

Fungal Diversity in Coffee Fruits and Beans and its Impact on Beverage Sensory Quality

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ABSTRACT

Coffee quality is determined by physical, chemical, and sensory attributes, which can be influenced by filamentous fungi present throughout the production cycle. The objective of this study was to isolate and identify fungi associated with two coffee cultivars (Catiguá MG2 and Paraíso MG H419-1), grown in organic and conventional systems, with or without cover crops, at two stages of harvest (cherry and dry). The predominant genera identified were *Cladosporium*, *Geotrichum*, *Penicillium*, *Rhizopus*, and *Fusarium*. It is important to note that no ochratoxin A (OTA) was detected in any isolate, and only one (*Aspergillus parasiticus*) produced aflatoxins B₁ and B₂. The dried fruits presented good beverage quality. The main nuances observed were fruity, caramel, chocolate, and floral. The production system influenced fungal diversity and sensory attributes, with the organic system presenting greater fungal richness and more complex sensory profiles. The harvest stage also affected fungal composition, but did not compromise beverage quality, indicating that harvesting at advanced maturity (dry fruits) may be a viable strategy without quality losses. These findings highlight that the production system and harvest stage influence fungal diversity and sensory characteristics without increasing the risk of mycotoxin contamination.

Key words: Fungal diversity; sensory analysis; content analysis.

1 INTRODUCTION

Coffee is one of the most consumed beverages in the world. The specialty coffee sector represents approximately 12% of the international market and 15% of the Brazilian market (Pimenta, Angélico, & Chalfoun, 2018). Coffee quality is related to a set of physical, chemical and sensory attributes. Quality can be affected by preharvest factors, such as species and variety, place of cultivation, crop treatment and plant nutrition, fruit maturation and incidence of microorganisms. In addition, postharvest factors, such as unwanted fermentation, drying, storage, roasting and preparation of the beverage, can influence quality (Iamanaka et al., 2014; Bemfeito et al., 2021). Agronomic techniques and environmental conditions directly influence the nutritional and toxicological properties of coffee beans (Consonni, Polla, & Cagliani, 2018), reinforcing the importance of integrated management strategies.

Among the main microorganisms associated with fruits and coffee beans, filamentous fungi stand out. Filamentous fungi are present throughout the production cycle and can, under specific conditions, cause quality losses, producing unpleasant odors and flavors, and in some cases lead to the production of mycotoxins, thus compromising the safety of the final product (Chalfoun, 2010). Mycotoxins are defined as secondary metabolites produced by certain fungi that can colonize crops in the field and/or occur during food storage

(Gomiero, 2018). In coffee crops, ochratoxin A (OTA) and aflatoxin stand out, especially in dry-processed coffees (Khaneghah et al., 2019). OTA is the most studied mycotoxin in coffee crops and is produced by several fungi of the genera *Aspergillus* and *Penicillium* (Alves da Silva et al., 2020). The main species of OTA-producing fungi in coffee are *A. ochraceus*, *A. carbonarius* and *A. niger* (López-Rodríguez et al., 2024), which belong to the Circumdati and Nigri sections (Chalfoun, 2010).

Recent advances in microbial ecology, including metagenomics and high-throughput sequencing, have revealed a much greater diversity of fungi and bacteria in coffee ecosystems than previously identified by culture-dependent methods (Neto et al., 2018; Lu et al., 2025). These approaches are expanding our understanding of fungal community dynamics, their interactions with beneficial microorganisms, and their impacts on coffee safety and sensory attributes (Lu et al., 2022).

The comparison between organic and conventional production systems is also of paramount importance, as it highlights relevant ecological differences. In conventional systems, chemical control of pathogens is often used, which can reduce the microbial diversity present. In contrast, organic systems, due to the absence of chemical pesticides, tend to favor greater diversity of microorganisms, including beneficial fungi and yeasts that play an important role in fermentation

and improving coffee quality (Velmourougane, 2016; Consonni, Polla, & Cagliani, 2018; Andrade et al., 2023). Thus, understanding the interaction between beneficial and harmful microorganisms is essential to guide management practices that reconcile sustainability and excellence in coffee production.

It is known that the adoption of good agricultural practices, such as harvesting during the mature stage, avoiding using cherry fruits or grains that have had contact with soil and adequately drying harvests, is desirable and recommended to reduce contamination of coffee fruits and grains by filamentous fungal species that have toxigenic potential (Iamanaka et al., 2014), thus providing better beverage quality. Despite the harmful effects of fungi on coffee quality, naturally dried coffees show potential for specialty production. Studies indicate that dry-stage harvested coffee has more sucrose and fewer chlorogenic acids, enhancing beverage quality (Santos, R. et al., 2018). However, little is known about how harvesting at advanced stages of ripeness influences fungal contamination and its relationship with beverage quality. Furthermore, the implications of this contamination for SCA classification and the sensory profile of specialty coffees remain largely unexplored.

Based on the information gathered, it is assumed that:

(i) organic systems have greater diversity of filamentous fungi and a greater variety of sensory nuances; (ii) the use of cover crops contributes positively to the composition of the fungal community; and (iii) the production of ochratoxin A and aflatoxin will be restricted to the organic system. Thus, the objectives of this study were: (i) to evaluate the effect of organic and conventional production systems, with and without the use of cover crops, on the diversity of filamentous fungi; (ii) to verify the occurrence of toxigenic species and the production of mycotoxins; and (iii) to analyze the sensory quality of coffee beverages.

2 MATERIAL AND METHODS

2.1 Characterization of the area and experimental design

The study was conducted in the municipality of Santo Antônio do Amparo - MG, in two areas where Arabica coffee is grown: one area that uses organic management (20°88'78.35"S 44°95'12.36"W) and conventional management (20°90'42.20"S 44°94'59.51"W). The predominant climate is the Cwa type (humid subtropical) according to the Köppen classification, with a dry season in the winter, an average annual temperature of 19.4 °C and an average annual rainfall of 1,530 mm. The soil was classified as a Red-Yellow Latosol (Santos, H. et al., 2018).

The experiment was conducted in a randomized block design with a factorial scheme (2x2x2) that included 2

cultivars (Catiguá MG2 and Paraíso MG H419-1), 2 types of production systems (organic and conventional) and 2 different management practices (the presence and absence of cover crops); four replicates were used for a total of 32 plots.

The conventional Arabica coffee plantations were established in 2016/2017, and the organic plantations were established in 2015/2016, both with a spacing of 3.80 x 0.70 m, resulting in stands with 3,759 plants ha⁻¹. The experiment was implemented in November 2018, when cover plants sowed included sunflower (*Helianthus annuus* L.), pearl millet (*Pennisetum glaucum*), buckwheat (*Fagopyrum esculentum* Moench) and sunn hemp (*C. ochroleuca*, *C. breviflora* and *C. juncea*). Cutting was performed later in February 2019, and a second planting was performed in November 2019. The second cutting was performed in February 2020. In both years, the cover plants were not incorporated and were used only as mulch.

2.2 Evaluation of the percentage of contamination by filamentous fungi in coffee fruits and grains

Coffee samples from fruits at two maturation stages were analyzed: natural cherry (NC) fruits harvested in the cherry stage and taken to a yard for sun drying under sieves until reaching 11% moisture content, and dried fruits (DF) fruits harvested in the dry stage and taken to a yard for sun drying under sieves until reaching 11% moisture content.

A total of 25 NC coffee fruits and 25 DF were selected, superficially disinfected with 70% alcohol for 1 min followed by 1% sodium hypochlorite at 30 sec and washed with sterile distilled water three consecutive times. After disinfection, the fruits were plated directly on filter paper. The plates were incubated at a temperature of 25 °C for a period of 7 days.

After this period, the fruits were analyzed to evaluate the percentage of contamination. After counting, the colonies were transferred to malt extract agar (MA) culture medium for subsequent identification and incubated at 25 °C for a period of 7 days. The fungal isolates were identified to the genus level and in some cases at the species level based on the macroscopic and microscopic characteristics of the filamentous fungi using the standard method of inoculated Petri dishes and the standard keys of Klich (2002), Pitt (2000) and Samson et al. (2000).

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2.3 Phenotypic identification of fungal species of the genera *Aspergillus* and *Penicillium*

From pure cultures, fungi belonging to the genera *Aspergillus* and *Penicillium* were identified according to

references from Klich (2002) and Pitt (2000). The isolates were incubated in Czapek yeast agar culture medium at 25 °C and malt extract agar. After seven days of incubation, the macroscopic and microscopic characteristics of the filamentous fungi were observed.

2.4 Detection of the production of ochratoxin A by fungi of the genus *Aspergillus* belonging to Sections Nigri and Circumdati

The potentially toxigenic species of the genus *Aspergillus* belonging to Section Nigri were inoculated in CYA medium for 7 days at 25 °C. The potentially toxigenic species belonging to the Circumdati Section were inoculated in sucrose yeast extract (SYE). Thin-layer chromatography plates (Merk silica gel 60, 20x20) were used, and TEF-ethyl toluene acetate and 90% formic acid were used as the mobile phase. (60:30:10). After elution, the plates were dried in a laminar flow hood. The confirmation of the production of OTA was performed in ultraviolet light with the λ value set to 366 nm in a CAMAG chromatograph (UF-BETRACHTER).

2.5 Detection of the production of aflatoxins B₁ and B₂ by fungi of the genus *Aspergillus* belonging to the Flavi Section

The potentially toxigenic species of the genus *Aspergillus* belonging to the Flavi Section were inoculated in SYE medium for 7 days at 25 °C according to methods described by Filtenborg and Frisvad (1980). The confirmation of the production of aflatoxins was performed in ultraviolet light with the λ value set to 366 nm in a CAMAG chromatograph (UF-BETRACHTER). The isolates considered to be AFB1 and AFB2 producers had retention factors (RFs) and fluorescence spots similar to those of the standard.

2.6 Quality of beverages

The coffee was harvested in May 2019 and June 2020, when the fruits were predominantly in the cherry stage. The coffee fruits were washed to eliminate the poorly formed, broken and impure fruits, and an eight-liter sample of ripe fruits was taken from each experimental site. The samples were dry processed and dried on polyethylene mesh sieves, suspended on wooden supports (Borém et al., 2014).

After the coffee fruits reached 11% water content, they were stored in a cold chamber for 30 days at 16 °C to standardize moisture. After the rest period, the coffee beans were processed and prepared for sensory analysis.

The sensory analysis was performed by three certified and calibrated judges according to protocols of the ASC (Specialty Coffee Association - SCA, 2021) and the methodology proposed by Lingle (2011) for sensory

evaluation of specialty coffees. Five cups representing each sample were prepared under standard conditions, and scores (ranging from 0 to 10) were given for the following characteristics: uniformity, absence of defects, sweetness, fragrance/aroma, flavor, acidity, body, finish (aftertaste), balance and overall impression. The total scores of the sensory analysis consisted of the sum of the average scores of all the attributes, and those with scores equal to or above 80 were considered special coffees (Bemfeito et al., 2021). In the DF coffee treatment, the fruits were harvested at the dry mature stage. All processing was similar to that used for the NC coffee samples.

2.7 Statistics

Data on the percentage of contamination by filamentous fungi in coffee fruits and grains are presented in percentages as the means of the two collection years (2019 and 2020). The percentage of contamination of coffee fruits and beans by filamentous fungi was evaluated by calculating the average percentage of contamination for two consecutive years (2019 and 2020).

The statistical analyses were performed with R software (3.3.1); analysis of variance was performed for the variables flavor, acidity, body, aroma, balance, completion, overall impression and total score through the following interactions: cultivars x production systems, cultivars x management and systems x management. The grouping of means was performed by the Scott–Knott test ($p < 0.05$) when a significant effect of a factor was identified by analysis of variance.

To complement the sensory evaluations of the beverages by scaled scores, the sensory profiles of the beverages were analyzed descriptively considering the sensory scores of each treatment in a single radar diagram, called a “sensoriogram”. Content analysis was also used, according to the adaptation proposed by Sobreira et al. (2015), for the sensory characterization of specialty coffees regarding the quality and intensity of the flavor and aroma nuances perceived by the judges in the sensory analysis.

3 RESULTS

3.1 Percentage of contamination by filamentous fungi

Seven genera of filamentous fungi were found and identified in Arabica coffee fruits and grains. Under a conventional production system in the presence of cover crops, regardless of the cultivar adopted and in the absence of disinfection, the most frequent genera were *Fusarium*, *Penicillium* and *Geotrichum* (Table 1).

Table 1: Percentage of contamination by filamentous fungi in coffee fruits at the natural cherry stage (NC) in different cultivation systems (conventional and organic) and cultivars (Paraíso MG H419-1 and Catiguá MG2) in the presence and absence of cover crops, with (D) and without disinfection (WD).

Genera/species	Conventional				Conventional				Organic				Organic			
	Paraíso MG H419-1				Catiguá MG2				Paraíso MG H419-1				Catiguá MG2			
	Presence of cover crops		Absence of cover crops		Presence of cover crops		Absence of cover crops		Presence of cover crops		Absence of cover crops		Presence of cover crops		Absence of cover crops	
	WD	D	WD	D	WD	D	WD	D	WD	D	WD	D	WD	D	WD	D
<i>Aspergillus parasiticus</i> section flavi	0.4															
<i>Aspergillus</i> section circumdati	2		1	38	4		3				3		0.3		1	
<i>Aspergillus</i> section Nigri	2		2		2		1	100	5		5	14			2	27
<i>Cladosporium</i> sp.									16		25	2			0.4	6
<i>Fusarium</i> sp1.	30	60	28	25	34		34		58	68	59	52	30	8	18	12
<i>Fusarium</i> sp2.	21		20		15		18				1		26	58	35	46
<i>Geotrichum</i> sp.	20		22		21		18		11	10	10	6				
<i>Penicillium</i> sp.	22	20	20		16		23		2	2	7	2	18	17	30	9
<i>Rhizopus</i> sp.													5	17	6	
Unidentified	2	20	7	38	9		4		24	4	17	2	18		9	

When the fruits were disinfected, there was a reduction in the occurrence of fungi. In the Paraíso MG H419-1 cultivar treatment and in the presence of cover crops, the predominant genera were *Fusarium* (60%), *Penicillium* (20%) and unidentified (20%). In the absence of cover crops, the most frequent genera were *Aspergillus* and unidentified (75%) and *Fusarium* (25%) (Table 1). For the cultivar Catiguá MG2, in the presence and absence of cover crops, there were no fungi and only *Aspergillus* (Section Nigri), respectively (Table 1). In the organic system, regardless of whether cover crops were planted and the cultivar studied, the most common genus was *Fusarium* (Table 1), with or without disinfection.

Tables 2 and 3 show the contamination percentages for fruits and coffee beans in the NC coffee. For the Paraíso MG H419-1 cultivar in the conventional production system and with the presence of cover crops, the fungi *Cladosporium* and *Penicillium* (71%) and *Cladosporium* and *Fusarium* sp 1 and sp 2 (94%) were the most frequent in the absence and presence of disinfection, respectively. In the absence of cover crops, regardless of whether disinfection occurred, the most frequent genera were *Cladosporium*, *Fusarium* and *Penicillium*.

Considering grains (Table 3) grown in the presence of cover crops, regardless of whether disinfection occurred, the most frequent genera were *Cladosporium*, *Fusarium* and *Penicillium*. In the absence of cover crops, the genera that showed greater prevalence were *Cladosporium*, *Fusarium* sp 1 and *Penicillium* in the absence of disinfection and *Cladosporium* and *Fusarium* sp 2 in the presence of disinfection.

For the Catiguá MG2 cultivar, regardless of whether cover crops were present, the predominant genera were

Cladosporium and *Penicillium* in the absence of disinfection and *Cladosporium* and *Fusarium* sp 2 in the presence of disinfection (Table 2). In the grains, the most frequent genera were *Cladosporium* and *Penicillium* in the absence of disinfection, regardless of whether cover crops were planted (Table 3). When disinfection was performed, no filamentous fungi were observed.

For the Paraíso MG H419-1 cultivar in the organic production system with and without cover crops, the fungi *Cladosporium* and *Penicillium* and *Cladosporium*, *Fusarium* and *Penicillium* were the most frequent in the absence and presence of disinfection., respectively (Table 2). In turn, the predominant fungi in the grains were *Cladosporium*, *Fusarium* sp 1 and *Penicillium* (Table 3). For the Catiguá MG2 cultivar in the organic system in the presence and absence of the cover crop, the fungi *Cladosporium* and *Penicillium* and *Cladosporium* and *Fusarium* sp 1 were the most frequent in the absence and presence of disinfection, respectively (Table 2). Among the grains (Table 3) from plants grown in the presence of cover crops, the genera *Cladosporium* and *Penicillium* were the most frequent without disinfection, and the genus *Penicillium* was the most frequent with disinfection (76%). In the absence of cover crops, regardless of whether disinfection occurred, the predominant genus was *Cladosporium*. It is worth noting that the fungi *Aspergillus ostianus* Section Circumdati, *Aspergillus* Section Nigri and *Rhizopus* sp. occurred exclusively in the conventional production system. The fungus *Penicillium funiculosum* occurred only in the Catiguá MG2 treatment in the organic production system.

Table 2: Percentage of contamination by filamentous fungi in parchment coming from NC coffee dried in the yard in different cultivation systems (conventional and organic) and cultivars (Paraíso MG H419-1 and Catiguá MG2) in the presence and absence of cover crops, with (D) and without disinfection (WD).

Genera/species	Conventional				Conventional				Organic				Organic			
	Paraíso MG H419-1				Catiguá MG2				Paraíso MG H419-1				Catiguá MG2			
	Presence of cover crops		Absence of cover crops		Presence of cover crops		Absence of cover crops		Presence of cover crops		Absence of cover crops		Presence of cover crops		Absence of cover crops	
	WD	D	WD	D	WD	D	WD	D	WD	D	WD	D	WD	D	WD	D
<i>Aspergillus</i> section flavi	6		2		3		2		0.4		1		1			
<i>Aspergillus</i> section circumdati	5	2	2		3		3	3	6	4	3	6	6	7	6	8
<i>Aspergillus ostianus</i> section circumdati			1													
<i>Aspergillus</i> section Nigri	3		3		7	1	3	1	5		6		5		9	
<i>Cladosporium</i> sp.	34	31	40	31	37	42	40	34	42	45	47	33	52	60	50	51
<i>Fusarium</i> sp1.	11	20	15	18	10	13	12	27	14	15	11	24	6	12	6	16
<i>Fusarium</i> sp2.	5	43	9	48	7	42		31	5	21	6	31				13
<i>Penicilium</i> sp.	37	2	29	1	33	1	38	1	27	15	26	3	30	11	30	13
<i>Rhizopus</i> sp.		2		3		2	1	3		1		2		10		

Table 3: Percentage of contamination by filamentous fungi in yard-dried coffee beans from NC coffee samples in different cultivation systems (conventional and organic) and cultivars (Paraíso MG H419-1 and Catiguá MG2) in the presence and absence of cover crops, with (D) and without disinfection (WD).

Genera/species	Conventional				Conventional				Organic				Organic			
	Paraíso MG H419-1				Catiguá MG2				Paraíso MG H419-1				Catiguá MG2			
	Presence of cover crops		Absence of cover crops		Presence of cover crops		Absence of cover crops		Presence of cover crops		Absence of cover crops		Presence of cover crops		Absence of cover crops	
	WD	D	WD	D	WD	D	WD	D	WD	D	WD	D	WD	D	WD	D
<i>Aspergillus westerdijkiae</i> section circumdati			3		2				5				18		4	
<i>Aspergillus ostianus</i> section circumdati			3													
<i>Aspergillus</i> section circumdati	4		4		3		25		6		13		6	5	16	
<i>Aspergillus</i> section Nigri	3		3		7		2								12	
<i>Cladosporium</i> sp.	23	24	54	57	28		49		30	33	60	33	27	19	47	100
<i>Fusarium</i> sp1.		29	19		10		5		10	33					6	
<i>Fusarium</i> sp2.	28	29		43	4				16		14	33	10			
<i>Penicilium</i> sp.	41	19	15		47		19		33	33	13	33	39	76	15	
<i>Penicilium funiculosum</i>													1			
<i>Rhizopus</i> sp.	1															

Tables 4 and 5 show the contamination percentages for DF and beans. Table 4 shows that there was a greater diversity of fungal contaminants in the organic coffee than in the conventionally grown coffee. In the organic system, the predominant fungi in the Paraíso MG H419-1 cultivar were *Cladosporium* and *Geotrichum*, while *Penicillium*, *Rhizopus* and *Fusarium* sp 1 and sp 2 were predominant in the grains. Conversely, when considering the

beans of the cultivar Catiguá MG2, the predominant fungi were *Cladosporium* and *Penicillium brevicompactum*, regardless of whether cover crops and disinfection were implemented. In the conventional system, considering grains and coffee beans (Tables 4 and 5), regardless of the cultivar used, the presence or absence of planting cover crops and disinfection, the predominant genera were *Cladosporium*, *Fusarium* and *Penicillium*.

Table 4: Percentage of filamentous fungi contamination in parchment coffee from dried coffee (DF) in different cultivation systems (conventional and organic) and cultivars (Paraíso MG H419-1 and Catiguá MG2) in the presence and absence of cover crops, with (D) and without disinfection (WD).

Genera/species	Conventional				Conventional				Organic				Organic			
	Paraíso MG H419-1				Catiguá MG2				Paraíso MG H419-1				Catiguá MG2			
	Presence of cover crops		Absence of cover crops		Presence of cover crops		Absence of cover crops		Presence of cover crops		Absence of cover crops		Presence of cover crops		Absence of cover crops	
	WD	D	WD	D	WD	D	WD	D	WD	D	WD	D	WD	D	WD	D
<i>Aspergillus</i> section circumdati					10		7	4	2		3		6		1	
<i>Aspergillus</i> section Nigri	1		1	3	1		1	1					1		1	
<i>Cladosporium</i> sp.	44	7	38	22	54	23	58	40	49	28	44	36	36	35	40	37
<i>Fusarium</i> sp1.	10	75	24	21	13	47		27	4	20	9	21	2	6	3	17
<i>Fusarium</i> sp2.	15	15		32		6	2	5	2	4		3		11	3	12
<i>Geotrichum</i> sp.									14	39	8	17	15	6	20	
<i>Penicilium</i> sp.					7	12	6		18	1	11	1	6	8		
<i>Penicilium brevicompactum</i>	23	4	21		14	13	26	23	8	7	24	23	30	33	32	32
<i>Rhizopus</i> sp.	7		16	22					1				1			1
<i>Talaromyces</i>									4				3			1

Table 5: Percentage of filamentous fungi contamination in coffee beans from DF coffee in different cultivation systems (conventional and organic) and cultivars (Paraíso MG H419-1 and Catiguá MG2) in the presence and absence of cover crops, with (D) and without disinfection (WD).

Genera/species	Conventional				Conventional				Organic				Organic			
	Paraíso MG H419-1				Catiguá MG2				Paraíso MG H419-1				Catiguá MG2			
	Presence of cover crops		Absence of cover crops		Presence of cover crops		Absence of cover crops		Presence of cover crops		Absence of cover crops		Presence of cover crops		Absence of cover crops	
	WD	D	WD	D	WD	D	WD	D	WD	D	WD	D	WD	D	WD	D
<i>Aspergillus parasiticus</i> section flavi					25											
<i>Aspergillus westerdijkiae</i> section circumdati	7	9							5							
<i>Aspergillus</i> section circumdati		9	7						3							
<i>Aspergillus</i> section Nigri			3				22		1				10		21	
<i>Cladosporium</i> sp.	15		41		15		33		16		37		27		10	
<i>Fusarium</i> sp1.	2								67							
<i>Fusarium</i> sp2.	56	55	10	100	12	44	3	50	31	33		50		13	5	17
<i>Penicilium</i> sp.	2		39		26	19	42	50	16		63	50	28		64	83
<i>Penicilium brevicompactum</i>	15	27			47	13			28				35	87		
<i>Rhizopus</i> sp.	2															

3.2 Toxicogenic capacity of species of the genus *Aspergillus*

The toxigenic potentials of a total of 56 isolates belonging to the genus *Aspergillus* obtained from Arabica coffee beans and fruits grown in organic and conventional production system in the presence and absence of cover crops in 2019 and 2020 were tested.

No isolate produced the OTA toxin. Only one fungal isolate (*A. Parasiticus* Section Flavi) from the Paraíso MG

H419-1 cultivar NC treatment in the organic system and in the presence of cover crops produced aflatoxins B₁ and B₂.

3.3 Quality of beverages

3.3.1. Cultivars x production systems

In the sensory analysis, it was observed that the cultivar Paraíso MG H419-1 in the conventional system and

Catiguá MG2 in the organic system had the highest scores for NC coffees (Figure 1a). The grouping test was not able to differentiate the means for the DF treatment (Figure 1b).

Tables 6 and 7 show the means of the characteristics evaluated in the sensory analysis. The mean test did not distinguish between the NC treatments (Table 6), and for the DF treatment (Table 7), the analysis of variance showed a lower mean for the equilibrium attribute in the Catiguá MG2 cultivar in the organic system and the interaction between cultivars and production systems.

3.3.2 Cultivars x management

In the sensory analysis, the cultivar Paraíso MG H419-1 grown with cover crops and Catiguá MG2 grown without cover crops had the highest sensory scores for the NC treatment (Figure 2a). The grouping test applied did not differentiate the means of the DF treatment (Figure 2b).

3.3.3. System x management

Regarding the sensory analysis, coffee grown in the conventional production system in the presence of

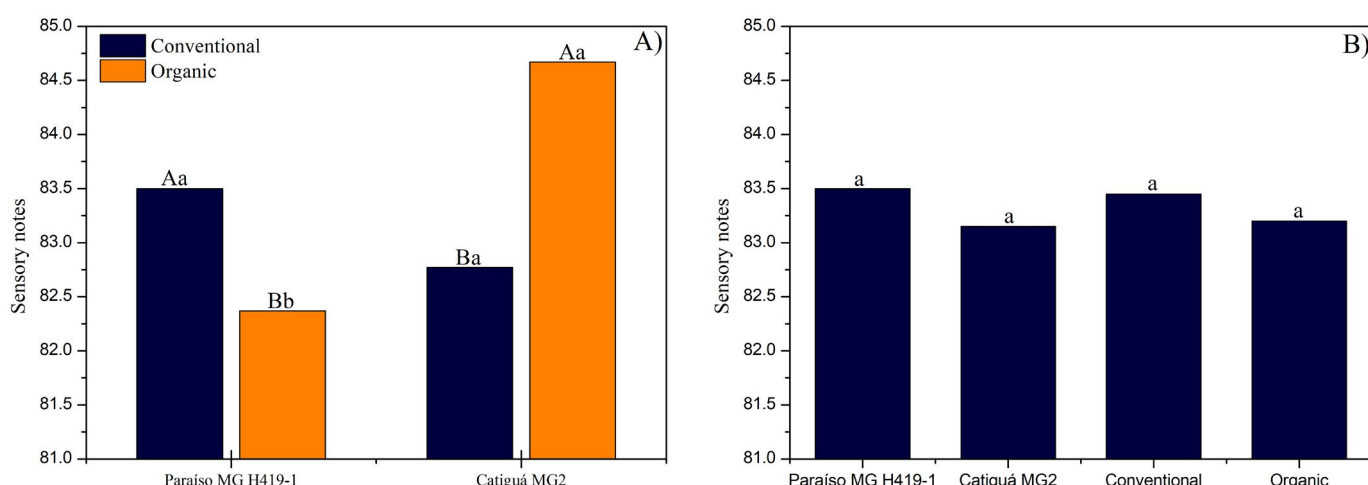


Figure 1: Beverage quality of natural cherry (NC) (a) and dried fruits (DF) (b). Lowercase letters indicate the comparison between cultivars within the same production system and uppercase correspond to the comparison of the same cultivar in different production systems. Averages followed by the same letter belong to the same group according to the Scott-Knott test ($p < 0.05$).

Table 6: Statistical analysis for sensory attributes considering cultivars and production systems for NC coffee.

Treatments	Sensory attributes						
	Flavor	Acidity	Body	Aroma	Balance	Finish	Overall impression
Paraíso MG H419-1	7.72 a	7.58 a	7.75 a	7.48 a	7.50 a	7.52 a	7.5 a
Catiguá MG2	7.25 a	7.05 a	7.22 a	6.97 a	6.93 a	7.13 a	6.93 a
Conventional	7.15 a	7.00 a	7.20 a	6.90 a	6.85 a	6.98 a	6.85 a
Organic	7.82 a	7.63 a	7.77 a	7.55 a	7.58 a	7.67 a	7.58 a
CV %	8.77	8.34	8.62	8.54	8.45	8.83	8.45

Averages followed by the same letter belong to the same group according to the Scott-Knott test ($p < 0.05$).

Table 7: Statistical analysis for sensory attributes considering DF coffee cultivars and production systems.

Treatments	Sensory attributes						
	Flavor	Acidity	Body	Aroma	Finish	Overall impression	Balance
Paraíso MG H419-1	7.8 a	7.60 a	7.87 a	7.5 a	7.67 a	7.53 a	Cultivares
Catiguá MG2	7.8 a	7.62 a	7.72 b	7.47 a	7.65 a	7.47 a	
Conventional	7.8 a	7.63 a	7.80 a	7.52 a	7.68 a	7.53 a	Production systems
Organic	7.8 a	7.58 a	7.78 a	7.45 a	7.63 a	7.47 a	
CV %	1.77	1.68	1.37	1.07	2.21	1.07	1.15

Lowercase letters indicate the comparison between cultivars within the same production system and uppercase correspond to the comparison of the same cultivar in different production systems. Averages followed by the same letter belong to the same group according to the Scott-Knott test ($p < 0.05$).

cover crops had the lowest mean scores (Figure 3a). There were no significant differences within the DF treatment (Figure 3b).

3.3.4 Sensory profile and content analysis

It was observed that all the cultivars, regardless of the production type and management system, had scores higher than 80 points (Figures 1, 2 and 3) and were then classified as specialty coffees. According to the sensory profiles of the coffees harvested in the NC stage, the Catiguá MG2 cultivar in the conventional system showed the highest scores for the different attributes evaluated regardless of whether cover crops were used (Figure 4a). Among the coffee harvested in the dry maturation stage (Figure 4b), the Catiguá MG2 cultivar grown in the organic system without cover crops stood out in terms of flavor and acidity.

The content analysis was performed based on descriptions of flavors identified by the judges in the sensory analysis, aiming to differentiate the treatments according to the quality and intensity of the nuances in the coffees (Figure 5). A total of 35 and 17 nuances/flavors were reported for the NC and DF treatments, respectively.

For NC and DF treatment, the nuances were grouped into the following categories: fruity (yellow fruits, tropical fruits, citrus fruits, black fruits, mango, vanilla, lemon, passion fruit, lemon with honey, cherry, lime, papaya, star fruit, and pineapple), caramelized (caramel, golden, honey, brown sugar, burnt sugar, and molasses), chocolate (milk chocolate, dark chocolate, milk chocolate with almonds, and dark chocolate with almonds), and others (cereal, floral, herbal, light fat, wine, chestnut, sugarcane juice, mold, and hops).

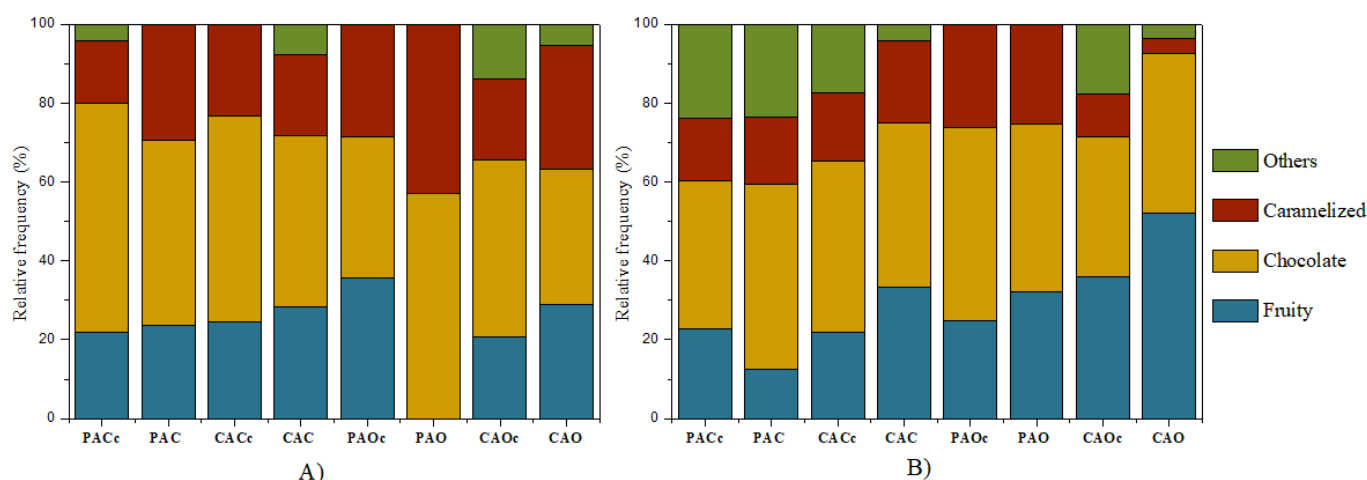


Figure 2: Beverage quality of natural cherry (NC) (a) and dried fruits (DF) (b). Lowercase letters indicate the comparison between cultivars within the same management and uppercase correspond to the comparison of the same cultivar in different managements. Averages followed by the same letter belong to the same group according to the Scott-Knott test ($p < 0.05$).

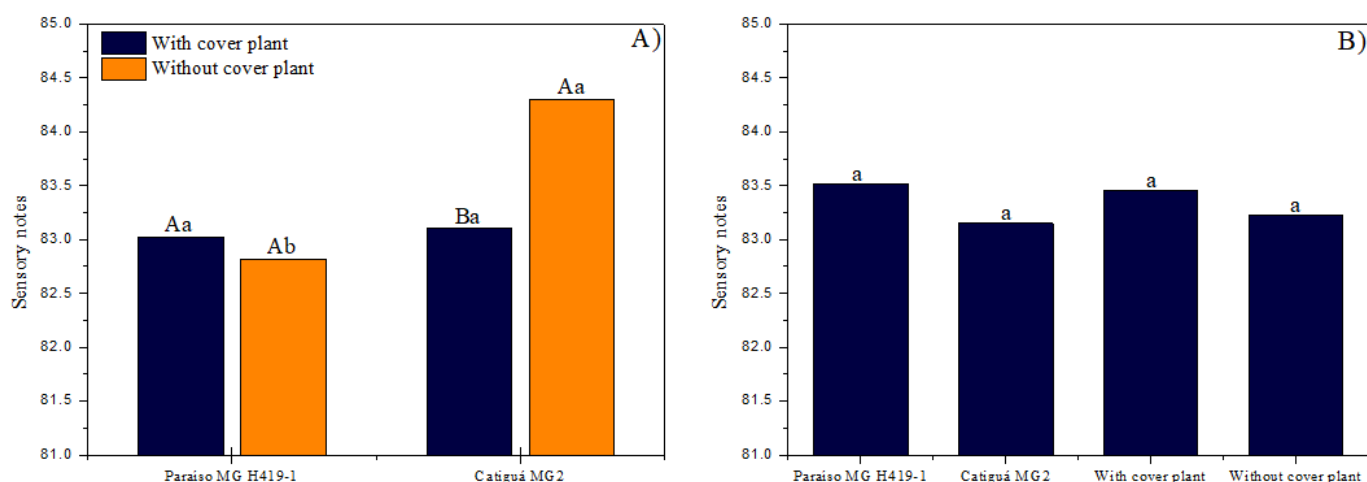


Figure 3: Beverage quality of natural cherry (NC) (a) and dried fruits (DF) (b). Lowercase letters indicate the comparison between production systems within the same management and uppercase correspond to the comparison of the same production system in different managements. Averages followed by the same letter belong to the same group according to the Scott-Knott test ($p < 0.05$).

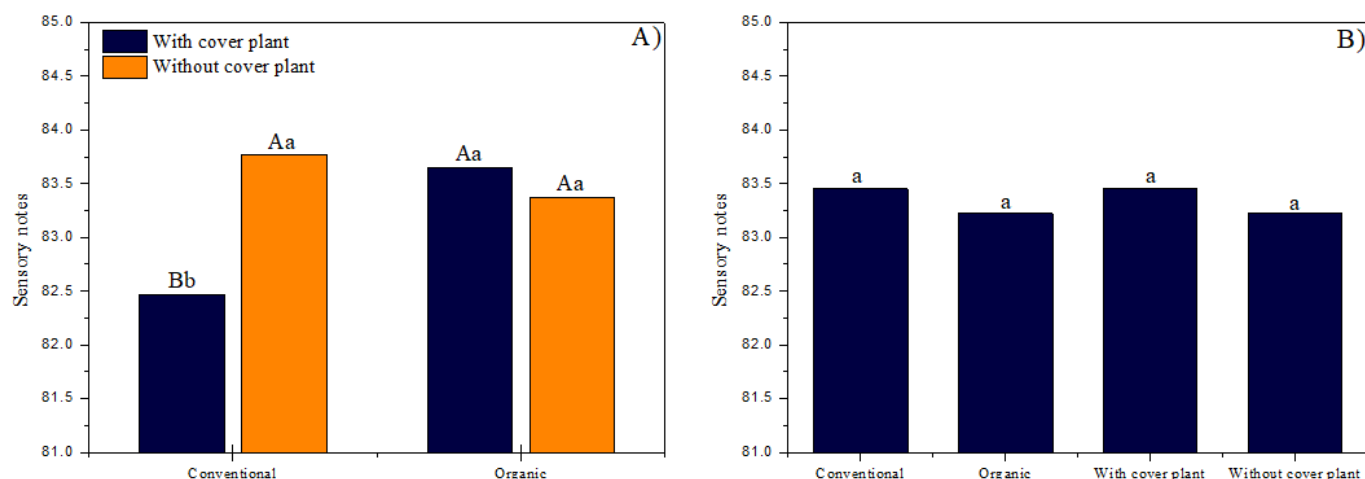


Figure 4: Sensory profile (sensoriogram) of natural cherry (NC) (a) and dried fruits (DF) (b) coffees considering cultivars, production systems and management and the characteristics flavor, acidity, body, finish and balance. Treatments are identified as PACc, PAOc, CACc, and CAOc (with cover crops), and PAC, PAO, CAC, and CAO (without cover crops). The cultivars evaluated were Paraíso MG H419-1 (PA) and Catiguá MG2 (CA), each under conventional (C) and organic (O) production systems.

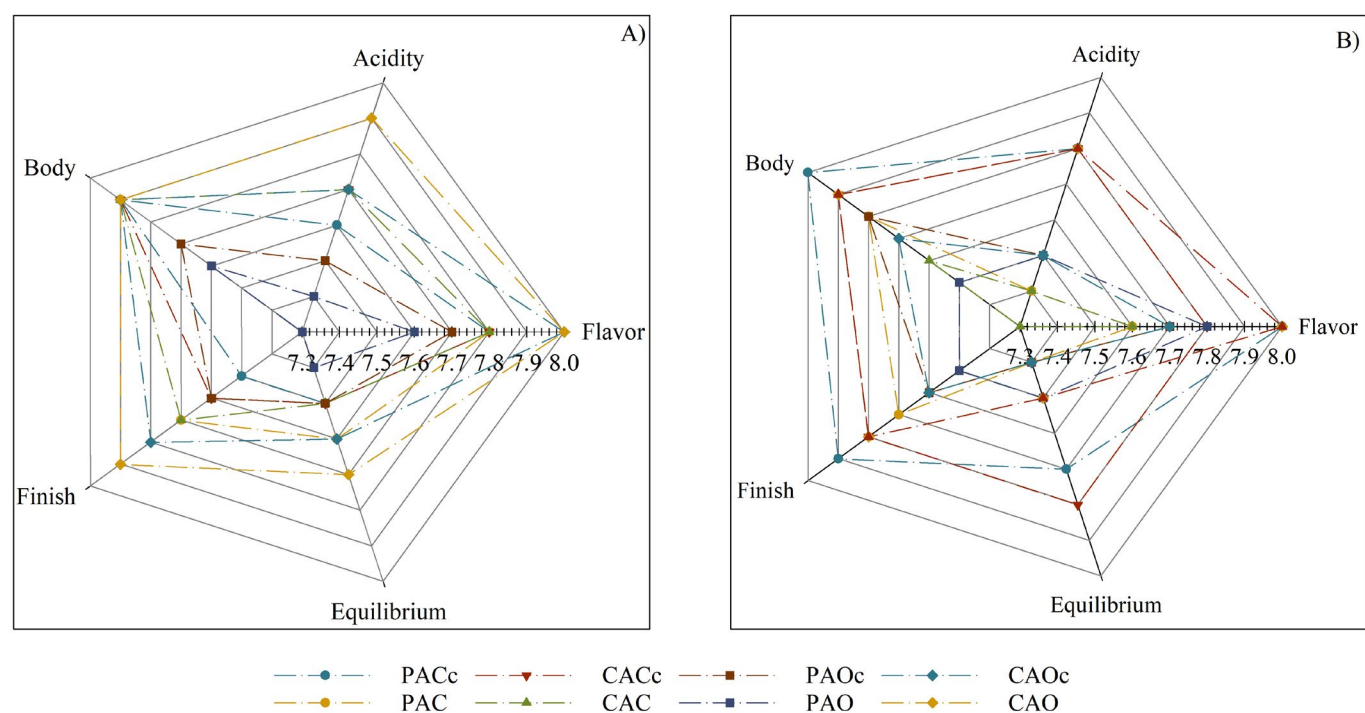


Figure 5: Content analysis of flavor and aroma nuances in natural cherry (NC) (a) and dried fruits (DF) (b) coffees. Bars represent the relative frequencies of descriptors. Treatments are identified as PACc, PAOc, CACc, and CAOc (with cover crops), and PAC, PAO, CAC, and CAO (without cover crops). The cultivars evaluated were Paraíso MG H419-1 (PA) and Catiguá MG2 (CA), each under conventional (C) and organic (O) production systems.

The greatest variety and frequency of nuances were observed in the Catiguá MG2 cultivar grown in the organic farming system with cover crops; in addition to common nuances such as fruity, caramel, golden, and chocolate, nuances were perceived that were not found in the other treatments, such as star fruit, dark chocolate with almonds,

herbs, and hops (Figure 5a). The greatest diversity of nuances was observed in the Paraíso MG H419-1 cultivar grown in the conventional system with cover crops. The nuances of brown sugar, citrus fruits, lemon with honey, and milk chocolate with almonds were observed exclusively in this treatment (Figure 5b).

4 DISCUSSION

Considering the coffee harvested in the NC stage, the most frequent fungi were *Cladosporium*, *Fusarium* and *Penicillium*, regardless of the production and management systems used. The genera *Cladosporium*, *Fusarium*, *Aspergillus* and *Penicillium* were consistently present in all the different treatments and combinations. The predominance of these genera may be associated and influenced by different growth needs, competition for space and the production of enzymes that have antifungal activity against bacteria and yeasts (Silva et al., 2021).

A greater diversity of contaminating fungi was observed in the organic system than in the conventional system, especially in coffee beans from the DF treatment. This finding is justified by the restriction of the use of pesticides in organic systems, which may represent an advantage in the preservation of desirable microorganisms such as some fungi and yeasts.

In the organic system, the most important fungi were *Cladosporium*, *P. brevicompactum*, *Geotrichum*, *Penicillium*, *Rhizopus* and *Fusarium*, regardless of whether cover crops were used and disinfection was performed. In the conventional system, the predominant genera were *Cladosporium*, *Fusarium* and *Penicillium*. The differences in the genera and fungal species identified between coffee harvested in the NC stage and as DF can be explained by the difference in exposure times to environmental factors, fermentation and drying processes. The exposure time and the difference in the drying location, combined with dry processing, may favor the proliferation of fungi. As in the present study, these factors may have affected the fungal percentage and diversity, since the DF were dried on the plant and the NC coffee underwent the drying process described by Borém et al. (2014).

Two species occurred exclusively in the production systems: *P. funiculosum* occurred only in NC coffee beans grown in the organic system, and *A. ostianus* Section *Circumdati* occurred only in coffee plants and NC coffee beans grown in the conventional production system. *P. funiculosum* is considered a ubiquitous fungus and is commonly found in soil and fruits (Yadav et al., 2018). Although the presence of OTA-producing species such as *A. ostianus* warrants attention, their occurrence was isolated and not associated with detectable mycotoxin production. In contrast, *Cladosporium* was consistently found in all stages and systems and is often associated with good beverage quality, as it can inhibit the colonization of toxigenic or spoilage fungi in coffee beans (Chalfoun, 2010). These fungi are widely distributed in soil, plant debris, and the atmosphere and can grow even with low water availability (Bensch et al., 2018).

Only one isolate, *A. parasiticus*, produced aflatoxins B₁ and B₂ in the Paraíso MG H419-1 cultivar grown in an organic system with cover crops. The production of

aflatoxins, especially from the fungi *A. flavus*, *A. paraciticus* and *A. nominous*, occurs in coffee crops (Azam et al., 2021). Mycotoxins are by far the most important contaminants in the food chain. Several studies have suggested that there is no evidence to support the claim that organic agriculture would lead to an increased risk of contamination by mycotoxins due to the nonuse of chemical fungicides. Similarly, there is no evidence that organic foods are more susceptible than conventionally grown foods to bacterial and fungal contamination (Mie et al., 2017).

It is known that in coffee, there is a relationship between the presence of fungi, bacteria and yeasts and the diversity of aromas and flavors, with low-quality coffees tending to have lower nuance diversity (Silva et al., 2021). The negative effects of microorganisms on coffee quality over time can be attributed to the fermentation process, mainly carried out by pectinolytic microorganisms that degrade the pulp and mucilage of coffee, producing alcohols and acids that affect the final quality of the beverage.

Regarding the sensory analysis, significant effects of the cultivar and production system were observed only for the balance attribute, which is understood as the complementation and contrast between the attributes flavor, finish, acidity and body of the beverage (SCA, 2021). Only the cultivar Catiguá MG2 grown in the organic system had a lower mean for this attribute. This shows the imbalance of the components of the balance attribute and may indicate that the coffee had a very delicate acidity or little body. Thus, the production system, the adoption of cover crops and the cultivars studied do not have a predominant direct influence on the evaluated attributes.

All the evaluated treatments had a total score above 80 points, being classified as “Premium” specialty coffees (SCA, 2021), these results are similar to those found by Sobreira et al. (2015) and Barbosa et al. (2020) for the cultivars Paraíso MG H419-1 and Catiguá MG2. Regardless of the adoption of cover crops, according to the sensorigram, the Catiguá MG2 cultivar in the NC treatment had superior characteristics. In the DF treatment, the cultivars Catiguá MG2 and Paraíso MG H419-1 stood out, considering the flavor and acidity attributes, as observed for the Paraíso MG H419-1 cultivar (Barbosa et al., 2020).

Increased scores and ability to classify coffees according to the SCA criteria reflect better remuneration for producers (Silva et al., 2021). Coupled with the increase in the final score, the search by consumers for coffees with naturally differentiated flavors and aromas has grown (Guimarães et al., 2019). In this sense, the study of nuances through content analysis can assist in the differentiation of specialty coffees and help producers meet certain market niches. It was found that fruity notes (fruity, yellow fruits, citrus fruits, tropical fruits, passion fruit, lemon with honey and pineapple), caramelized flavors (caramel, brown, honey, brown sugar and molasses), chocolate flavors

(chocolate, milk chocolate, dark chocolate and milk chocolate with almonds) and floral flavors occurred in the NC coffee and DF, but at different levels. These nuances were also observed by Sobreira et al. (2015) and Freitas et al. (2020).

Considering the treatments evaluated in the present study, a greater variation in nuances was observed in the NC treatment, especially in the Catiguá MG2 cultivar grown in the organic system with cover crops, where common nuances and more complex nuances, such as carambola, dark chocolate with almonds, herbal and hops, were observed. Among the DF, the Paraíso MG H419-1 cultivar grown in a conventional system with cover crops showed a greater diversity of nuances than the other treatments. Planting cover crops may be beneficial for improvements in beverage quality and nuances.

Although the maturation stage considered ideal for harvesting is the NC stage, under specific conditions, harvesting fruits in the dry stage did not cause losses in quality. Considering that the maturation process and, consequently, the harvest occur over a short period of time, the possibility of harvesting at a more advanced maturation stage could extend the harvesting period. This is because the traditional method of harvesting all the fruits at once results in the harvesting of fruits at different stages of maturation. This represents a great challenge to achieve high uniformity of fruit maturation in coffee cultivation.

5 CONCLUSION

Fungal diversity and coffee quality were influenced by the production system, cover crops, and harvest stage. *Cladosporium*, *Fusarium*, and *Penicillium* predominated in all treatments, with greater diversity in the organic system. Coffees harvested in the dry phase maintained premium quality, with distinct fruity, caramelized, and chocolate notes. These results demonstrate that harvesting in the dry phase and managing cover crops can maintain coffee quality without increasing fungal contamination, contributing to more sustainable and profitable coffee production systems.

6 AUTHOR CONTRIBUTION

Conceptual idea: Barros, V.M.S.; Chalfoun, S.M.; Carvalho, G.R. Methodology design: Barros, V.M.S.; Chalfoun, S.M.; Carvalho, G.R. Data collection: Barros, V.M.S.; Lira, N.A.; Passamani, F.R.F.; Data analysis and interpretation: Barros, V.M.S.; Lira, N.A.; Passamani, F.R.F.; Pereira, D.G.C.; Andrade, V.T.; Writing and editing: Barros, V.M.S.; Pereira, D.G.C.; Luz, S.R.O.T.; Chalfoun, S.M.; Carvalho, G.R.

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8 DATA AVAILABILITY STATEMENT

Data available upon request to authors.

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