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Short communication

Potential of volatile compounds produced by fungi to influence sensory quality of coffee beverage

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ABSTRACT

Fungi are known producers of a large number of volatile compounds (VCs). Several VCs such as 2,4,6 trichloroanisole (TCA), geosmin and terpenes have been found in coffee beverages, and these compounds can be responsible for off-flavor development. However, few studies have related the fungal contamination of coffee with the sensory characteristics of the beverage. The aim of this research was to investigate the production of VCs by fungi isolated from coffee and their potential as modifiers of the sensory coffee beverage quality. Three species were isolated from coffee from the southwest of São Paulo state and selected for the study: *Penicillium brevicompactum*, *Aspergillus luchuensis* (belonging to section *Nigri*) and *Penicillium* sp. nov. (related to *Penicillium crustosum*). VCs produced by the fungal inoculated in raw coffee beans were extracted and tentatively identified by SPME–GC–MS. Different VCs that may interfere in the coffee beverage quality were detected in the raw coffee beans inoculated with these fungal species (mainly *A. luchuensis*). Oct-1-en-3-ol was detected in the raw coffee inoculated with *A. luchuensis*. This compound, which is characterized by earthy and moldy/mushroom aroma, can be related to negative characteristics of coffee beverage in sensory analysis. On the other hand, the presence of some fungal species in the coffee, even at a high percentage of infection, did not necessarily result in loss of the sensorial quality of the beverage, since the samples with a high infection of *P. brevicompactum* showed positive sensory evaluation.

1. Introduction

Several VCs, such as monoterpenes, sesquiterpenes, alcohols, aldehydes, aromatic compounds, esters, furans, hydrocarbons, ketones, as well as nitrogen and sulfur compounds are produced by filamentous fungi and formed during their primary and secondary metabolism (Jelen & Wasowicz, 1998; Larsen & Frisvad, 1995; Müller et al., 2013). The fungal VCs have been identified as off-flavors and since they can be detected before visible microbial growth, they are used as food and feed spoilage indicators (Borjesson, 1993; La Guerche, Dauphin, Pons, Blancard, & Darriet, 2006; Parinet, Rodriguez, & Sérodes, 2010).

Currently, gas chromatography coupled with mass spectrometry (GC–MS) is the main method for detecting fungal VCs due to its powerful separation and highly sensitive detection (Bennett, Hung, Lee, & Padhi, 2012; Morath, Hung, & Bennett, 2012; Polizzi, Adams, De Saeger, et al., 2012; Polizzi, Adams, Malysheva, et al., 2012). The headspace technique and the use of solid adsorbents, such as Tenax and solid-phase microextraction (SPME) followed by thermal desorption into the GC–MS, have been used to extract VCs from different samples. The use of SPME has the advantage of providing the desorption in the GC injector which reduces the preparation time, and can also be used for fungal cultures (Morath et al., 2012). SPME coupled with GC–MS has also been used to detect VCs and off-flavors from coffee (Agresti, Franca, Oliveira, & Augusti, 2008; Bicchi, Iori, Rubiolo, & Sandra, 2002; Toci & Farah, 2008).

VCs such as 2,4,6-trichloroanisole (TCA), geosmin, methylisoborneol, 2-methyl-isobutanol, 2-methoxy-3-isopropylpyrazine, 2-methoxy-3-isobutylpyrazine and dimethyl sulfide were identified in coffee beverages and related to off-flavors and negative notes (Cantergiani et al., 2001; Spadone & Liardon, 1988; Spadone, Takeoka, & Liardon, 1990; Toci & Farah, 2008). Furthermore, few studies have indicated that these off-flavor compounds can be related to the presence of microorganisms and microbial fermentation in the coffee beverage. Spadone et al. (1990) found that TCA and 2,4,6-trichlorophenol (TCP), the precursor of TCA, as the compounds most responsible for Rio flavor in Rio coffee, which is characterized by medicinal and phenolic taste. These authors also suggested that the molds from Rio coffee beans can be capable of converting TCP to TCA and develop these characteristics in the beverage. The quality of coffee beans was also related to the presence of defective beans (Agresti et al., 2008; Toci & Farah, 2008). According to Toci and Farah (2008) the PVA, which is defined as black (P), green (V) and sour (A) defective beans, contribute to the cup quality decrease and the

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defects black and sour are originated when the beans have contact with the soil, which can result in a high fungal infection. Iamanaka et al. (2014) found that overripe cherries from the trees and cherries from the ground showed negative sensorial evaluation with attributes such as moldy, dirty and fermented and a high contamination by *Aspergillus* section *Nigri* and *Aspergillus westerdijikiae*. Thus it is possible that the sensory characteristics of the beverage can also be affected by the presence of filamentous fungi in coffee beans since they have been shown to be able to produce a large number of VCs.

In this sense, the objective of this study was to investigate the influence of VCs produced by fungi on sensory coffee quality.

2. Material and methods

2.1. Fungal isolates

Three fungal species isolated from coffee beans from the southwest of São Paulo state were selected for this study: *Aspergillus luchuensis* (belonging to *Aspergillus* section *Nigri*), *Penicillium* sp. nov. (closely related to *Penicillium crustosum*) and *Penicillium brevicompactum*. The production of VCs was investigated in the coffee inoculated with these species and in the culture medium.

2.2. Coffee sample

Arabica coffee beans from the southwest of São Paulo state were collected from a storage facility. In order to promote only the growth of the specific fungal species, the coffee beans were sterilized using Gamma radiation at 3 kGy dose (gamma rays Co-60).

2.3. Inoculation of fungi in coffee

The strains were inoculated on malt extract agar (MEA) and incubated at 25 °C for 5 days. Coffee was contaminated using an inoculum of 10^6 spores/mL concentration of each fungal species. For contamination of the previously sterilized coffee beans, 2 mL of the initial suspension was added to 150 g of raw coffee, followed by the addition of 3 mL of sterile water in order to increase the water activity of the sample and to allow fungal growth. After 7 days at 25 °C, the samples (inoculated and control) were submitted to sensory analysis according to Section 2.4.

2.4. Sensory analysis

Sensory analyses were carried out in the beverages prepared with irradiated and non-irradiated coffee beans. The beverage was evaluated by 5 trained judges who were coffee experts from the Assicafé tasters´ group (São Paulo). A single session with a maximum of dozen samples was carried out. The tests were conducted in an isolated room, free from any external interferences, at 25 °C (temperature controlled by air conditioning). Infusion, diluted espresso and espresso tasting tests were carried out to evaluate the characteristics of the beverage according to the methodology described by Illy and Viani (2005). The raw coffee was roasted at 220 °C for 5–6 min in a Probat gas roaster BRZ4 (Emmerich, Germany). For the infusion test, 10 g of powder was added to 100 mL of water at 90 °C, and for the espresso test, the beverage was prepared with 13 g of powder and 50 mL of water at 90 °C under pressure (9 bar) with a leaching time of 30 s (La Marzocco) (EE-2G model, Florence, Italy). Diluted espresso was obtained by diluting the espresso beverage with water at 80 °C (1:2, v/v).

The following attributes were evaluated by the experts by a 10 point structured scale: body, aroma, acidity, bitterness, sweetness and astringency. The final assessment of the beverage was described as positive or negative after the team consensus decision. Moreover, the presence of positive flavors and aromas such as caramel, chocolate, almond, fruity and floral, and negative attributes including immature, fermented, woody, rancid, moldy, "riado", "rio" (these two denominations indicate chemical taste) and smoky was noted.

2.5. VCs produced by fungi

VCs were determined in the coffee inoculated with the fungal species (*A. luchuensis*, *Penicillium* sp. nov. and *P. brevicompactum*). VCs produced by these species were also detected in the Czapek Yeast Extract (CYA).

2.5.1. Volatile compound extraction

The extraction of the VCs from raw coffee beans was carried out using 1.0 g of the sample, extraction temperature of 65 °C and extraction time of 30 min. Raw coffee beans were weighed in a 50 mL vial with a silicone septum and kept in a bath at 65 °C. A DVB/Carboxen™/PDMS Supelco fiber (Pennsylvania, USA) was used to extract the VCs. The exposure of the fiber to the volatiles was performed with the aid of a holder. After insertion of the needle through the silicone septum, the fiber w exposed to the headspace during the time defined for each experiment. After the adsorption times, the fiber was inserted directly into the gas chromatograph injector. Fungal species were inoculated onto Czapek Yeast Autolyzate agar and incubated at 25 °C for 7 days in tubes with silicone septum. To extract the fungi VCs from the culture medium, the fiber was directly exposed to the headspace of the tube maintained at room temperature (25 °C, controlled by air conditioning). After absorption the fiber was inserted directly into the gas chromatograph injector. The VCs from the culture medium (CYA) were not considered.

2.5.2. Identification of volatile compounds in coffee with GC–MS

Conditions for GC analysis were: injector at 270 °C, operating in splitless mode with purge valve opening after 0.7 min. SUPELCOWAX column (60 m × 0.25 mm × 0.25 μm Supelco) and helium as carrier gas at a flow rate of 1.0 mL/min, were used. The oven temperature program used was: beginning at 40 °C, raising it by 5 °C/min up to 250 °C and holding for 3 min at this temperature. Detection was performed in full scan mode after positive electron ionization (70 eV). The default of the equipment was used with m/z range of 10–700. The gas chromatography used was Agilent model 6890 coupled to a mass detector Agilent model 5973. Tests were carried out in duplicate.

For the identification of the VCs, the mass spectra obtained for each compound was compared to the NIST 98 Library and high similarity (>90%) was considered. In addition, an alkane series (C5–C24) was used to calculate the retention index (RI) for each compound and compared with RI values found in the literature for columns with the same polarity (Acree & Arn, 2013). For those compounds whose RI was not found in the literature, the identification was carried out only by comparison with the mass spectra. All the compounds were considered tentatively identified.

3. Results and discussions

3.1. Coffee inoculated with the fungal species

The 3 kGy of ionizing radiation Co-60 was sufficient to eliminate all fungal growth without developing negative effects on the sensory quality of the beverage.

After the inoculation, coffee beans showed 100% infection by the species *A. luchuensis*, *P. brevicompactum* and *Penicillium* sp. nov.

Table 1 shows the results of the sensory evaluation of the beverage prepared with the coffee beans inoculated with *A. luchuensis*, *P. brevicompactum*, *Penicillium* sp. nov. and with control (non-inoculated raw coffee).

Undesirable characteristics such as fermented, moldy and earthy were present in the sample inoculated with *A. luchuensis*. This species was considered similar to *Aspergillus kawachii* and *Aspergillus acidus*

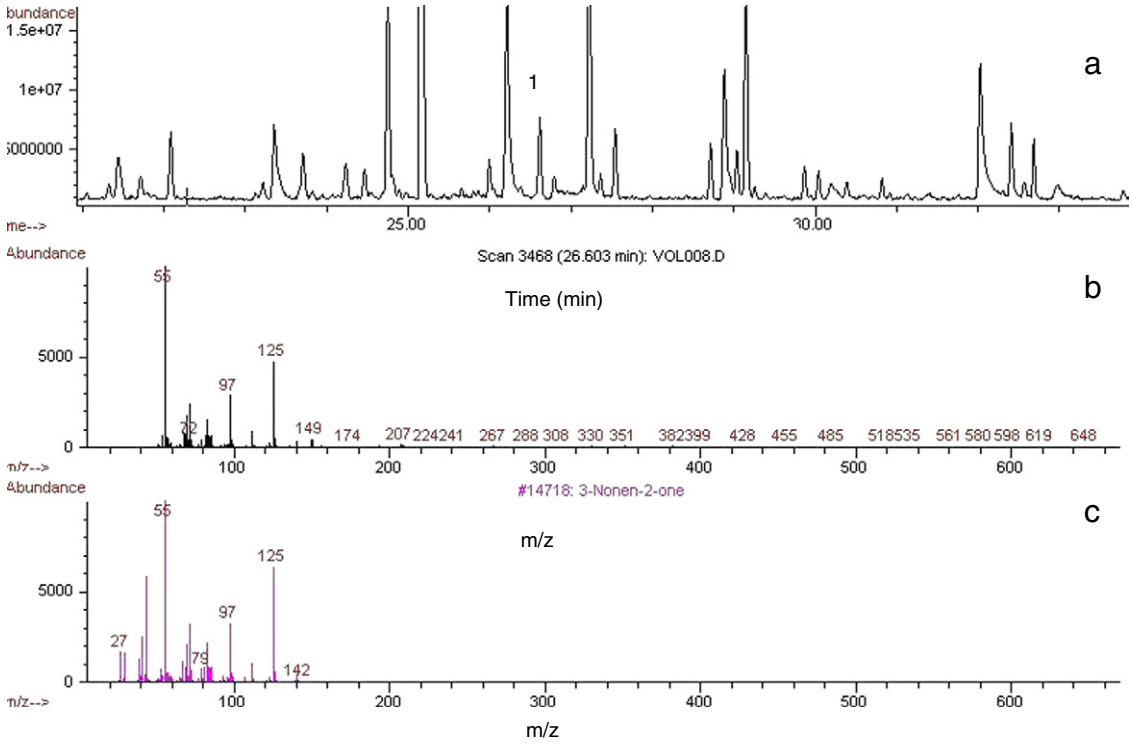

Fig. 1. Chromatogram, obtained by SPME–GC–MS, of the volatile compounds extracted from *Penicillium brevicompactum* in Czapek yeast autolyzate agar. Legend: (a) Peak 1 is non-3-en-2-one; (b) mass spectrum of the non-3-en-2-one present in the sample; and (c) mass spectrum of non-3-en-2-one according to the NIST 98 Library.

and is an important *black Aspergilli* in Asian countries related to the fermenting processes (Hong et al., 2013). The aroma characteristics of *Penicillium* sp. nov. were strongly fermented and immature. The beverage had negative sensory evaluation. The opposite occurred with the coffee inoculated with *P. brevicompactum*. This sample showed a positive final evaluation and desirable sensory characteristics such as floral aroma and caramel and sweet tastes. The presence of floral aroma was also observed in the beans inoculated with this species, even before the preparation of the beverage. The control sample (non-inoculated raw coffee) showed positive final evaluation. In the espresso test, this sample presented characteristics such as chocolate taste, bitterness and astringency, indicating that increasing the water activity of the raw coffee did not interfere negatively in the sensory characteristics of the final beverage.

As the results showed, the characteristics described in the sensory evaluation could be related with the presence of the fungi, since they have shown to be producers of diverse off-flavor VCs and have been used as food spoilage indicators. Several VCs, such as trichloroanisole acid (TCA), geosmin, methylisoborneol and dimethylsulfide have been found in coffee beverages and are responsible for undesirable flavor development also known as off-flavor (Cantergiani et al., 2001; Spadone & Liardon, 1988; Spadone et al., 1990). However, they were not correlated with the fungal species.

Table 2 shows the VCs found in the coffee beans inoculated with the studied species and in the control (non-inoculated raw coffee).

The production of ketones, such as octan-3-one (herbal odor) and pentadecan-2-one (floral aroma) was observed in the coffee inoculated with *P. brevicompactum*. These compounds can be responsible for the positive characteristics of the beverage, observed in the sensory analysis. In addition to these compounds, alcohols, such as 2-methylpropan-1-ol and hexan-1-ol and other compounds such as phenol, 2,6-bis(1,1-dimethylethyl)-4-ethyl and 7-methyl-3-methylene-1,6-octadiene (myrcene) were also identified. The former compound, which has a pleasant odor, is found in plants and used in the perfume industry.In the culture medium, *Penicillium brevicompactum* produced several aldehydes, such as 2-octenal and decanal, which are characterized by citrus, fresh and herbal flavors, and ketones, such as nonan-2-one, non-3-en-2-one, undecan-2-one, which are characterized by fruity aroma. Fig. 1 shows the chromatogram of the volatile compounds from *P. brevicompactum* (a) and the mass spectra of non-3-en-2-one (peak 1) from the sample (b) and from the Nist library (c).

Alcohols and 1,2,3,4-tetrahydronaphthalene (turpentine), which have a mixture of benzene and menthol odors, were detected in the coffee inoculated with *Penicillium* sp. nov. Only alcohols, such as methylpropan-1-ol, oct-1-en-3-ol and 3-methyl-1-butanol were detected in the coffee inoculated with *A. luchuensis*. The oct-1-en-3-ol is

Table 1
Sensory evaluation of non-inoculated coffee beans (control sample) and of coffee beans inoculated with *Aspergillus luchuensis*, *Penicillium brevicompactum* and *Penicillium* sp. nov.

Samples	Sensorial evaluation results
Control (Arabica coffee)	Infusion: regular Espresso: chocolate aroma, a little bitter and soft Final evaluation: positive
Arabica coffee + *Aspergillus luchuensis*	Infusion: fermented, moldy, earthy Espresso: stinker, moldy, earthy Final evaluation: negative
Arabica coffee + *Penicillium* sp. nov.	Infusion: strongly fermented Espresso: strongly fermented, astringent, immature Final evaluation: negative
Arabica coffee + *Penicillium brevicompactum*	Infusion: clean Espresso: floral aroma, caramel, sweet, a little astringent Final evaluation: positive

All the coffee samples were irradiated with a 3 kGy dose for sterilization before fungi inoculation.

Table 2
Volatile compounds identified in raw Arabica coffee sterilized with 3 kGy and non-inoculated and inoculated with fungi.

Samples	Volatile compounds	Retention index	Identification method
Control (non-inoculated Arabica coffee)	Alcohol		
	1,7-Octadien-3-ol,2,6-dimethyl	1543	MS + RI[a]
	Aldehydes		
	3-Methylbutanal		
	Hexanal	922	MS + RI
	Nonanal		MS[a]
	2-Octenal	1093	MS + RI
	Octadecenal	1402	MS + RI
			MS + RI
		1442	
		2400	
	Others		
	1-Methyl-4-(1-methylethenyl)-cyclohexene	1212	MS + RI
	Pyrazine, 2-methoxy-3-(2-methylpropyl)	1500	MS + RI
			MS + RI
	Benzaldehyde	1525	
Penicillium brevicompactum	Ketone		
	Octan-3-one	1270	MS[b]
	Pentadecan-2-one	2218	MS
	Alcohol		
	2-Methylpropan-1-ol	1101	MS + RI
	Hexan-1-ol	1362	MS + RI
	Others		
	Phenol,2,6-bis(1,1-dimethylethyl)-4-ethyl	1957	MS
	7-Methyl-3-methylene-1,6-octadiene	1184	MS + RI
Penicillium sp. nov.	Ketone		
	Pentadecan-2-one	1189	MS
	5,9-Undecadien-2-one, 6,10-dimethyl	1889	MS
	Alcohol		
	6-Octen-1-ol, 3,7-dimethyl	1778	MS + RI
	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)	1695	MS
	6-Hepten-1-ol	1430	MS
	Hexan-1-ol	1362	MS + RI
	1-Propanol, 2-methyl	1100	MS + RI
	Benzene derivatives		
	1,2,3,4-Tetrahydronaphthalene	1645	MS
Aspergillus luchuensis	Alcohol		
	2-Methylpropan-1-ol	1100	MS + RI
	1-Octen-3-ol	1459	MS + RI
	3-Methyl-1-butanol	1217	MS + RI

[a] MS + RI — tentatively identified by comparison of the MS spectrum of the sample with the library (NIST 98) and of the calculation retention index with values from the literature.
[b] MS — tentatively identified by comparison of the MS spectrum of the sample with the library (NIST 98).

characterized by having a mushroom flavor and earthy and moldy odors. Borjesson (1993) studying compounds produced by fungi in culture medium, identified *Aspergillus* and *Penicillium* species as producers of a lot of volatiles with unpleasant odor in oatmeal agar, among them, dimethyl bisulfite, octen-3-ol and 2-methylisoborneol, with an earthy odor, and geosmin, 1-methoxy-3-methylphenol and methylbenzene with an unpleasant odor. Several compounds derived from benzene were also detected when this specie was inoculated in CYA, including 1-ethyl 2,4,5- trimethylbenzene, 1,1-dimethyl propylbenzene, 1-ethyl 3,1-methylethylbenzene and 1-ethyl 3,1-methyl ethylbenzene 1, 2, 3, 4, 5-pentamethylbenzene. In addition to these compounds, 1-octen 3-one, with a mushroom and earthy flavor, benzene methoxy naphthalene and 1,3-ciclopentadiene, both with camphor aroma, 1,6-octadien 3-ol with floral aroma, and pentylcyclopropane with petroleum ether aroma, were identified.

The presence of 2,4,6 trichloroanisole (TCA) and geosmineol in coffee was reported as being the responsible for earthy and moldy defects by some authors (Cantergiani et al., 2001; Spadone et al., 1990). Cantergiani et al. (2001) studied the volatile compounds in coffee from Mexico by GC/MS and olfactometry and identified a few compounds with earthy odor (geosmin, 2-methylisoborneol, 2,4,6 trichloroanisole and derivatives of methoxypyrazines). However, these authors did not relate the sensory defects with the presence of fungi but suggested that the cause of the sensory problem could be related to failures in the drying process. These compounds were not detected in the present study. Nevertheless, the presence of oct 1-en 3-ol was confirmed in the coffee inoculated with *A. luchuensis*, which showed the same features of moldy/mushroom odor that may be related to a negative final evaluation of the beverage. In a study carried out by Fiedler, Schültz, and Geh (2001), *Aspergillus niger*, which was morphologically similar to *A. luchuensis*, was able to produce the following compounds in malt extract agar: 1-octen-3-ol (mushroom and earthy odor), 3-octanol (wax and lipid odors) acetone and 3-octanone (herbal odor). The compound 1-octen-3-ol has been reported to be one of the major fungal VCs emitted by various fungi species (*Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Stachybotrys*, etc.) regularly found in moldy and water-damaged buildings or from composting facilities (Claeson, Levin, Blomquist, & Sunesson, 2002; Fisher & Wolfgang, 2003). In this study, *Aspergillus luchuensis* in CYA produced several compounds with negative sensory characteristics, such as 2-butenal (pungent smell), 1,3bis (1,1-dimethyl etyl benzene (rotten plant odor), nonan-2-ol (pepper odor), acetic acid, 2-pentylfuran (earthy odor) and methylsulfonylmethane (garlic odour).

The fungal inoculation test in coffee beans, followed by sensory evaluation, showed that fungi are able to produce few compounds in the raw coffee which may be related to the characteristics of the beverage. In the case of *A. luchuensis* it is possible that these compounds are responsible for conferring negative sensory characteristics to the beverage. On the other hand, the inoculation of *P. brevicompactum* in the raw coffee was not detrimental to the beverage. Coffee beans in contact

with the ground and those which are dried on the tree, had higher fungal infection by *Aspergillus* section *Nigri* (the same group of *A. luchuensis*) and the sensory evaluation of the beverage with a high infection of this species was affected negatively (Iamanaka et al., 2014). Coffee with high infection by *Aspergillus* section *Nigri* (16 to 64%) and *Aspergillus westerdijkiae* (0 to 30%) had the characteristics of moldy, "riado", fermented, highly bitter and immature. In this study, *A. luchuensis* was able to produce the compound 1-octen-3-ol, with a moldy/mushroom odor.

More studies should be performed to explore the potential beneficial influence of the presence of *Penicillium* species such as *P. brevicompactum*, on the coffee aroma.

In this study, it was possible to verify the changes in the volatile profile and in the sensory characteristics of the beverage due to the different fungal species tested even without evaluation of the volatile compounds of the final beverage, by analyzing the raw coffee inoculated by the fungi. However, more investigations are required with the aim of studying the volatile compounds of the final beverage. Also the influence of roasting on the identified compounds in this study, in order to assess whether these compounds are chemically modified.

4. Conclusions

The changes in the sensory characteristics of the coffee beverage possibly caused by some fungal species and their VCs were verified in this study. However, it was only an initial work and more studies are needed to consider the volatile compounds of the final coffee beverage and also a quantitative approach for the VCs.

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