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Research article

Impact of immature coffee fruits and water addition during spontaneous fermentation process: Chemical composition and sensory profile ☆

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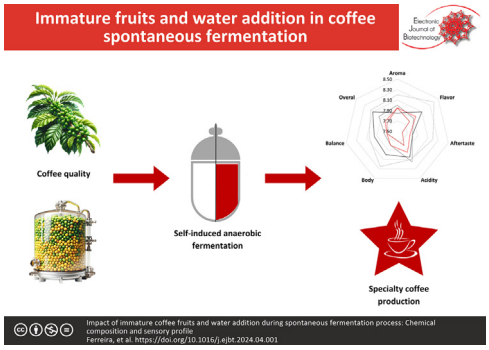
GRAPHICAL ABSTRACT



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ABSTRACT

Background: Coffee fermentation process influences the final coffee composition and the sensory aspects which define the quality of the beverage. In this study, coffee fruits underwent spontaneous self-induced anaerobic fermentation using samples with two percentages of immature fruits in submerged and solid-state processing. The effects on the physicochemical composition and sensory quality of coffees were evaluated.

Results: The two percentages of immature fruits corresponded to 11.0 and 0.3% of unripe fruits. The percentage of immature fruits significantly altered the initial content of sugars (sucrose, glucose, and fructose), ash, and titratable acidity. The water addition during the fermentative process did not significantly influence final moisture, proteins, citric acid, and propionic acid concentrations. Compared to the solid-state, the submerged process gave rise to coffees with lower concentrations of ethanol, glycerol, ash, lipids, succinic, and acetic acids. Coffee fermented with 0.3% of immature fruits showed higher lactic acid production in submerged fermentation (67.44 mg/g), and higher concentrations of ethanol (42.84 mg/g) and glycerol (1.68 mg/g) in solid-state fermentation. All coffees produced were classified as specialty coffees with a score above 84 points. However, the submerged fermented coffee with 11% immature fruit stood out with notes of caramel, brown sugar, honey, orange, lemon, floral, nut, yellow and red fruits.

Conclusions: This study confirmed that spontaneous fermentation can be used to produce specialty coffees. Differentiation in sensory attributes can be achieved through the addition of water and varying the

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percentage of green fruits during the fermentation process. Up to 11% of immature fruits did not compromise coffee quality.

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1. Introduction

Coffea arabica is the primary species related to the production of superior-quality coffees worldwide. Several factors, including genetic characteristics of the plant, cultivation methods, and postharvest processing, can impact coffee quality [1]. The coffee fruit is composed of skin, pulp, mucilage, parchment, silverskin, and seed [2]. According to Elías [3] and Schwan and Wheals [4], 5% of the fruit corresponds to mucilage on a dry basis. The mucilage is the soft layer found between the peel and the parchment, formed by water, sugars, complex polysaccharides, pectic substances (mainly pectin), organic acids, holocellulose, lipids, proteins, and minerals. Due to its rich composition, it is directly related to the fermentation process [5]. The coffee bean has in its composition up to 53% of insoluble polysaccharides, among them mainly cellulose, up to 18% of lipids, and 12% of proteins [6]. It is important to know the initial composition of coffee fruits because the changes that occur during roasting depend on volatile and non-volatile precursors, which can be altered during the postharvest processing steps [7,8].

The maturation stage causes impact on the chemical compounds and physical properties of coffee [9,10]. During the development and ripening of fruits, alterations occur in malic acid, citric acid, oxalic acid, and quinic acid. Citric acid is at its highest amount in seeds, whereas malic acid is more concentrated in pericarps [11]. The mucilage thickness varies according to the maturation of the grain, from 0.5 to 2.0 mm [12]. During the harvest, mature and immature coffee fruits are collected. This occurs because in a single coffee crop, several blooms can occur, what results in a harvest with heterogeneous maturation [13]. For this reason, the ripe coffee fruits are processed with a variable percentage of unripe fruits. The sensory characteristic of green coffee is considered detrimental to the final quality of the coffee beverage [14]. Immature coffee fruits produce roast coffees with lower scores than those produced from mature cherries according to sensory analyses [15]. However, the influence of immature coffee fruits during a fermentation process is unknown.

Fermentation has been used to improve coffee quality and promote sensory differentiation. During a spontaneous fermentation, the epiphytic microorganisms from coffee fruit, soil and environment are responsible for mucilage consumption and metabolite production [16,17,18]. Different biochemical processes occur by which enzymes produced by yeasts and bacteria degrade sugars, lipids, proteins, and acids present in the mucilage, transforming them into alcohols, acids, esters, and ketones [19]. The postharvest process and the way of conducting the fermentation influence the final composition and sensory characteristics of coffee [20,21,22,23]. During a Self-induced Anaerobic Fermentation (SIAF), the process can be carried out by conditioning the whole coffee fruits in an appropriate container, with or without the addition of water [24,25,26,27]. However, the interference of water during the coffee fermentation process, including its solubilizing role in substrates and products, and its influence on the resulting beverage, is not yet well studied.

There are still no studies that compare the combined effect of water and immature coffee fruits with mature coffees during fermentation. The sensory aspects that define the quality of a coffee are associated with the chemical composition of the grain. Thus, the present study aimed to conduct the physicochemical characterization and sensory evaluation of Arara coffee fruits from the Cerrado Mineiro, submitted to spontaneous SIAF in submerged and solid-state processes using two different percentages of immature fruits.

2. Materials and methods

2.1. Coffee fruits

Coffea arabica variety Arara was harvested in Patos de Minas, Brazil (latitude 18°35′15″, altitude 1040 m, and longitude 46°25′35″) in 2022. Mechanical harvesting was followed by processing with a horizontal washing machine (Ecoflex, Pinhalense, São Paulo, Brazil), which removed overripe fruits, sticks, stones, and leaves. After that, a sample with 11% of immature fruits was obtained. A manual sorting stage was carried out to remove unripe fruits, resulting in a new sample with 0.3% of immature fruits. The quantification of immature fruits followed a standard agronomic procedure commonly employed in agricultural practices. This method entailed a quintuplicate analysis where one liter of fruits was randomly withdrawn from each sample. Subsequently, trained farm technicians meticulously hand-counted the green (immature) fruits in accordance with established farm protocols.

2.2. Coffee fermentation process

Fermentation treatments were performed using whole coffee fruits, without starter inoculation, in solid-state or submerged process, and with different percentages of immature fruits, as described in Table 1. Fermentations were conducted employing the Self-Induced Anaerobic Fermentation (SIAF) process in closed polyethylene bioreactors (31 cm × 31 cm × 31 cm) equipped with an airlock valve, for 96 h without moisture control. In solid-state fermentations, the bioreactors were filled with approximately 10.4 kg of coffee fruits, equivalent to a height of 21 cm, resulting in a working volume of approximately 20 L. In submerged fermentations, in addition to the fruits, 6 L of water was added. Temperature and pH were measured at different coffee mass points at regular time intervals (0, 24, 40, 48, 64, 72, 88, and 96 h). Temperature and pH measurements were performed using as Akso AK95 portable pH meter equipped with a digital rod thermometer. The thermometer has a measurement range of 0 to 80°C and offers a

Table 1
Treatment identification according to the fermentation process and percentage of immature fruits.

ID	Process	Percentage of immature fruits (%)
SB11	Submerged	11.5 ± 2.96
SB0.3	Submerged	0.358 ± 0.0366
SS11	Solid-state	11.5 ± 2.96
SS0.3	Solid-state	0.358 ± 0.0366
Control	Without fermentation	11.5 ± 2.96

precision of ±0.5°C with a resolution of 0.1°C. The pH meter has a pH measurement range of 0 to 14, a precision of ±0.04 pH units and a resolution of 0.01 pH units. Each fermentation was conducted in triplicate. After fermentation, the cherries were transferred to suspended terraces until obtaining 11.0–12.0% moisture. Samples of cherries (100 g) were collected before and after fermentations and frozen until chemical analyses. A sample with 11% of immature fruits was taken directly to drying in a concrete yard, without fermentation, and used as a control to assess the influence of fermentation.

2.3. Chemical proximate composition analyses and High-Performance Liquid Chromatography (HPLC) analyses

The frozen samples were freeze-dried and ground in a knife mill (SOLAB) to a fine granulometry of 20 mesh. Physicochemical analyses were performed on the powdered samples in accordance with the methods of the Association of Official Analytical Chemists [28]. The following determinations were performed: moisture (method 925.09B), protein content (method 920.87, using 6.25 as correction factor), lipid content (method 920.97), ash content (method 923.03), and titratable acidity (method 942.15).

Organic acids (citric, malic, lactic, succinic, acetic, propionic, and butyric), sugars (sucrose, glucose, and fructose), glycerol and ethanol were analyzed. Samples were extracted using MilliQ water according to Elhalis et al. [29] with modifications. The modifications consisted of a reduction in the crushing step duration, increase in the mass of samples and volume of water that were mixed, changes in the centrifugation parameters, removal of the sediment washing step, and the addition of a filtration step. In the present study, 10 g of fruits were mixed with 100 mL MilliQ water and blended in a domestic blender (Oster 1400 W) for 2 min. The slurry was filtered through two layers of organza polypropylene and then centrifuged (Heal Force Neofuge 18R) at 10.000 rpm for 15 min at 17°C. The supernatants were filtered through a 0.45 μm nylon filter and injected into a liquid chromatography system (Shimadzu Corp., Japan). A Supelcogel C-610H (7.8 mm × 30 cm) column was used with a 0.1% solution of phosphoric acid, with a flow rate of 0.5 mL per min as the mobile phase. The oven temperature was kept at 30°C. Acids were detected with a 210 nm UV detector (SPD), while sugars were detected with a refractive index detector (RID). The results were processed using the LC-Solutions software (Shimadzu Corp., Japan) from linear calibration curves of each compound. Calibration curves ranged from 0.01 to 10 g/L for sugars, ethanol and glycerol, and from 0.05 to 1 g/L for organic acids.

2.4. Sensory analyses

The sensory analyses were performed following the recommendations of the Specialty Coffee Association [30]. A panel of five expert coffee tasters, with a Q-Grader Coffee Certificate, performed the sensory analyses using five cups for each sample (a predetermined ratio of 8.25 ± 0.25 g per 150 mL of water). The attributes evaluated were fragrance/aroma, flavor, aftertaste, acidity, body, balance, sweetness, clean cup, uniformity, overall, and final score, besides the sensory descriptors. Each attribute was scored from 0 to 10, and the final score consisted of the sum of the scores.

2.5. Statistical analyses

Data on carbohydrates, organic acids, ethanol, glycerol concentrations, and proximate composition were evaluated using analysis of variance (ANOVA), and the Duncan test was utilized for comparing means ($p < 0.05$).

The fermentation experiments were carried out in randomized design. A 2^2 factorial experimental design was used to analyze the compounds identified at 96 h: two types of process (solid-state [SS] and submerged [SB]) and two percentages of immature fruits in sample (0.3% [S0.3] and 11% [S11]). Data were analyzed under [**Equation 1**]:

$$X = mean + Proc_i + Imm_j + (Proc \times Imm)_{ij} + e_{ij} \quad (1)$$

where mean = global mean; $Proc_i$ = process effect (i = SS, SB); Imm_j = immature fruits effect (j = S0.3 and S11); $(Proc \times Imm)_{ij}$ = effect of interaction between process and immature fruits; e_{ij} = experimental error. Significance was defined at $p < 0.05$ level.

3. Results and discussion

3.1. Influence of immature fruits in the initial sample composition

After mechanical separation in a horizontal machine, the coffee sample contained 11% immature fruits, and after manual selection to remove the remaining immature fruits, the coffee showed 0.36% immature fruits. The maturation stages of the coffee fruit are marked by the color transition from intense green to cane green and finally red or yellow, depending on the coffee variety [11,31]. Fruit coloring is the simplest way to identify the coffee maturation stage. This identification is crucial as a low incidence of immature fruits is desirable, given their potential negative impact on production yield and coffee beverage quality [32,33].

Propionic acid, butyric acid, lactic acid, ethanol, and glycerol were not found in natural coffee fruits (0 h). There were no significant differences in moisture, lipids, protein, succinic acid, and acetic acid between the coffees with different percentages of immature fruits (Table 2). Although it is not considered a specific acid from coffee, in this study, acetic acid was identified in natural coffee (1.12 and 1.26 mg/g, in S11 and S0.3, respectively). Ribeiro et al. [34] also found acetic acid in initial samples of pulped coffee: 1.6 and 2.3 mg/g, in Ouro Amarelo and Mundo Novo varieties, respectively. There are epiphytic microorganisms that can start fermentation in the fruit still on the tree, as demonstrated by Evangelista et al. [35] and Vilela et al. [12]. Probably, the acid detected in the initial samples comes from the metabolism of these microorganisms.

The initial percentage of immature fruits significantly affected the initial concentration of sugars (sucrose, glucose, and fructose), ash, titratable acidity, and citric acid ($p < 0.05$). Initial sucrose, glucose, and fructose concentrations were significantly higher in S0.3 than in S11 samples ($p < 0.05$) (Table 2). This is expected because in ripe fruits, sucrose is found in pulp and mesocarp, and its concentration increases during ripening when hexoses are converted to sucrose [36].

The ash content is related to minerals present in different compositions and concentrations in the different parts of coffee fruit [3,6]. The major initial ash content of 6.99 ± 0.30% was observed in the sample with a major percentage of ripe fruits (Table 2). It may have occurred because immature fruits have less mucilage completely formed, and they did not yet absorb the maximum minerals of the soil [37].

Regarding the acidity, the highest value was identified in samples with the highest percentage of immature fruits (190.0 ± 3.11 mL of NaOH 0.1 N/100 g (Table 2)). This result agrees with Torres et al. [38] that found higher values of total titratable acidity in immature coffee beans when compared to coffee beans originated from ripe fruits. The acidity is naturally present in coffee fruits, and it comes from various organic acids mainly citric acid, malic acid, and phosphoric acid [39]. Citric and malic acids are the acids most prominent in green coffee [40]. The initial citric acid concentration

Table 2
Effects of water addition and immature fruits percentage on carbohydrates, organic acids, ethanol, glycerol, and proximate composition of coffee fruits submitted to SIAF processing.

Compounds and Processing	Treatments						p value[e]/Effect[f]			
	Natural coffee (0 h)		SB (96 h)		SS (96 h)		Processing (P)	Immature % (Im)	P x Im	ER[g]
Sucrose (mg/g)							<0.01[e]	<0.01[e]	<0.01[e]	0.08[g]
S0.3	33.15 ± 1.69	d	0.97 ± 0.10	a	1.93 ± 0.38	ab	0.61[f]	1.15[f]	1.57[f]	
S11	18.19 ± 1.66	c	3.69 ± 0.41	b	1.51 ± 0.10	a				
Glucose (mg/g)							<0.01[e]	<0.01[e]	0.03[e]	0.22[g]
S0.3	49.24 ± 2.54	e	8.29 ± 1.03	a	11.29 ± 0.21	ab	−4.12[f]	6.14[f]	−1.12[f]	
S11	32.33 ± 0.35	d	13.31 ± 0.15	b	18.56 ± 1.09	c				
Fructose (mg/g)							<0.01[e]	<0.01[e]	<0.01[e]	0.58[g]
S0.3	76.26 ± 3.62	d	31.32 ± 1.04	a	32.48 ± 0.60	a	−6.71[f]	6.09[f]	−5.55[f]	
S11	54.30 ± 0.04	c	31.86 ± 2.05	a	44.12 ± 3.24	b				
Citric Acid (mg/g)							0.73[e]	0.81[e]	0.15[e]	0.03[g]
S0.3	0.65 ± 0.02	a	0.64 ± 0.20	a	0.52 ± 0.01	a	0.02[f]	−0.01[f]	−0.09[f]	
S11	1.06 ± 0.02	b	0.53 ± 0.00	a	0.60 ± 0.01	a				
Malic Acid (mg/g)							<0.01[e]	<0.01[e]	0.062[e]	0.04 [g]
S0.3	10.11 ± 1.19	d	0.98 ± 0.16	a	1.65 ± 0.05	ab	−0.85[f]	0.58[f]	−0.18[f]	
S11	8.80 ± 0.19	c	1.38 ± 0.05	a	2.42 ± 0.24	b				
Latic Acid (mg/g)							<0.01[e]	<0.01[e]	0.67[e]	0.69[g]
S0.3	1.31 ± 0.64	a	67.44 ± 1.53	d	51.27 ± 0.12	b	15.57[f]	−11.72[f]	−0.60[f]	
S11	0.00 ± 0.00	a	55.12 ± 1.67	b	40.15 ± 4.18	c				
Succinic Acid (mg/g)							<0.01[e]	0.35[e]	0.09[e]	0.06[g]
S0.3	1.84 ± 0.52	c	0.41 ± 0.12	ab	0.78 ± 0.07	ab	−0.61[f]	0.12[f]	−0.23[f]	
S11	1.87 ± 0.50	c	0.30 ± 0.00	a	1.14 ± 0.41	bc				
Acetic Acid (mg/g)							<0.01[e]	0.12 [e]	0.39 [e]	0.16[g]
S0.3	1.26 ± 0.03	a	4.15 ± 0.21	b	5.63 ± 0.34	c	−1.78[f]	−0.58[f]	−0.30[f]	
S11	1.12 ± 0.17	a	3.27 ± 0.16	b	5.35 ± 1.05	c				
Propionic Acid (mg/g)							0.26 [e]	0.51[e]	0.39 [e]	0.01[g]
S0.3	0.00 ± 0.00	b	0.22 ± 0.06	a	0.20 ± 0.05	a	0.03[f]	−0.02[f]	0.02[f]	
S11	0.00 ± 0.00	b	0.22 ± 0.04	a	0.20 ± 0.01	a				
Butiric Acid (mg/g)							–	–	–	–
S0.3	ND		ND		ND		–	–	–	
S11	ND		ND		ND					
Ethanol (mg/g)							<0.01[e]	<0.01[e]	0.65 [e]	0.75[g]
S0.3	0.00 ± 0.00	a	21.80 ± 2.28	b	42.84 ± 0.73	d	−20.34[f]	−17.34[f]	0.69[f]	
S11	0.00 ± 0.00	a	5.16 ± 0.29	c	24.80 ± 4.63	b				
Glycerol (mg/g)							<0.01[e]	<0.01[e]	<0.01[e]	0.02[g]
S0.3	0.00 ± 0.00	a	0.36 ± 0.05	b	1.68 ± 0.17	d	−1.06[f]	−0.60[f]	0.27[f]	
S11	0.04 ± 0.04	a	0.02 ± 0.00	a	0.81 ± 0.02	c				
Moisture (g/100 g)							0.09 [e]	0.69 [e]	0.43[e]	0.95[g]
S0.3	66.59 ± 2.89	b	72.20 ± 1.19	a	69.59 ± 1.89	ab	1.82[f]	−0.43[f]	−0.79[f]	
S11	66.30 ± 0.32	b	70.99 ± 2.39	a	69.95 ± 0.49	a				
Ash (g/100 g)							<0.01[e]	0.35 [e]	0.32[e]	0.12[g]
S0.3	6.99 ± 0.30	c	5.19 ± 0.37	b	6.04 ± 0.17	a	−0.99[f]	−0.12[f]	−0.13[f]	
S11	5.95 ± 0.05	a	4.93 ± 0.09	b	6.05 ± 0.10	a				
Lipid (g/100 g)							<0.01[e]	<0.01[e]	0.22 [e]	0.09[g]
S0.3	4.22 ± 0.12	a	3.39 ± 0.03	bc	4.21 ± 0.33	a	−0.69[f]	−0.41[f]	0.13[f]	
S11	4.27 ± 0.37	a	3.11 ± 0.03	b	3.67 ± 0.06	c				
Protein (g/100 g)							0.60 [e]	0.06 [e]	0.07 [e]	0.26[g]
S0.3	9.96 ± 0.14	ab	9.96 ± 0.34	ab	10.65 ± 0.64	b	−0.14[f]	−0.58[f]	0.55[f]	
S11	9.89 ± 0.11	a	9.93 ± 0.37	a	9.53 ± 0.38	a				
Acidity (mL NaOH 0.1 N/100 g)							<0.01[e]	<0.01[e]	0.83 [e]	2.06[g]
S0.3	179.47 ± 2.58	b	286.98 ± 3.15	e	270.78 ± 5.76	a	15.73[f]	−9.98[f]	−0.47[f]	
S11	190.06 ± 2.20	c	276.53 ± 0.49	a	261.27 ± 2.81	d				

Data are presented as means followed by their respective standard deviations. For each compound, mean values with different lowercase letters are statistically different at $p < 0.05$ by the Duncan test. [e]p-value. [f]Effects values. [g]Standard error of the means. SB, submerged fermentation process; SS, solid-state fermentation process; S0.3, coffee sample with 0.3% of immature fruits; S11, coffee sample with 11% of immature fruits. ND: not detected.

was significantly higher in the S11 treatment (1.06 mg/g), while malic acid was higher in the S0.3 treatment (10.11 mg/g). There is concern about the difference in composition of coffee fruits harvested at different degrees of ripeness, and some studies investigate chlorogenic acids and volatile compounds [41,42]. However, studies about organic acids variations' during ripeness were not found.

3.2. Influence of immature coffee fruits and water during coffee fermentation

3.2.1. Physicochemical characterization

Temperature monitoring during SIAF fermentation is widely applied and can indicate microbiological activity [25,27]. During fermentation, energy is released by the biochemical reactions that form alcohols, acids, and other compounds [22]. In this study, the mass temperature ranged from 17.8 to 21.0°C, and the environmental temperature ranged from 16.0 to 29.0°C (night/day). The temperature in the solid-state process remained higher than in the submerged state for most of the fermentation time. Despite the heat loss during the night, it was possible to observe microbiological activity capable of increasing the temperature during the day (Fig. 1).

The pH of the aqueous coffee extract specifies its perceived acidity and indicates the progress of acid production during fermentation [32]. There was a reduction in pH during the fermentation for all treatments. After 64 h, lower pH values were observed for the submerged processes. The final values were 3.9 and 4.2 in

the submerged and in the solid-state process, respectively. This behavior may be indicative of high acid dissolution in the aqueous phase during the process (Fig. 1).

3.2.2. Sugars

Reductions in sugar concentrations were detected in all processing types; however, no sugar was completely consumed by the end of the fermentations (Table 2). The reduction in sugars is a result of the microbiological activity, consuming the mucilage of the grains [18,29]. The lower final sucrose concentration found in this study indicates the action of the enzyme β-fructofuranosidase, which acts in the generation of monosaccharides glucose and fructose [43].

Both factors studied had significant effects on the final glucose, and fructose concentrations. On average when the process changed from solid-state to submerged, the final concentrations of glucose and fructose decreased, and sucrose increased, while increasing the percentage of immature fruit in the initial sample (11%) favored higher residual values in all sugars. After submerged fermentation, the sucrose and glucose concentrations were significantly lower in S0.3 (0.97 and 8.29 mg/g, respectively), and the highest fructose concentration (44.12 mg/g) was identified in the solid-state process and S11 (Table 2). Knopp et al. [21] found similar results. After submerged fermentation, only low amounts of fructose and glucose were present, while concentrations of those sugars were significantly higher after the solid-state process.

Although the initial fructose concentration was about 1.7 higher than glucose, the residual fructose was about 3-fold higher than residual glucose (Table 2). That is because glucose is the preferential molecule used in the fermentative process. The metabolism of the fruits in post-harvest changes from aerobic to anaerobic pathways, when the lactic and alcoholic fermentation require glucose as the main substrate [29]. The residual sucrose found in this study is probably from the endosperm and it is an interesting result because sucrose is an import precursor of flavor compounds during roasting and can contribute to sweetness in coffee beverage [29,44,45].

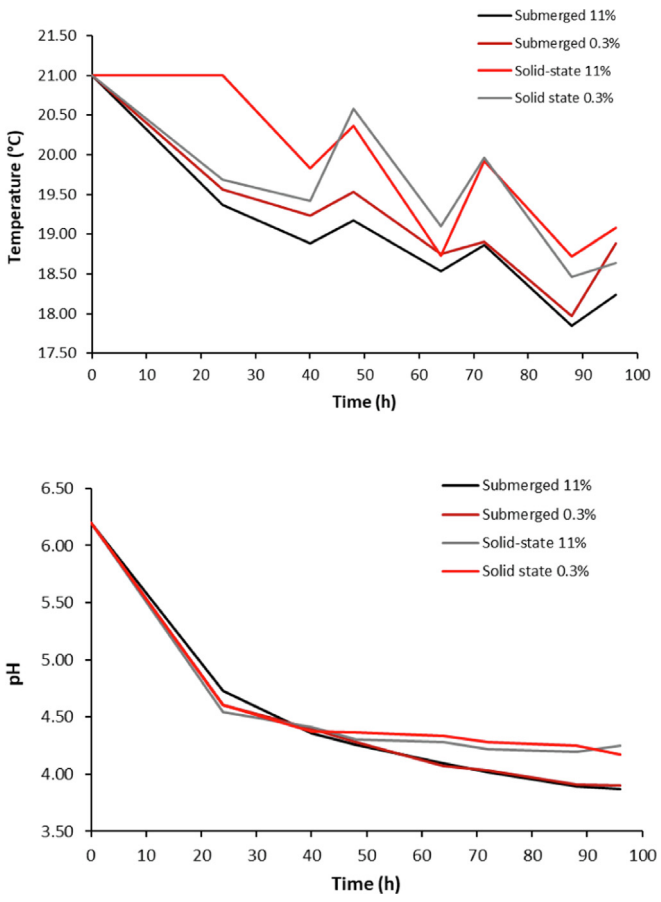

Fig. 1. Temperature and pH variation during the fermentative processes.

3.2.3. Organic acids

Organic acids are frequently determined after the coffee fermentation process. Citric, malic, and succinic acids are normally naturally found in coffee fruits. On the other hand, acetic, lactic, butyric, and propionic can be produced during the fermentative process [18,22,24,46,47].

The butyric acid was not identified in any treatment. The final concentrations of citric and propionic acids were not influenced by the studied factors and did not show significant differences in any of the four treatments ($p > 0.05$). After the fermentation process, the citric acid concentration ranged from 0.52 to 0.64 mg/g. Compared to the initial sample, a significant decrease in citric acid was observed only for S11 (Table 2). Similarly, Bressani et al. [46] observed a decrease in citric acid concentrations after 40 h of fermentation of Bourbon Amarelo, obtaining values from 0.53 to 1.66 mg/g. The reduction can be attributed to the fact that citric acid can serve as a precursor to other acid decomposition products, such as glutaric, fumaric, and maleic acids [40].

Propionic acid, which was not detected in the natural fruit, achieved a concentration of 0.2 mg/g after fermentation in all treatments (Table 2). Propionibacteria and several other genera of anaerobic bacteria such as *Veillonella*, *Selenomonas*, and *Clostridium*, especially *Clostridium propionicum*, produce propionic acid as a main fermentation product. It can be produced from lactic acid [48,49]. Since high concentrations of lactic acid were identified at the end of all treatments, the presence of this acid is justified. It is known that butyric acid and propionic acid develop in the later stages of coffee fermentation; however, concentrations below 2.09 mg/g are not considered prejudicial to coffee quality [19,44]. This suggests that 96 h was a satisfactory time to avoid over-fermentation in the environmental conditions of this study.

The final concentrations of succinic and acetic acids were only significantly affected by the addition of water during the fermentative process. Succinic acid is a dicarboxylic acid naturally present in coffee fruit, and it is also produced by several microorganisms [50]. It is an intermediate of the tricarboxylic acid (TCA) cycle and one of the end products of anaerobic metabolism, and it is synthesized in almost all microbes, plants, and animal cells [51]. After fermentation, succinic acid decreased significantly in all treatments except in SS11 varying from 0.30 to 1.14 mg/g. On average, the final concentration of succinic acid was major in the solid-state processes. Ribeiro et al. [18] found succinic acid concentrations of 2.61 to 4.42 mg/g in initial coffee; and after fermentation, concentrations of 0.74 to 3.85 mg/g. Bressani et al. [46] observed after spontaneous fermentations succinic acid contents varying from 0.18 to 1.01 mg/g.

In this study, there was an increase in the acetic acid content in all types of fermentations. The initial immature fruit percentage did not significantly interfere in the acetic acid formation. On average, when the process changed from solid-state to submerged, acetic acid decreased. After fermentation, the acetic acid increased significantly to 4.15 and 5.63 mg/g (SB0.3 and SS0.3, respectively), and 3.27 and 5.35 mg/g (SB11 and SS11, respectively). Similar findings in SIAF spontaneous coffee fermentation have been reported elsewhere. Bressani et al. [24] found that the highest acetic acid concentration was found in the SIAF spontaneous fermentation (7.59 mg/g). Ribeiro et al. [18] observed an increase of acetic acid in Mundo Novo coffee after fermentation, whose concentration

changed from 2.23 to 7.0 mg/g. Acetic acid is identified as a beneficial product in some fermented foods, as well as coffee [48]. Bacterial sequences assigned to the genera *Acetobacter* were detected during the spontaneous SIAF process [17,29]. *Acetobacter* can produce acetic acid by oxidizing ethanol under aerobic conditions; however, in the SIAF method, the presence of oxygen is low and only available for the first few hours [48]. Therefore, the production of acetic acid can be attributed to the heterolactic pathway performed mainly by lactic acid bacteria and Enterobacteriaceae [19].

Lactic and malic acids had the final concentration affected by the two studied factors. Malic acid content decreased significantly in all treatments, and it was lower in submerged processes (0.98 and 1.38 mg/g to S0.3 and S11, respectively). An increase in the percentage of immature fruits promoted an increase in final malic acid concentration. Malic acid is naturally present in coffee fruit, and it is possibly produced by endogenous microorganisms present in the soil [39]. The reduction of malic acid after coffee fermentation process is reported by several authors [24,25,44]. Cassimiro et al. [25] found a reduction of malic acid from 3.48 to 0.39 (mg/g) after 72 h of SIAF spontaneous fermentation. Malolactic fermentation is responsible for the conversion of malic acid into lactic acid and CO_2. This fermentation can occur during or after alcoholic fermentation, and it is carried out by lactic acid bacteria, which are able to grow at low pH values [52].

The lactic acid is not naturally found in coffee fruits [40,53]. The lactic acid is produced mainly by lactic acid bacteria present in coffee fruits, and it significantly influences the final coffee quality [17,18,35,54]. Lactic acid content increased significantly in all treatments, and it was higher in submerged fermentation with less immature fruits, 67.44 mg/g. On average, when the process changed from solid-state to submerged, lactic acid concentration increased. On the other hand, when the percentage of immature fruits changed from 0.3% to 11%, lactic acid concentration decreased. The lactic acid production was favored by higher sugar concentrations. Just like in this study, the lactic acid is a common acid found at the end of coffee fermentative processes. Pereira et al. [53] found that lactic acid was the major end-metabolite of carbohydrate metabolism during submerged fermentation for 24 h. In the study of Ribeiro et al. [55], lactic acid concentration ranged from 0.71 to 8.14 mg/g after wet fermentation for 48 h. Bressani et al. [24] found 1.27 mg/g of lactic acid at the end of a spontaneous solid-state SIAF process. The homofermentative LAB usually uses hexose and pentose sugars via the Embden-Meyerhof pathway. Homofermentative LAB produces two lactic acid molecules as a major end-product per mole of consumed glucose [56]. In addition to sugars, LAB species have the ability of metabolizing citrate present in high concentration in the coffee pulp [57].

3.2.4. Ethanol and glycerol

Although it was not identified in the initial sample, ethanol was identified in all treatments after fermentation. Ethanol accumulation after fermentation has been reported previously by Elhalis et al. [58] and confirmed in our study. The highest final concentration (42.84 mg/g) was found in coffee processed by solid-state fermentation and minor immature fruits (Table 2). On average when the process changed from solid-state to submerged, ethanol concentration decreased. Likewise, when the percentage of immature fruits changed from 0.3% to 11% there was a decrease in ethanol concentration. Ethanol can be produced by yeasts during the alcoholic fermentation and by LAB in the heterofermentative process [57,59]. Ethanol is one of the most abundant alcohols found after coffee fermentation, it has an essential role in the beverage viscosity, and it serves as a solvent for volatile compounds [58]. Ethanol in coffee beans shows an alcoholic sensory description [16].

Glycerol concentration increased in all treatments after fermentation, except for SB11, in which concentration remained constant (Table 2). The final concentration was 0.36 and 1.68 mg/g (SB0.3 and SS0.3, respectively), and 0.02 and 0.81 mg/g (SB11 and SS11, respectively). Glycerol concentration decreased significantly when the process changed from solid-state to submerged, and when the percentage of immature fruits changed from 0.3% to 11%. According to Elhalis et al. [58], the endosperm did not contain glycerol initially, but it was detected as the fermentation progressed, reaching a maximum concentration of 0.08%. Yeasts are the microbial group responsible for producing glycerol during coffee fermentation [58]. High sugar concentration favors glycerol production, because this compound protects yeast from high osmotic stress [59]. The glycerol has an exceptional role in Maillard reactions, and it has the potential to act as an active flavor precursor. In addition to its role of solvent, glycerol actively contributes to the formation of proline-specific compounds in Maillard model systems [60]. Glycerol has a sweet taste and smooth mouthfeel [59]. The highest value of glycerol found in this study occurred in the same process where the highest value of ethanol was found, in SS0.3 (1.68 and 42.84 mg/g, respectively). This may suggest some relationship between the production of these compounds. The water in fermentative process may be responsible for solubilizing some compounds.

3.2.5. Proximate composition

Neither the type of process nor the percentage of immature fruits had a significant effect on the final coffee's protein and moisture content ($p > 0.05$) (Table 2). The moisture increased in all treatments, except in SS0.3. Since the final moisture was statistically equal in all treatments, the application of submerged fermentation may not extend the drying time, when compared to solid-state fermentation.

The final ash content was not affected by the immature fruits during the fermentation. The highest ash concentration was found after solid-state fermentation. After fermentation, the final ash concentration was 5.19 and 6.04 mg/g (SB0.3 and SS0.3, respectively), and 4.93 and 6.05 mg/g (SB11 and SS11, respectively). On average, when the process changed from solid-state to submerged, ash decreases by 0.98 mg/g. It may be because of the solubilization of the minerals of the coffee in the water.

Lipid content decreased in all processes except in SS0.3 (Table 2). After fermentation, the final lipid concentrations were 3.39 and 4.21 mg/g (SB0.3 and SS0.3, respectively), and 3.11 and 3.67 mg/g (SB11 and SS11, respectively). The lipid concentration decreased when the process changed from solid-state to submerged, and when the percentage of immature fruits changed from 0.3% to 11%. Lipid consumption by microorganisms was higher in coffee that had fewer sugars as substrates.

The titratable acidity increased significantly in all samples after fermentation ($p < 0.05$). After fermentation, the titratable acidity was 286.98 and 270.78 mL NaOH 0.1 N/100 g (SB0.3 and SS0.3, respectively), and 276.53 and 261.27 mg/g (SB11 and SS11, respectively). Similarly, Elhalis et al. [29] showed that the coffee spontaneous fermentation was responsible for increasing the titratable acidity. On average, the titratable acidity increased when the process changed from solid-state to submerged, and it was higher in S0.3 samples. These results are correspondents with the lactic acid, the main acid quantified in this study, showing the relationship between organic acid production and titratable acidity.

3.2.6. Sensory analysis

The SIAF spontaneous fermentations were able to modify the sensory descriptions and the final score of the coffees as shown in Fig. 2 and Table 3. According to the SCA protocol, all coffees produced in this study can be classified as specialty coffees and showed final scores above 84 points. The scenario with 11% of

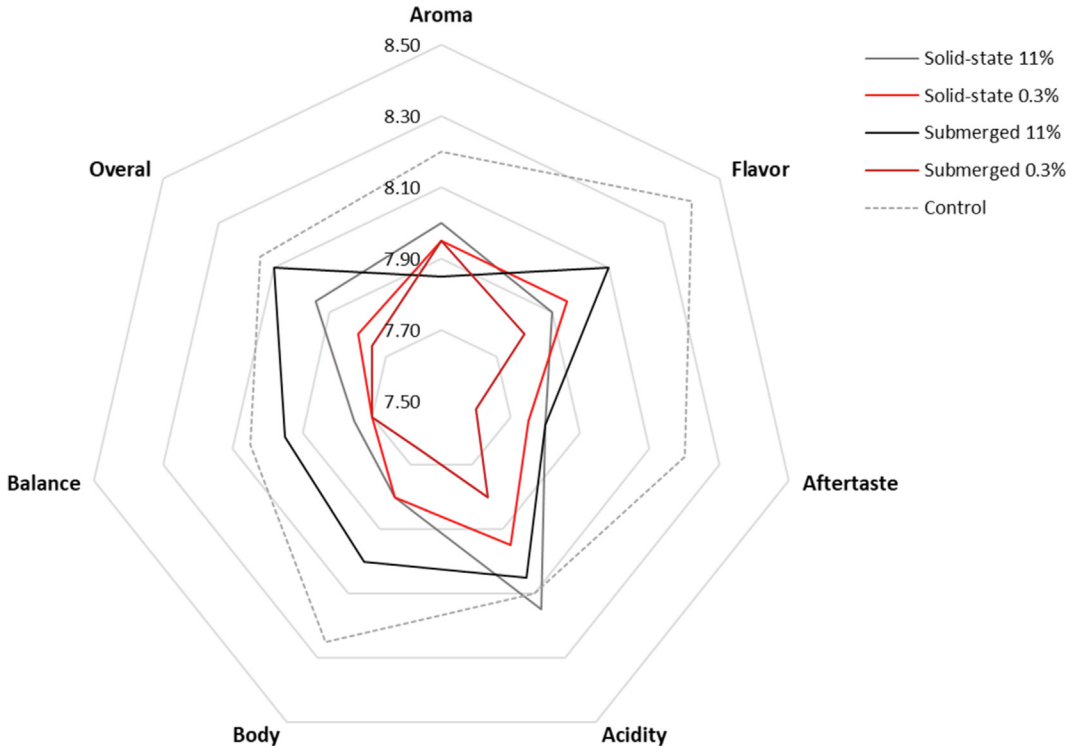

Fig. 2. Results of the sensory evaluation of coffees according to the SCA protocol: average scores for each attribute. The attributes uniformity, sweetness, and clean cup were omitted from the figure because in all treatments, they receive a score of 10.

Table 3
Coffee beverage sensory descriptions and final score by SCA protocol.

Treatment	Sensory Descriptions	Final Score
SB11	Caramel, brown sugar, honey, orange, lemon, floral, nut, yellow fruits, red fruits	86.50 ± 0.53[a]
SB0.3	Chocolate, floral, brown sugar, honey, nuts, orange, grape, pineapple, apple, spices	84.25 ± 0.92[a]
SS11	Caramel, molasses, wine, red fruits, orange, bitter	85.35 ± 1.43[a]
SS0.3	Chocolate, nuts, wine, alcoholic, dry fruits, orange, strawberry, passion fruit	85.75 ± 1.55[a]
Control	Chocolate, brown sugar, honey, yellow fruits, vanilla	87.35 ± 1.46[a]

Data are presented as means followed by their respective standard deviations. SB11: coffee from submerged fermentation process carried out with 11% of immature fruits; SB0.3: coffee from submerged fermentation process carried out with 0.3% of immature fruits; SS11: coffee from solid-state fermentation process carried out with 11% of immature fruits; SS0.3, coffee from solid-state fermentation process carried out with 0.3% of immature fruits; Control, coffee with 11% of immature fruits from conventional drying and without fermentation. Using the Scott Knot test, there were no significant differences in the final scores of the coffees obtained by the different treatments ($p > 0.05$).

immature fruits in the submerged process (SB11) exhibited the best sensory results: aroma (8.0), flavor (7.9), after taste (7.8), acidity (8.15), body (7.8), balance (7.75), and overall (7.95) (Fig. 2). The spontaneous fermentation using SIAF method in solid-state process has been related to specialty coffee production [24,61]. Differently from Cassimiro et al. [25], our results showed specialty coffee production using natural coffee and submerged process in spontaneous fermentation using the SIAF method.

The sensory analysis results were consistent with the chemical analyses and confirmed that immature fruits and water addition can modify the sensory perceptions of coffee beverage. The changes in the chemical composition of the coffee caused by each process variable analyzed resulted in beverages with a specific sensory profile, such as lemon in SB11, grape, pineapple, apple, and spices in SB0.3, bitter in SS11, and dry fruits, strawberry, and passion fruit in SS0.3. The fruit sensory perception and orange were found in all treatments and probably are related to the Arara coffee variety. The chocolate sensory perception was found only in S0.3 treatments. The concentration of ethanol interferes with the tasters' sensory perceptions. Only the solid-state treatments presented alcoholic descriptions, as well as the major concentrations of ethanol at the final of fermentations (Table 3).

The acidity in sensory analyses can be related to total titratable acidity [32]. The best score for sensory acidity was found in SS11 and the worst was found in SB0.3 (Fig. 2). The sample with the lowest value of titratable acidity, corresponding to SS11, was more valued during the sensory analysis. On the other hand, the highest value of titratable acidity, corresponding to SB0.3, was penalized during the sensory analysis. Although the initial titratable acidity was higher in S11 fruits, organic acids produced during fermentation seemed to have a greater influence in the perception of acidity in the sensory tests.

It is known that the incomplete maturation of coffee beans can be related to a metallic and astringent flavor caused by high concentrations of chlorogenic acids and phenolic compounds [31,62]. However, according to the results found in this study, the fraction of 11% of immature fruits during processing may not have a negative influence on the final score of the drink evaluated by the SCA protocol. There is no information in the literature about immature fruits' influence during coffee fermentation. This work is thus the

first to show that the immature fruits can modify the results of fermentation process. Therefore, the immature fruit percentage and the fermentation method (solid-state or submerged) influenced the sensory results.

4. Conclusions

The percentage of immature fruits directly influences the initial concentration of glucose, sucrose, fructose, ash, citric acid, and titratable acidity. During the fermentative process, this variable was able to significantly modify the lactic acid, ethanol, and glycerol production. It also altered the final concentration of sugars, lipids, and titratable acidity. The lower percentage of unripe fruits (S0.3) was related to lower consumption/degradation of lipids during fermentation. This shows that the presence of immature fruit during fermentation significantly alters the availability of substrates for fermentation, mainly sugars, and consequently the capacity for metabolite production.

The presence of water during the fermentation process did not significantly influence the final concentration of citric acid, propionic acid, moisture, and protein. The submerged process favored the production of lactic acid and gave rise to coffees with lower concentrations of ethanol, glycerol, malic acid, acetic acid, and succinic acid. These results suggest that water may have a solubilizing action during the coffee fermentation process.

The presence of 11% immature fruits during processing may not negatively impact the final score of the beverage evaluated using the SCA protocol. Furthermore, there was no significant difference ($p < 0.05$) in the sensory analysis scores between the coffees produced through solid-state fermentation and submerged fermentation. All coffees were classified as specialty coffees with a score above 84 points. Based on the findings of this study, if the harvest results in a composition where a maximum of 11% of the fruits are green, there is no need for the removal of immature fruits to ensure the production of high-quality coffees. This provides a significant advantage for the producer, eliminating the fruits' selection step and allowing the utilization of green fruits, traditionally considered of lower quality, in the production of specialty coffees. Although the processing conditions assessed in this study did not result in significant differences ($p < 0.05$) in the scores obtained from sensory evaluations, there was a notable variation in chemical compounds after fermentation, as well as in the description of sensory attributes perceived by the panel of tasters. In other words, each processing method yielded a specialty coffee with distinct sensory perceptions. Therefore, producers can obtain and offer differentiated products to the market by introducing a fermentation step in coffee post-harvest processing or by using different percentages of immature fruits.

Ethical approval

The study protocol was approved by the Ethics Committee of Federal University of Uberlândia, with the approval number 73864723.0.0000.5152.

Author contributions

- Study conception and design: L Santos, L Oliveira, L Ferreira.
- Data collection: L Ferreira, I Casé.
- Analyses and interpretation of results: L Ferreira, P Bertarini, L Santos, L Oliveira.
- Draft manuscript preparation: L Ferreira.
- Revision of the results and approval of the final version of the manuscript: L Santos, L Oliveira, L Ferreira, P Bertarini, I Casé.

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Declarations of interest

None.

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Supplementary data

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