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RESEARCH

Coffee Ozonization in a Microbubble System: Ozone Reaction Kinetics, Inactivation of Mesophilic Bacteria and Fungi, and Grain Quality

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Abstract

The objective of this study was to characterize the reaction kinetics of ozone in water in a microbubble system and to determine the potential of ozonated water in the inactivation of mesophilic bacteria and fungi and preservation of coffee sensory quality (*Coffea arabica* L.). For coffee treatment, 10 kg samples were immersed in water with microbubbles without ozone and microbubbles with ozone for periods of 40, 60, and 90 min for pulped natural coffee and immersion periods of 60, 120, and 180 min for natural coffee. The treatment for pulped natural coffee with immersion in microbubbles with ozone for 40 min was responsible for reducing contamination by filamentous fungi and yeasts by 1.13 log CFU g^{-1}. In natural coffee, the treatment with immersion in microbubbles with ozone for 60 min led to a reduction of 2.98 log CFU g^{-1} in contamination by filamentous fungi and yeasts. The results of the sensory analysis for coffee did not show statistically significant differences ($P < 0.05$), with an average score of 80.29. The immersion water of pulped natural coffee in microbubbles with ozone for 90 min showed values of 46.63 ± 1.62 and 93.43 ± 3.91 mg L^{-1} for BOD (biochemical oxygen demand) and COD (chemical oxygen demand), respectively, complying with the current environmental legislation. It is concluded that the application of ozonated water as a pretreatment in coffee drying is a technology that allows microbiological decontamination and maintains the quality of specialty coffees.

Keywords *Coffea arabica* L. · Post-harvest · Ozone · Microbubble system · Sensory quality

Introduction

Coffee is one of the most consumed beverages worldwide due to its flavor, nutritional value, and unique functionalities. Brazil stands out on the international scene in coffee production and export. In 2024, coffee production in Brazil was 54.79 million bags (CONAB, 2024). Variations in coffee yield from year to year and fluctuations in market value make coffee production profitability vulnerable since coffee is priced according to the quality, type, and beverage that the product presents. One way to make this production more sustainable is to invest in technologies that add value to the product, such as the production of specialty coffees. Defined by scores of 80 points or higher in the cup test, these coffees have shown growing market demand and command premium prices (Borém et al., 2019a).

Specialty coffee production has become a relevant activity for producers. In addition, it has mobilized researchers to conduct scientific investigations with the aim of proposing improvements to maintain product quality in the stages involving production and post-harvest (Brazil Specialty Coffee Association, 2025). Factors such as climatic conditions of the cultivation site, altitude, cultural treatments, harvesting and post-harvest operations, processing, storage conditions, and type of packaging used influence coffee quality (Borém et al., 2008, 2019b; Livramento et al., 2017; Worku et al., 2023).

Coffee quality is also influenced by the microbial population present in the fruits and the processing conditions that may favor the development of one species or another (Nakayama et al., 2020). The presence of some groups of

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microorganisms, mainly filamentous fungi, may be associated with reduced coffee quality (Silva et al., 2000; Haile and Kang, 2019). The fruit and coffee beans may present a great diversity of microorganisms (Bruyn et al., 2017; Ferreira et al., 2011), among bacteria, yeasts, and filamentous fungi. Contamination by microorganisms occurs naturally during fruit development, during coffee harvest, and during post-harvest. Immediately after harvesting and during drying, molds, as filamentous fungi are popularly called, have been reported as one of the main groups of microorganisms that make up the microbiota present in fruits (Nakayama et al., 2020).

The development of microorganisms generates, in addition to post-harvest losses, risks to human health, especially due to the development of filamentous fungi that can produce mycotoxins, more specifically, ochratoxin A (Batista & Chalfoun, 2007; Oueslati et al., 2022). Some groups of microorganisms, such as bacteria and yeasts, can cause other effects such as the production of enzymes that act in the degradation of mucilage and coffee pulp (Haile & Kang, 2019). Due to the problems related to contamination by microorganisms, scientific investigations have been conducted with the aim of developing technologies to inactivate these microorganisms. Among the technologies under development, it is possible to mention the use of ultraviolet radiation (UV-C) (Byun et al., 2020), use of cold plasma (Casas-Junco et al., 2019), chlorine dioxide (Lee et al., 2020), and ozone gas (Akbar et al., 2020).

Ozone is a gas resulting from the rearrangement of oxygen atoms and can be artificially generated by electrical discharges or by the incidence of high-energy electromagnetic radiation (ultraviolet light) in the air (Hafeez et al., 2021; Khadre et al., 2001). It is a highly reactive oxidizing agent with great disinfection and sterilization capacity (Pandiselvam et al., 2019). Due to this characteristic, the application of ozone has been efficient in controlling and inactivating microorganisms in grains (Uzoma et al., 2023), fruits, vegetables, and greens (Botondi et al., 2021). Ozone used in food preservation and environmental sanitation can be applied in gaseous form (Akbar et al., 2020), dissolved in water (Premjit et al., 2022), or even through ozonated mist (Cabral et al., 2023).

Ozone gas can be incorporated into water using porous stones (Baquero-Rodrigues et al., 2018), Venturi injectors (Zhou & Smith, 2000), or even microbubble generators (Hashimoto et al., 2021). Considering the applications involving the use of ozone dissolved in water, it is worth mentioning that the stability and solubility of ozone are influenced by factors such as temperature, pH, and the presence of organic matter (Gardoni et al., 2012). The disinfectant properties of ozone dissolved in water are associated with the formation of free radicals that damage the cell envelope of microorganisms due to induced oxidative stress (Aslam et al., 2020; Pandiselvam et al., 2022). The food industry has used ozone as an oxidizing agent in food processing and as a potential substitute for chlorine-based disinfectants, extending the shelf life of fruits, vegetables, grains, and their by-products (Pandiselvam et al., 2022). The process of food sanitization and water treatment with chlorine-based compounds has some disadvantages as a function of the formation of organochlorine compounds, trihalomethane, and haloacetic acid, which can be mutagenic, carcinogenic, and toxic (Ferreira et al., 2017).

Given the potential of ozone dissolved in water for microbiological control and disinfection of products, and considering that coffee processing uses water for several stages, it is relevant to investigate the application of ozonated water in the microbiological decontamination of coffee as a drying pretreatment. It is expected that by using ozonated water, it will be possible to achieve decontamination levels of coffee fruits sufficient for slow drying on a suspended drying rack without loss of quality due to the action of deteriorating microorganisms. This study aimed to evaluate the application of ozonated water generated by a microbubble system as a pre-drying treatment for natural and pulped natural coffee, focusing on ozone reaction kinetics, microbial inactivation, coffee quality, and water physicochemical characteristics.

Material and Methods

Experiment Location

Coffee was harvested and dried on Aparecida Farm, located in the state of Minas Gerais, Brazil. On this farm, the coffee was processed, immersed in ozonated water, and dried. The area where the coffee was harvested had an altitude of 920 m and the following geographic coordinates (Fig. 1): latitude: 20° 47′ 17″ S and longitude: 42° 11′ 47″ W. Microbiological and physical analyses were performed at the Post-Harvest Laboratory of the Technical Area for Storage and Processing of Agricultural Products of the Department of Agricultural Engineering of Universidade Federal de Viçosa (UFV). Sensory quality was analyzed at Instituto Federal do Espírito Santo (IFES), Venda Nova do Imigrante campus of Espírito Santo. The analyses of BOD, COD, and turbidity related to the water used in immersion were performed at the ANALAG laboratory, Viçosa (MG).

Ozone Reaction Kinetics in Water

Figure 2 shows the layout of the equipment used to generate ozonated water and immerse coffee in water with microbubbles without ozone and microbubbles with ozone (pilot-scale). The reaction kinetics of ozone in water was characterized by determining the saturation curve and the

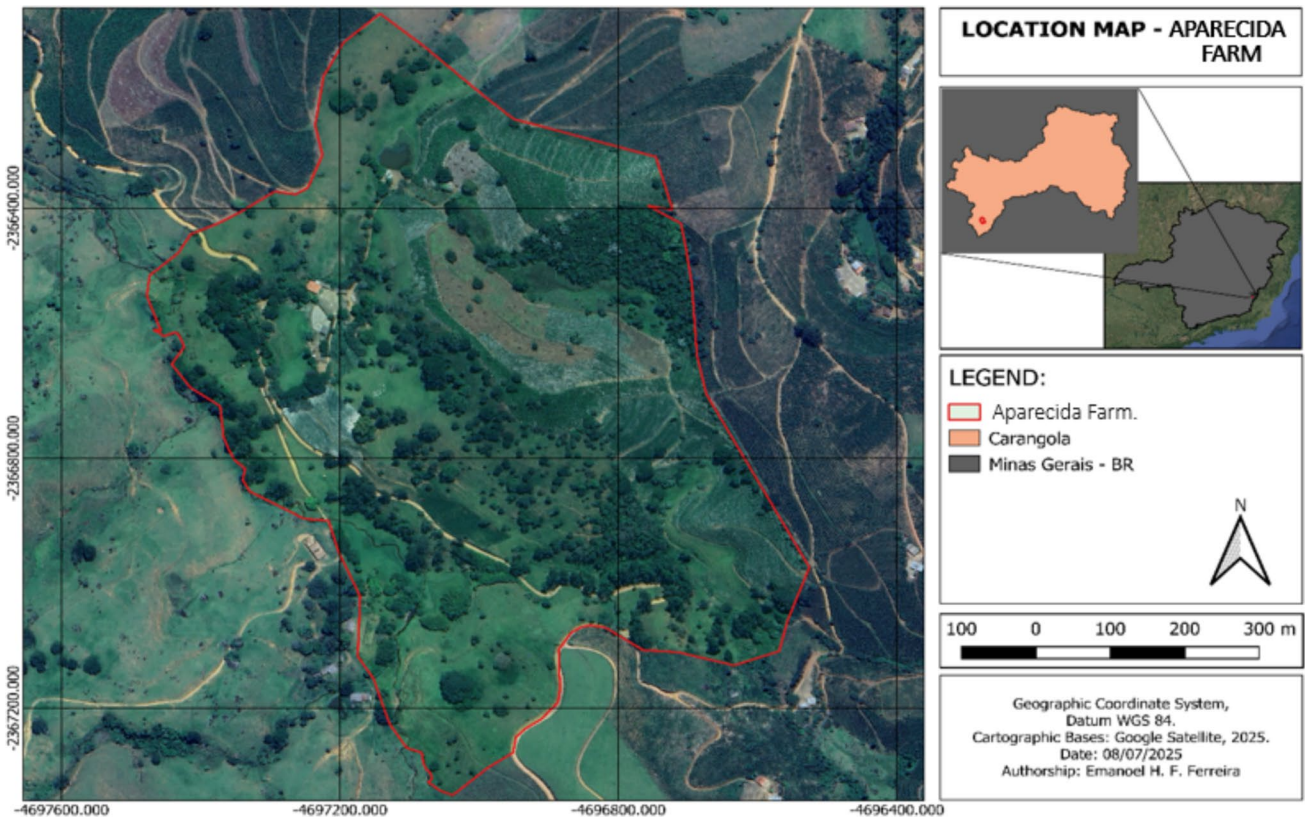

Fig. 1 Satellite image of Aparecida Farm in Carangola (MG)

decay curve of the ozone concentration in water. Saturation and decomposition kinetics curves were determined for input concentrations equal to 15.77, 25.18, and 37.20 mg L^{-1}. To determine the saturation curve, the concentration was monitored by the iodometric method in the incorporation tank (Fig. 2a) (Rakness et al., 1996). Concentration readings were taken every 3 min until it remained constant, indicating that the water had reached the saturation state. After saturation, the ozone injection into the microbubble generator was stopped, and the concentration decay of ozone gas dissolved in water in the immersion tank was monitored until concentrations below 0.1 mg L^{-1} were reached. Fifty milliliter water samples were used for concentration readings.

The assays related to the saturation curve and decay curve of the ozone concentration over time were performed in duplicate. Equation 1 was adjusted to model the water saturation kinetics in the incorporation tank. The decomposition kinetics was modeled using the exponential decay model (Eq. 2). The half-life of the ozone gas (Eq. 3) in the water contained in the immersion tank was obtained from the decomposition rate constant (k) of the first-order reaction kinetics model (Eq. 4) (Wright, 2005).

$$\widehat{C} = y_0 + a \bullet exp\left[\frac{-0.5 \bullet \left(\frac{ln(t/b)}{c}\right)^2}{t}\right] \tag{1}$$

$$C = ae^{-bt} \tag{2}$$

$$ln(C) = ln(C_0) - kt \tag{3}$$

$$t_{1/2} = ln\frac{k}{2} \tag{4}$$

where

C = Concentration of ozone gas dissolved in water (mg L^{-1});
C_0 = Initial concentration of ozone gas dissolved in water (mg L^{-1});
t = Time (min);
a, b, c = Model constants;
k = Decomposition rate constant (min^{-1}).

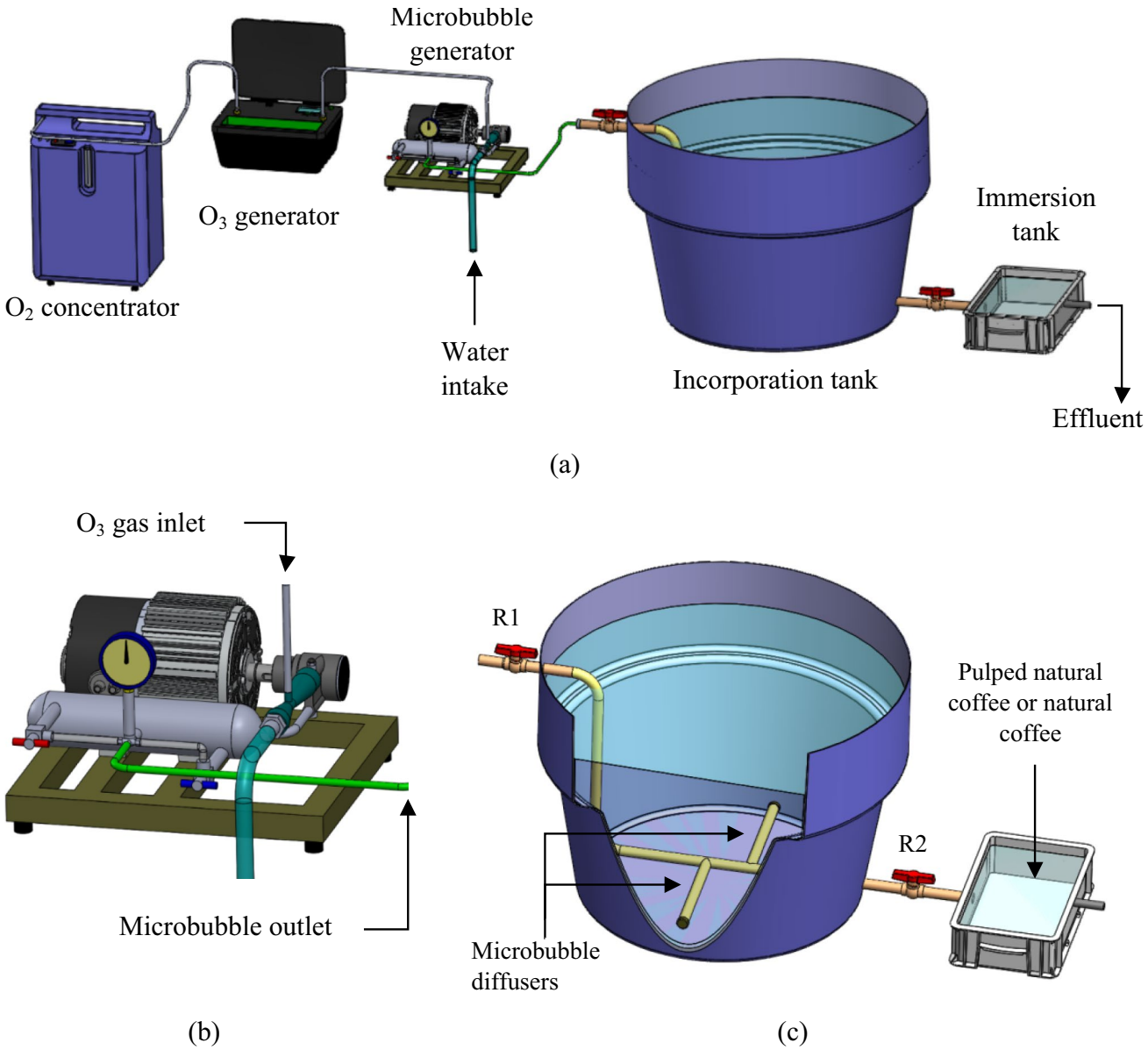

Fig. 2 **a** Isometric view showing the arrangement of the equipment used for coffee immersion in water with microbubbles without ozone and microbubbles with ozone, **b** microbubble generator, and **c** cross-sectional view of the incorporation tank with details of the diffusers used to distribute the microbubbles

Coffee Harvesting and Processing

"Catuaí vermelho" Arabica coffee (*Coffea arabica* L.) was harvested manually in the mountainous region of Matas de Minas, in a plot with an altitude of approximately 920 m, on two different dates: natural coffee, on 06/13/2024, and pulped natural coffee, on 07/16/2024. The coffee harvesting was carried out on tarps, and transportation to the processing site was performed by an agricultural tractor on the same day as the harvest. At the processing unit, the natural coffee was subjected to reception and density separation steps. Meanwhile, the pulped cherry coffee underwent reception, density separation, and pulping steps, as described below.

The harvested coffee beans were placed in a fruit receiving pit and then transported by a bucket elevator model EVU-07 (Pinhalense, Espírito Santo do Pinhal, São Paulo, Brazil) to the mechanical separator for coarse impurities (sticks, stones, and leaves) model ABC-10 (Pinhalense, Espírito Santo do Pinhal, São Paulo, Brazil). Subsequently, natural coffee underwent hydraulic separation by a mechanical washer model LSC-05 (Pinhalense, Espírito Santo do Pinhal, São Paulo, Brazil). The density separation process divided the coffee berries into

two batches: one overripe fruit (which was not used in the experiment) and the other of ripe and green natural coffee. The ripe natural coffee was manually selected to standardize the samples. The pulped natural coffee underwent the same process, with separation of coarse impurities and separation by density. The ripe and green natural coffee, separated in the washing process, were sent to the ECONOFLEX-6 mechanical pulper (Pinhalense, Espírito Santo do Pinhal, São Paulo, Brazil), where the ripened fruits were pulped and separated from unripe fruits and husks. The methods used to process the coffee ensured sample homogenization for the next immersion stage in water with microbubbles without ozone and in water with microbubbles containing ozone. A detailed flow chart of coffee processing is shown in Fig. 3.

Coffee Immersion in Water with Microbubbles Without Ozone and Microbubbles with Ozone

The water used for the experiment came from spring water on the Aparecida Farm. The water was stored in a thousand-liter tank to ensure a regular water flow throughout the experiment. The water from the mine was subjected to laboratory analysis for characterization:

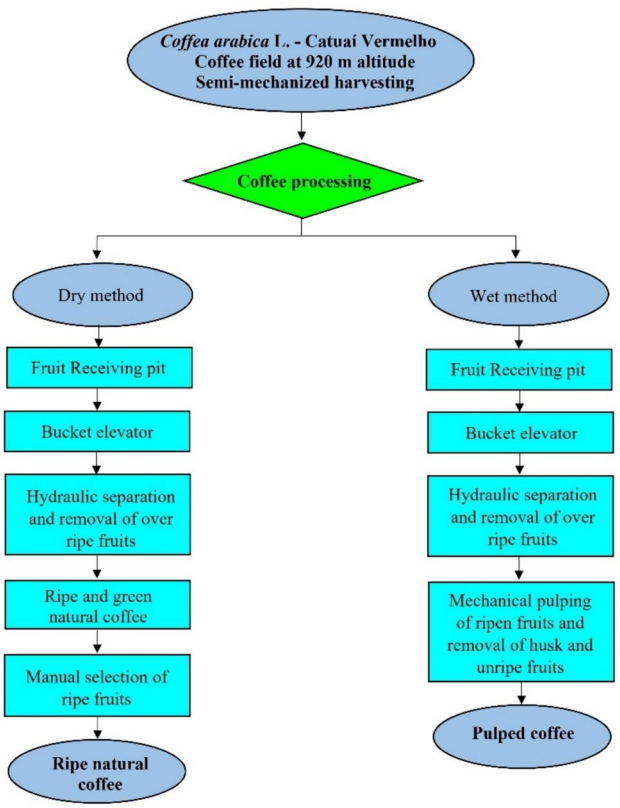

Fig. 3 Coffee processing flow chart following the dry and wet method

- Microbiological assays: Total coliforms 2.2×10^2 NMP/100 mL (lower limit: 1.5×10^2; upper limit: 3.4×10^2); *Escherichia coli* < 1.0 NMP/100 mL (less than one).
- Physical assays: Apparent color < 1.0 µH (less than one); total hardness 8.2 mg L^{-1}; pH 5.77; turbidity 1.12 UNT; total iron < 0.08 mg L^{-1} (less than 0.08).

An MB 600 microbubble generator (Fig. 2b) (myOZONE, Jaguariúna, São Paulo, Brazil) was used to generate microbubbles without ozone and microbubbles with in water. Ozone was obtained from an M10 ozone generator (Fig. 2a) (myOZONE, Jaguariúna, São Paulo, Brazil) supplied with oxygen from an EverFlo oxygen concentrator (Philips Respironics, Murrysville, Pennsylvania, USA). Ozone gas was injected into the microbubble generator at a volumetric flow rate of 1 L min^{-1} and a concentration of 37.20 mg L^{-1}. This incorporation was carried out in a 500 L incorporation tank (Fig. 2a). A diffuser (Fig. 2c) was inserted at the bottom of this tank and connected directly to the microbubble outlet of the microbubble generator. This diffuser was made of perforated PVC pipe to evenly distribute the microbubbles inside the incorporation tank. To generate microbubbles without ozone, the microbubble generator was fed directly from the oxygen concentrator. The treatments with immersion in water with microbubbles without ozone were carried out considering that previous studies reported that only the physical action of the microbubbles is sufficient to cause a reduction in microbiological contamination (Hou et al., 2022). The coffee was immersed in water with microbubbles without ozone and microbubbles with ozone in a polypropylene tank with dimensions ($0.15 \times 0.35 \times 0.75$ m) and 30 L capacity (Fig. 2c).

The microbubble generator pump was responsible for circulating the water at a volumetric flow rate of 0.43 m^3 h^{-1}. This volumetric flow rate corresponded to a residence time of the ozonated water inside the oxidation tank of 1.16 h and a residence time of 0.33 h in the immersion tank. Thus, the water used in coffee immersion had a renewal rate inside the immersion tank of three times per hour. In each immersion treatment in ozonated water, 10 kg of pulped natural coffee and 10 kg of natural coffee were used.

Immersion assays were conducted in water with microbubbles without ozone and in water with microbubbles containing ozone for periods of 40, 60, and 90 min for pulped natural coffee and for periods of 60, 120, and 180 min for natural coffee. These periods were set based on preliminary tests. Shorter immersion periods were adopted for pulped natural coffee in order to avoid negatively affecting the sensory quality of the beverage. To demonstrate the antimicrobial effect of the water with microbubbles, a control treatment was analyzed with both types of coffee undergoing only conventional processing (mechanical washing). All

effluent generated during coffee preprocessing and the process of immersing coffee in ozonated water was directed to a compacted lagoon, meeting the regulatory standards established by the National Environment Council – CONAMA and in accordance with the Certifica Minas – Café Program.

Coffee Drying

Immediately after immersing the coffee in water with microbubbles without ozone and microbubbles with ozone, the coffee samples were distributed on raised drying beds. This type of drying system was chosen since it is a widespread method for specialty coffees (Borém et al., 2019a). Drying on raised drying beds provides more uniform drying, lower risks of undesired fermentation, and a lower drying rate, which maintains coffee chemical compounds intact, thus ensuring its quality. The raised drying beds measured 0.90 m wide and 2.5 m long and were located 0.6 m above the ground. In this drying system, the coffee was in contact with a shade cloth, and the floor on which these raised drying beds were placed was concrete.

Drying for both natural and pulped natural coffees followed the same parameters. Initially, while the coffees had a high moisture content, they were spread in a thin layer, fruit by fruit. The coffee mass was continuously turned (every 30 min) until the end of drying, with the temperature of the coffee beans controlled by a mercury bulb thermometer. To ensure uniformity and continuous monitoring of the moisture content during drying, a G610i moisture content meter was used (GEHAKA, São Paulo, Brazil). After reaching the half-dry stage, which corresponded to a water content of 25% w.b. for pulped natural coffee and 30% w.b. for natural coffee, a new fold was made in the coffee mass, increasing the thickness of the layer. From this point on, the coffee was covered at 3:00 p.m. each day and uncovered at 9:00 a.m. the following day to take advantage of periods with the highest incidence of solar radiation. The drying period was 30 days for pulped natural coffee and 38 days for natural coffee. After drying, the samples were wrapped in two types of packaging: the first made of kraft paper and the second of more resistant plastic. They were then stored in a cold chamber at a temperature of 10 °C.

Microbiological Analysis of Pulped Natural Coffee and Natural Coffee

Immediately after treatment with or without ozonated water, 10 g of coffee was collected from each experimental plot and transferred to sterilized plastic bags for microbiological analyses to count filamentous fungi, yeasts, and mesophilic aerobes. In these plastic bags, a 10^{-1} dilution was prepared and used for subsequent dilutions. As a diluent, 90 mL of 0.9% autoclaved saline solution were added to the plastic bags, and the coffee beans were macerated to release the microorganisms by 2 min. Dilutions from 10^{-2} to 10^{-6} were considered for filamentous fungi and yeasts, and from 10^{-1} to 10^{-6} for mesophilic aerobes. For both filamentous fungi and yeasts, as well as for mesophilic aerobes, dilutions from 10^{-2} to 10^{-6} were considered.

Filamentous fungi and yeasts were plated using the Spread-Plate method, and aerobic mesophiles were plated using the Pour-Plate method. For filamentous fungi and yeasts, 0.1 mL (100 µl, µL) aliquots were removed from each dilution, pipetted, and spread with a Drigalski loop onto Petri dishes filled with solidified culture medium (Potato Dextrose Agar-PDA) acidified with 10% tartaric acid. For aerobic mesophiles, 1 mL aliquots were removed from each dilution and pipetted into empty Petri dishes, where liquid culture medium (Plate Count Agar-PCA) was added. The plates were left at room temperature until completely solidified.

The procedures were performed in a PCR FLV-1266/4 vertical laminar flow chamber (Filterflux, Piracicaba, São Paulo, Brazil). The plates were incubated in chamber (New Química, Belo Horizonte, Minas Gerais, Brazil) for 120 h (5 days) at 25 °C for filamentous fungi and yeasts and 48 h (2 days) at 30 °C for aerobic mesophiles. After completing the necessary incubation time, the colony-forming units (CFU) present on the plates were counted, considering the ranges of 15 to 150 colonies for filamentous fungi and yeasts and 25 to 250 colonies for aerobic mesophiles, with the final results expressed in log CFU g^{-1}. The criteria adopted in microbiological analyses follow the recommendations of the American Public Health Association-APHA, (2015), and normative instructions (ISO) numbers 6887–1/1999, 4833–1:2013, and 21,527–1/2:2008 (Salfinger & Tortorello, 2015).

Coffee Quality Analysis

Sensory Analysis

Sensory analyses were performed by an expert panelist trained by Coffee Design professionals at the Instituto Federal do Espírito Santo (IFES), Venda Nova do Imigrante campus of Espirito Santo, Brazil, where the coffee was lightly roasted. The temperature of the drying air during roasting was monitored to ensure that the roasting time did not exceed 12 min per sample.

The processing of ground coffee samples for sensory analysis began after 8–24 h, when the coffee was ground in a G3AHD grinder (Bunn, Springfield, Illinois, United States), with a particle size between 70 and 75% and passed through a 0.074-mm mesh sieve. The sensory analysis was performed and evaluated by six panelists certified as Q-Graders by the Specialty Coffee Association of America (SCAA), following the recommendations of Pereira et al. (2020). The sensory

analysis was approved by the institution's ethics review committee (IFES) under Certificate of Submission for Ethics Review No 31567320.2.0000.5072. The samples were prepared according to the SCAA recommendations. The coffee samples were placed in five cups, containing 8.25 g of coffee and 150 mL of mineral water at 93 °C.

The six panelists (certified Q-Graders) began their evaluations when the cups reached 55 °C (132 F) and the tasting time was 4 min after infusion. The sensory analysis variables presented according to the SCAA protocol were fragrance/aroma, flavor, aftertaste, acidity, body, balance, sweetness, clean cup, uniformity, overall, and a final score.

Five cups were provided for each sample during tasting. This set was randomly arranged on the tasting table and coded differently, preventing the panelists from knowing which treatment was applied to each sample. Furthermore, only the origin of the coffee was disclosed to the panelists, thus preventing any external factors from interfering with the final result. These analyses were used to characterize the samples and compare the different coffee treatments.

Water Content

Water content was determined by the oven method, at 105 °C, for 16 h, according to ISO 6673 (International Organization for Standardization – ISO, 2003), expressed on a wet basis (% w.b.). Three replicates were performed for each sample.

Color

The color of pulped natural coffee and natural coffee was analyzed immediately after the treatments and after drying on raised drying beds with 30 days of storage in a cold chamber at 10 °C and relative humidity of 80%. Three replicates were made per sample. Coffee color was evaluated by direct reflectance reading of the coordinates L^* (intensity from white to black), a^* (intensity from red to green), and b^* (intensity from yellow to blue) using a CR410 colorimeter (Konica Minolta, Osaka, Japan).

From the values of L^*, a^*, and b^*, the color saturation or chroma (Cr^*, Eq. 5), the color hue or hue angle (h^*, Eq. 6), and the total color difference (ΔE^*, Eq. 7) were determined. To calculate the total color difference, the L^*, a^*, and b^* coordinates of the coffee samples not exposed to ozone or oxygen, that is, the "control" samples of each type of coffee, were used as a reference.

$$Cr^* = \sqrt{a^{*2} + b^{*2}} \tag{5}$$

$$h^* = arctang\left(\frac{b^*}{a^*}\right) \tag{6}$$

$$\triangle E^* = \sqrt{\left(L^* - L_0^*\right)^2 + \left(a^* - a_0^*\right)^2 + \left(b^* - b_0^*\right)^2} \tag{7}$$

where

L_0^*, a_0^* and b_0^* = Coordinates obtained from coffee samples that were not immersed in water with microbubbles without ozone or in microbubbles with ozone.

Defect Quantification

The number of defects was determined 40 days after drying, when samples were being prepared for sensory analysis. Initially, 300 g of green coffee bean from each sample was weighed and divided into three 100 g portions. After this step, each defect in the samples was separated based on the Official Brazilian Classification (COB). Three replicates were made per sample. The following defects were quantified: black, green, burnt, pomegranate, broken, barley, and ladle.

Physicochemical Analysis of Water from Coffee Immersion

To characterize the quality of the water generated during coffee immersion, electrical conductivity, pH, and redox potential were analyzed, with three replicates each during the process. Water quality was determined only in treatments with the longest immersion period, which corresponded to 90 min for pulped natural coffee and 180 min for natural coffee. The choice of the longest immersion periods for conducting water physicochemical analyses was based on a prior visual analysis of the effluent generated. Electrical conductivity was determined using a MCAA150 conductivity meter (Tecnopon, São Paulo, Brazil). pH was determined with a Q-400A digital pH meter (QUIMIS, Diadema, São Paulo, Brazil). Redox potential was determined using an OPR/Redox Tester with a sensitivity range of 0 to 1999 mV (169E, Ningbo Sinotester Biological VO., Ltda). During ripe natural coffee immersion, three 500 mL water samples were collected at 0, 22.5, 45.0, 67.5, and 90.0 min. During pulped coffee immersion, three 500 mL water samples were collected at 0, 45, 90, 135, and 180 min. For BOD, COD, and turbidity analyses, water samples were collected only at the beginning and end of the immersion periods. The American Public Health Association-APHA (2012) method was followed in the analyses, with SMEWW 5210 B for Biochemical Oxygen Demand (BOD), SMEWW 5220 D for Chemical Oxygen Demand (COD), and SMEWW 2130 B for Turbidity.

Experimental Design and Statistical Analysis

For each type of coffee, a factorial $(3 \times 2) + 1$ design experiment was conducted, with three immersion times and two treatments, and control samples in a completely randomized design with three replications. The immersion times adopted for pulped natural coffee were 40, 60, and 90 min and for natural coffee, they were 60, 120, and 180 min. The two treatments corresponded to the immersion of the two types of coffee in water with microbubbles without ozone and microbubbles with ozone. The control samples were subjected only to conventional washing.

Data on the ozone reaction kinetics during filling the ozone incorporation tank and during ozone decomposition in the immersion tank for the different concentrations were subjected to regression analysis. The model selection was based on the significance of the regression coefficients, which were determined by the *t*-test at 5% probability and the coefficient of determination (R^2). Data related to microbiological and quality analyses were subjected to analysis of variance (ANOVA). For the sensory analysis, ANOVA was performed in a randomized block design (RBD) using Tukey's test, with comparisons of means at 1% probability. Using Dunnett's test, treatments were compared with the control at 5% significance. For water physicochemical analysis, electrical conductivity, pH, and redox potential values were subjected to regression analysis as a function of time. For the data related to biochemical oxygen demand (BOD), chemical oxygen demand (COD), and turbidity, descriptive statistics were performed with mean values and standard deviation. For both tests, the SAEG 9.1 software (SAEG, "Sistema para Análises Estatísticas, (2007)" UFV, Viçosa-MG) was used. To prepare the graphs and regression analyses, the SigmaPlot software, version 14.5 (n.d.) (Systat Software Inc., Erkrath, Germany) was used.

Results

Ozone Reaction Kinetics in Water

Figure 1S(a) shows the variation in the concentration of dissolved ozone in water as a function of the filling time of the incorporation tank and Fig. 1S(b) shows the decay of ozone concentration in the immersion tank for inlet concentrations equal to 15.77, 25.18, and 37.20 mg L^{-1}. Table 1 shows the regression models with their respective coefficients of determination (R^2) and standard error of estimate (SEE). The curves presented in Fig. 1S(a) demonstrate that higher ozone concentrations at the inlet of the incorporation tank imply higher ozone concentrations in the water. The variation in ozone concentration in the water inside the incorporation tank was monitored during filling. Higher dissolved ozone concentrations in water were observed in the first 15 min of tank filling for all concentrations tested.

The filling time and residence time of the ozonated water in the incorporation tank was 70 min. At the end of this period, ozone concentrations in the water in the incorporation tank were equal to 1.89, 2.60, and 3.78 mg L^{-1} for ozone gas inlet concentrations of 15.77, 25.18, and 37.20 mg L^{-1}, respectively. This corresponded to an incorporation percentage equal to 11.9% for the concentration of 15.77 mg L^{-1}, 10.32% for the concentration of 25.18 mg L^{-1}, and 10.16% for the concentration of 37.20 mg L^{-1}.

Table 1 Regression equations relating to the variation in ozone concentration during the filling of the incorporation tank and ozone decomposition in the water in the immersion tank for inlet concentrations equal to 15.77, 25.18, and 37.20 mg L^{-1}

Initial O_3 concentration	Adjusted model	R^2	SEE
Ozone incorporation tank			
37.20 mg L^{-1}	$\widehat{C} = 3.4379^{**} + 52.2370^{**} \bullet exp\left[\frac{-0.5 \bullet \left(\frac{ln\left(\frac{t}{27.5147^{**}}\right)}{0.7353^{**}}\right)^2}{t}\right]$	0.9394	0.2619
25.18 mg L^{-1}	$\widehat{C} = 2.2605^{**} + 42.5159^{**} \bullet exp\left[\frac{-0.5 \bullet \left(\frac{ln\left(\frac{t}{43.0061^{*}}\right)}{1.2832^{**}}\right)^2}{t}\right]$	0.9456	0.1990
15.77 mg L^{-1}	$\widehat{C} = 1.06856^{**} + 25.2741^{**} \bullet exp\left[\frac{-0.5 \bullet \left(\frac{ln\left(\frac{t}{25.4592^{**}}\right)}{0.6531^{**}}\right)^2}{t}\right]$	0.9737	0.0928
Immersion tank			
37.20 mg L^{-1}	$\widehat{C} = 2.0158^{**} exp(-0.0150^{**} t)$	0.9876	0.0745
25.18 mg L^{-1}	$\widehat{C} = 1.7911^{**} exp(-0.0149^{**} t)$	0.9943	0.0451
15.77 mg L^{-1}	$\widehat{C} = 1.0810^{**} exp(-0.0235^{**} t)$	0.9909	0.0235

*Significant at 5% probability by the *t*-test

**Significant at 1% probability by the *t*-test

Figure 1S(b) and Table 1 present the decay curves of the ozone concentration in the water inside the immersion tank for the three concentrations tested. The ozone concentration in the immersion tank was monitored after 20 min, which was the time required to fill the immersion tank. The initial dissolved ozone concentration in the immersion tank water was 1.16, 1.89, and 2.03 mg L^{-1} for ozone gas inlet concentrations of 15.77, 25.18, and 37.20 mg L^{-1}, respectively. The ozone concentration decay was monitored until the readings reached values below 0.1 mg L^{-1}, which corresponds to the detection limit of the iodometric method. For inlet concentrations equal to 15.77, 25.18, and 37.20 mg L^{-1}, the incorporation percentage in the immersion tank was 7.65; 7.50 and 5.45% respectively.

Figure 2S and Table 2 show the adjusted first-order reaction kinetic models for the three concentrations studied. The ozone half-lives in the immersion tank in pure water alone were 30.00, 46.52, and 63.59 min for the concentrations of 15.77, 25.18, and 37.20 mg L^{-1}, respectively. No major differences in the percentage of incorporation were observed as a function of input concentration. However, it is evident that higher ozone input concentrations imply longer half-lives of the gas in the water, which consequently represents greater ozone stability in the water.

Microbiological Analysis of Pulped Natural Coffee and Natural Coffee

Table 3 shows the mean values of filamentous fungi, yeasts, and aerobic mesophilic counts for pulped natural coffee after conventional processing (control), immersion in water with microbubbles without ozone, and microbubbles with ozone for 40, 60, and 90 min. Table 3 also presents the mean values of filamentous fungi, yeasts, and aerobic mesophilic counts for pulped natural coffee after hydraulic separation in a mechanical washer (control), immersion in water with microbubbles without ozone, and microbubbles with ozone for 60, 120, and 180 min. It was observed that, for pulped natural coffee, the treatments were significant at 5% significance for filamentous fungi, yeasts, and aerobic mesophilic counts. For natural coffee, the treatments were significant at 1% for filamentous fungi, yeasts, and aerobic mesophiles.

Table 3 Mean values of counts of filamentous fungi and aerobic mesophilic (log CFU g^{-1}) in pulped natural coffee and natural coffee

Treatment	Filamentous fungi and yeasts (log CFU g^{-1})	Aerobic mesophiles (log CFU g^{-1})
Pulped natural coffee		
Control	5.87 a	7.39 a
Microbubbles without O_3/40 min	4.96 *ab	6.37 *b
Microbubbles with O_3/40 min	4.74 *b	6.85 ab
Microbubbles without O_3/60 min	4.94 *ab	7.12 ab
Microbubbles with O_3/60 min	4.95 *ab	6.84 ab
Microbubbles without O_3/90 min	5.55 ab	7.35 a
Microbubbles with O_3/90 min	5.19 ab	6.97 ab
Natural coffee		
Control	7.42 a	8.11 a
Microbubbles without O_3/60 min	4.75 *b	8.08 a
Microbubbles with O_3/60 min	4.44 *b	8.06 a
Microbubbles without O_3/120 min	4.86 *b	7.96 a
Microbubbles with O_3/120 min	4.82 *b	8.19 a
Microbubbles without O_3/180 min	7.15 a	7.66 ab
Microbubbles with O_3/180 min	5.45 *b	7.10 *b

Means followed by an asterisk in the column differ from the control at 5% probability using Dunnett's test. Means followed by at least one of the same letter in the column do not differ from each other at 5% probability using Tukey's test

For pulped natural coffee, there was a significant difference, according to Dunnett's test at 5% probability, for the treatments in water immersion with microbubbles without ozone and microbubbles with ozone for 40 and 60 min, compared to the control. According to the results presented in Table 3, for pulped natural coffee, the treatment with water immersion in microbubbles with ozone for 40 min was responsible for a 1.13 log CFU g^{-1} reduction in contamination by filamentous fungi and yeasts. For aerobic mesophiles, the same 40-min immersion treatment was responsible for a 0.54 log CFU g^{-1} reduction. For natural coffee, the treatment with water immersion in microbubbles with ozone for 60 min led to a 2.98 log CFU g^{-1} reduction in contamination by filamentous fungi and yeasts. Still for natural coffee, only the treatment with water immersion for 180 min

Table 2 Adjusted first-order reaction kinetic models for the decay of ozone concentration in the immersion tank for inlet concentrations equal to 15.77, 25.18, and 37.20 mg L^{-1}

Initial O_3 concentration	Adjusted model	$t_{1/2}$ (min)	R^2	SEE
37.20 mg L^{-1}	$ln\left(\hat{C}\right) = 0.4921^{**} - 0.0109^{**}t$	63.59	0.96	0.1819
25.18 mg L^{-1}	$ln\left(\hat{C}\right) = 0.5806^{**} - 0.0149^{**}t$	46.52	0.99	0.0745
15.77 mg L^{-1}	$ln\left(\hat{C}\right) = 0.0698^{**} - 0.0231^{**}t$	30.00	0.99	0.0576

**Significant at 1% probability by the *t*-test

in microbubbles with ozone was sufficient to significantly reduce ($P < 0.05$) the aerobic mesophile count.

Coffee Quality Analysis

Sensory Analysis

Table 4 presents the mean values for the sensory attributes of pulped natural coffee, natural coffee, and the final score. It was found that, for pulped natural coffee, none of the evaluated factors presented a significant difference and for natural coffee, only acidity was significant at 1%. It was observed that the immersion of natural coffee in ozonated water in a microbubble system for 120 min presented the highest acidity value (7.63), differing statistically from the other treatments and the control at 5% probability using Dunnett's test. Despite the significant difference observed in acidity values, the effects of the treatments were not sufficient to cause a significant change in the coffee final score. The final score for the coffees from all treatments ranged from 80.29 to 82.25, characterizing the coffees as specialty. The treatment with natural coffee immersion in ozonated water for 120 min presented the highest final score (Table 4).

Color

Table 5 presents the results regarding color difference (ΔE), color hue (h*), and color saturation (Cr*) for pulped natural coffee and natural coffee on the day immediately after treatments and after drying the beans (30 days of storage). For both coffee beans immediately after treatments, a significant difference was observed at 5% probability using Dunnett's test for the color difference between the control and the other treatments. This color difference may be associated with the coffee immersion process in water with microbubbles with and without ozone. No statistical differences ($P > 0.05$) were observed for color difference between the treatments with microbubbles without ozone and microbubbles with ozone.

Regarding color hue, Dunnett's test showed a statistical difference ($P > 0.05$) between the control and the other treatments. Furthermore, it was evident that the color hue of pulped natural coffee was higher in the treatments with immersion in ozonated water compared to the coffee samples immersed in water with microbubbles without ozone. Although the color hue was higher in the ozonated water treatments, the differences were not perceptible to the human eye. The color saturation values for pulped natural coffee immediately after the treatments did not show statistically significant differences. However, for natural coffee, a significant reduction in color saturation values was observed with immersion in ozonated water for 180 min.

For the pulped and natural coffee, after drying, statistical differences in color were observed compared to the control using Dunnett's test at 5% probability. However, there was no statistical difference ($P > 0.05$) in color difference between treatments. The color hue of both pulped and natural coffee did not vary significantly ($P > 0.05$). Regarding color saturation, only pulped natural coffee showed significant variation, with the control treatment having the highest chroma value. Despite the statistical difference observed in chroma values, this change was not perceptible to the human eye.

Quantification of Defects and Water Content

Table 6 presents the mean values of defects related to black, immature, sour, withered/malformed, insect-damaged, shell, and water content for both pulped and natural coffee. The treatments were significant for the presence of immature, sour, and insect-damaged beans. For natural coffee, no statistical differences ($P < 0.05$) were observed regarding the types of defects. However, the water content of pulped natural coffee varied significantly ($P < 0.01$) as a function of the treatments.

For pulped natural coffee, the immersion in microbubbles with ozone for 60 min differed statistically ($P < 0.05$) from the control according to Dunnett's test and was responsible for the highest number of immature beans (17.00). The same treatment also recorded the highest number of sour beans (19.99). Furthermore, the treatment with immersion for 60 min in water with microbubbles without ozone for pulped natural coffee presented the highest number of broken beans (23.00), differing statistically ($P < 0.05$) from the control and the other treatments. For the same coffee type, the variation in water content was not significant ($P > 0.05$) between treatments, presenting values between 9.40 and 9.79% w.b. For natural coffee, no statistical differences were observed ($P > 0.05$) for any of the defects. Only the water content showed a significant change ($P < 0.05$), with the lowest value recorded of 11.00% for the control and 11.28% for the treatment with water immersion with microbubbles without ozone for 60 min.

Physicochemical Analysis of Water from Coffee Immersion

Table 7 presents the regression equations for the variation in electrical conductivity, pH, and redox potential of the effluent generated during the treatment by immersing pulped natural coffee and natural coffee in water with microbubbles without ozone and microbubbles with ozone. For the water used to immerse pulped natural coffee in microbubbles without ozone, treatment time influenced the variation in electrical conductivity and redox potential, while pH was not influenced by time, with an average value of 4.55. When analyzing electrical conductivity and redox potential, it is possible to observe that for every 1 min of treatment, there

Table 4 Average values of some sensory attributes and final score of pulped natural coffee and natural coffee

Treatments	Aroma	Flavor	Aftertaste	Acidity	Body	Balance	Overall	Final score
Pulped natural coffee								
Control	7.25 a	7.17 a	7.21 a	7.17 a	7.29 a	7.17 a	7.17 a	80.42 a
Microbubbles without O_3/40 min	7.33 a	7.29 a	7.25 a	7.33 a	7.17 a	7.21 a	7.17 a	80.75 a
Microbubbles with O_3/40 min	7.33 a	7.29 a	7.21 a	7.29 a	7.33 a	7.25 a	7.29 a	81.00 a
Microbubbles without O_3/60 min	7.54 a	7.46 a	7.33 a	7.42 a	7.38 a	7.42 a	7.38 a	81.92 a
Microbubbles with O_3/60 min	7.54 a	7.38 a	7.21 a	7.33 a	7.25 a	7.29 a	7.25 a	81.25 a
Microbubbles without O_3/90 min	7.33 a	7.08 a	7.13 a	7.13 a	7.17 a	7.25 a	7.21 a	80.29 a
Microbubbles with O_3/90 min	7.38 a	7.38 a	7.29 a	7.33 a	7.38 a	7.29 a	7.29 a	81.33 a
Natural coffee								
Control	7.58 a	7.42 a	7.38 a	7.42 ab	7.38 a	7.33 a	7.46 a	81.96 a
Microbubbles without O_3/60 min	7.38 a	7.46 a	7.29 a	7.38 b	7.42 a	7.29 a	7.38 a	81.58 a
Microbubbles with O_3/60 min	7.46 a	7.42 a	7.33 a	7.46 ab	7.46 a	7.42 a	7.50 a	82.04 a
Microbubbles without O_3/120 min	7.42 a	7.38 a	7.29 a	7.42 ab	7.42 a	7.29 a	7.38 a	81.58 a
Microbubbles with O_3/120 min	7.46 a	7.63 a	7.25 a	7.63*a	7.54 a	7.33 a	7.42 a	82.25 a
Microbubbles without O_3/180 min	7.46 a	7.42 a	7.38 a	7.46 ab	7.58 a	7.42 a	7.38 a	82.08 a
Microbubbles with O_3/180 min	7.50 a	7.42 a	7.25 a	7.33 b	7.38 a	7.17 a	7.38a	81.42 a

Means followed by an asterisk in the column differ from the control at 5% probability by Dunnett's test. Means followed by at least one of the same letter in the column do not differ from each other at 5% probability by Tukey's test

Table 5 Average values of color difference (ΔE), color tone or hue angle (h*), and color saturation or chroma (Cr*) of pulped natural coffee and natural coffee, after treatments and after drying with 30 days of storage

Treatments	After treatments			After drying		
	(ΔE)	(h*)	(Cr*)	(ΔE)	(h*)	(Cr*)
	Pulped natural coffee					
Control	0.00 b	83.33 b	16.12 a	0.00 b	83.95 a	10.48 a
Microbubbles without O_3/40 min	7.01 *a	88.91 *ab	19.19 a	3.56 *a	85.51 a	10.16 ab
Microbubbles with O_3/40 min	7.27 *a	91.43 *a	21.21 a	2.00 *a	84.34 a	9.04 *b
Microbubbles without O_3/60 min	8.13 *a	89.00 *ab	18.79 a	2.65 *a	85.05 a	9.00 *b
Microbubbles with O_3/60 min	7.31 *a	90.25 *a	18.62 a	1.73 *a	87.32 a	9.19 *ab
Microbubbles without O_3/90 min	7.75 *a	88.73 *ab	20.53 a	1.80 *a	86.13 a	9.86 ab
Microbubbles with O_3/90 min	8.36 *a	91.00 *a	22.76 a	2.23 *a	83.89 a	10.14 ab
	Natural coffee					
Control	0.00 b	38.21 a	14.20 ab	0.00 b	84.46 a	11.19 a
Microbubbles without O_3/60 min	9.19 *a	39.21 a	16.57 a	2.96 *a	85.00 a	11.72 a
Microbubbles with O_3/60 min	7.23 *a	35.65 a	18.94 a	2.98 *a	86.58 a	11.40 a
Microbubbles without O_3/120 min	9.64 *a	42.68 a	16.15 ab	2.63 *a	85.74 a	11.09 a
Microbubbles with O_3/120 min	10.19 *a	36.94 a	14.42 ab	3.35 *a	83.59 a	10.78 a
Microbubbles without O_3/180 min	8.93 *a	38.70 a	18.23 a	2.43 *a	86.56 a	11.52 a
Microbubbles with O_3/180 min	9.24 *a	40.19 a	13.17 b	2.29 *a	84.66 a	11.30 a

Means followed by an asterisk in the column differ from the control at 5% probability by Dunnett's test. Means followed by at least one of the same letter in the column do not differ from each other at 5% probability by Tukey's test

Table 6 Average values of different types of defects and water content of pulped natural coffee and natural coffee

Treatments	Black	Immature	Sour	Withered/ malformed	Broken	Insect-damaged	Shell	Water content (%)
Pulped natural coffee								
Control	2.67 a	8.00 ab	14.33 ab	8.33 a	14.33 ab	15.67 a	4.67 a	9.74 a
Microbubbles without O_3/40 min	1.33 a	13.33 ab	6.67 *bc	7.00 a	17.33 ab	20.00 a	6.00 a	9.58 a
Microbubbles with O_3/40 min	0.00 a	5.33 b	4.00 *c	6.67 a	12.67 ab	14.33 a	4.67 a	9.60 a
Microbubbles without O_3/60 min	3.33 a	9.00 ab	5.67 *c	8.33 a	23.00 *a	19.33a	6.67 a	9.79 a
Microbubbles with O_3/60 min	3.33 a	17.00 *a	19.00 a	10.67 a	18.67 ab	18.33 a	5.00 a	9.56 a
Microbubbles without O_3/90 min	0.67 a	7.00 ab	6.33 *bc	6.67 a	21.00 a	15.00 a	4.33 a	9.54 a
Microbubbles with O_3/90 min	1.33 a	6.67 ab	4.33 *c	6.00 a	9.00 b	16.33 a	6.00 a	9.40 a
Natural coffee								
Control	0.00 a	1.33 a	1.33 a	9.33 a	23.00 a	37.67 a	2.33 a	11.00 c
Microbubbles without O_3/60 min	0.33 a	0.67 a	4.00 a	5.67 a	35.67 a	48.00 a	2.33 a	11.28*a
Microbubbles with O_3/60 min	0.00 a	2.00 a	5.33 a	13.33 a	35.67 a	48.33 a	2.67 a	11.26*ab
Microbubbles without O_3/120 min	0.33 a	1.00 a	4.00 a	13.67 a	24.33 a	31.67 a	2.67 a	11.13*bc
Microbubbles with O_3/120 min	0.00 a	1.33 a	5.00 a	11.67 a	21.67 a	40.33 a	2.67 a	10.99 c
Microbubbles without O_3/180 min	0.00 a	0.67 a	3.00 a	9.00 a	20.33 a	45.00 a	3.00 a	11.04 c
Microbubbles with O_3/180 min	0.00 a	0.67 a	5.00 a	9.00 a	21.00 a	41.33 a	4.00 a	11.07 c

Means followed by an asterisk in the column differ from the control at 5% probability by Dunnett's test. Means followed by at least one of the same letter in the column do not differ from each other at 5% probability by Tukey's test

was a 1.44-fold reduction in electrical conductivity values and a 2.40-fold reduction in redox potential values.

Regarding pulped natural coffee immersion water, it was found that only redox potential was not influenced by treatment time, presenting an average value of 874.93 mV (Table 7). The water electrical conductivity and pH were influenced by ozone incorporation into the water. The electrical conductivity was reduced by 1.95 times with each minute of treatment, while pH increased by 0.012 times with each minute of treatment in microbubbles with ozone.

Table 7 Regression equations and respective coefficients of determination (R^2) and standard error of estimate (SEE) referring to the variation of electrical conductivity, redox potential, and pH of the immersion water of pulped natural coffee and natural coffee as a function of immersion time in water with microbubbles without ozone and microbubbles with ozone

Treatment	Quality variable	Adjusted model	SEE	R^2
Pulped natural coffee				
Microbubbles without ozone	Electrical conductivity	$\hat{y} = 172.246 - 1.446^{**}t$	10.92	0.96
	pH	$\hat{y} = 4.558$	-	-
	Redox potential	$\hat{y} = 730.60 - 2.400^{**}t$	23.60	0.94
Microbubbles with ozone	Electrical conductivity	$\hat{y} = 194.474 - 1.957^{**}t$	18.95	0.94
	pH	$\hat{y} = 4.114 + 0.012^{**}t$	0.12	0.94
	Redox potential	$\hat{y} = 874.93$	-	-
Natural coffee				
Microbubbles without ozone	Electrical conductivity	$\hat{y} = 82.071 + 0.105^{**}t - 0.001^{*}t^2$	1.04	0.99
	pH	$\hat{y} = 4.746 + 0.004^{**}t$	0.048	0.97
	Redox potential	$\hat{y} = 919.886$	-	-
Microbubbles with ozone	Electrical conductivity	$\hat{y} = 62.934$	-	-
	pH	$\hat{y} = 4.912$	-	-
	Redox potential	$\hat{y} = 962.730$	-	-

*Significant at 5%, according to the *t*-test

**Significant at 1%, according to the *t*-test

For natural coffee immersion water, it is possible to observe that only electrical conductivity and pH of the water with microbubbles without ozone underwent significant variations over the treatment. Electrical conductivity presented a quadratic behavior described by a second-degree polynomial equation, with a maximum electrical conductivity of 83.80 μS cm^{-1} in the 33-min time period. Regarding pH, a 0.0036-fold increase was observed for each minute of treatment. The immersion time of natural coffee in microbubbles with ozone did not significantly influence the values of electrical conductivity, pH, and redox potential, which recorded average values equal to 62.93 μS cm^{-1} and 4.99 and 962.73 mV, respectively.

Table 8 presents the mean values and standard deviations for BOD, COD, and water turbidity at the beginning and end of the immersion treatment of pulped natural coffee and natural coffee in water with microbubbles without ozone and microbubbles with ozone. At the end of the treatment period, a reduction in BOD, COD, and water turbidity was observed for both pulped natural coffee and natural coffee. This reduction was more significant in the immersion water of pulped natural coffee in microbubbles with ozone, where the final BOD and COD values were 46.63 ± 1.62 and 93.43 ± 3.91 mg L^{-1}, respectively. Regarding the effluent generated from the immersion treatment of natural coffee in microbubbles with ozone, the final BOD and COD values were 185.20 ± 5.16 and 377.00 ± 12.64 mg L^{-1}, respectively. The turbidity values of the immersion water from the treatment with microbubbles without ozone and microbubbles with ozone in pulped natural coffee were 14.47 ± 0.59 and 5.12 ± 0.48 UNT, respectively. The turbidity values of the water from the treatment with microbubbles without ozone and microbubbles with ozone in natural coffee were 8.75 ± 0.14 and 7.17 ± 0.46 UNT, respectively.

Table 8 Mean values and standard deviation of biochemical oxygen demand (BOD), chemical oxygen demand (COD), and turbidity of the water used in the treatments

Treatments	Sample collection	DBO (mg L^{-1})	DQO (mg L^{-1})	Turbidity (UNT)
Pulped natural coffee				
Microbubbles without ozone	Beginning of the immersion period—00:00 h	387.70 ± 92.60	675.65 ± 3.65	70.00 ± 11.06
Microbubbles with ozone	Beginning of the immersion period—00:00 h	459.80 ± 38.36	879.97 ± 29.13	61.57 ± 5.46
Microbubbles without ozone	End of the immersion period—01:30 h	62.23 ± 4.35	159.23 ± 15.06	14.47 ± 0.59
Microbubbles with ozone	End of the immersion period—01:30 h	46.63 ± 1.62	93.43 ± 3.91	5.12 ± 0.48
Natural coffee				
Microbubbles without ozone	Beginning of the immersion period—00:00 h	314.15 ± 8.35	609.20 ± 7.90	40.70 ± 5.81
Microbubbles with ozone	Beginning of the immersion period—00:00 h	221.07 ± 30.20	431.03 ± 25.10	28.27 ± 2.33
Microbubbles without ozone	End of the immersion period—03:00 h	171.30 ± 19.19	355.47 ± 17.28	8.75 ± 0.14
Microbubbles with ozone	End of the immersion period—03:00 h	185.20 ± 5.16	377.00 ± 12.64	7.17 ± 0.46

Discussion

Ozone Reaction Kinetics in Water

There are some studies in the literature that describe how the reaction kinetics of gaseous ozone (McClurkin et al., 2013) and ozone dissolved in water (Gardoni et al., 2012) occur. The reaction kinetics of ozone in water are influenced by the initial concentration of ozone gas, pH, temperature, hardness, alkalinity, concentration of metals, anions/cations, presence of organic matter in the water, and hydrodynamic flow conditions (Gardoni et al., 2012). Due to the complexity involved in the reaction kinetics of ozone in water and the absence of a mathematical approach that equates the influence of all these variables, previous studies suggest that experiments be carried out under conditions that are as close as possible to real-scale applications (Gardoni et al., 2012).

The characterization of ozone reaction kinetics is of fundamental importance to understand the magnitude of mass transfer from the gaseous phase to the liquid phase and decomposition reactions. Based on this understanding, it is possible to design treatment systems, define the power of ozone generators, and define the incorporation technology appropriate to the volume of water and quantity of product for a given process on a commercial scale. In this study, a microbubble generation system was used as the technology for incorporating ozone gas into water. Microbubbles are characterized by having a diameter of less than 100 µm, while common bubbles have a diameter between 2 and 5 mm (Khuntia et al., 2012). Thus, microbubbles have a smaller volume, larger specific surface area, and lower movement speed compared to common bubbles. All these factors favor the incorporation of ozone gas into water (Khuntia et al., 2012; Wei et al., 2017). The incorporation of ozone into water through microbubbles has been extensively investigated and has demonstrated good results regarding the efficiency of incorporating ozone gas into water (John et al., 2025).

Figure 1S(a) shows a greater incorporation of ozone into the water in the first 15 min of filling the incorporation tank. This can be explained by the fact that, in the first minutes of filling the tank, there was a smaller water volume and, consequently, greater proximity between the microbubble diffusers (Fig. 2c) and the water sample collection point. As the water volume inside the incorporation tank increased, the sample collection point became further away from the microbubble diffusers. The increase in the height of the water column over the diffusers favored the dispersion of microbubbles in a larger water volume, in addition to observing a tendency to stabilize the mass transfer from the gaseous phase (ozone) to the liquid phase (water).

Regarding the percentage of ozone gas incorporation into the water in the immersion tank, lower concentration values were observed in the immersion tank compared to the ozone concentrations in the incorporation tank (Fig. 2a, b). This difference is due to the decomposition that occurred due to the hydrodynamics of the water flow during the filling of the immersion tank with ozonated water. Previous studies have reported that the hydrodynamic conditions of the flow influence ozone decomposition in the water (Gardoni et al., 2012). Higher flow stirring speeds increase ozone decomposition (Sheffer & Esterson, 1982; Sotelo et al., 1989).

Ozone saturation in the water of the incorporation tank occurred in approximately 40 min. This time was sufficient for the concentration of dissolved ozone in water to stabilize in a volume of 500 L of water. Higher ozone concentration values implied higher concentrations of dissolved ozone in water. Similar results were found by Galdeano et al. (2018), when they investigated the concentrations and time of ozone saturation in water at different temperatures and pH values. In the study conducted by these authors, for an initial ozone concentration equal to 22.3 mg L^{-1}, pH equal to 6.0 and temperature of 25 °C, saturation time and concentration were equal to 27.94 min and 5.26 mg L^{-1}. The decay of the ozone concentration in water observed in this study was also dependent on the initial ozone concentration. Higher initial ozone concentration values implied longer half-lives. For a concentration of 15.77 mg L^{-1}, the half-life time was 30 min; a similar result was also found by Ferreira et al. (2021), who evaluated the kinetics of ozone decomposition in water at an initial concentration of 13.0 mg L^{-1}.

Microbiological Analysis of Natural Coffee and Pulped Natural Coffee

In the present study, it was verified that ozone has the potential to reduce the count of mesophilic bacteria and fungi in coffee. The presence of these microorganisms, such as filamentous fungi, can result in the contamination of coffee beans by mycotoxins (Batista et al., 2009; Lee et al., 2024). In a study conducted by Akbar et al. (2020), the potential of ozone gas in the control of mycotoxigenic fungi and in the degradation of ochratoxin A in stored coffee was evident. The purpose of this study was to investigate the potential of ozonated water in a microbubble system for microbiological decontamination of coffee immediately after harvest. Recent scientific investigations have demonstrated the great potential of ozonated water in a microbubble system in the microbiological decontamination of agricultural products (Chen et al., 2025; Hou et al., 2022; Silva et al., 2024).

Ozone inactivates microorganisms through the oxidation of cellular components. The principal reason for the high antimicrobial potential of ozone gas is due to its oxidation potential (2.07 mV), which is higher than the oxidation

potential of other commonly used oxidizing agents (hydrogen peroxide: 1.78 mV, hypochlorous acid: 1.49 mV, chlorine: 1.36 mV, chlorine dioxide: 1.27 mV) (Karaca et al., 2007). Oxidation reactions can be caused directly by molecular ozone dissolved in water, reactive oxygen species (ROS), and hydroxyl radical (OH) (Hunt & Mariñas, 1997; Gardoni et al., 2012; Premjit et al., 2022). When interacting with microbial cells, ozone oxidizes polyunsaturated fatty acids to acid peroxides and causes the oxidation of sulfhydryl groups and amino acids of enzymes, proteins, and peptides. This compromises the integrity of the cell wall of microorganisms (Victorin, 1992). When subjected to oxidative stress, this leads to leakage of intracellular contents and deleterious effects on nucleic acid (Rangel et al., 2021; Yang et al., 2025). In fungi and molds, ozone has proven effective in inhibiting conidia germination, morphological changes in hyphae, and fungal cell metabolism, thus resulting in the inactivation of these microorganisms (Xue et al., 2023).

In this study, ozonated water in a microbubble system had a significant effect on reducing the counts of filamentous fungi, yeasts, and aerobic mesophiles (Table 3). More significant reductions in the counts of these microorganisms were observed in natural coffee. These reductions may be related to treatment time, the initial sample contamination, and the physical characteristics of the two types of coffee from the two processing methods, with and without the husk. This study did not investigate the viability of spores after treating coffee with immersion in ozonated water. However, some studies demonstrate the potential of ozone in inactivating fungal spores (Vijayanandraj et al., 2006; Wen et al., 2020). Vijayanandraj et al. (2006) observed that, although some fungal spores were not completely inactivated, some fungal colonies that developed from ozone-treated spores did not produce viable spores. Treatments involving immersion of coffee in water with microbubbles without ozone also showed a reduction in microbiological contamination (Table 3). This reduction in contamination may be associated with washing the coffee by the immersion process in water and also by the physical action of the microbubbles when they break on the surface of the fruits. Hou et al. (2022), when evaluating the effectiveness of ozonized water in a microbubble system for microbiological decontamination of tomatoes, found that the physical action of the microbubbles contributed to promoting the detachment of bacterial cells adhered to the surface of the fruits.

The immersion times in ozonated water for natural coffee (0 to 180 min) were longer than the immersion times for pulped natural coffee (0 to 90 min). Contamination in the control samples for filamentous fungi and yeasts (7.42 log CFU g^{-1}) and mesophiles (8.11 log CFU g^{-1}) was higher in natural coffee compared to the control contamination in pulped natural coffee (Table 3). This difference in the control counts can be associated with the mechanical removal of the husk from the pulped natural coffee, which possibly contributes to the reduction in microbiological contamination. In addition, it was observed that, when immersed in water, pulped natural coffee released the mucilage that surrounds the endocarp. This mucilage is rich in proteins (4 to 12%), lipids (1 to 2%), minerals (6 to 10%), and total carbohydrates (45 to 89%) (Franca & Oliveira, 2009; Klingel et al., 2020). The presence of this mucilage in the immersion water accelerates the decomposition of ozone, which helps to explain the smaller reduction observed in the counts of filamentous fungi, yeasts, and mesophiles in the pulped natural coffee.

It is important to emphasize that, in the present study, the antimicrobial effect of ozonated water was assessed through the quantification of mesophilic bacteria and fungi, without isolation or taxonomic identification of individual microorganisms. As a result, specific growth rates and microbial growth kinetics were not evaluated. Other microbial groups commonly associated with coffee post-harvest processing, such as acetic acid bacteria and lactic acid bacteria, were not considered. Future studies should therefore investigate the effects of ozone on these additional microbial groups, including their growth kinetics under controlled conditions. Such information is particularly relevant for subsequent post-ozonation processes, including coffee fermentation, in which the selective control of undesirable microbiota while preserving beneficial microorganisms plays a critical role in process efficiency and beverage quality. Fermentation has recently been investigated as a strategy to improve coffee sensory quality (Elhalis et al., 2023).

The microbial groups that compose the microbiota associated with coffee beans are influenced by several factors, including the coffee species, altitude, climatic conditions, crop management practices, and the technological level adopted during post-harvest processing (Bruyn et al., 2017; Hamdouche et al., 2016; Veloso et al., 2020, 2023). Hamdouche et al. (2016) evaluated the impact of post-harvest processing methods on coffee microbial ecology and reported marked differences between wet and dry processing. In wet processing, lactic acid bacteria were predominantly identified, including *Weissella* spp., *Lactobacillus* spp. (with *Lactobacillus fermentum* highlighted), *Leuconostoc mesenteroides*, and *Lactococcus lactis*, as well as environmental bacteria such as *Ralstonia* spp. and *Acidovorax* spp. In dry processing, bacteria belonging to the genera *Chryseobacterium* spp. and *Citrobacter* spp. were identified. Regarding fungi, dry processing was associated with microorganisms belonging to the class Sordariomycetes, as well as the species *Meyerozyma caribbica*, *Meyerozyma guilliermondii*, and *Cladosporium sphaerospermum*. In wet processing, fungi of the genus *Penicillium* spp. and yeasts belonging to the class Saccharomycetes were identified. These microbial groups are commonly associated with coffee post-harvest environments and may play distinct roles,

ranging from quality deterioration to beneficial biochemical transformations during processing and storage, depending on the processing conditions.

Quality Analysis of Pulped Natural Coffee and Natural Coffee

Sensory Analysis

The results obtained in the sensory analysis (Table 4) show that, although ozone is a strong oxidizing agent (Karaca & Velioglu, 2007), the treatment with immersion in microbubbles with ozone preserved the sensory quality of pulped natural coffee and natural coffee. All samples remained with a final score above 80 points and were classified as specialty coffees (Lingle & Menon, 2017). The treatments with microbubbles without ozone and microbubbles with ozone, during the periods studied, according to the sensory analysis, did not lead to the oxidation of flavor precursor compounds. In addition, it is noteworthy that pulped natural coffee does not have the protection of the fruit skin and, even so, did not present sensory changes that would harm the beverage.

In the sensory analysis, a significant change was observed only in natural coffee acidity. The highest acidity intensity occurred in the treatment with immersion for 120 min in microbubbles with ozone. Despite presenting a statistical difference, the increase in acidity did not cause a significant difference in the final coffee score. Furthermore, it is worth noting that the treatments with the highest final scores were those that presented the highest acidity values, in which natural coffee was immersed in microbubbles with ozone for 60 and 120 min (Table 4). For specialty coffees, even though no statistical differences were observed, an increase of one point in the final score of the coffee implies a higher commercial value of the product.

The preservation of coffee sensory quality is a strong indication that the treatment with immersion in ozonated water did not cause chemical changes in the coffee beans. The results obtained in this investigation indicate that the oxidation caused by immersing coffee beans in ozone microbubbles only affected the coffee beans superficially, promoting microbiological decontamination (Table 3) and changes in color (Table 5). However, the action of ozonized water in a microbubble system as a pre-drying treatment did not penetrate the parchment and the interior of the coffee bean, thus allowing the maintenance of the coffee's sensory quality.

Some studies show that, during storage, coffee is subject to chemical changes directly linked to respiration and other oxidative processes (Borém et al., 2019b, 2021). The intensity at which these chemical changes occur in coffee beans depends on the type of processing, the initial coffee quality, the type of packaging used in storage, the relative humidity, and temperature of the storage environment (Borém et al., 2021, 2023). Among the chemical changes that can occur, the oxidation of carbohydrates, proteins, and lipids stands out (Borém et al., 2019b, 2021). Lipid oxidation has been identified as one of the main reasons for the aging of coffee beans and for the presence of flavors such as cardboard, paper, and past-harvest in the sensory analysis (Borém et al., 2021). Although ozone is a strong oxidizing agent, according to the sensory analysis, the absence of flavors such as cardboard, paper, and past-harvest is an indication that the treatments did not promote lipid oxidation. This study did not characterize the nutritional composition of coffee after immersion in ozonated water. However, it is important to highlight that some scientific investigations have already demonstrated that the use of ozone does not alter the nutritional composition of food (Glowacz et al., 2015; Santos et al., 2018). This reinforces the potential of this technology for coffee microbiological decontamination.

Color

The significant changes observed for color difference in pulped natural coffee and natural coffee immediately after the treatments (Table 5) were greater than the color difference values observed for coffee after drying. Color difference values ranged from 7.01 to 8.36 for pulped natural coffee and from 7.23 to 10.19 for natural coffee immediately after the treatments (Table 5). Color difference values for pulped natural coffee after drying ranged from 1.73 to 3.56 for natural coffee, and after drying, they ranged from 2.43 to 2.98. Color difference values greater than 3 already imply a color difference that can be perceptible to the human eye (Altmann et al., 2022; Francis & Clydesdale, 1975). The results obtained for color differences in this study (Table 5) show that the effect of the treatments was more pronounced immediately after immersing the coffee in water with microbubbles without ozone and in microbubbles with ozone. In pulped natural coffee, the highest color hue values were observed immediately after treatments involving coffee immersion microbubbles with ozone. These changes are possibly associated with the effect of removing the mucilage that surrounds the coffee beans during immersion treatments.

In the treatment with natural coffee immersion in microbubbles with ozone for 180 min, a significant reduction in color saturation was observed (Table 5). The ozone dissolved in water possibly caused the oxidation of some pigments present in the coffee husk. The same behavior has already been observed in other studies carried out with chestnuts (Ferreira et al., 2021; Freitas-Silva et al., 2013). Although significant changes were observed in the color saturation of natural coffee husk, they did not have an impact on the color of the coffee beans after drying. This indicates that the immediate effect of the oxidation of pigments in the fruit husk does

not influence the color of the coffee beans after drying, processing, or storage. Despite having presented a statistical difference between treatments, the color saturation of pulped natural coffee after drying, as well as the longest period of immersion in ozonated water, was statistically equal to that of the control. It is of fundamental importance to develop post-harvest technologies that do not cause changes in coffee color. The quality of the coffee beverage is closely related to the color of the bean and undesirable changes can lead to the depreciation of a given coffee batch (Borém et al., 2013; Coraldi et al., 2008).

Number of Defects and Water Content

Significant differences were observed in defects only in pulped natural coffee for immature, sour, and broken beans (Table 6). Immature defects are related to the uneven maturation of coffee which, according to the 4th Harvest Survey carried out by the National Supply Company (CONAB), there was a high percentage of immature coffee in the 2024 harvest (CONAB, 2024). The coffee used in the experiment came from a plantation in which no type of ripening agent was used on the coffee plants. Passing through the mechanical pulper allowed the separation of the immature fruits, but this separation was not fully efficient, which may explain the presence of this type of defect in the samples. The presence of broken beans may be related to the adjustment of the machine used in pulping and to the water content of the beans. Pulped natural coffee had lower water content values compared to natural coffee (Table 6). Drier grains are more susceptible to mechanical damage; thus, it is suggested that the machine itself may have been responsible for breaking the grains during the processing operation (Brighenti & Cirilo, 2018).

According to Normative Instruction No. 8 of June 11, 2003, "sour" beans are those whole or pieces of beans that have a brown color as their predominant color, which can be light or dark brown (various shades). These shades of brown come from fermentation processes. Fermentation is a metabolic process facilitated by the presence of microorganisms present in the natural microbiota of coffee fruits (Silva et al., 2008). In this study, it was observed that the treatment with immersion of pulped natural coffee for 60 min in microbubbles with ozone presented the highest number of burnt beans (Table 6). However, it is important to emphasize that the treatment with immersion in microbubbles with ozone for the longest time (90 min) presented the lowest number of burnt beans. This is an indication that the treatments were not responsible for the unwanted fermentation and that the presence of sour beans may be related to sample heterogeneity since the fermentation of these fruits may have occurred in the crop.

Physicochemical Analysis of Water from the Coffee Immersion Process

The physicochemical analyses of the water during coffee immersion were electrical conductivity, pH, and redox potential. The electrical conductivity of a solution measures the capacity of this solution to conduct electrical current and is directly related to the presence of ions in solution (Bhateria & Jain, 2016; Gupta & Paul, 2013). In this study, it was evident that the electrical conductivity of the immersion water of pulped natural coffee with microbubbles without ozone and microbubbles with ozone reduced during the treatment. The most significant reduction occurred in the water with microbubbles with ozone, as evidenced by the angular coefficient (1.95) of the adjusted equation (Table 7). The immersion of pulped natural coffee in water causes the release of mucilage into the water, which explains the higher electrical conductivity values in relation to the immersion water of natural coffee. The immersion water of natural coffee presented lower electrical conductivity values since the coffee husk that surrounds the fruit limits the release of mucilage into the immersion water.

The redox potential is an indicator of the oxidizing capacity of water (Linnik et al., 2023). Therefore, a higher redox potential value implies a higher water oxidation potential. The redox potential values for surface waters are around 300 to 500 mV (Søndergaard, 2009). In this study, the redox potential value of the immersion water of pulped natural coffee and natural coffee was between 874.93 and 962.73 mV. These results are close to the redox potential values found by Santos et al. (2024) in distilled water (1013 mV) and mineral water (1059 mV). The average pH values of the immersion water of pulped natural coffee in microbubbles without ozone were 4.55 (Table 7). The pH values for coffee wastewater reported in the literature are around 3.88 to 4.21 due to the presence of organic acids present in the coffee husk and mucilage (Rattan et al., 2015; Selvamurugan et al., 2010). In the treatment with immersion of pulped natural coffee in microbubbles with ozone, an increase in pH values was observed (Table 7). This increase may be related to the potential of ozone in the degradation of organic acids present in the water.

BOD refers to the amount of dissolved oxygen that is consumed by microorganisms to decompose the organic matter in a given wastewater. The higher the BOD values for a wastewater, the greater the potential of that water to pollute a given watercourse, limiting the amount of dissolved oxygen for other organisms (Bhateria & Jain, 2016). According to Normative Deliberation COPAM-CERH/MG No. 8, of November 21, 2022, the maximum allowable limit for BOD and COD is 60 and 180 mg L^{-1}, respectively. In this study, the most significant reduction in BOD and COD values occurred in the immersion water

from the treatment of pulped natural coffee, with average values of 46.63 ± 1.62 and 93.43 ± 3.91 mg L^{-1}, respectively, which meets the established limits. In addition to the reduction observed in BOD and COD values, a reduction in turbidity values was also observed. Turbidity is related to the resistance that water offers to the passage of light as a function of suspended particles.

Research into more sustainable production processes that limit the generation of effluents has been increasing (Sengupta et al., 2020). Within this context, the potential of ozone in the treatment of water resulting from coffee sanitization is evident and opens up possibilities for the reuse of this water, including in other coffee processing stages. The use of ozonated water in a microbubble system for microbiological decontamination of coffee before drying is characterized as a promising technology. This treatment strategy contributes to the production of coffee batches with beverage quality and food safety. The process of treating coffee with ozonated water may in the future be associated with fermentation. One of the great challenges of fermentation is to produce coffee batches with standardized quality. Good fermentation performance is ensured when the group of microorganisms present in the medium is controlled. In this context, it is suggested that the use of ozonated water in the microbiological decontamination of coffee may guarantee greater control of the initial population of microorganisms in fermentation.

Although ozonation is recognized as an effective oxidative treatment, the potential formation of compounds resulting from the degradation of mycotoxins or pesticide residues in coffee beans or in process water was not specifically investigated in the present study. The assessment of water quality was limited to physicochemical parameters, such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), and turbidity. Therefore, the occurrence of oxidative degradation compounds cannot be ruled out. Future studies should include targeted analytical approaches to identify and quantify these compounds in both coffee beans and water, particularly under different ozonation intensities and exposure times, in order to ensure process safety and to support subsequent post-ozonation steps, such as coffee fermentation.

Although the pilot-scale approach represents the strength of this study compared with laboratory experiments, scale-up effects remain critical in gas–liquid processes. Future research should investigate industrial-scale reactors, advanced monitoring of ozone mass-transfer coefficients, integration with existing washing and drying systems, and modeling of ozone demand in high-organic-load environments. Multi-season and multi-origin trials, combined with sensory panels and storage studies, will help establish robustness across commercial conditions. Finally, techno-economic and life-cycle assessments are required to evaluate the feasibility and sustainability of large-scale adoption.

Conclusion

The percentages of ozone incorporation into the water did not show major differences in relation to the initial concentration of ozone gas. However, the half-life of ozone in the water was influenced by the initial concentration. Higher initial ozone concentrations resulted in a longer half-life of ozone gas in the water. The treatments with immersion in water with microbubbles without ozone and microbubbles with ozone were effective in reducing the counts of filamentous fungi, yeasts, and mesophilic bacteria. These reductions were more significant in the treatments with immersion in microbubbles with ozone. The results obtained in the quality analyses showed that the treatment with immersion in microbubbles with ozone preserved the sensory quality of pulped natural coffee and natural coffee. In addition to preserving the sensory quality, the incorporation of ozone into the water in a microbubble system has great potential in the treatment of water resulting from coffee immersion.

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Author Contribution F. G. A. Lacerda: conceptualization, data curation, methodology, writing, review, and editing. M. V. A. Silva: project guidance, supervision, conceptualization, methodology, formal analysis, investigation, writing original draft preparation. L.R.A. Faroni: resource provision, conceptualization, project guidance, review, and editing. P. R. Cecon: Formal analysis and writing. E. R. Alencar: methodology and writing (review and editing). C. G. Magalhães: methodology, formal analysis and investigation. L. Haeberlin: methodology and writing (review and editing). E. H. F. Ferreira: methodology and investigation.

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Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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