 5.8 % more than the previous year, of which 102.2 million bags will be Arabica coffee. Colombia is the third largest coffee producer in the world by volume, behind Brazil and Vietnam (FAO), and is the world's largest producer of Arabica coffee (FNC) [2]. In Colombia, coffee production represents the direct support of 540 thousand families and two million people indirectly [3]. Producing countries like Colombia strive all time to improve post-harvest processes of the coffee beans, and position themselves in the changing and demanding market. According to Iriondo-DeHond et al. [4], the coffee industry has nine stages: planting, harvesting, processing, drying, husking, marketing, roasting, grinding, and brewing. Commonly, the four first stages correspond to pre-harvest (planting/harvesting) and post-harvest (processing and drying) work, which the coffee grower performs on the farm. These processes transform the bean from cherry to dry parchment coffee, market-processing grade. Processing can be carried out by dry, semi-dry, or wet methods, the latter being the most used by Colombian coffee farmers [5].

The coffee fermentation process is part of the coffee cherry processing, and its functional objective is to help the detachment and elimination of the mucilage layer of the bean, to reduce the risk of proliferation of fungi by high humidity in the tropical region [6]. At present, this stage is given more importance; due to the chemical changes that can help to improve the quality of coffee beverages [7]. Coffee fermentation is carried out by microorganisms (bacteria, yeasts, and fungi) and enzymes produced by these, which generate alcohols (ethanol, 2,3-butanediol, 2-heptanol, phenylethyl-alcohol, and benzyl alcohol) and acids (citric, malic, succinic, lactic, oxalic, isobutyric, and propionic) from the degradation of the mucilage [8,9]. Other authors have mainly identified LAB, acetic acid bacteria, enterobacteria, and yeasts in coffee fermentation [10]. Coffee fermentation is carried out in hermetically closed tanks with CO_2 exhaust, generally, which fosters an anaerobic and anoxic environment that favors the growth of the groups of microorganisms aforementioned [11]. However, the diversity of microorganisms present during the fermentation of the coffee bean is subject to factors such as the physicochemical composition of the cherries, the fermentation method, the asepsis of the equipment and processing utensils, the handling and operational practices throughout the process line, the edaphoclimatic and topographic conditions of the crop, and agricultural cultivation practices [6,10,12].

Haile & Kang [13] suggested that control of fermentation conditions favors the growth of specific microorganisms that promote the development of favorable organoleptic and structural characteristics. In this sense, controlled coffee fermentations are not limited to using starter organisms [14–16]. These also includes the use of appropriate techniques for post-harvest handling and hygiene of the raw material, which allow reducing the high natural microbial load, especially pathogenic, that adheres to the coffee beans from the cultivation and harvesting (microorganism presents in: coffee farm soil, leaves, cherries collected on the ground, over-ripe cherries, equipment and utensils of the harvesting and processing). This microbiota is transferred to the fermenting coffee mass, affecting the homogeneity of the process or the growth of desirable microorganisms during this stage [17].

In the food industry, various physical and chemical techniques are used to control, reduce, or eliminate the microbial load, mainly focussed to pathogenic, both in process lines and on the product. Heat treatments, irradiation, drying or dehydration, filtration, refrigeration, freezing, the addition of chemical agents, sanitization, and cleaning procedures, among others, are applied for this purpose [18,19]. Of these, disinfecting agents are a practical and effective alternative for reducing microbial load in agricultural raw materials and equipment. Chemical substances and commercial preparations based on different compounds have been tested for sanitizing agricultural raw materials, utensils, equipment, and processing facilities in the food industry with remarkable results [20–22]. However, there is no reported research on the application of specific methodologies for the disinfection of coffee cherries before processing to eliminate pathogens microorganisms, and their toxins in coffee along to process, and much less information on the use of chemical agents for this purpose on coffee, which represents a gap in the literature.

Different factors influence the final quality of coffee, which is understood in this article as the evaluation of the raw material (sensory analysis), such as the balance in the cup (flavor, aroma, body, mouthfeel and texture), in addition to being safe for consumption [23]. Between these factors, the processing stage highlights (fermentation, drying, packaging, storage). It is during the processing stages that the chemical composition of the coffee beans is preserved or enhanced, ultimately influencing the sensory profile [24]. In this sense, another important aspect of coffee fermentation is the time required for this process and its effect on the final quality characteristics of the bean. Typically, on-farm coffee fermentation and wet processing take between 12 and 36 h [5]. However, recent studies have shown the benefits and improvements offered at the sensory level by prolonged coffee fermentations (between 48 and 72 h), where the productions of essential metabolites and flavor/aroma precursor compounds are major due to the significant variation in the metabolism of microorganisms [25]. Likewise, the effect of fermentation time, especially long times, in coffee beans subjected to wet processing, with or without control of variables such as temperature, pH and the microbiota present in the fermenting coffee mass, on the kinetics has been evaluated. Of mucilage removal, the behavior of the microorganisms that participate in this stage (identification/presence/absence/counts), and on quality parameters of the coffee bean or the fermenting mass such as the content of different organic acids, sugars, alcohols, content humidity and final sensory profile in cup [23,26–29]. These studies have shown various results of interest where they show variability in the quality of the coffee bean depending on the fermentation conditions, especially the time used, the spontaneity or control of the process and the microbiota present at this stage.

Under this scenario, with the development of this research, the aim is to explore for the first time the use of coffee cherry sanitization methods, seeking to demonstrate the beneficial effects of their application on the coffee fermentation process at different times and the final quality in the cup, monitoring the autonomous behavior of the temperature and pH of the coffee mass during this processing stage. Thus, this work aimed to evaluate the dual effects of the use of a quaternary ammonium compound (Timsen®) as a

chemical disinfection agent in the coffee cherry, and the incidence of the coffee fermentation process time, on the physicochemical quality, microbiological and sensory of the coffee bean, Castillo variety, from the Huila Region (Colombia).

2. Materials and methods

2.1. Collection and conditioning of vegetal material

Ripe cherries of *Coffea arabica* – Castillo variety, from combined harvests of two specific farms in the region of Huila – Colombia, were collected manually through color selection. Due to the experimental conditions, 360 kg of coffee cherry were needed for the entire experimental work. This sample size was determined based on the available yield of the selected farms and was processed from coffee cherry to roasted and ground forms, for physicochemical, microbiological, and sensory analysis, at the pilot plant and laboratories of the Centro Surcolombiano de Investigación en Café - CESURCAFÉ, from the Universidad Surcolombiana, Huila-Colombia.

2.1.1. Georeferencing of coffee sample collection areas for the study

Palermo town (Vereda Líbano, farm Bella Vista 1, altitude 1708 m.a.s.l., average temperature: 22 °C. Coordinates N 2°52′24.1″ y W 75°35′52.4″) and Hobo town (Vereda Batan, farm Monte frío, altitude 1580 m.a.s.l., average temperature 25 °C. Coordinates N 2°29′58.7″ y W 75°25′44.1″) in the region of Huila – Colombia.

2.2. Experimental design

For coffee cherry sanitization trial I, we applied an experiment that falls under the Single-Factor Experimental Design with Three Levels. The established treatments were: ST1: unclassified cherries directly processed, ST2: cherries classified by density and sanitized with drinking water from the municipal supply, and ST3: cherries classified by density and disinfected with Timsen® (1 g/L of water for 4 min; Restrepo Gómez S.A.S., Barranquilla, Colombia). Subsequently, each replicate per treatment (n = 3) was pulped and fermented for 24 h at room temperature (approx. 24 ± 1 °C), which was monitored and reported for the experiments. For trial II of different fermentation times in coffee, based on preliminary results and others reported in the literature [30], we applied an experiment that falls under the Single-Factor Experimental Design with five Levels in triplicate (n = 3), the treatments were evaluated applying a geometric sequence with the equation $a_n = a_1\, r^{(n-1)}$ with time in hours: FT1: 12 h, FT2: 24 h, FT3: 48 h, FT4: 72 h, and FT5: 96 h. The coffee cherry beans used in this second test and its treatments were conditioning by applying the treatment that yielded the best results in the sanitization test. Subsequently, the pulped coffee was fermented at the time indicated for each treatment.

2.3. Coffee fermentation tests

For the sanitization test of the coffee cherry (in total: 135 kg of the coffee cherry, from Palermo-Huila) and the different fermentation times test (in total: 225 kg of the coffee cherry, from Hobo-Huila), for each replicate of treatment to evaluate, 7.1 ± 0.5 kg of pulped coffee were deposited in plastic containers, properly clean and labeled. The coffee was then mixed with potable water (30 %, w/w) using a stainless steel helical type agitator for each treatment replicate for manual homogenization. The containers were hermetically closed, and the mixture's pH, temperature, and dissolved oxygen values were recorded every 10 min, from time 0 until completing 24 h of fermentation for the treatments of trial I and, for trial II, until completion of each fermentation time evaluated (12, 24, 48, 72, and 96 h). For this, multiparameter measurement equipment (ORION Star A Series A3265, Thermo Scientific, Indonesia) and their respective sensors (Accurity relative: pH [±0.002], Temperature [±0.1 °C] y RDO [±2 % sat. ≤ 200 % sat]), previously installed on the lid of each container were used. The coffee masses were mechanically stirred during the first 3 h of the fermentation and from 16 h until the end, for 50 s every hour. The temperature and relative humidity of the room were also monitored over time.

2.4. Washing, drying and roasting of fermented coffee beans

Both for the sanitization test and for the test of different fermentation times and their respective treatments (ST1, ST2 and ST3/FT1, FT2, FT3, FT4 and FT5), the fermented coffee was washed with drinking water (three washes with removal of empty coffee beans, cherry residue and mucilage), and 1.6 kg of drained coffee was weighed per replicate of each treatment. The washed coffee was dried in trays made with high-density polyethylene mesh and metal edges (Dimensions 29 × 39 × 5 cm). The coffee was dried in a forced convection oven (Memmert UF55, Schwabach, Germany) at 40 °C, with homogenization and rotation every 20 min during the first 2 h and then every hour until the end of drying (moisture content between 10 and 12 % (wet base - w.b.) [31], Moisture meter, KETT, PM-450, California, USA). The oven method determined the final moisture content according to the NTC2325 standard [32]. The dry parchment coffee beans were threshed, and the green almond was passed through a No. 13 sieve with (ø = 5 mm). Green coffee beans without defects are used for roasting, according to the conditions of the Specialty Coffee Association (SCA) protocol. Pots of 150 g of green coffee were roasted at an inlet temperature between 180 and 200 °C. The conditions of apparent density, humidity, and activity of the bean were taken into account before roasting.

2.5. Total soluble solids (TSS)

The TSS was determined in liquid mucilage samples (0.5 mL) at the beginning and the end of the fermentation process using a

digital refractometer (Atago PR-201α, Fukaya-shi, Japan) [33]. The results were expressed in °Brix scale.

2.6. Chromatographic analysis

The determination of caffeine and chlorogenic acids in green and roasted coffee beans followed the ISO 20481:2008 procedure with modifications [34]. 1 g of sample (green or roasted coffee) was mixed in 200 mL of UHQ water (type 1, 18.2 MΩ cm), extracted for 20 min in a water bath at 90 °C, and cooled to room temperature, and brought to a volumetric volume of 250 mL. Finally, the extracts were filtered through a 0.20 μm nylon membrane filter and stored in 2 mL amber vials. Chromatographic conditions for quantification were as follows: 10 μl of filtered extract was injected into the HPLC system (Infinity II 1260 manufactured by Agilent Technologies, California, USA). The flow condition was isocratic with methanol:water type 1 (Merck, Darmstadt, Germany) 50:50. A 120 EC-C18 Poroshell column, 150 × 4.6 mm and 4 μm (Agilent Technologies, California, USA), a flow rate of 1 mL/min was used with a system equipped with a quaternary pump (G7111A), integrated autosampler with a temperature-controlled column oven at 30 °C (G7129A). Caffeine was detected at 272 nm and chlorogenic acid at 325 nm with a diode array detector (G7117C). The reference materials used were: caffeine (CAS: 58-08-2; Merck, Darmstadt, Germany) and chlorogenic acid (CAS: 327-97-9; Sigma Aldrich Inc., St. Louis, USA). The chromatographic data were processed with OpenLab ChemStation software version LTS 01.11.

2.7. Microbial count

Using a vortex mixer (Benchmark Scientific Inc, Taiwan), samples of 10 mL of mucilage were homogenized with 90 mL of buffered peptone water (Scharlab S. L., Barcelona, Spain), using a vortex mixer (Benchmark Scientific Inc, Taiwan, China) and serially diluted. The enumeration of yeasts, aerobic mesophilic microorganisms, and LAB was carried out using the technique of deep plating using chloramphenicol agar (YGC agar) for yeasts [33], MRS agar for LAB [16] and standard method agar (PCA) for mesophiles [35], all acquired from Laboratorios Conda S.A. (Madrid Spain). The plates were incubated at 30 °C (yeasts), 37 °C (aerobic mesophiles), and 37 °C (LAB) for 48 h in an incubator (Thermo Scientific, Langenselbold, Germany), and the number of Colony-Forming Units was quantified (CFU).

2.8. Physical and sensorial analysis

Physical analysis tests were carried out under NTC 2324.2002 [36] and the Specialty Coffee Association (SCA) standards [37]. The tests were applied to coffee samples with a moisture content between 10 and 12 % (w.b.) [31]. The apparent density of dry and green parchment coffee was determined using a test tube. The water activity (aw) in green, roasted, and ground coffee was measured using a vapor sorption analyzer (VSA Aqualab Decagon Devices, Inc. Pullman, WA) with 1 and 3 g of sample. The sensory analysis was developed following Cuping Protocol SCA [37], this methodology indicated for special coffees according to the indicated standards. Roasting was performed in the Prisma sample roaster and grinding in the mahlkonig M EK43s roaster. The sensory analysis panel consisted of 4 expertly trained tasters.

2.9. Statistical analysis

The two fermentation tests employed random screening-type designs with three repetitions per treatment. Sanitization effects were assessed with repeated measures ANOVA, supported by Mauchly's test for sphericity. Microbiological, physicochemical, and sensory analyses used univariate/multivariate statistics with Fisher's/Tukey's tests and PLS-DA for correlations. To determine significant differences between treatment means, mean separation was performed using Fisher's Least Significant Difference (LSD) test for pairwise comparisons and Tukey's Honestly Significant Difference (HSD) test for multiple comparisons. Results are presented as mean ± standard deviation (n = 3 for sensory, n = 4 for others), with α = 0.05 significance. Analyses were done using Statgraphics Centurion

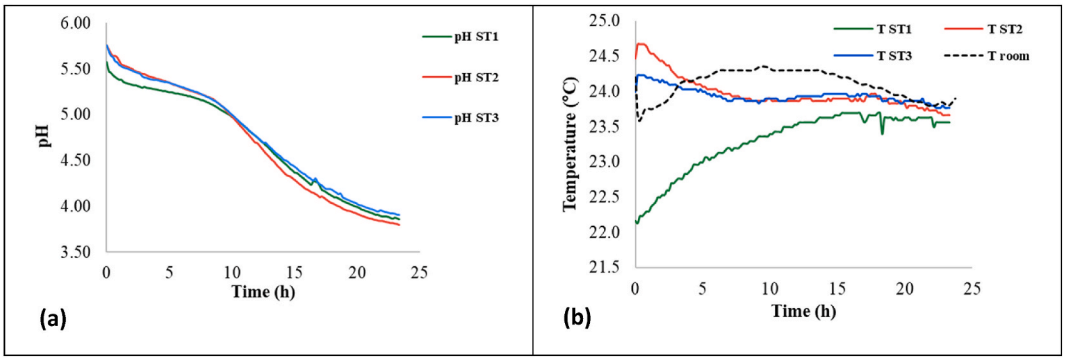


Fig. 1. Evolution of pH and temperature during the fermentation of the coffee mass subjected to different sanitization treatments of the coffee cherries. Parameters: (a) pH and (b) Temperature. The data shown are the means of three replicates (n = 3).

XVI (United States).

3. Results and discussion

3.1. Fermentative process of coffee beans

The significant change in the pH values during the fermentation time was evidenced for the three sanitization treatments evaluated in coffee (see Table S1). Three differentiated periods related to a different rate of change in pH were observed for the three treatments (Fig. 1a). A smooth and constant drop is observed up to an approximate pH of 5.0 around 10 h of fermentation, followed by a marked and constant decrease to a pH of 4.3 at approximately 15 h of the process; and ending with an exponential decline to a pH of 3.8 at the end of the process. In wet fermentations of coffee beans, researchers have found that once the fermenting mass reaches pH 5, this stage can be completed within the next 2 h [38]. However, spontaneous fermentation processes at room temperature (25 °C) can be completed between 12 and 19 h (15 h, average) [29]. Likewise, during the first 15 h of fermentation, the microbial count increases and the production of organic acids as well, due to the consumption of simple sugars by these microorganisms, which generates the degradation of the mucilage and a decrease in pH [39]. Thus, pH decrease behavior until the final of the fermentation is attributed to the acidification of the medium due to the effect of the reaction products of the microbial and enzymatic activity together with the

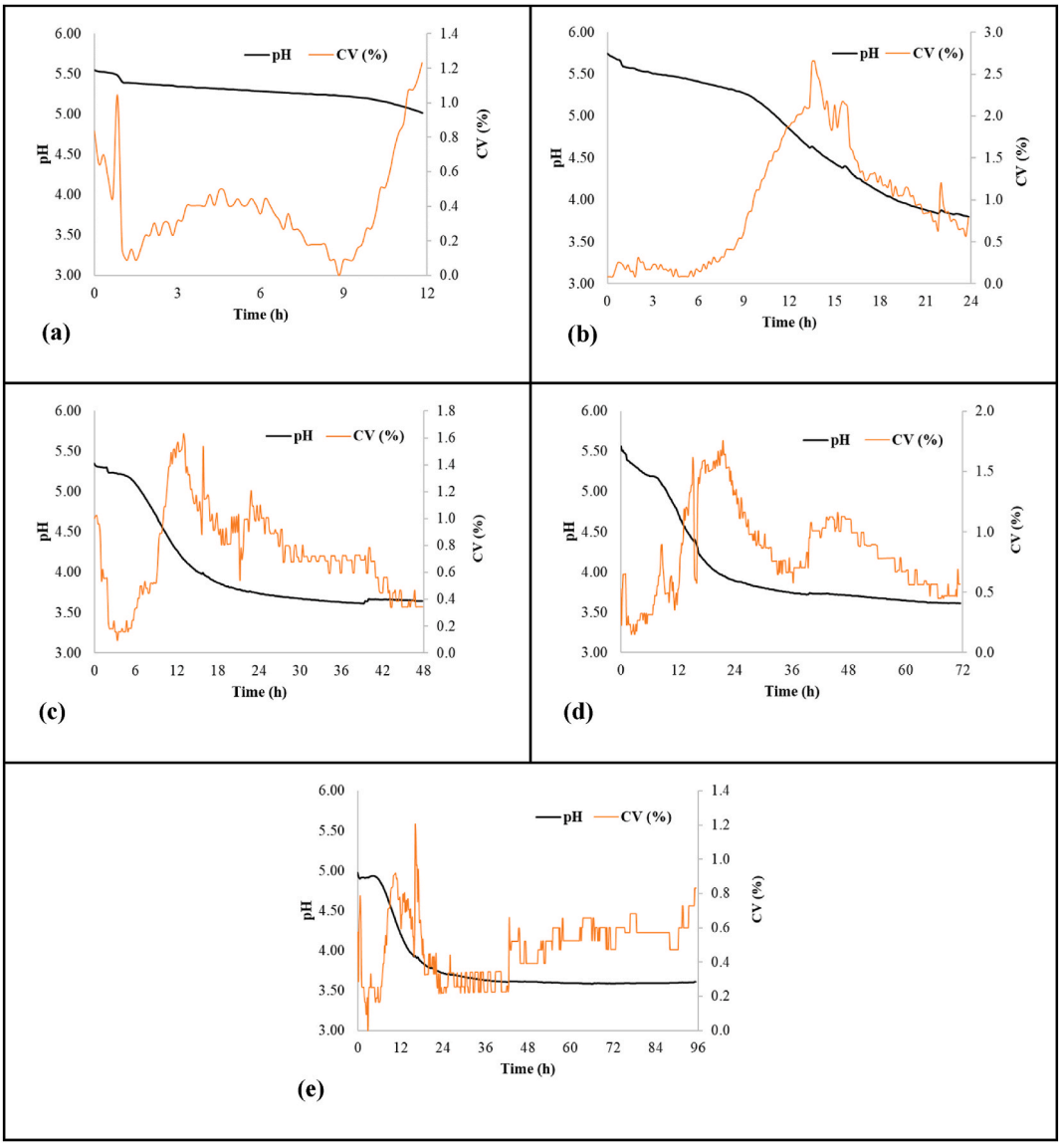


Fig. 2. Evolution of pH at different fermentation times evaluated for coffee. [FT1 (a), FT2 (b), FT3 (c), FT4 (d), and FT5 (e)]. CV: % Coefficient of variation. The data shown are the means of three replicates (n = 3).

nature of the products or metabolites generated over time (lactic, butyric, acetic, and other higher carboxylic acids, ethanol and enzymes) [13,26,40].

The comparison of the simple repeated measures analysis did not show statistical differences between the three treatments: ST1, ST2 and ST3 (see Table S2); therefore, the effect of sanitization did not significantly influence the evolution of pH during coffee fermentation ($p > 0.05$). However, in ST3 (Fig. 1a), the rate of pH change is slightly lower after the second period (from 10 h of the process) compared to ST1 and ST2, yielding lower values of standard deviation and coefficient of variation of pH towards the end of the process. That can be related to the lower concentration of LAB in ST3 at the end of fermentation, as described in the microbiological counts performed. The wide non-specific but random biocidal effect of Timsen® [22,41], can reduce to a small extent the growth and action of endogenous LAB from the second fermentation period, which decreases the lactic acid production generating a lower rate of pH change in ST3 [42]. Then, the sanitization of coffee cherries with a chemical agent such as Timsen® allows the process to be maintained under more controlled conditions.

For the three sanitization treatments, the energy in the form of heat generated is not enough to achieve a significant increase ($p > 0.05$) in the temperature inside the reactors (Fig. 1b). The temperature value then tends to stabilize in all treatments at room temperature (T room average: 24 ± 0.2 °C). This trend has been reported in spontaneous coffee fermentations (without the addition of microorganisms and other specific additives, or control of variables), where the temperature of the fermenting mass depends on the

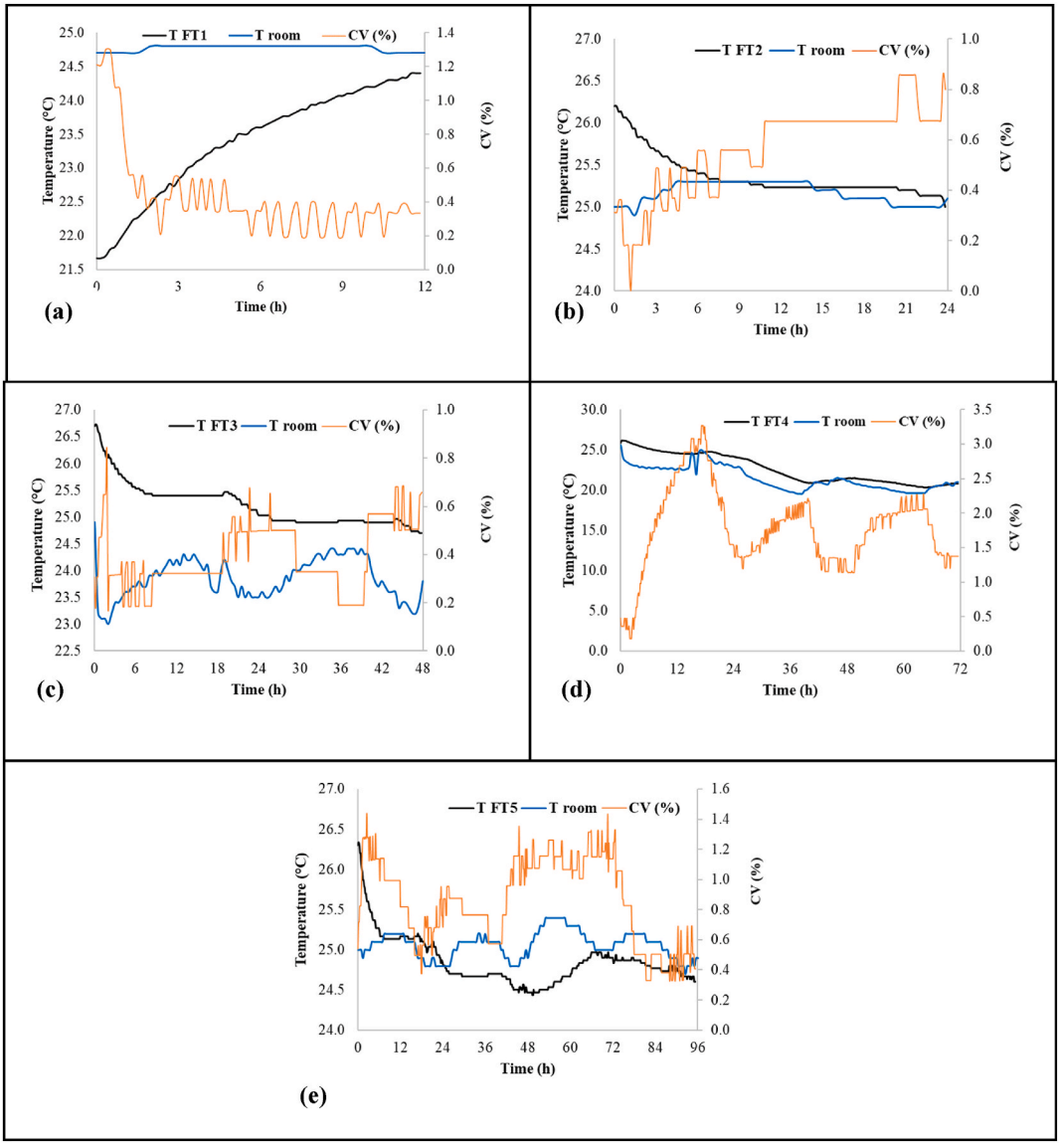


Fig. 3. The temperature recorded inside the reactor during the fermentation process of coffee subjected to different process times [FT1 (a), FT2 (b), FT3 (c), FT4 (d), and FT5 (e)]. T room: Room temperature; CV: % Coefficient of variation. The data shown are the means of three replicates (n = 3).

ambient temperature [29]. The type of sanitization treatment had a significant effect ($p < 0.05$) on the temperature of the fermenting dough. ST1 was significantly different from ST2 and ST3, treatments that showed homogeneity with each other (see Table S3). The temperature of coffee mass tended to increase and stabilize with the room temperature for ST1; while, for ST2 and ST3 this variable tended to decrease and balance with the environment. An unclassified or undisinfected coffee such as ST1 presents a higher content of defective cherries (dry, green, and with phytosanitary problems) and a major amount and variability of endogenous microflora. This results in higher rates of bean degradation and microbial growth, which increases the temperature in the fermentation for coffee masses such as ST1 [43]. Taking into account that during spontaneous fermentation on mucilage, a typical temperature increase occurs in this type of processes, which in turn is an indicator of the activity of the microorganisms [44]. These results highlight the positive effect of the sanitization of coffee cherry on the stability of the fermentation temperature and microbial activity.

The evolution of the pH at different fermentation times shows that when the coffee mass presents an initial pH value above 5.5 ± 0.1, a slight and constant decrease can be observed during the first 9 h of the process, reaching an approximate pH of 5.2 ± 0.1 (Fig. 2a, 2 b and 2 d). Meanwhile, if the coffee mass starts the process with pH values lower than 5.5, the period of constant decrease is short (less than 6 h) (Figs 2c and 2e). After 36 h of the process, the pH tends to stabilize at an approximate value of 3.6 for the FT3, FT4, and FT5 treatments. Osorio-Pérez et al. [45] report similar pH values at time zero and at 36 h of fermentation, while Zhang et al. [46], who also carried out wet fermentations of coffee beans at 24 h, 48 h and 72 h, obtained the greatest drop in pH after 36 h of fermentation.

For Colombian coffee fermentations of up to 48 h, de Oliveira et al. [42] report the prevalence at the beginning of fermentation of LAB and bacteria belonging to the Enterobacteriaceae and Acetobacteraceae families; followed by a drastic decrease in the latter at 12 h of the process, being surpassed mainly by LAB, *Leuconostoc*, and *Lactobacillus*. The acid lactic is the main metabolic product of LAB by the demucilagination effect of the coffee pulp [47]. It is the predominant metabolite at the end of the coffee fermentation, responsible for the acidification of the medium and the subsequent decrease in pH [48]. The stabilization of the pH after 36 h, despite the prolongation of the fermentation time, can then be attributed to the acid saturation of the medium that slows down bacterial activity, favoring the predominance of yeasts at the end of the process, typical behavior of the fermentation natural of coffee [40].

Likewise, in all the coffee fermentation treatments at different times (Fig. 3a to 3e), the temperature of the coffee mass tends to stabilize with the room temperature (Range: 24–25 °C). It showed that the coffee masses do not generate enough heat to alter the thermodynamic equilibrium with the environment during fermentation. In this trial, the coffee cherry was classified by density and disinfected with Timsen® for all treatments, which explains the similar behavior of the temperature of the fermenting coffee mass evidenced in the ST3 treatment of the sanitization trial. Therefore, the tendency to a slight increase or decrease in the temperature inside the reactors can be attributed to I) the differential of temperature between the masses and room temperature at the beginning and during fermentation, which tends to balance; and II) the typical activity of the microorganisms whose growth and metabolism at each stage of coffee fermentation can affect the behavior of this variable [49].

Finally, for the sanitization treatments experiments and different fermentation times, dissolved oxygen within the fermenting coffee mass (% RDO) was consumed between the first 15–60 min of the process, falling from 100 % to 0.0 %. This behavior of the % RDO is attributed to the gradual production of CO_2 by microbial metabolism and to the guarantee of anaerobiosis desired for the coffee fermentation system [40].

3.2. Determination of caffeine and chlorogenic acid content by HPLC

For the two tests carried out and their green and roasted coffee samples, the results of caffeine and chlorogenic acids analyses are found in Table 1. Caffeine remained thermostable in the states from green to roasted for both experiments (11–12 mg/g). Similar values were reported by Girma et al. [50]. De Luca et al. [51] found caffeine contents in Colombian arabica coffee between 9.33 ± 0.26

Table 1
Caffeine and chlorogenic acid concentrations of coffee subjected to different sanitization treatments and fermentation times.

Assay	Coffee state	Treatment	Clorogenic acid (mg/g)	CV (%)	Caffein (mg/g)	CV (%)
Sanitization	**Green**	ST1	35.17 ± 0.98a	2.79	11.51 ± 0.25a	2.17
		ST2	32.07 ± 1.45 b	1.40	11.17 ± 0.12a	1.07
		ST3	35.78 ± 2.37a	3.83	10.52 ± 0.34a	3.23
	Roasted	ST1	24.34 ± 2.31a	9.49	11.33 ± 0.14a	1.24
		ST2	24.43 ± 1.4a	5.73	11.07 ± 0.24a	2.17
		ST3	25.3 ± 3.5a	5.93	11.53 ± 0.92a	7.98
Fermentation times	**Green**	FT1	34.59 ± 1.65a	4.77	11.56 ± 0.93a	8.04
		FT2	32.62 ± 3.05a	9.35	11.62 ± 0.91a	7.83
		FT3	35.07 ± 2.59a	7.39	11.63 ± 0.85a	7.31
		FT4	33.21 ± 3.19a	3.58	11.7 ± 1.06a	9.06
		FT5	35.87 ± 3.83a	5.10	11.52 ± 1.05a	9.11
	Roasted	FT1	20.63 ± 1.43a	6.93	11.70 ± 1.07a	9.15
		FT2	21.58 ± 2.54 ab	7.14	11.21 ± 1.11a	9.90
		FT3	23.14 ± 2.74 ab	7.52	11.01 ± 1.60a	9.63
		FT4	24.45 ± 1.00 ab	4.09	11.33 ± 0.67a	5.91
		FT5	23.01 ± 2.68 b	7.30	11.12 ± 1.52a	9.44

Values are expressed as means ± standard desviation (n = 3). CV (%): Coefficient of variation in percentage. Different letters in the same column by treatment group of the same trial indicate statistically significant differences ($p < 0.05$).

and 16.47 ± 0.25 (mg/g). According to the Turkey HSD test, no statistically significant differences ($p > 0.05$) exist between the treatments for the green and roasted coffee samples, in any of the two evaluated trials. In other words, the caffeine concentration is not affected significantly ($p > 0.05$) by performing a sanitization treatment before processing the coffee cherry, nor by the fermentation process time. Regarding the results obtained from chlorogenic acids, the green coffee samples showed contents between 32 and 35 (mg/g). Similar contents are reported by Kulapichitr et al. [52] in Colombian arabica coffee. It was evidenced that chlorogenic acids, which are thermolabile [50,53], were affected by the roasting process in all treatments, reducing their concentrations. Thus, no significant changes ($p > 0.05$) in the total content of chlorogenic acids in green or roasted coffee were observed for the sanitization process or the fermentation time used. This is because the analyzed compounds may be sensitive to other factors not investigated, such as exposure of the fermentation mass to direct sunlight, strong temperature changes, or the presence of oxygen. However, all of these factors were not encountered during coffee processing due to controlled conditions. Therefore, the sanitization process did not influence significant changes. Other authors mention that these compounds can be altered by various factors, such as roasting temperature, which plays a crucial role in altering chlorogenic acid levels. Higher temperatures lead to a reduction in chlorogenic acids due to the formation of melanoidin compounds [54]. Furthermore, different postharvest processing can lead to modifications in the protein fraction of coffee beans, which could affect the interaction between proteins and phenolic compounds, including chlorogenic acids [55]. Similarly, caffeine levels can vary due to the dry, washed, and semi-dry post-harvest processes in green beans as well as in roasted ones [56] Additionally, selective harvesting may influence the chlorogenic acid content of coffee beans [57].

3.3. Microbiological analysis

It was established that coffee fermentation is related to the diversity of microorganisms present naturally or added during post-harvest handling of the coffee cherries [14], whose process develops spontaneously by the action of the microbiota present during processing, with variations linked to regional conditions, cherry composition, and processing method [58]. Considering the fermentation conditions, a first statistical approach with multivariate analysis was performed for coffee from treatments ST1, ST2, and ST3. The data matrix was constructed with the sensory analysis results, TSS, and microbiological data, and a PLS-DA. The analysis on the results helped to evaluate the impact of disinfection application, as well as the variables that most influenced the data analyzed. The characteristics analyzed in the matrix included calculated data corresponding to the "growth delta," which was obtained from the difference between the microbial count between 0 h and 24 h of mesophiles, LAB, and yeasts. The measured data did not allow for the separation of the three treatments due to the overlapping distribution of microbial counts observed at 0 h and 24 h for mesophiles, LAB, and yeasts. Despite the application of treatments at three different times during coffee cherry collection (ST1, ST2, and ST3), the resulting distributions did not show distinct clusters for each treatment. However, it is evidenced that the distribution of the points for the samples treated with Timsen® (ST3) presented the lowest dispersion (Fig. 4) and, therefore, greater homogeneity in the measured parameters.

Table 2 shows the growth of microorganisms with vast diversity, which generate metabolites that can be transferred to the coffee beans and subsequently influence the sensory characteristics of flavor and aroma in the beverage [59,60].

In the trial I, the cell count corresponding to LAB at 0 h did not show statistical differences between treatments. The measurement at 24 h in this trial showed differences; mainly, ST3 (Timsen® application combined with coffee cherry selection) showed the lowest microbial counting. Statistically, the LAB-24 h count for ST1 and ST2 had no differences. Since in ST1, there was no kernel selection, but in ST2, it was made with coffee cherries selection by density (Flotation) with water washing, it is inferred that the lack of disinfection influenced this result.

Yeast counts were lower than those reported for mesophiles and LAB. It can be due to the low level of TSS, as reported in other works such as Udeagha et al. [61]. Additionally, a lower variation was observed between the initial and final counts (growth delta

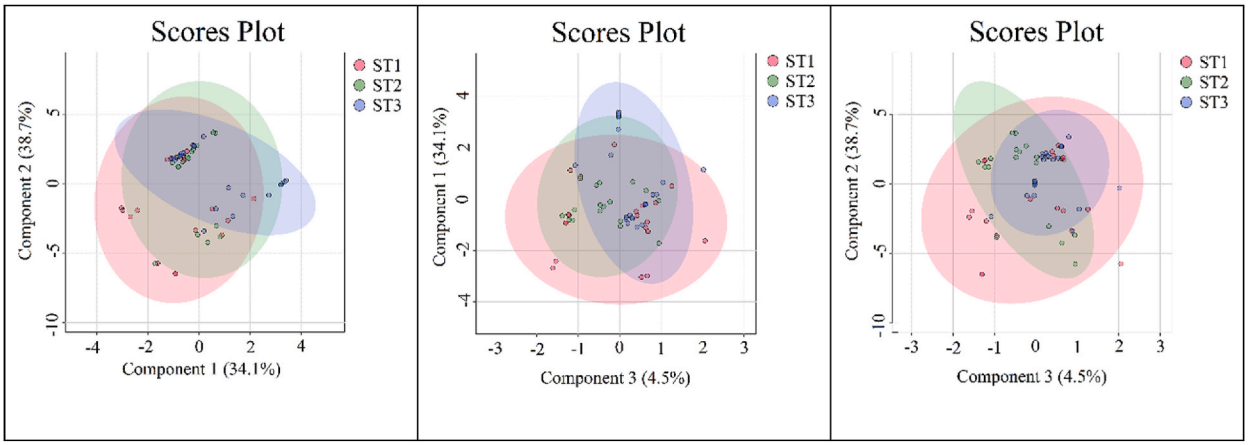


Fig. 4. Data distribution by treatment with the PLS-DA model for the cherry coffee sanitization trial. The Fig.s show the combination of the first three components that explain 78.2 % of the data.

Table 2
Microbiological and physical parameters evaluated in coffee subjected to different sanitization treatments and fermentation times.

Treatment	Fermentation time (h)	LAB	Yeasts	Mesophiles	TSS (°Brix)	IMC (%)	FMC (%)	GC*aw*	RC*aw*	GRC*aw*
		Log_{10} CFU/mL								
C*	–	–	–	–	15.21 ± 0.90 g					
ST1	0	5.33 ± 0.38a	5.13 ± 0.32 ab	6.42 ± 0.97a	6.79 ± 0.88a	50.22 ± 0.62	11.1 ± 0.39	0.59 ± 0.01	0.36 ± 0.03	0.36 ± 0.02
	24	7.69 ± 0.27c	5.31 ± 0.45 b	7.48 ± 0.40 b	8.87 ± 0.34c					
ST2	0	5.26 ± 0.55a	5.00 ± 0.20a	6.04 ± 0.29a	7.79 ± 0.53 b	52.71 ± 0.63	10.48 ± 0.62	0.47 ± 0.11	0.39 ± 0.04	0.37 ± 0.04
	24	7.64 ± 0.26c	5.07 ± 0.38 ab	7.55 ± 0.40 b	8.76 ± 0.39c					
ST3	0	5.31 ± 0.22a	5.12 ± 0.20 ab	6.33 ± 0.52a	6.48 ± 0.71a	51.01 ± 0.51	12.33 ± 0.74	0,51 ± 0.2	0.42 ± 0.02	0.34 ± 0.02
	24	7.27 ± 0.31 b	5.09 ± 0.24 ab	7.41 ± 0.71 b	7.53 ± 0.67 b					
FT1	0	5.12 ± 0.05a	5.08 ± 0.20 d	5.61 ± 0.37a	6.75 ± 0.16c	52.11 ± 1.07	10.41 ± 0.88	0.59 ± 0.04	0.37 ± 0.02	0.41 ± 0.01
	12	6.63 ± 0.15 d	5.14 ± 0.12 d	7.62 ± 0.80 d	8.19 ± 0.24f					
FT2	0	5.20 ± 0.76 ab	4.49 ± 0.24 b	5.31 ± 0.29a	4.97 ± 0.45a	51.74 ± 0.26	12.01 ± 0.51	0.60 ± 0.02	0.29 ± 0.02	0.38 ± 0.05
	24	9.52 ± 0.12 g	4.26 ± 0.17a	8.44 ± 1.30e	7.35 ± 0.33 d					
FT3	0	6.56 ± 0.39 d	5.08 ± 0.22 d	6.41 ± 0.67c	4.81 ± 0.23a	51.86 ± 0.71	10.63 ± 0.53	0.53 ± 0.02	0.32 ± 0.04	0.35 ± 0.04
	48	7.19 ± 0.29ef	4.42 ± 0.26 ab	7.34 ± 0.51 d	7.28 ± 0.12 d					
FT4	0	5.60 ± 0.34bc	5.45 ± 0.13e	6.24 ± 0.33bc	6.43 ± 0.18 b	52.02 ± 0.4	12.28 ± 0.85	0.51 ± 0.03	0.34 ± 0.04	0.34 ± 0.04
	72	7.51 ± 0.40f	4.84 ± 0.20c	7.91 ± 0.53de	7.80 ± 0.16e					
FT5	0	5.88 ± 0.19c	5.88 ± 0,10f	5.75 ± 0.43 ab	6.32 ± 0.65 b	54.23 ± 1.93	10.86 ± 1.14	0.56 ± 0.04	0.32 ± 0.02	0.33 ± 0.03
	96	7.10 ± 0.72e	6.31 ± 0.20 g	6.37 ± 0.49c	6.47 ± 0.13bc					

C*: Pulped coffee mucilage without adding water. IMC: Initial Moisture Content of washed coffee; FMC: Final Moisture Content of dry parchment coffee; GCaw: Green Coffee Water Activity; RCaw: Roasted Coffee Water Activity; GRCaw: Ground Roasted Coffee Water Activity. Values are expressed as means ± standard deviation (n = 3 y n = 4). Different letters in the same column by microbiological analysis indicate statistically significant differences ($p < 0.05$).

defined before), although there were no statistical differences between the treatments.

Mesophilic aerobes showed the highest growth count results. Although the methodology is not selective, the culture media could allow the growth of facultative microorganisms. However, the methodology allows having an overall estimate of the microbiota present in the treatments. Even though we did not find statistical differences, the average count shows the lowest mesophilic count in ST3 at 24 h. This tendency can be attributed to the non-specific biocidal action of the sanitizing chemical agents like the quaternary ammonium of Timsen® [41].

Considering the anaerobic conditions reached in the bioreactors, according to the dissolved oxygen sensor records, we understood that the environment would be favoring the growth of LAB, which are viewed chiefly as facultative microorganisms, and whose conditions allow increasing the production of organic acids, alcohols, and volatile compounds, which contribute to the formation of flavor, fragrance, and aroma [26]. The tendency of the ST3 treatment to show lower microbial counts and to coincide with better sensory scores is highlighted.

The dynamics of LAB, mesophilic, and yeast counts at the beginning and end of each fermentation were examined in trial II (Table 2). The mesophilic and LAB counts at the beginning and end of the fermentation were higher than the yeast counts. This behavior was similar to the findings in Trial I, and also previously reported by Pereira et al. [40], who evidenced that microbial successions in coffee are initiated by bacteria and subsequently due to the growth rate, by yeasts. These similarities indicate that part of the population fluctuation results from the intrinsic characteristics of the raw material (pulped coffee) and the variation in nutrient availability during fermentation. The highest mesophile counts at the end of fermentation were constant for all treatments, in agreement with previous reports [59,60]. According to Mahingsapun et al. [14], the rise of bacteria and yeasts CFU prompts the tendency to increase along the fermentation. This microbial fluctuation phenomenon relates to more significant variability and complexity of flavors plus aromas, and boosts the sensory evaluation score. In this way, the findings described previously allow justifying the findings in the present research, where all treatments with specialty sensory characteristics were highlighted in the category of "very good" according to SCA, with prevalence to the best scores for treatments FT4 and FT5.

The TSS (Table 2) presented a decrease in concentration at the beginning of the process caused by adding water to condition the

coffee mass. This parameter tended to increase towards the end of the fermentation for all the trials, similar to that reported by Puerta-Quintero [62], who indicates that a possible explanation for this phenomenon is the dissolution of compounds enveloped and present in the mucilage of the coffee beans in submerged fermentations.

Table 2 has the reports for the results of the raw material characterization for moisture content in the washed coffee beans (before drying) and dried parchment coffee for the two evaluated trials. The values were similar to those reported by Tripetch & Borompichaichartkul [63] for moisture content (ranges between 8 and 12 %) and by Cortés-Macías et al. [64] for water activity in wet-processed coffees with 18 h of fermentation.

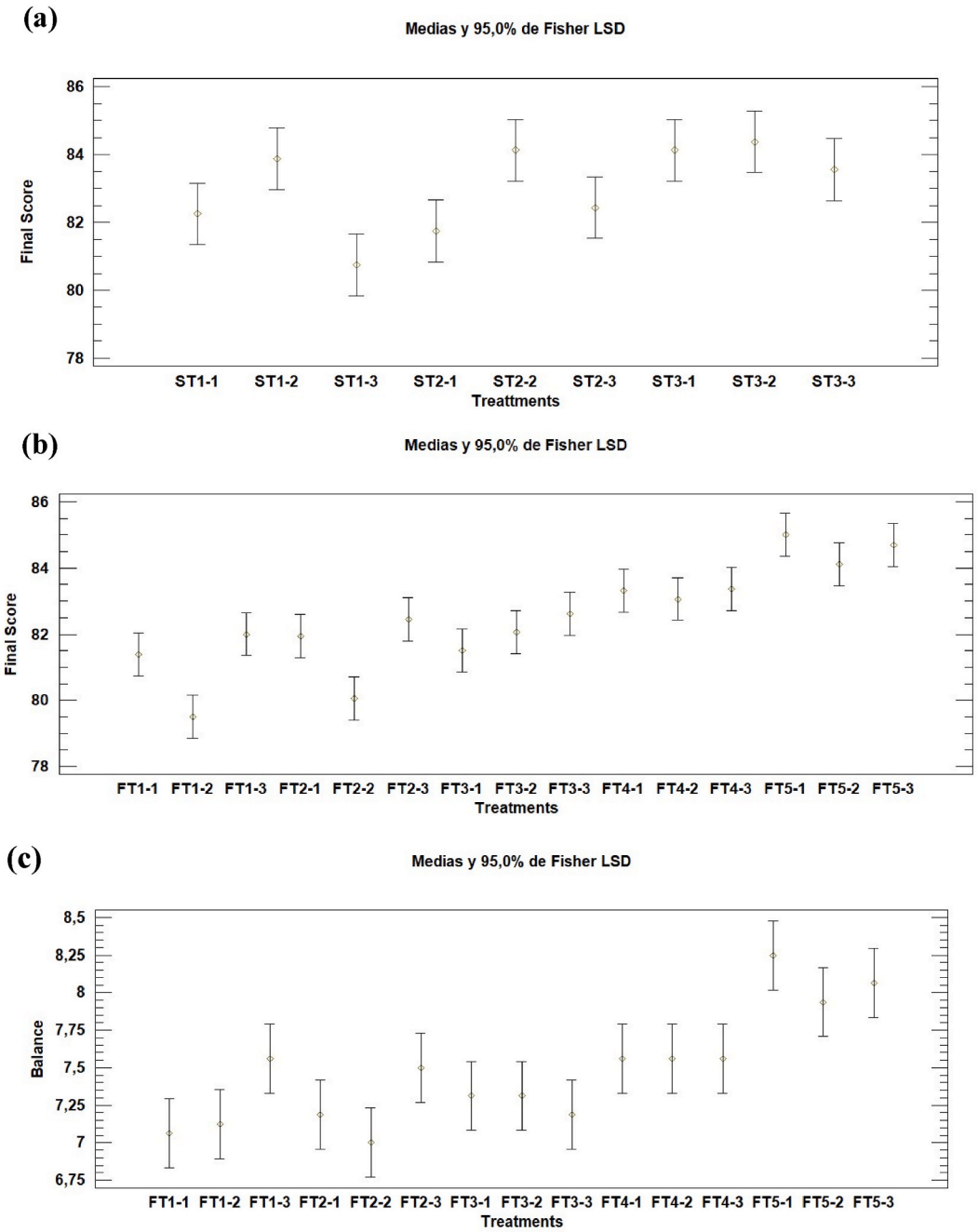


Fig. 5. Simple Anova on sanitization treatments in coffee cherries (a) and coffee fermentation treatments at different processing times (b) as a function of the final score SCA methodology and the evaluation parameter balance (c).

3.4. Sensory results

Similar to microbiological results, ST3 shows greater homogeneity in the final score of the sensory analysis versus the other sanitization treatments (Fig. 5a). Thus, no statically significant differences ($p > 0.05$) were evident between the sanitization treatments tested. However, variations between their means yes [ST1 (82.21 ± 1.73), ST2 (82.73 ± 1.7), and ST3 (83.85 ± 1.04)]. These sensory results in the final coffee drink reaffirm, with a difference of 1.64 points in the final score, the positive effect of sanitizing cherry coffee with the use of a disinfectant chemical agent such as Timsen® (ST3), compared to not carrying out no sanitization (ST1), and a difference of 1.12 points versus to carrying out washings with only drinking water and classification by the density of the coffee cherries (ST2).

Fig. 5b shows the results of the final score in the SCA methodology sensory analysis for the test of different fermentation times in coffee, where the treatment with 96 h (FT5) presents significant differences ($p < 0.05$) versus the FT1, FT2, and FT3. Likewise, in Fig. 5c it can be seen that, for the sensory parameter evaluated, balance, which involves the harmony between flavor, residual flavor, acidity, and body, there are significant differences ($p < 0.05$) of the FT5 versus FT1, FT2, and FT3. Thus, the final scores and average balances are respectively FT1 (80.96 ± 1.26, 7.25 ± 0.32); FT2 (81.48 ± 1.39, 7.23 ± 0.34); FT3 (82.06 ± 1.23, 7.27 ± 0.25); FT4 (83.25 ± 0.76, 7.56 ± 0.11) y FT5 (84.60 ± 0.76, 8.08 ± 0.5), where the treatments with the lowest standard deviation, highest final score and best balance correspond to the prolonged fermentations of 72 h and 96 h. Similar results for prolonged fermentations of between 60 and 180 h have been reported where, under optimal conditions without oxygen, coffees with higher final sensory scores in the cup have been generated [49,65].

Additionally, a lower number of positive descriptors was required for FT1 and FT2, and a greater of positive attribute numbers such as cocoa liquor, cocoa, purple fruits (blueberries, raisins, plums), red fruits, and vinous notes in FT4 and FT5. Similarly, in natural and pulped coffees with fermentation times of up to 60 h, Pereira et al. [40] found that descriptors such as wine, sugar cane, and chocolate were present with greater dominance in coffees fermented without oxygen and prolonged fermentations.

4. Conclusions

This study investigates the dual effects of Timsen® as a disinfectant for coffee cherry and the influence of fermentation time on the physicochemical, microbiological and sensory attributes of coffee. As we announced, the coffee industry faces challenges with microbial contamination, which affects product safety and quality. Limited or no research has focused on effective disinfection methods for coffee cherries, highlighting the need for extensive research. Furthermore, understanding the optimal fermentation time to improve coffee quality remains an essential area of exploration. Our key findings included:

- In a first trial that sanitization with Timsen® did not significantly influence the evolution of pH during fermentation ($p > 0.05$), but it can reduce to a small extent the action of LAB at the end of the process. The thermal balance of the coffee mass with the ambient temperature was observed, noting significant effects ($p < 0.05$) of the sanitization of the cherries (ST2 and ST3) on the stability of this variable during fermentation. The use of Timsen® affected microbial populations and this behavior correlates with the improvement of sensory profiles in the cup.
- In the second test we found that for fermentations of more than 24 h, such as: (FT3, FT4 and FT5), the pH of the coffee mass tended to stabilize after 36 h, regardless of the process time, attributable to the predominance of yeasts due to the acid saturation of the medium, typical of the natural fermentation of coffee.
- Neither the sanitization process nor the fermentation time significantly affected the caffeine and chlorogenic acid contents of the coffee, both in its green and roasted states because these components are the own of the coffee bean and generated during photosynthesis.
- Microbiologically, LAB and mesophilic populations predominated over yeasts in both assays. A correlation was found between higher microbial loads, better final sensory scores, and balanced coffee flavors from prolonged fermentations.

These results underline the importance of disinfecting the coffee cherry with Timsen® and optimizing the fermentation time for microbial control and improvement of sensory quality. The study provides valuable information on the safety and final quality of coffee products, emphasizing the importance of effective microbial management practices in coffee processing.

Ethics declarations

This article does not contain animal studies. This article included the participation of humans as trained tasters, who participated voluntarily and were knowledgeable about the entire experiment. These experiments were conducted according to established ethical guidelines, and informed written consent obtained from the participants. The sensory analysis of coffee samples by trained cuppers was developed following the Specialty Coffee Association (SCA) Cupping Protocol. This methodology is appropriate for the analysis of specialty coffees and also protects the rights and privacy of all the panelists.

Data availability statement

The data associated with the present study has not been deposited into a publicly available repository. The data associated with the present study is included/referenced in the article and supplementary material. Additional data will be available upon request.

CRediT authorship contribution statement

Andrea Milena Sánchez-Riaño: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Carolina Vega-Oliveros:** Writing – review & editing, Visualization, Supervision, Methodology. **Wilmer Licerio Ladino-Garzón:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Dayana Alejandra Orozco-Blanco:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Andrés Felipe Bahamón-Monje:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Nelson Gutiérrez-Guzmán:** Visualization, Supervision, Project administration. **Claudia Milena Amorocho-Cruz:** Writing – review & editing, Supervision, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank the Universidad Surcolombiana and the Colombian National Planning Department through the General System of Royalties of the Science, Technology and Innovation Fund, for facilitating and financing the scientific and administrative execution of the research project, BPIN code 2020000100460. They would also like to thank the coffee growers who provided the plant material for the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33508.

References

[1] International Coffee Organization - ICO, Coffee Market Report -December (2023). https://www.icocoffee.org/documents/cy2023-24/cmr-1223-e.pdf.
[2] E. Ciravegna, V. Ingram, M.A. Naranjo, S. Cocchini, N. Herrera, T. Weitkamp, Coffee sector in Colombia - statistics and overview of the value chain – November. https://edepot.wur.nl/651860, 2023.
[3] Federación Nacional de Cafeteros de Colombia - FNCC, Informe de Gestión 2021, 2021. https://federaciondecafeteros.org/app/uploads/2022/05/IG-2021-FNC-Web.pdf.
[4] A. Iriondo-DeHond, N. Aparicio García, B. Fernandez-Gomez, E. Guisantes-Batan, F. Velázquez Escobar, G.P. Blanch, M.I. San Andres, S. Sanchez-Fortun, M. D. del Castillo, Validation of coffee by-products as novel food ingredients, Innovat. Food Sci. Emerg. Technol. 51 (2019) 194–204, https://doi.org/10.1016/j.ifset.2018.06.010.
[5] S. Das, Post-harvest processing of coffee: an overview, Coffee Science 16 (2021) e161976, https://doi.org/10.25186/.v16i.1976.
[6] F. De Bruyn, S.J. Zhang, V. Pothakos, J. Torres, C. Lambot, A.V. Moroni, M. Callanan, W. Sybesma, S. Weckx, L. De Vuyst, Exploring the impacts of postharvest processing on the microbiota and metabolite profiles during green coffee bean production, Appl. Environ. Microbiol. 83 (2017) e02398, https://doi.org/10.1128/AEM.02398-16, 16.
[7] M.C.S. da Silva, J.M.R. da Luz, T.G.R. Veloso, W. dos S. Gomes, E.C. da S. Oliveira, L.M. Anastácio, A. Cunha Neto, A.P. Moreli, R.C. Guarçoni, M.C.M. Kasuya, L. L. Pereira, Processing techniques and microbial fermentation on microbial profile and chemical and sensory quality of the coffee beverage, Eur. Food Res. Technol. (2022), https://doi.org/10.1007/s00217-022-03980-6.
[8] A.P.P. Bressani, S.J. Martinez, A.B.I. Sarmento, F.M. Borém, R.F. Schwan, Organic acids produced during fermentation and sensory perception in specialty coffee using yeast starter culture, Food Res. Int. 128 (2020) 108773, https://doi.org/10.1016/j.foodres.2019.108773.
[9] L.J. Carvalho-Ferreira, M. de Souza Gomes, L. Maciel de Oliveira, L. Diniz Santos, Coffee fermentation process: a review, Food Res. Int. 169 (2023) 112793, https://doi.org/10.1016/j.foodres.2023.112793.
[10] S.J. Zhang, F. De Bruyn, V. Pothakos, J. Torres, C. Falconi, C. Moccand, S. Weckx, L. De Vuyst, Following coffee production from cherries to cup: microbiological and metabolomic analysis of wet processing of Coffea arabica, Appl. Environ. Microbiol. 85 (2019) 1–22, https://doi.org/10.1128/AEM.02635-18.
[11] B. Girma, A. Sualeh, A review of coffee processing methods and their influence on aroma, International Journal of Food Engineering and Technology 6 (2022) 7, https://doi.org/10.11648/j.ijfet.20220601.12.
[12] T.G.R. Veloso, M. de C.S. da Silva, W.S. Cardoso, R.C. Guarçoni, M.C.M. Kasuya, L.L. Pereira, Effects of environmental factors on microbiota of fruits and soil of Coffea arabica in Brazil, Sci. Rep. 10 (2020) 14692, https://doi.org/10.1038/s41598-020-71309-y.
[13] M. Haile, W.H. Kang, The role of microbes in coffee fermentation and their impact on coffee quality, J. Food Qual. (2019) 1–6, https://doi.org/10.1155/2019/4836709.
[14] R. Mahingsapun, P. Tantayotai, S. Panyachanakul, Titiporn Samosorn, W. Dolsophon, Kulvadee Jiamjariyatam, Rossaporn Lorliam, N. Srisuk, S. Krajangsang, Enhancement of arabica coffee quality with selected potential microbial seed culture under controlled fermentation in wet process, Food Biosci. 48 (2022) 101819, https://doi.org/10.1016/j.fbio.2022.101819.
[15] S.J. Martinez, A.P.P. Bressani, D.R. Dias, J.B.P. Simão, R.F. Schwan, Effect of bacterial and yeast starters on the formation of volatile and organic acid compounds in coffee beans and selection of flavors markers precursors during wet fermentation, Front. Microbiol. 10 (2019) 1–13, https://doi.org/10.3389/fmicb.2019.01287.
[16] G.V. de M. Pereira, D.P. de Carvalho Neto, A.B.P. Medeiros, V.T. Soccol, E. Neto, A.L. Woiciechowski, C.R. Soccol, Potential of lactic acid bacteria to improve the fermentation and quality of coffee during on-farm processing, Int. J. Food Sci. Technol. 51 (2016) 1689–1695, https://doi.org/10.1111/ijfs.13142.
[17] V.B. Pregolini, G.V. de Melo Pereira, A. da Silva Vale, D.P. de Carvalho Neto, C.R. Soccol, Influence of environmental microbiota on the activity and metabolism of starter cultures used in coffee beans fermentation, Fermentation 7 (2021) 278, https://doi.org/10.3390/fermentation7040278.
[18] A.S. Shaker, M.A. Ali, H.M. Fathy, D.A. Marrez, Food Preservation: comprehensive overview of techniques, applications and hazards, Egypt. J. Chem. 65 (2022) 345–353, https://doi.org/10.21608/EJCHEM.2022.110711.5043.
[19] E. Stavropoulou, E. Bezirtzoglou, Predictive modeling of microbial behavior in food, Foods 8 (2019) 654, https://doi.org/10.3390/foods8120654.

[20] C. Chaidez, J. López, N. Castro-del Campo, Quaternary ammonium compounds: an alternative disinfection method for fresh produce wash water, J. Water Health 5 (2007) 329–333, https://doi.org/10.2166/wh.2007.009.

[21] A.V. Chinchkar, A. Singh, S.V. Singh, A.M. Acharya, M.G. Kamble, Potential sanitizers and disinfectants for fresh fruits and vegetables: a comprehensive review, J. Food Process. Preserv. 46 (2022) e16495, https://doi.org/10.1111/jfpp.16495.

[22] V. Lansini, D.S.V. Maia, D. da Fontoura Prates, A.S. de Lima, W.P. da Silva, Antibacterial activity of Timsen® (n-alkyl dimethyl benzyl ammonium chloride-40%) in scalding and precooling water in poultry slaughterhouses, J. Food Sci. Technol. 54 (2017) 2607–2612, https://doi.org/10.1007/s13197-017-2660-2.

[23] M. Banti, T. Atlaw, Physicochemical and cup quality of coffee as affected by processing methods and growing locations, CyTA - J. Food 22 (2024) 2329760, https://doi.org/10.1080/19476337.2024.2329760.

[24] V.V. Freitas, L.L. Rodrigues, V. Borges, Márcia Cristina Teixeira Ribeiro, M.H. Dos Santos, P.C. Stringheta, Coffee: a comprehensive overview of origin, market, and the quality process, Trends Food Sci. Technol. 146 (2024) 104411, https://doi.org/10.1016/j.tifs.2024.104411.

[25] A. da Silva Vale, G. Balla, L.R.S. Rodrigues, D.P. de Carvalho Neto, C.R. Soccol, G.V. de Melo Pereira, Understanding the effects of self-induced anaerobic fermentation on coffee beans quality: microbiological, metabolic, and sensory studies, Foods 12 (2023) 37, https://doi.org/10.3390/foods12010037.

[26] M.C.B. da Mota, N.N. Batista, D.R. Dias, R.F. Schwan, Impact of microbial self-induced anaerobiosis fermentation (SIAF) on coffee quality, Food Biosci. 47 (2022) 101640, https://doi.org/10.1016/j.fbio.2022.101640.

[27] H. Elhalis, J. Cox, J. Zhao, Yeasts are essential for mucilage degradation of coffee beans during wet fermentation, Yeast 40 (2023) 425–436, https://doi.org/10.1002/yea.3888.

[28] R.A.R. Rocha, M.A.D.d. Cruz, L.C.F. Silva, G.X.R. Costa, L.R. Amaral, P.L.L. Bertarini, M.S. Gomes, L.D. Santos, Evaluation of arabica coffee fermentation using machine learning, Foods 13 (2024) 454, https://doi.org/10.3390/foods13030454.

[29] A.E. Peñuela-Martínez, S. Moreno-Riascos, R. Medina-Rivera, Influence of temperature-controlled fermentation on the quality of mild coffee (Coffea arabica L.) cultivated at different elevations, Agriculture (Switzerland) 13 (2023) 1132, https://doi.org/10.3390/agriculture13061132.

[30] B.Z. Agnoletti, W. dos S. Gomes, G.F. de Oliveira, P.H. da Cunha, M.H.C. Nascimento, Á.C. Neto, L.L. Pereira, E.V.R. de Castro, E.C. da S. Oliveira, P. R. Filgueiras, Effect of fermentation on the quality of conilon coffee (Coffea canephora): chemical and sensory aspects, Microchem. J. 182 (2022) 107966, https://doi.org/10.1016/j.microc.2022.107966.

[31] P. Ghosh, N. Venkatachalapathy, Processing and Drying of Coffee, Drying and Roasting of Cocoa and Coffee - A Review, vol. 3, 2014, pp. 784–794, https://doi.org/10.1201/9781315113104-6.

[32] I.C. de, N.T. ICONTEC, NTC 2325. Café verde, Determinación de la pérdida de masa a 105 °C (2005). Colombia.

[33] W.L. Ladino-garzon, Y.F. Barrios-rodríguez, C.M. Amorocho-cruz, Revista Brasileira de Engenharia Agrícola e Ambiental Inoculation of Saccharomyces cerevisiae with sugar cane juice, Brazilian Journal of Agricultural and Environmental Engineering 28 (2024) e272094, https://doi.org/10.1590/1807-1929/agriambi.v28n1e272094.

[34] International Organization for Standardization, ISO 20281:2006 "Coffee and Coffee Products — Determination of the Caffeine Content Using High Performance Liquid Chromatography (HPLC) — Reference Method iTeh,", 2015.

[35] L.S. Ribeiro, M.G. da Cruz Pedrozo Miguel, S.J. Martinez, A.P.P. Bressani, S.R. Evangelista, C.F. Silva e Batista, R.F. Schwan, The use of mesophilic and lactic acid bacteria strains as starter cultures for improvement of coffee beans wet fermentation, World J. Microbiol. Biotechnol. 36 (2020) 186, https://doi.org/10.1007/s11274-020-02963-7.

[36] I.C. de, N.T. ICONTEC, Café verde, examen olfativo y visual y determinación de materia extraña y defectos, 2002. Colombia.

[37] M.R. Fernández, P. Giulano, Coffee Sensory and Cupping Handbook, first ed., Specialty Coffee Association - SCA, New Orleans, 2021.

[38] S.C. Jackels, C.F. Jackels, Characterization of the coffee mucilage fermentation process using chemical indicators: a field study in Nicaragua, J. Food Sci. 70 (2005) 321–325, https://doi.org/10.1111/j.1365-2621.2005.tb09960.x.

[39] H. Elhalis, J. Cox, J. Zhao, Coffee fermentation: expedition from traditional to controlled process and perspectives for industrialization, Applied Food Research 3 (2023) 100253, https://doi.org/10.1016/j.afres.2022.100253.

[40] T.S. Pereira, N.N. Batista, L.P. Santos Pimenta, S.J. Martinez, L.S. Ribeiro, J.A. Oliveira Naves, R.F. Schwan, Self-induced anaerobiosis coffee fermentation: impact on microbial communities, chemical composition and sensory quality of coffee, Food Microbiol. 103 (2022) 103962, https://doi.org/10.1016/j.fm.2021.103962.

[41] P. Gilbert, A.J. McBain, Potential impact of increased use of biocides in consumer products on prevalence of antibiotic resistance, Clin. Microbiol. Rev. 16 (2003) 189–208, https://doi.org/10.1128/CMR.16.2.189-208.2003.

[42] A.C. de oliveira Junqueira, G.V. de M. Pereira, J.D.C. Medina, M.C.R. Alvear, R. Rosero, D.p. de C. Neto, H.G. Enríquez, C.R. Soccol, First description of bacterial and fungal communities in Colombian coffee beans fermentation analysed using Illumina-based amplicon sequencing, Sci. Rep. 9 (2019) 8794, https://doi.org/10.1038/s41598-019-45002-8.

[43] A.E. Peñuela-Martínez, C.E. Oliveros-Tascón, J.R. Sanz-Uribe, Remoción del mucílago de café a través de fermentación natural, Cenicafé, 159–173, https://biblioteca.cenicafe.org/bitstream/10778/494/1/arc061%2802%29159-173.pdf, 2010.

[44] V. Kumar, V. Ahluwalia, S. Saran, J. Kumar, A.K. Patel, R.R. Singhania, Recent developments on solid-state fermentation for production of microbial secondary metabolites: challenges and solutions, Bioresour. Technol. 323 (2021) 124566, https://doi.org/10.1016/j.biortech.2020.124566.

[45] V. Osorio-Pérez, C.I. Alvarez-Barreto, L.G. Matallana, J.R. Acuña, L.F. Echeverri, L.C. Imabachí, Effect of prolonged fermentations of coffee mucilage with different stages of maturity on the quality and chemical composition of the bean, Fermentation 8 (2022) 1–16, https://doi.org/10.3390/fermentation810051.

[46] S.J. Zhang, F. De Bruyn, V. Pothakos, G.F. Contreras, Z. Cai, C. Moccand, S. Weckx, L. De Vuyst, Influence of various processing parameters on the microbial community dynamics, metabolomic profiles, and cup quality during wet coffee processing, Front. Microbiol. 10 (2019) 2621, https://doi.org/10.3389/fmicb.2019.02621.

[47] A. Endo, L.M.T. Dicks, Physiology of the LAB, in: W.H. Holzapfel, B.J.B. Wood (Eds.), Lactic Acid Bacteria: Biodiversity and Taxonomy, Wiley-Blackwell, New Jersey, 2014, pp. 13–30.

[48] D.P. de Carvalho Neto, G.V. de Melo Pereira, A.M.O. Finco, L.A.J. Letti, B.J.G. da Silva, L.P.S. Vandenberghe, C.R. Soccol, Efficient coffee beans mucilage layer removal using lactic acid fermentation in a stirred-tank bioreactor: kinetic, metabolic and sensorial studies, Food Biosci. 26 (2018) 80–87, https://doi.org/10.1016/j.fbio.2018.10.005.

[49] M.C.B. da Mota, N.N. Batista, M.H.S. Rabelo, D.E. Ribeiro, F.M. Borém, R.F. Schwan, Influence of fermentation conditions on the sensorial quality of coffee inoculated with yeast, Food Res. Int. 136 (2020) 109482, https://doi.org/10.1016/j.foodres.2020.109482.

[50] B. Girma, A. Gure, F. Wedajo, Influence of altitude on caffeine, 5-caffeoylquinic acid, and nicotinic acid contents of arabica coffee varieties, J. Chem. 2020 (2020) 1–7, https://doi.org/10.1155/2020/3904761.

[51] S. De Luca, E. Ciotoli, A. Biancolillo, R. Bucci, A.D. Magrì, F. Marini, Simultaneous quantification of caffeine and chlorogenic acid in coffee green beans and varietal classification of the samples by HPLC-DAD coupled with chemometrics, Environ. Sci. Pollut. Control Ser. 25 (2018) 28748–28759, https://doi.org/10.1007/s11356-018-1379-6.

[52] F. Kulapichitr, C. Borompichaichartkul, M. Fang, I. Suppavorasatit, K.R. Cadwallader, Effect of post-harvest drying process on chlorogenic acids, antioxidant activities and CIE-Lab color of Thai Arabica green coffee beans, Food Chem. 366 (2022) 130504, https://doi.org/10.1016/j.foodchem.2021.130504.

[53] C.L.O. Corrêa, E. das Merces Penha, M.R. dos Anjos, S. Pacheco, O. Freitas-Silva, A.S. Luna, L.M.F. Gottschalk, Use of asparaginase for acrylamide mitigation in coffee and its influence on the content of caffeine, chlorogenic acid, and caffeic acid, Food Chem. 338 (2021) 128045, https://doi.org/10.1016/j.foodchem.2020.128045.

[54] M. Misto, N.P. Lestari, E. Purwandari, Chlorogenic acid content of local Robusta coffee at variations of roasting temperature, Jurnal Pendidikan Fisika Indonesia 18 (2022) 25–32, https://doi.org/10.15294/jpfi.v18i1.27889.

[55] G.A. Figueroa Campos, J.G.K.T. Kruizenga, S.T. Sagu, S. Schwarz, T. Homann, A. Taubert, H.M. Rawel, Effect of the post-harvest processing on protein modification in green coffee beans by phenolic compounds, Foods 11 (2022) 159, https://doi.org/10.3390/foods11020159.

[56] Y. Yulianti, D.R. Adawiyah, D. Herawati, D. Indrasti, N. Andarwulan, Detection of markers in green beans and roasted beans of kalosi-enrekang arabica coffee with different postharvest processing using LC-MS/MS, International Journal of Food Science (2023) 1–12, https://doi.org/10.1155/2023/6696808.
[57] C.S. da Silva, A.P. de F. Coelho, C.F. Lisboa, G. Vieira, M.C. de A. Teles, Post-harvest of coffee: factors that influence the final quality of the beverage, Revista Engenharia Na Agricultura - REVENG 30 (2022) 49–62, https://doi.org/10.13083/reveng.v30i1.12639.
[58] S.R. Evangelista, M. Miguel, C.F. Silva, A.C.M. Pinheiro, R.F. Schwan, Microbiological diversity associated with the spontaneous wet method of coffee fermentation, Int. J. Food Microbiol. 210 (2015) 102–112, https://doi.org/10.1016/j.ijfoodmicro.2015.06.008.
[59] H. Elhalis, J. Cox, J. Zhao, Ecological diversity, evolution and metabolism of microbial communities in the wet fermentation of Australian coffee beans, Int. J. Food Microbiol. 321 (2020) 108544, https://doi.org/10.1016/j.ijfoodmicro.2020.108544.
[60] H. Elhalis, J. Cox, D. Frank, J. Zhao, The crucial role of yeasts in the wet fermentation of coffee beans and quality, Int. J. Food Microbiol. 333 (2020) 108796, https://doi.org/10.1016/j.ijfoodmicro.2020.108796.
[61] E.C. Udeagha, C.N. Ishiwu, C.U. Obiora, J.O. Iwouno, Effects of yeast concentration and total soluble solids on the quality of wine produced from pineapple, Current Journal of Applied Science and Technology 39 (2020) 28–42, https://doi.org/10.9734/cjast/2020/v39i3030968.
[62] G.I. Puerta-Quintero, Factores, procesos Y controles en La fermentación del café, avances técnicos cenicafé, 1–12, https://www.cenicafe.org/es/publications/avt0461.pdf, 2012.
[63] P. Tripetch, C. Borompichaichartkul, Effect of packaging materials and storage time on changes of colour, phenolic content, chlorogenic acid and antioxidant activity in arabica green coffee beans (Coffea arabica L. cv. Catimor), J. Stored Prod. Res. 84 (2019) 101510, https://doi.org/10.1016/j.jspr.2019.101510.
[64] E.T. Cortés-Macías, C.F. López, P. Gentile, J. Girón-Hernández, A.F. López, Impact of post-harvest treatments on physicochemical and sensory characteristics of coffee beans in Huila, Colombia, Postharvest Biol. Technol. 187 (2022) 111852, https://doi.org/10.1016/j.postharvbio.2022.111852.
[65] E.J. Martinez-Jimenez, P.M. Machado-Martins, A.L. de Oliveira-Vilela, N. Nara-Batista, S.D. Veiga- Franco da Rosa, D. Ribeiro-Dias, R. Freitas-Schwan, Influence of anaerobic fermentation and yeast inoculation on the viability, chemical composition, and quality of coffee, Food Biosci. 51 (2023) 102218, https://doi.org/10.1016/j.fbio.2022.102218.