Effect of Fermentation of Arabica Coffee on Physicochemical Characteristics and Sensory Analysis

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Abstract

This work studied the physicochemical characteristics and final sensory quality of the Yellow Catuai IAC 62 fermented with *Saccharomyces cerevisiae*. An experimental design was done by a Composite Central Rotational Design (CCRD) to evaluate the fermentation time and temperature. The responses were: fermentative mass pH, fruit moisture (just as processes were conducted), sugars, organic acids content (HPLC), and sensory quality by Specialty Coffee Association (SCA) methodology on two different roasts levels (light-medium roast and medium roast). It was observed that coffees, even in extreme time and temperature, behave similarly related to final scores (in general from 81 to 85 in SCA score), all classified as specialty coffees. Fructose content varies only with fermentation time regardless of temperature, decreasing its levels as time increases, from 12g/L to near 5g/L. In contrast, sucrose content decreased while time and temperature advanced, from 4g/L to 2g/L in lowers time or temperature for sucrose, similar behavior for glucose from 7g/L to zero, however it shows some concentration increase on lowers time or temperature. Glycerol was formed depending on both time and temperature, and its content increased with time and temperature increase, showing optimum formation conditions between 24°C and 32°C and 35 to 45 hours. pH behaved just as described by literature, decreasing with time advance and more intensely as temperature increases, beginning from around 5 and getting to lower than 4 in extreme cases. Organic acids such as acetic, propionic, succinic, and lactic were measured at final fermentation and presented in different concentrations.

1. Introduction

Coffee is one of the most popular and consumed beverages in the world. According to the Food and Agriculture Organization of United Nations, Brazil was the highest coffee producing country in the world between 1994–2019 (FAOSTAT, 2019), with a production of more than 58 million of 60kg bags in 2019–2020 crop year (WCO 2021a) and exported, only from May to October of 2021, more than 17 millions of 60kg bags (WCO 2021b). This beverage has a rich and complex flavor of olfactory sensations, making it a more excellent added value product. The taste of a freshly prepared coffee cup is a final expression and a noticeable result of a long chain of operations that transforms the bean and takes it to the cup (Joët et al., 2010). The definition of the coffee's quality as a beverage is extensive, depending on the beans' chemical composition, determined by several factors: harvesting, processing methods, preparation, and roasting of the coffee (Pimenta et al., 2018). The flavor is a decisive parameter and is significantly influenced by the processing to transform the cherries into green beans (Wang et al., 2019). Elhalis, Cox and Zhao (2020) believe that post-harvest processing has a significant impact on the final taste, directly affecting alcohols, sugars, and acids concentration, but with still unknown effects of the entire process.

The coffee fermentation process can occur in a submerged medium or solid-state. Bioreactors can provide controlled environmental conditions for fermentation development (Tang et al., 2021). Solid fermentation is a process carried out on solid materials in the absence or low moisture of water that acts as physical support and a source of nutrients for microorganisms. Due to this low water availability, a limited number of microorganisms can develop for solid fermentation.

In the dry process, coffee cherries are fermented and dried simultaneously immediately after harvest, taking 20 days (Brando and Brando, 2014; Silva, 2014). This process is generally totally aerobic and can retain the highest glucose and fructose concentration in the fruits (Knopp et al., 2006). These coffees have a chemical characteristic of natural coffees, with a higher soluble solids and total sugars (Ribeiro et al. 2011). Consequently, the beverage has a superior body and more remarkable sweetness.

Several authors evidenced the use of microorganisms that initiate fermentation (Evangelista, Silva et al. 2014; Elhalis, Cox, Frank & Zhao et al. 2020, Evangelista, Miguel et al. 2014; Martinez et al. 2017; Pereira et al. 2015; Ribeiro et al. 2017) as conductors of an appropriate fermentative process producing unique coffees, with scores above 80 points, according to Cupping Specialty Coffee protocols-SCA (Specialty Coffee Association of America, 2020). During fermentation, microorganisms produce several metabolites. The microbial activity and the extent of fermentation determine the concentration of free sugars (fructose and glucose, for example) and free amino acids, which remain in the bean and subsequently contribute to the production of the compounds of the Maillard reactions and volatile compounds during the roasting process (Haile and Kang, 2019b).

The aroma of coffee is associated with yeast metabolism. It is evident from several studies that the addition of selected yeasts to wild yeasts from the coffee cherry microbiota affects coffee aroma. *Saccharomyces cerevisiae* is an important microorganism that adapts well to solid fermentation conditions and can be used worldwide to produce food and beverages (Santos da Silveira et al., 2019). The yeast *Saccharomyces cerevisiae* has been reported to improve the sensory quality of coffee, contributing to coffee's fruity aroma at the end of the roast (Bressani et al., 2018; Wang et al., 2020).

Most coffee fermentation processes are still carried out worldwide without environmental control and spontaneously produce coffee beans with inconsistent and unpredictable quality (Elhalis, Cox and Zhao 2020). This study aimed to analyze the effects of fermentation of Arabica coffee, the Catuaí Amarelo strain, on the physical-chemical characteristics and the product's sensory result, to be a first step on applying bucket method of coffee fermentation to apply it at farm conditions and made optimized process to increase coffee quality

2. Material And Methods

2.1 Source of Material

The rural property chosen to harvest the fruits is located in the greater coffee production region in Carmo do Paranaíba, Minas Gerais - Brazil. The coffee field, taken from the samples, is in the geographical coordinates Lat.: *18 57'37"S*/ Long.:*46 36'17"O*, with an altitude of 1,080 meters. The cultivar, from the area mentioned above, is the Arabica coffee of the Catuaí Amarelo strain, IAC 62, which, according to the Coffee Research Consortium (2020), is a variety

that produces most fruits with beans called flat beans, medium, retained in #16 sieve, without mochas, leaving to sensory evaluation only big flat beans. Generally, it has more than 82% of flat beans, with excellent beverage quality.

Samples were from the crop year 2019/2020, randomly harvested a final amount of 500 liters of coffee fruits (for 220 L of ripe coffee fruits) in about 5 different times, always running through the entire coffee field.

2.2 Sample collection and preparation

The coffee was harvested manually to select only the ripe fruits, having more significant potential for quality in the cherry stage, which, yellow fruits, has brass color and no greenish color in any part of it. The harvested fruits were placed in plastic containers, and after harvesting, a new classification was carried out to separate fruits of higher quality. All the fruits were submerged in water using a 500 L reservoir to simulate the same process performed by the coffee washer, which separates the dried fruits or those with granulation problems. Dried fruits or those with grainy problems (float) are less dense than water and float, and these fruits were discarded. Immature fruits were selected manually and discarded due to their astringency. Cherry fruits were considered for fermentation, with a bronze-yellow color throughout the skin's length. The selected fruits were placed in plastic containers with cold water and placed in a cool box with ice to keep the temperature low (< 4ºC), according to the method used by Carvalho Neto et al. (2018). This procedure avoids the fermentation process from the farm to the laboratory during coffee transportation, where the experiments and analyses were carried out.

2.3 Incubation

The commercial yeast *Saccharomyces cerevisiae* of the commercial brand Lallemand ORO® (10^{10} UFC/g) was kindly provided by Lallemand. The manufacturer's ratio was used for the incubation, with 12 g (0,012 kg) of yeast for each 10 kg of coffee. The yeast was weighed on an analytical balance (Shimadzu, model BL-3200H), dissolved in distilled water, and applied to the mass coffee ratio of 10 g of commercial yeast for 500 mL distilled water. There were used Neubauer chamber method for cell count, with previously prepared solution, diluted 1 ml for 100 ml, according to Eq. 1, were C_1 and C_2 were quadrant mean counts: After Neubauer chamber count, were found about 2.55×10^8 cell/mL of solution.

$$Cellcount\left(\frac{Cell}{ml}\right) = \left(\frac{C_1 + C_2}{2}\right) \times 2{,}5 \times 10^5 \times \frac{1}{100} \quad (1)$$

2.4 Solid fermentation

The fermentation was carried out in a batch process. Each batch was fermented a volume of 6 L of coffee, divided into open beakers of 2 L, with approximately 2.4 kg of mass. An incubator with temperature control was used for fermentation (Tecnal, model TE-421). The temperature was determined, for each experiment, according to the experimental plan. Samples of 200 g were taken every 4 hours to measure moisture, pH, sugars, and organic acids. Manual turning over the entire bean mass was realized to ensure the aerobic fermentation environment during the sampling.

2.5 Drying and final samples treatment

The beans' final drying was carried out in an oven, with forced air circulation (Ethik Technology, Model 400/8D) at a controlled temperature and intermittent drying processes, traditionally applied to coffee in conventional processing. After half drying (darkening of the fruit peel), the process occurred in cycles of 8 hours of drying with a temperature of 37.5ºC, with rest intervals of 8 hours of the bean mass at room temperature (Isquierdo et al., 2013).

After drying, the samples were adequately packed in high-strength low-density polyethylene bags, identified, and packed in a BOD Incubator, at 25°C (Solab, Model SL-225/364) hermetically sealed to prevent the entry of light and contamination. For 60 days, the coffees were stored and later removed for milling. A mechanical coffee mill carried out the exocarp and dry endocarps' complete removal for samples located on the farm (Model DRC; Pinhalense). This stage transformers the dry cherries into green raw coffee without any classification (this is a raw coffee, without proper separation by size and removal of defects, just as done on commercial coffees evaluation). After this step, samples were completely placed in plastic packaging, traditionally used to store coffee samples. Thus, the samples were sent directly to the classification for the separation of defects (impurities and imperfect beans) and separation of only large beans (beans retained in the 16-coffee sieve, without mochas, without clubs, for their sensory analysis (SCA, 2020a).

2.6 Sample’s classification

Samples were classified based on the standards established by IN 08/2003 (Brasil, 2003). All intrinsic and extrinsic defects were removed entirely. For the separation of big beans, the number 11 oblong sieves (separation of mocha beans) and round classification sieve of size 16/64” were used (this sieve is the same as Sieve #16 of coffee classification). Only the beans retained in the 16/64” sieve without straws went through the classification stage.

2.7 Physicochemical analysis of moisture and pH during the fermentation process

Moisture measurement evaluation consisted of placing in previously weighed crucibles and taking to the oven (Nova Ética, model 402-3N) at 105ºC for 24 hours and placing in desiccators to cool weighed (Brasil, Ministério da Agricultura, 2009).

Part of the coffee fruit sample was crushed with an industrial blender for a minute (Camargo, 2L 800W / 22000rpm) until a uniform paste was formed and subjected to pH measurement (Instrutherm, model PH-1700), adequately calibrated, according to the methodology described by Velmourogane (2013). All analyzes were performed in triplicate.

2.8 Influence of temperature and fermentation time variables for carbohydrates, residual acids, and sensory evaluation

A Central Composite Rotatable Design (CCRD) was proposed to analyze the coffee fermentation process and find the best process conditions in the studied ranges. The variables studied were temperature (x_1) and fermentation time(x_2) in the process.

The temperature range used in the CCRD was from 15 to 30ºC, considering an average temperature range of the Cerrado Mineiro Region and other works in the literature (Evangelista, Silva et al. 2014). The fermentation time was defined based on at least 12 hours to 48 hours, as mainly used by Avallone et al. (2001) and Pereira et al. (2015).

The experimental design consisted of 11 central replicates and α equal to 1.4142. The temperature variable (°C) was studied in the range of 11.89 (-α) to 33.10 (α), and the variable fermentation time (hours) was studied in the range of 4.42 (-α) to 55.50 (α). In response to this investigation, the dependent variables were carbohydrates (sucrose, glucose, and fructose), organic acids (acetic, propionic, succinic, and lactic), glycerol concentration, and sensory evaluation. Multiple regression was performed to assess the influence of the independent variables studied ($x_1$and x_2) on the dependent variables, and the parameters that showed a significance level greater than 10% were disregarded, that is, in the hypothesis test with *Student's t*-table was considered the maximum error probability of 10%. In the regression analysis of the coffee fermentation results, the independent variables were transformed into the dimensionless form according to Eq. 2 for temperature (*T*) and Eq. 3 for fermentation time (*t*). On the fast-drying process, fermentation was stopped just after fermentation time defined on design.

$$x_1 = \frac{T\left(^\circ C\right) - 22.5}{7.5}$$

2

$$x_2 = \frac{t(h) - 30}{18}$$

3

2.9 Determination of sugars, glycerol, and organic acids

For the analyses of sugars and organic acids, the fermented coffee mass (entire fruit: exocarp, mesocarp, endocarp, and bean) was placed in aluminum trays and taken to the sun radiation for natural drying until it was dry enough to be ground in an analytical mill (about 12 hours of sun drying, to facilitate mass processing and posterior extraction, with day temperature of 23.2°C, once the oven was full of fruits, with fermented coffee mass, oven moisture were near 100%, and so, did not dry coffee fruits for sensory analysis. The mill used was the Willye knife (STAR FT-50, Fortinox), and the sample was ground using a 20 mesh sieve to separate the material used for extraction. The crushed samples of the fermented dough were weighed (6 g) and placed in a 125 mL Erlenmeyer with 40 mL of ultrapure water removed from the purifier (Gehaka, Model Master System All), maintaining the same proportion described by Ribeiro et al. (2017). This mixture was taken to a magnetic stirrer for 10 minutes at room temperature to extract the samples. The extract was decanted and centrifuged (10,000 rpm, 4°C for 10 min) in the centrifuge (Heal Force, Model Neofuge 15R) according to the methodology used by (Ribeiro et al. 2017). The supernatant was filtered through a 0.22 µm cellulose acetate filter (for particles smaller than 0.22 µm) for reading in the High Precision Liquid Chromatography (HPLC) after centrifuging.

The sugars and glycerol concentrations were determined by HPLC (Shimadzu LC-20A) equipped with a refractive index detector, a Hi-plex Ca column (7.0 × 300 mm, Agilent, CA, USA), operated at 85°C and ultra-pure water as the mobile phase at a flow rate of 0.6 mL min^{-1}. HPLC (Shimadzu LC-20A) determined the organic acid concentrations equipped with a diode array detector, a Shim-pack VP-ODS C8 phenyl column (150 × 4.6 mm), operated at 30°C and a flow rate of 0.6 mL min^{-1}. The mobile phase A consisted of 0.01 M potassium dihydrogen phosphate solution (pH 2.50 with H3PO4), and B was acetonitrile. The elution was programmed as the following: 0.00–3.00 min, 0% B; 3.00–5.00 min, 0–15% B; 5.00–8.00 min, 15 – 0% B; 8.00–10.10 min, 0% B; 10.10–15.00 min, 0–15% B.

2.10 Sensory analysis

For cupping (sensory analysis of coffee), samples were classified and judged according to Cupping Specialty Coffee protocols from the Specialty Coffee Association of America (2015). Two Q-Graders (Specialty Coffee Professional Grader) panels were formed for sensory analysis, totaling eight certified judges. One of the panels was done in Patos de Minas town – at Farroupilha Group's Coffee Laboratory, a kindly-given place by the group's Board. This one had the participation of 3 trained judges. The second panel was done in Carmo do Paranaíba town – at Veloso Coffee's Coffee laboratory, a place kindly given by the group's Board, with a participation of 5 trained judges.

The roast was performed on a Specialty Coffee Roaster for samples (LABORATTO, Carmomaq, São Paulo, Brazil) before 24 hours of coffee cupping. Each sample was roasted in two different roast levels: light-medium roast and medium roast (#65 and #55 Agtron Measurement, as described ABIC, 2018), and tasted at each different roast level for every experiment. Roasted coffee beans were ground just before brewing in a coffee mill (ML1-NA, Pinhalense, São Paulo, Brasil), grinding a small amount of each sample before grinding samples.

The coffee tasting was performed ‘at dark’; each sample had a code, and none of the tasters knew what process each code represented. Ground coffee is first evaluated to evaluate its fragrance, then brews with hot water around 90°C, and then evaluates the aroma of brewed coffee. After olfactory analysis, cups were cleaned (excess of ground material withdrawn) and samples were tasted several times to evaluate gustatory analysis. Samples were sensory were performed according Specialty Coffee Association (SCA) Protocol, which evaluate 10 coffee attributes (Fragrancy and Aroma, Flavor, Aftertaste, Acidity, Body, Balance, Sweetness, Clean Cup, Uniformity, Overall, from 0 to 10, and it final note is sum of all ten) (Specialty Coffee Association of America, 2020). and different coffee nuances, from Coffee Wheel of Flavors (Association and World Coffee Research, 2016).

3. Results And Discussion

3.1 Moisture and pH

The moisture of the fermentative mass did not vary during the process. The samples' average initial moisture was 70.62 ± 1.03, and at the end of the process was 68.67 ± 1.39 (wet basis). In Fig. 1 is shown the initial and final pH of the fermentation process. The fermentative mass's pH had its initial values with an average of 5.4, with a standard deviation of 0.25.

The pH values observed in this work were similar to those found by Elhalis, Cox and Zhao (2020) in its spontaneous fermentation (without inoculation), around 5.5. Other studies have shown that the initial pH of coffee beans ranges from 5.0 to 7.0 (Haile and Kang, 2019a; Kwak et al., 2018).

With the advancement of the fermentative process, microorganisms’ metabolism uses the sugars available to form soluble organic acids as metabolites (Avallone et al., 2001; Kwak et al., 2018). It thereby promotes the reduction of pH, a fact also observed by Haile & Kang (2019a). In this work, it was evidenced that the pH reduction varied with the test temperature. This fact was also verified by Avallone et al. (2001). They found a relationship between the decrease in pH and temperature, describing a lower reduction in pH at night due to low temperature. Figure 1 shows a graph comparing the initial pH with the final pH of the fermentation process.

As we can see in Fig. 1, tests 5 and 6 (same fermentation time), there is possible to see temperature effect on pH, and greater temperature provides pH decrease. Beyond this, as shown in tests 8 and 9 (same temperature), longer times also provide a pH decrease, which shows us a pH relationship with both time and temperature. pH variation can be shown at the central point without variation.

3.2 Influence of temperature and fermentation time variables for carbohydrates, residual acids, and sensory evaluation

The results of the CCRP are shown in Table 1. The responses evaluated were sensory analyses of fermented coffee with light-medium roast and medium roast. Other responses evaluated were the concentrations of sugars (sucrose, glucose, and fructose), glycerol, and final organic acids that interfere in the grade of the sensory evaluation of fermented coffee.

Table 1
– Experimental planning with the evaluated responses (sugars, glycerol, organic acids and final grade of sensory analysis) concerning the variables studied.

	Real Value (Coded Value)		Carbohydrates			Alcohols	Organic Acids				Final Grade	
	Temperature (x_1) (°C)	Time Fermentation (x_2) (h)	Sucrose (g/L)	Glucose (g/L)	Fructose (g/L)	Glycerol (g/L)	Acetic (mg/g)	Propionic (mg/g)	Succinic (mg/g)	Lactic (mg/g)	Light Medium Roast (SCA)	Medium Roast (SCA)
1	15.00 (-1)	12.00 (-1)	4.5382	6.6934	10.5473	0.1634	2.0000	3.2733	27,667	0.0000	81.30	81.19
2	15.00 (-1)	48.00 (1)	3.0540	7.0421	6.0881	0.5641	9.3333	4.2200	18.000	0.0000	83.55	83.11
3	30.00 (1)	12.00 (-1)	2.6863	7.2341	11.1752	0.3914	13.333	3.3600	0.0000	0.0000	83.53	83.88
4	30.00 (1)	48.00 (1)	0.5159	1.8150	5.4760	0.7682	0.0000	4.6133	0.0000	0.0000	82.06	81.00
5	11.89 (-α)	30.00 (0)	4.0506	8.0709	8.8953	0.1800	48.000	0.0000	4.2133	5.8867	83.54	82.01
6	33.10 (α)	30.00 (0)	1.7347	4.6283	9.5563	0.9095	42.000	0.0000	0.0000	7.5933	84.42	82.98
7	22.50 (0)	4.42 (-α)	4.4759	7.1148	12.2048	0.0000	73.333	0.0000	0.0000	9.4000	82.25	83.44
8	22.50 (0)	55.50 (α)	0.6205	3.0667	5.1005	0.7244	28.000	0.0000	0.0000	3.0667	82.56	82.54
9	22.50 (0)	30.00 (0)	3.8526	5.0698	8.9730	0.7844	22.666	0.0000	0.0000	8.4000	84.47	82.31
10	22.50 (0)	30.00 (0)	3.1957	6.1205	8.7715	0.7071	28.000	0.0000	0.0000	9.1333	84.32	82.41
11	22.50 (0)	30.00 (0)	3.8671	6.7429	9.3328	0.7846	25.333	0.0000	0.0000	8.1333	84.39	82.27

Sugars and glycerol

Figure 2 and 3 presents reduced model surfaces for fructose and glucose concentration at the end of the fermentation process, respectively. Observing Fig. 2 is possible to know that temperature did not influence the fructose concentration results at the end of the process. As for the fermentation time, it was

observed that a reduction in the concentration of fructose is evident in longer times. On the other hand, the glucose concentration-response showed a different profile than fructose concerning the variables studied. As shown in Fig. 3, the response surface was shaped like a saddle. It is possible to observe that the highest glucose concentration values are in the range of high temperatures and shorter fermentation times and, also, in longer fermentation times with lower temperatures. Eq. 5 shows a reduced model for glucose.

Initial sugars content was essential to determine the consumption and generation of mono and disaccharides. Fructose is sugar with greater initial concentration, showed a mean of 10.92 g/L (σ = 1.29), followed by glucose mean content 8.09 g/L (σ = 0.97) and sucrose with a mean content of 4.90 g/L (σ = 1.13). In the fermentative process of coffee, a reduction in sugars occurs due to microorganisms' metabolic activity, forming metabolites, such as acids alcohols, among others (De Bruyn et al. 2017; Elhalis, Cox and Zhao, 2020; Martinez et al. 2017). At the end of the fermentation process, the main sugars observed in this work were fructose, glucose, and sucrose; the last was found in lower concentrations.

In Figs. 4 and 5 is shown the surfaces of the reduced model for sucrose and glycerol concentration at the end of the fermentation process. In experimental design (Fig. 4), the response surface showed an optimum tendency for glycerol concentration in the fermentation time interval between 30 and 50 hours at temperatures above 24°C. The highest concentrations of glycerol occurred at the points considered central for both variables (22.5°C and 30 h) and, also, it was observed in experiment 6, where the highest temperature was used in all experimental design (33.1°C) and at the central point of the fermentation time (30 h).

Fructose, followed by glucose, were the carbohydrates observed in higher concentrations. Those sugars resulted from the sucrose's hydrolysis by the yeasts present, mainly *Saccharomyces cerevisiae*, transforming glucose and fructose into the monosaccharides. Yeast consumes glucose first and then fructose due to its metabolism.

As shown in Fig. 4, the sucrose concentration presented higher values in fermentation time and lower temperatures. Its degradation is due to microbiological activity, which may form monosaccharides as a result. Sucrose is the main sugar used in yeast metabolism.

The sugar concentration results showed that the lowest values were for the experiments with higher fermentation times and temperatures. Elhalis, Cox and Zhao (2020) reported in their works that sucrose and monosaccharides as fructose and glucose were practically degraded at the end of fermentation, which was conducted 24 h at room temperature (between 10 and 30°C). These sugars content reduction also was observed in other studies, as in De Bruyn et al. (2017) and Martinez et al. (2017).

In some parts of the tests conducted on this work, sugar's final concentration was still high, evidencing that fermentative mass' sugars were not entirely degraded. (Ribeiro et al. 2017) noticed an increase in glucose and fructose at the end of the fermentative process. This is related to enzymatic or hydrolytic reactions from the fermentative process. The polygalacturonase and pectinase enzyme activities, mainly produced by yeast fermentative processes, degrade pectinolytic polysaccharides (Masoud and Jespersen, 2006). This process has a great positive impact on coffee quality (Lee et al., 2015).

According to Avallone et al. (2001), the most easily metabolized sugars are prioritized by microorganisms before the hydrolysis of polysaccharides. This means that the coffee fermentation process is dynamic in consumption kinetics and sugar generation. De Bruyn et al. (2017) found in their study that despite the concentration of sucrose and monosaccharides having decreased, glucose and fructose showed peaks during the fermentation process, also corroborating the hypothesis that these sugars are both substrate and metabolites of biochemical reactions that occurred in the process fermentation. Other authors who also evidenced, in their work, an increase in monosaccharides' levels were Ribeiro et al. (2017). The authors were justifying the same due to the breakdown of polysaccharides. This fact was also discussed by Haile & Kang (2019b), in which they described that microbial activity and fermentation time determine the final concentration of sugars such as glucose and fructose.

Glycerol concentration present at the end of the process is another interesting response in the study of coffee fermentation. Glycerol is an essential metabolite for quality formation since it has a sweet taste and a smooth mouth sensation (Swiegers et al., 2005). In experiment 7, the glycerol concentration was zero. In this experiment, the fermentation time was the shortest, just 4.42 hours. This can be explained because glycerol is a metabolite of sugar degradation by yeasts. It is also not found in mechanically husked beans without fermentation (Elhalis, Cox and Zhao, 2020; Elhalis, Cox, Frank and Zhao, 2020). Elhalis, Cox and Zhao (2020) showed glycerol after 24 hours of fermentation, which may be explained by their process, which removes exocarp and mesocarp partially, reducing polysaccharides content.

As presented in the results section, the central points and experiment 6 for time and temperature favored the highest glycerol concentrations. Similar results were observed in De Bruyn et al. (2017), who found spikes in glycerol concentration during the dry process fermentation and not at the end of it.

Sensory analysis

The coffees fermented for 48 hours showed yellow (experiment 4) and yellowish (experiment 2). The other experiments had a green color, characteristic of normal, natural coffees, as defined in the MAPA Normative Instruction, nº 8 of 2003. Based on the same instruction, the same samples' appearance was defined as regular, and the others with a determined aspect were good. In the characteristic of physical defects, the burnt beans, those beans that present brown color in different tones due to fermentative processes, were only identified in experiment 4, at a level of 3% of the total mass. An interesting view is experiment 6, with higher temperatures but lower fermentation time, which shows an important relationship between time and temperature for quality degradation.

The second part was the sensory evaluation of the coffees, carried out with the roasted beans in two different roasting levels and tasted according to the SCA classification. The sensory analysis results of the fermented coffees, as shown in Table 3.1, showed slight variation in their grades, between 81.33 to

84.47 for coffees tasted in the light-medium roast (Agtron # 65 / SCA). For medium roast coffees (Agtron # 55 / SCA), the grades ranged from 81.00 to 83.88. The sensory analysis results of the different roasting types showed that regardless of the roasting level used; there was a significant dependence on the coffees' notes' values with the two variables, temperature (x_1) and fermentation time (x_2). Figure 6 shows the reduced model's response and contour surface for scoring the average clear roasting according to the SCA concerning the studied variables.

The central levels of both variables showed the best sensory results. The fermentation time ranged from 20 to 40h, and temperatures ranged from 16 ºC to 28°C for the sensory note's optimal range. Table 2 presents some works developed with the inoculation of microorganisms in the fermentation of coffee.

Table 2
– Inoculation in different cultivars and fermentation conditions

Author	Cultivar	Temperature	Fermentation Time	Method	Inoculation	Final Grade
Elhalis et al., 2020a	Bourbon	Air temperature (25–30°C - day e 10–15°C night)	36 h	Wet process	Spontaneous	89.50
					Natamycin (anti-Yeast)	84.75
					T. delbrueckii 084	85.50
					Spontaneous	91.50
Bressani et al., 2018	Yellow Catuai	Air temperature	16 h	Dry process	*S. Cerevisiae 0543*	84.00
					C. parapsilosis 0544	81.50
Carvalho Neto et al., 2018	Catuí	30°C	12h aerobic and 12h anaerobic	Wet process	Spontaneous	80.67
					Lactobacilus Plantarum	80.00
Ribeiro et al., 2017	Mundo Novo	Air Temperature	Over drying process 284h	Semi-dry process	*S. Cerevisiae 0200*	80.13
					S. Cerevisiae 0543	82.63
					Spontaneous	-
	Yellow Ouro				*S. Cerevisiae 0200*	83.25
					S. Cerevisiae 0543	82.88
					Spontaneous	81.38
Martinez et al., 2017	Yellow Catuaí	Air temperature 14.6°C – 28.2°C	352 h	Semi-dry process	*S. Cerevisiae 0543*	81.40
					C. parapsilosis 0544	81.30
					T. delbrueckii 084	81.00
					Spontaneous	81.40
Pereira et al., 2015	Catuí	Air temperature (24–32°C - day and 12–15°C night)	24 h	Wet process	*P. fermentaris YC.2*	89.00
					P. fermentaris YC.2 Sup.	87.50
					Spontaneous	89.00
Evangelista et al., 2014b	Acaiá	Air Temperature	Over Drying process	Semi-dry process	Controle	80.93
					S. cerevisiae YCN 724	79.33
					P. guillermondii YCN 731	74.17
					C. parapsilosis YCN448	80.00
					*S. cerevisiae *YCN 727*	81.08

*YCN 727 = CCMA 0543

Figure 7 shows the reduced model's response and contour surface for the average roasting score about the variables studied.

Before sensory analysis, the raw bean's physical evaluation was carried out, as recommended by the Ministério da Agricultura Pecuária e Abastecimento (MAPA) (Brasil, 2003), by the main author, trained as a Brazilian Grader. The physical evaluation consisted of the appearance, color, and percentage of burnt

beans, representing signs of undesirable fermentation processes.

The response surface shows that shorter fermentation times are required to better sensory evaluation when fermentation occurs at higher temperatures. When the fermentation temperature is lower, it must use a long time to reach the sensory evaluation's desired value. The best results were obtained in experiments 9, 10, and 11, from the central point (22.50°C and 30 h) and experiment 6 (33.10°C and 30 h), with sensory scores greater than 84 points. A control experiment called a "Control" was used to compare the sensory evaluation grade with experimental design results. In this experiment, inoculation with the yeast *Saccharomyces cerevisiae* was not performed from the control. The fermentation control was not performed, and the light-medium roast was performed because it is a commercial standard. According to SCA, the control (without fermentation) had a sensory score of 82.16. This result indicates that the experiments carried out in this work showed sensory results superior to those in control. Authors such as Bressani et al. (2018), Ribeiro et al. (2017), Martinez et al. (2017) obtained better results with coffees inoculated with *S. cerevisiae* when compared with the controls.

Similar results were observed by several authors who carried out experiments with the inoculation of microorganisms. Bressani et al. (2018) found a maximum score of 84 points, with inoculation of the *S. cerevisiae* strain CMA0543, in the dry process. Pereira et al. (2015), Neto et al. (2018), Elhalis, Cox and Zhao (2020) used 24h, 24h (12 aerobic and 12 anaerobic), and 26 hours of fermentation, respectively, only controlled the temperature at 30°C, with the other two jobs at room temperature, obtaining exceptional coffees in wet processes. Through these studies, it is possible to observe that fermentation at room temperature with inoculated microorganisms can benefit the sensory quality of coffee.

According to Illy and Viani (2005), soluble carbohydrates, proteins, and chlorogenic acids are degraded in melanoidins through Maillard and caramelization reactions in the roasting process. Moreover, there is the release of other volatile compounds. The best relationship found for aroma precursors in coffee is generally found during medium-light roasting, a statement that was also evidenced in this work. During the evaluation of the beverage potentials after fermentation, the coffee with the medium-clear roast presented better sensory results than medium roasting.

The best sensory results observed were in the fermentation time range between 25 to 35 hours and temperatures above 28°C. The response surface shows that shorter fermentation times can be used to achieve a good sensory evaluation when the fermentation process occurs at higher temperatures. The best result was obtained in experiment 3 (30°C and 12 h) with sensory notes close to 84 points (Table 3.1). The comparison with the control was not performed because the medium roasting is not considered commercial and was only used to analyze the Q-Graders. Based on the results of the two types of toast, it can be seen that the medium-light roast showed a better sensory result when purchased the medium roast in conditions of lower temperature and longer fermentation time.

Lee et al. (2016) used different roasting levels to evaluate the quality of coffee, fermented greens, dark and light roasting in notes of 0–5 for various attributes (sweet, fruity, buttery, caramel, chestnuts, roasted, smoky, spicy, sulfurous). The sensory analysis results showed a higher quality for roasted coffee in dark roasting than in light roasting, different from what was evidenced in this work.

Organic acids

As we can observe in Fig. 8, acetic and lactic acids were the primary acids found in the analysis performed just as done by other authors in fermented coffee with *S. cerevisiae* (Bressani et al., 2018; Evangelista et al., 2015, 2014b; Neto et al., 2018; Pereira et al., 2015). There was found succinic acid in lower concentrations and less representativity (except for tests 1 and 2, in which succinic is the main acid).

Acetic acid shows concentrations from 0 to 73.33 mg/g, which lower concentration was in experiment 4, which was 30°C for 48hours and higher concentration on the shorter fermentation time (4,42h), finding values between 0 and 73.33 mg/g with at least 7 tests with concentrations above 20 mg/g of this acid. Succinic acid also showed adverse concentrations among different experiments and did not correlate with time or temperature. Succinic acid greater concentration was found at experiment 1 (27.67mg/g), and this acid was not found in 8 of 11 tests conducted. This acid was found in every other experiment, also in lower concentrations (Bressani et al., 2018; Evangelista et al., 2014b; Martinez et al., 2017).

Lactic acid was the second most common acid, both in concentration at the end of the process and experiments, with absence only on Experiment 1 to 4. Its concentrations varied from 3.1 mg/g (longer time) to 9.4 mg/g (shorter time). Propionic acid was found only on the four first experiments (1 to 4), with concentrations varying from 3.2 to 4.6 mg/g. None of those acids were on all of the experiments, and none of them presented a correlation with fixed parameters of CCRP (Time and Temperature).

As can be seen in Fig. 8, at the end of fermentation, a considerable concentration of acetic and lactic acid was obtained in the fermentation process. On the other hand, other authors have not found those significant acetic acid concentrations at the end of the process. Bressani et al. (2018) and Evangelista et al. (2014) found near 10 mg/g of acetic acid, Martinez et al. (2017) found around 2 mg/g. Concerning about acetic acid, in the work of Neto et al. (2018), the authors observed that the lactic acid increased with the advance of fermentation. However, other authors found the lactic acid concentration near 2 mg/g (Evangelista et al. 2015; Martinez et al. 2017).

Propionic acid is sensorially it may be responsible for the taste and smell of onions (Pimenta, 2003). Evangelista et al. (2014) found concentrations near 2 mg/g and lower values were found by Martinez et al. (2017), and it was not found in many other works (Elhalis, Cox and Zhao, 2020; Evangelista et al. 2015; Ribeiro et al. 2017).

4. Conclusion

A slight variation in the coffees' sensory quality represents great possibilities for producing a higher quality coffee and can add value to the final product. Initiating microorganisms in fermentation can lead to the maintenance of coffee characteristics, showing that these microorganisms can inhibit undesirable metabolites' production. The yeast *Saccharomyces cerevisiae* (Lallemand ORO®) was well suited to the cultivar Catuaí Amarelo, IAC 62, in which it presented good sensory results.

Studying environmental conditions and fermentation time for coffee standardization is crucial to obtaining a quality product. From the point of view of applying the results obtained from the fermentation with the light-medium roast on coffee properties, we realized that the process could be easily employed, as they are consistent with the producing region's temperatures. This fact is already becoming more complex for coffee evaluated in medium roast since the best sensory results do not match the region's natural temperatures, requiring investments that can make the process unfeasible.

On the other hand, these data can be used for regions with different temperatures from the region where the coffee is being produced. The concentrations of sugars, glycerol, and acids in fermented coffee showed values close to that of the literature and the decrease in pH, indicating the completion of fermentation.

By simulating different farm temperature conditions and using different fermentation times, it was possible to evaluate how much time is needed to ferment coffee fruits for optimizing field conditions, once temperature control at farms is a not easily controllable variable, always demanding or investments or even energy coasts. With the equations obtained, it was possible to give the first step to applying new fermentation methods at the farms level, without significant investment in infrastructure or energy costs and improve beverage quality, increasing Brazilian competitiveness in world coffee market. Unlike sugars and sensory analysis, organic acids did not represent concentrations between literature values and showed no correlations with time/temperature parameters.

Declarations

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Authors' contributions: C.J.T.V.: Writing, Research, Methodology. L.S.M.: Writing, Review, Editing. R.C.S.: Validation, Software. M.A.S.: Formal analysis, Supervision, Validation, Review, Editing. M.F.Z.: Formal analysis, Supervision, Validation, Review, Editing. C.Z.G.: Formal analysis, Supervision, Validation, Review, Editing, Software.

Declarations of Interests: Carlos Johnantan Tolentino Vaz declares that he has no conflict of interest. Larissa Soares de Menezes declares that she has no conflict of interest. Ricardo Corrêa de Santana declares that he has no conflict of interest, Michelle Andriati Sentanin declares that she has no conflict of interest. Marta Fernanda Zotarelli declares that she has no conflict of interest and Carla Zanella Guidini declares that she has no conflict of interest.

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Compliance with Ethical Standards

The present work is consonance with the ethical regulations of the institution and the country, having been approved by the Ethics Committee in Research with Human Beings under N^{o}. CAAE 55909622.0.0000.5152.

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Figures

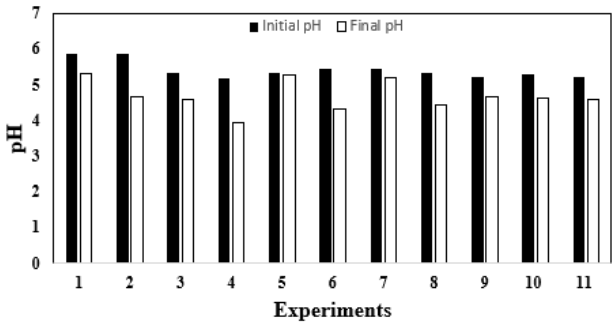

Figure 1

Initial and final pH of the fermentation process

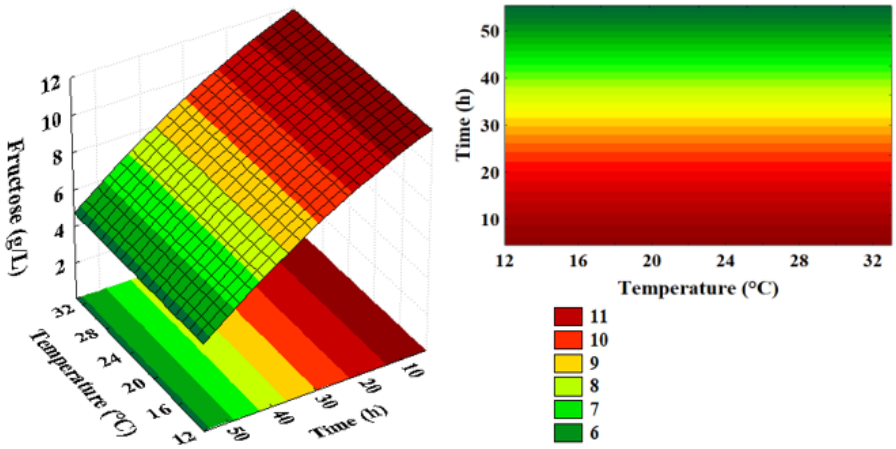

Figure 2

Reduced model surfaces for fructose concentration at the end of the fermentation process

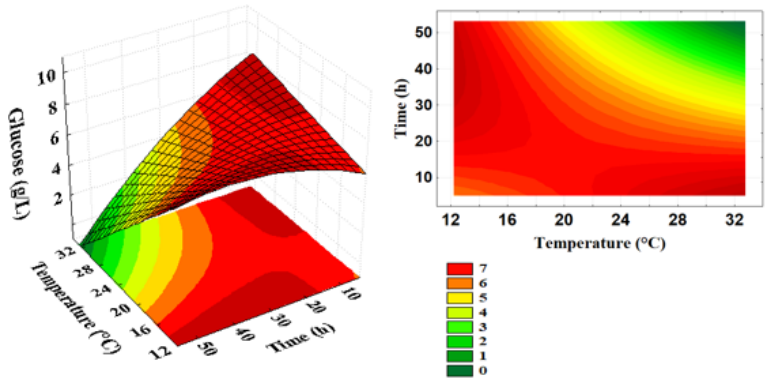

Figure 3

Reduced model surfaces for glucose concentration at the end of the fermentation process

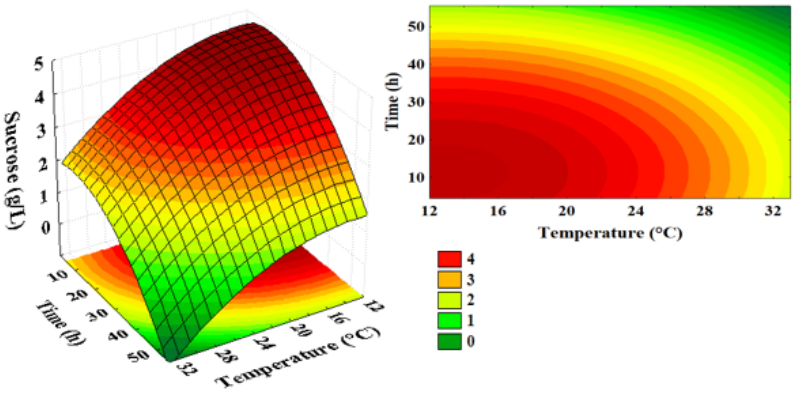

Figure 4

Surfaces of the reduced model for sucrose concentration at the end of the fermentation process

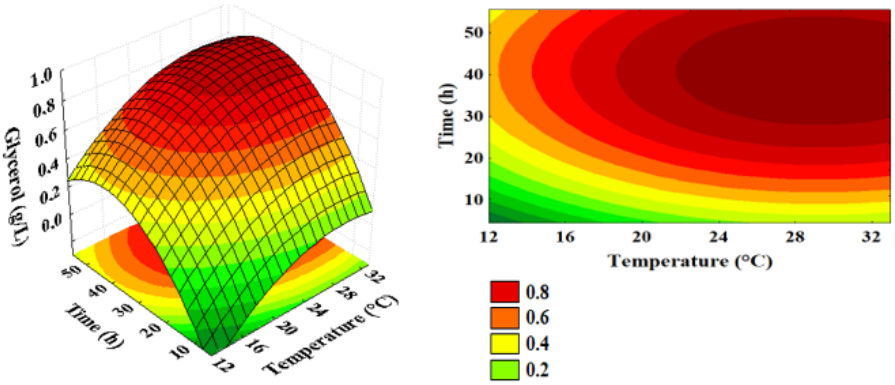

Figure 5

Surfaces of the reduced model for glycerol concentration at the end of the fermentation process

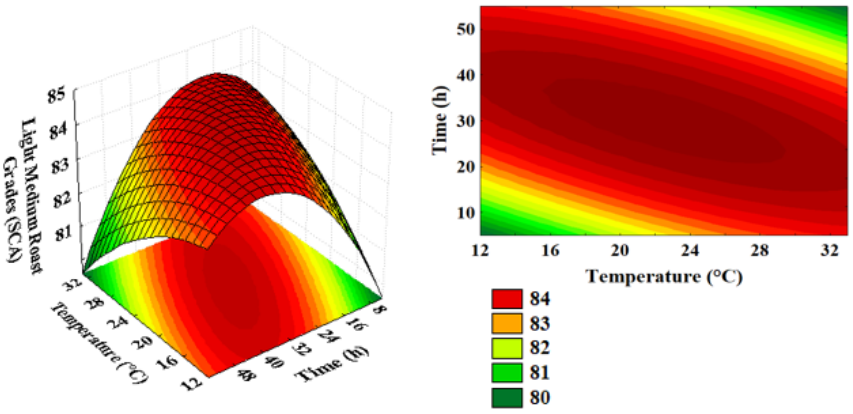

Figure 6

Reduced model surface for SCA sensory assessment for light medium roasting (#65, SCA)

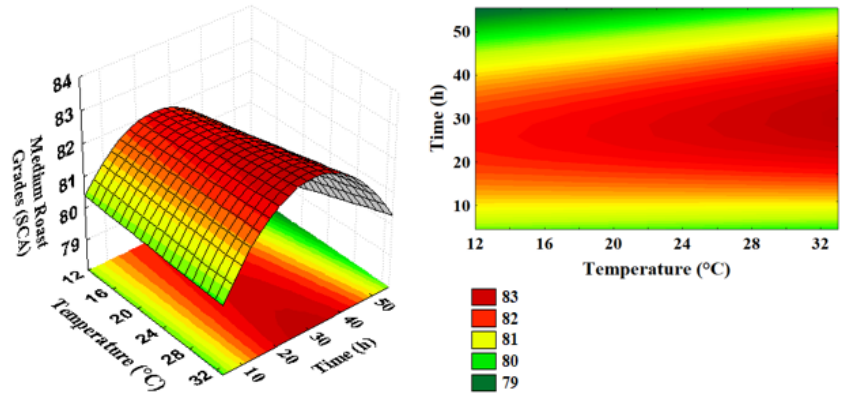

Figure 7

Reduced model surface for SCA sensory assessment for medium roasting (#55, SCA)

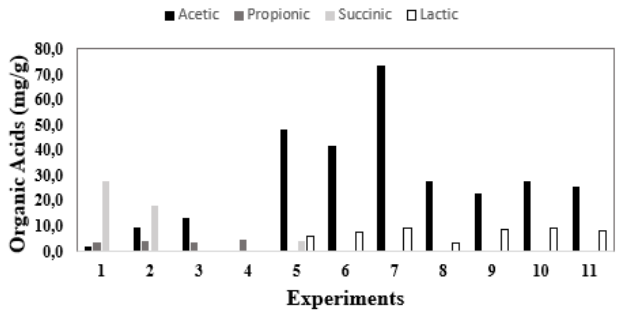

Figure 8

Final organic acids concentrations for each experiment

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