IMPACT OF MATURATION STAGE IN THE CHEMICAL COMPOSITION OF COFFEE FRUITS AFTER INDUCED FERMENTATION

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ABSTRACT

Coffee fermentation is a technique used to convert sugars of the fruit mucilage by the action of microorganisms, which can improve the sensory quality and increase chemical complexity in the beverage. As the maturation stage of fruits has direct influence on their chemical composition, it can also impact coffee fermentation. This work aimed to evaluate the fermentation of natural arabica coffee (Catiguá MG2 cv.) in solid-state with natural microbiota in self-induced anaerobiosis, comparing fruits separated in green-ripe and raisin-buoy maturation groups. HPLC analysis showed the degradation of sucrose, glucose and fructose after fermentation, the increase in glycerol and ethanol content and the behavior of some organic acids in the process. The results were also analyzed as a factorial experimental design and obtained a great general regression fitting ($R^2 \geq 0.920$) for most of the studied compounds. Fresh green-ripe fruits had higher content for all sugars. Fresh raisin-buoy fruits had higher contents of lactic and acetic acids. The post-fermentation comparison between green-ripe and raisin-buoy fruits showed that green-ripe fruits had higher content of ethanol and malic acid, while the raisin-buoy group had higher content of glycerol and succinic acid.

Keywords: Arabica coffee. Solid-state fermentation. HPLC analysis. Factorial experimental design.

1 INTRODUCTION

Coffee is a complex food matrix known worldwide as one of the most consumed beverages and the second most traded commodity. Brazil is considered the leading country in production and exportation of coffee, mainly for cultivation of species *Coffea arabica* and *C. canephora*. Arabica coffee is generally used for specialty coffee development due to better smoothness in the cupping and quality of aroma and flavor, representing 56% of the global market[1,2]. Alongside this, the Cerrado Mineiro region (a certified Protected Denomination of Origin location in the state of Minas Gerais, Brazil) has consolidated itself as a producing area of arabica coffee with distinct sensory characteristics, which provides a key factor for consumer appeal[3].

A concerning problem related to coffee quality is the homogeneity of maturation of the fruits in the plant. Climatic and physiological conditions such as the availability of water in the soil, evapotranspiration rate on the leaves, air temperature and irregular rainfall are determinant factors during blooming and crop ripening, which can induce insufficient development of the bean and change the chemical composition (e.g. sugars, alcohols, organic acids, chlorophyll, carotenoids, anthocyanins)[4,5]. After the mechanical harvesting process, washing the fruits in a hydraulic separator is a technique used to the select the immature, ripe and overripe cherries, which are usually prioritized over dried (raisin) and low-density (buoy) fruits[2,6].

The post-harvest processing is also important to determine and improve coffee overall sensory quality. Advances in coffee fermentation methods open the pathway for better management of controlled processes and for better description of variables: fruit treatment (natural and pulped), oxygen availability (open environment, self-induced anaerobiosis and carbonic maceration), water addition (solid-state and submerged), microbiota conditions (spontaneous fermentation and the use of starter culture)[6]. More than just simply the degradation of the mucilage by microorganisms, coffee fermentation has become a promising technique to produce specialty coffees with diverse flavors, aromas and chemical compounds[1,2].

Coffea arabica L. cv. Catiguá MG2 (Timor UFV 440-10 hybrid x Catuaí Amarelo IAC 86) is a cultivar of the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), which have small beans, medium productivity, and great potential for specialty coffee production. Its plant is a small tree, with medium crown diameter and high vegetative vigor, being rust resistant[1].

Regarding specialty coffee production and fruit maturation stage divergence, this work aimed to analyze the influence of an induced fermentation process on the chemical composition (sugars, alcohols and organic acids) of coffee fruits harvested in the Cerrado Mineiro region, comparing two maturation stage groups: green-ripe and raisin-buoy.

2 MATERIAL & METHODS

This study was carried out with *Coffea arabica* L. cv. Catiguá MG2, cultivated at an altitude of 1048 m in the Cerrado Mineiro region, located in Santiago de Minas, Presidente Olegário, Minas Gerais, Brazil (18°32'37"S 46°18'29"W). The coffee fruits were mechanically harvested and washed to separate green and ripe cherries (apparent specific mass of 0.865 ±0.0222 g/cm^3) from raisin and buoy fruits (apparent specific mass of 0.740 ±0.0164 g/cm^3).

Fermentation occurred with natural fruits in 200 L high-density-polyethylene bioreactors exposed to direct sunlight for a period of 60 h, using spontaneous self-induced anaerobic fermentation in solid-state with natural microbiota. The bioreactors were previously cleaned and sanitized with 0.3% peracetic acid solution. For both green-ripe and raisin-buoy fruits, samples were collected before and after the fermentation procedure for further analysis, in triplicate.

For the determination of sugars (sucrose, glucose, fructose), alcohols (glycerol, ethanol) and organic acids (citric, malic, succinic, lactic, acetic) content, a high-performance liquid chromatography (HPLC) system was employed[1]. For the extraction of the samples, 10 g of fruits were blended with 100 mL of Milli-Q deionized water for 2 min in a domestic blender (Oster©, 1400 W). Residual coarse material was filtered through two layers of organza (polypropylene) and then centrifuged for 15 min at 13,000 rpm and 17°C (Hermle Labortechnik©, Z 326 K). The supernatant was then collected and filtered through a 0.22 µm nylon filter. The filtrate was injected into the HPLC system (Shimadzu©, LC-20AT Prominence), using a Supelcogel© C-610H column (30 cm x 7.8 mm), a refractive index detector (RID-10A) for sugars and alcohols determination, and a photodiode array detector (SPD-M20A) for organic acids detection at 210 nm. The elution was conducted with 0.1% aqueous phosphoric acid as the mobile phase, at a flow rate of 0.5 mL/min. The oven temperature was set to 32°C, the sample run duration was 35 min and the injection volume was 20 µL. Results were processed with LabSolutions© 5.117 software based on each pure compound calibration curve.

The statistical difference between the samples was analyzed using ANOVA followed by Tukey's test (p-value < 0.05). For better understanding of the independent variables effects on the results, the experimental conditions were evaluated through a 2^2 factorial design[4], in which "fruit maturation type" was considered variable x_1 (having "green-ripe" as the lower -1 level and "raisin-buoy" as the higher +1 level) and "occurrence of induced fermentation" was considered as variable x_2 (having "non-fermented" as the lower -1 level and "fermented" as the higher +1 level). These data analysis was performed using Statistica© 12.5 software.

3 RESULTS & DISCUSSION

Figure 1 presents the results obtained in HPLC for the coffee fruits samples studied.

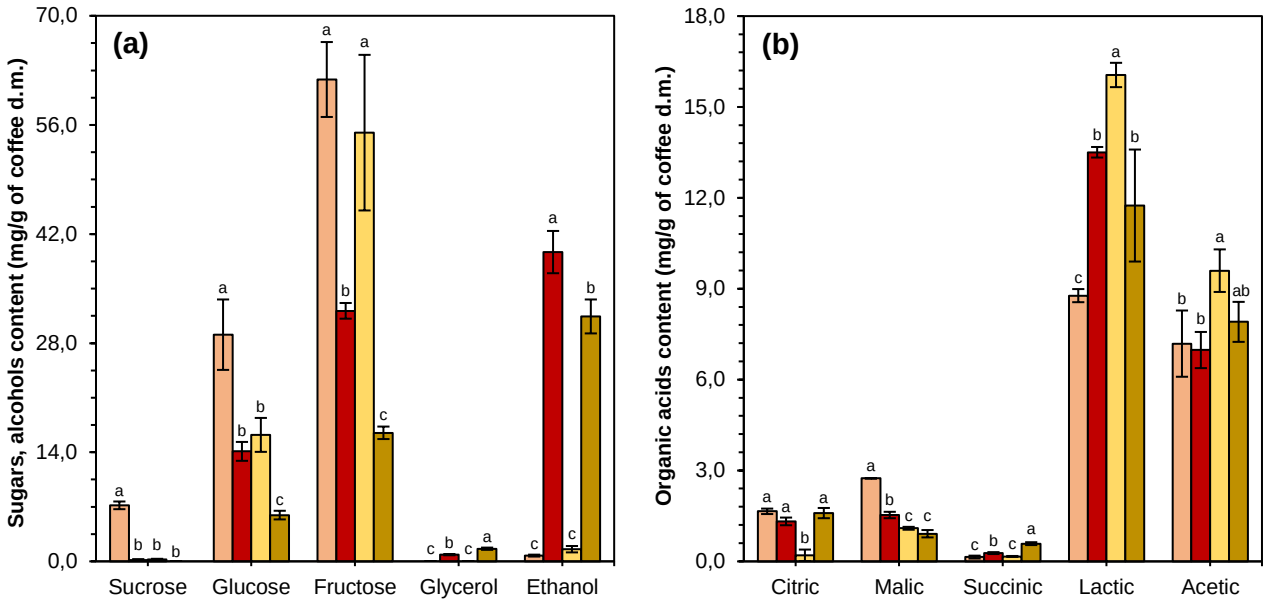

Figure 1 Chemical composition of coffee in terms of (a) sugars, alcohols and (b) organic acids for: fresh green-ripe fruits, fermented green-ripe fruits, fresh raisin-buoy fruits, and fermented raisin-buoy fruits.

Comparing the fresh samples, it was shown that green-ripe fruits presented higher content of sucrose and glucose, and for citric and malic acids, while raisin-buoy fruits had higher values for lactic and acetic acids and ethanol. This could be related to the greater maturation stage of this last sample group, since it had already started drying in the plant itself and it was more susceptible to enzymatic and fermentation reactions, which has led to the consumption of sugars and production of other compounds.

Fermentation showed almost a total consumption of the sucrose content. In regards of glucose and fructose contents, green-ripe fruits presented a consumption of around 50% for both compounds, while raisin-buoy fruits registered a higher consumption (around 67%), due to its lower initial values and low sucrose content. None of the fresh samples showed glycerol content, and the production during fermentation in raisin-buoy fruits (1.62 mg/g) was nearly twice the amount produced in green-ripe fruits (0.856 mg/g). For ethanol, green-ripe fermented fruits showed a content of 38.9 mg/g, higher than that for raisin-buoy fruits (29.9 mg/g).

For the organic acids, even though the initial content of citric acid in raisin-buoy fruits was the lowest (0.198 mg/g), after fermentation both samples reached the same level as the fresh green-ripe fruits (around 1.65 mg/g). Malic acid content decreased 44% after the fermentation of green-ripe fruits, which could be related to the increase of 54% in the lactic acid for this same sample, indicating evidence of malolactic fermentation. In the other hand, while the fermentation of raisin-buoy fruits did not affect the malic acid content, it was observed a degradation of 27% for lactic acid. Both samples had around the same initial succinic acid content, and they both showed an increase in this compound after fermentation, higher for raisin-buoy (0.578 mg/g) than for green-ripe (0.274 mg/g) fruits. For the acetic acid, only the contents of the fresh samples differed from each other with significance.

The results for the effects of process variables in the chemical compounds analyzed through a factorial design are presented in Table 1. All the compounds showed a great general regression fitting in this analysis ($R^2 \geq 0.920$) except for acetic acid, due to the lack of statistical difference between the samples after fermentation.

Table 1 Effects of fruit maturation type (x_1) and occurrence of induced fermentation (x_2) on the chemical compounds determined with HPLC.

Factor	Sugars			Alcohols		Organic acids				
	Sucrose	Glucose	Fructose	Glycerol	Ethanol	Citric	Malic	Succinic	Lactic	Acetic
Mean	1.90	16.3	41.4	0.619	18.4	1.19	1.57	0.287	12.5	7.91
x_1	-3.56	-10.5	-11.2	0.383	-3.72	-0.588	-1.13	0.161	2.76	1.67
x_2	-3.63	-12.63	-34.1	1.24	34.4	0.528	-0.696	0.277	-0.212*	-0.949*
x_1x_2	3.38	2.35*	-4.42	0.383	-4.53	0.866	0.516	0.143	-4.52	-0.741*
R^2	0.995	0.939	0.941	0.991	0.993	0.958	0.991	0.971	0.920	0.719

*Effect with no statistical significance identified (p-value ≥ 0.05).

For the sugars content, it can be noted that both sucrose, glucose and fructose tend to negative effects in the direction of raisin-buoy fruits and with fermentation operation, which indicates that there is a better consumption of sugars in these conditions. Despite the lower moisture content in raisin-buoy fruits and the potential accumulation of sugars in the fruit (which would led to a positive effect in variable x_1), this sample group already could have been submitted to a partial fermentation in the coffee plant (which justifies a previous consumption of some sugars and the final negative effect observed on this study). Furthermore, since the main factor in fermentation is the conversion of sugars in other compounds, it was expected that the effect for variable x_2 went negative from non-fermented to the fermented samples. For the interaction effects (x_1x_2) each sugar showed a different behaviour: sucrose has a directly proportional effect, glucose has no significance in the interaction of the variables, and fructose has an inversely proportional relation between variables x_1 and x_2.

For the alcohols content, variable x_2 (occurrence of induced fermentation) showed more expressive effects for both compounds. Glycerol showed a positive effect for all factors, which indicates a higher production of this compound for raisin-buoy fruits and with fermentation, since there was no glycerol found in any of the fresh samples. For ethanol, it is observed a preference in its production in green-ripe fruits (negative effect for variable x_1) and with fermentation (positive effect for variable x_2), which indicates a negative effect for the interaction effect (optimal x_1 equals to level -1, while x_2 equals to level +1).

For the organic acids, it was observed a positive interaction effect for citric, malic and succinic acids (levels directly proportional), and negative interaction effect for lactic acid (levels inversely proportional). Higher content of citric acid is observed in green-ripe fruits and with fermentation. Malic acid is more affected by the variable x_1 (fruit maturation type), showing a tendency for higher content in green-ripe fruits and without fermentation. Succinic acid production is optimized using raisin-buoy fruits and with fermentation. Variable x_2 (occurrence of induced fermentation) showed no significance in the isolated effect to produce lactic acid (only in interaction effect), due to the increase in the content of green-ripe fruits after fermentation and decrease in this compound for fermented raisin-buoy fruits. Acetic acid content was not affected by the isolated variable x_2 or even by the interaction factor, since only variable x_1 showed statistical difference in Figure 1, in the direction of raisin-buoy fruits.

4 CONCLUSION

The difference in maturation stage of coffee fruits influenced the fermentation process and the chemical composition of the final products. Generally, fresh green-ripe fruits showed higher content of sugars and the initial fermentation in the coffee plant for raisin-buoy fruits was confirmed by the higher contents of lactic and acetic acids. With the consumption of sugars during fermentation, green-ripe fruits registered higher production of ethanol while raisin-buoy fruits showed higher values for glycerol. Regarding the organic acids, both samples showed similar final values for citric, lactic and acetic acids, in ways that green-ripe fruits registered higher content of malic acid and raisin-buoy fruits presented higher content of succinic acid. Alongside analytical comparison, the fermentation was also be evaluated through a factorial design that estimated the direction and intensity of the effects of the independent variables (fruit maturation type and occurrence of induced fermentation) on the chemical composition.

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ACKNOWLEDGEMENTS

The authors wish to thank funding agencies Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, MaiDai 68/2022), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, APQ-04267-22) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 2021/06968-3), and also Ministry of Science, Technology and Innovation (MCTI) of Brazil, Dean of Research and Postgraduate Studies (PROPP) at Federal University of Uberlândia (UFU) and rural producer Elmiro Alves do Nascimento (on behalf of all collaborators at Santiago Farm).