Profile of the volatile organic compounds of pink pepper and black pepper

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Abstract. Black pepper (Piper nigrum L.) and pink pepper (Schinus terebinthifolius Raddi) are two plant-based spices, which despite having a common popular name, have a botanical family and distinct centers of origin. Its fruits are known worldwide in cuisine as condiments; in addition, the extraction of essential oil from these species is interesting from a pharmacological and industrial perspective. In this sense, the present study aimed to analyze the chemical profile of volatile organic compounds (VOC’s) present in black pepper and pink pepper. The solid phase microextraction method in headspace mode (HS-SPME) was used, using the fiber, polydimethylsiloxane-divinylbenzene (PDMS/DVB) for the extraction of VOCs. In the extraction of volatile compounds, 2g of the seeds of each sample were used, previously ground in an analytical mill, and placed in a 20 ml headspace flask. The adsorption of the compounds was carried out at a temperature of 60ºC, for 20 minutes, with the exposed PDMS/DVB fiber, after extraction, the desorption was carried...
out in the gas chromatograph injector coupled to mass spectrometry (CG-MS), where the fiber was exposed for 5 minutes. The identification of VOCs was performed by comparing the mass spectra obtained with data from the NIST library. Thirty-six volatile organic compounds (VOCs) were identified and quantified among pink pepper and black pepper seed samples. Of which 16 were found in black pepper, and 20 in pink pepper. These compounds are divided into monoterpenes, sesquiterpenes, and other classes such as alkaloids and sesquiterpenoids. The volatile organic compounds found in higher concentrations in black pepper were Carnegine with 36.32%, beyerene (30.84%), alphagurjunene (6.10%) and 1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo [5.3.1.0 (4.11)] undec-8-ene also with 6.10%. In pink pepper, the compounds with the highest concentrations were, phyllocladene (36.16%), 3-carene (12.49%), and 1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo [5.3.1.0 (4.11)] undec-8-ene (12.43%).

Keywords: Schinus terebinthifolius, Myracrodruon urundeuva, Headspace Solid Phase Microextraction (HS-SPME)

Introduction

The spices, or condiments, are present in food has additives, enriching the food with colors, flavors and aroma since, at least, 5000 b.C. Besides providing a better sensorial experience to the consumers, it is common the spices present physiologically beneficial effects to the human health too. These products uses for pharmacological purposes are known since the ancestral and indigenous societies, in different places on the planet, and can be seen has one of the first registered functional foods (Biazotto, 2014; Srividhyan, 2008). Spices are pointed too has one of the main economic factors that impulsionated the development of the maritime expansion, pioneered by the kingdom of Portugal at the beginning of the XV century. From that historic moment, spices started to be widely traded, by boats, worldwide, while new lands were being discovered, mainly by europeans. The “discovery” and the american territory colonization was one of the in this maritime expansionist race in search for spices for trading (Ramos, 2013; Roggenbach, 2017). In this work, two spices of vegetal origin, the black pepper and pink pepper, respectively known has Piper nigrum L. and Schinus terebinthifolius Raddi, will be studied.

The Piper nigrum L. (Piperaceae), known as black pepper, is one of the most ancient and historically coveted spices in the world, being crucial to many maritime historic events. It is a fruit of a perennial, semi-woody species with a liana habit, originally from India, being, however, cultivated in tropical and subtropical areas around the world. The fruits, of the berry type, are produced from inflorescences formed on secondary branches and, when ripe, they present a color ranging from light green or yellowish to shades of red. For the spice exploration, the fruits are dried and crushed, and can be consumed as powder, as well as by extracting their essential oils and/or oleoresins, which amplifies the possibility of its industrial uses as a condiment, for example, in beverage production, canned food, cosmetics and even pharmaceuticals (Almeida, 2017; Alves, 2015; Andrade, 2015; Biazotto, 2014; Carnevali & Araujo, 2015; Ferreira & Meirelles, 2002; Guimarães et al., 2015). Despite its high value and commercial importance to the kingdom of Portugal at the beginning of the colonization process of the Brazilian territory, the spice was only introduced in Brazil for cultivation in the 1930s, brought by Japanese immigrants. The national production established itself and maintained predominant in the Paraná state, in which its production is correspondent to 80% of the country total production. It was only after 1950 that Brazil became self-sufficient in the production on the condiment, and, shortly thereafter, became one of the world’s largest exporters of the product, reaching 1st place in the world ranking, both in production and exportation, of the spice (Almeida, 2017; Bendaoud et al., 2010; Bortolucci et al., 2019; Cole et al., 2014; Lourinho et al., 2014; Meireles, 2014; Melo et al., 1997).

The Schinus terebinthifolius Raddi (Anacardiaceae) known as pink pepper, is a vegetal species with a generally shrubby or arboreal habit of small to medium size, native to South America, especially Brazil, where it is present from the Northeast to the South of the country (Clemente, 2006; Dourado, 2012; Figueiredo et al., 2021; Silva-Luz & Pirani, 2015). Similar to the black pepper, its fruits are rounded and appear in bunches, from inflorescences, however, they have smaller size and, when ripe, their color varies in shades of red or pink, only. Due to its slightly sweet and spicy flavor, they are used in cooking and cocktail making, for example, despite being a species poorly explored in Brazil (Dourado, 2012). In addition, the astringent, antidiarrheal, depurative, diuretic and febrifugal properties of this species are known due to the presence of various chemical compounds, such as alcohols, ketones, acids, monoterpenes, sesquiterpenes and triterpenes, present not only in fruits but also in the stem and in the leaves (Andrade, 2015; Macedo, 2018; Oliveira et al., 2014; Santana et al., 2012). It is a typical plant from the Brazilian Cerrado, a biome whose great animal and plant biodiversity suffers some prejudice because it is a dry climate biome most of the year and is characterized by its vegetation, largely creeping and/or shrubby and by a soil naturally poor in fertility (Figueiredo et al., 2021; Klink & Machado, 2005; Pereira et al., 2011). However, there are several species native to the Cerrado whose potential, both as a spice, for medicinal use or in the food industry is promising, but poorly explored in Brazil, such as pink pepper spices (Schinus t.) or even monkey pepper (Xylopia aromatica (Lam.) Mart.), whose flavor and aroma is similar to black pepper (Piper n.) (Costa et al., 2021; Pontes Pires, 2019).

A quick and efficient way to analyze the industrial, gastronomic and/or pharmacological potential of these spices is the extraction of their volatile organic compounds through the solid phase
microextraction method (SPME). This method, developed by Arthur and Pawliszyn (1990), uses the principle of “green chemistry”, since it requires a small amount of sample and does not use organic solvents, and can be used direct extraction mode or in headspace mode (HS-SPME). A great advantage of HS-SPME is its high capacity for adsorption of different classes of compounds, due to the diversity of applicable coatings of SPME fibers, and it can be used to analyze complex matrices such as acerola, cagaita, callistemom, cambuí, beer, grumixama, pequi, corn, as well as spices such as black pepper and pink pepper, species which will be analyzed in this work (Franzin et al., 2020; Figueiredo et al., 2021; Srinivasan, 2008; García et al., 2019; Rodrigues et al., 2021; Silva et al., 2019; Silva et al., 2020; Silva et al., 2021; Oliveira Júnior, 2020; Silva et al., 2021; García et al., 2021; Pereira et al. 2021; Ramos et al., 2020; Assunção et al., 2020; Ramos et al., 2021a; Ramos et al., 2021b; Botti et al., 2019; Nascimento et al., 2021a; Nascimento et al., 2021b; Santos et al., 2020; Mariano et al., 2020; Rocha et al., 2019; Bueno et al., 2021; Mazzinghy et al., 2021; Viana et al., 2018).

**Volatile organic compounds identification**

Black pepper and pink pepper seed samples were analysed by a gas chromatography system (Trace GC Ultra) coupled to a mass spectrometer detector (Polaris Q model, Thermo Scientific, San Jose, CA, USA), with a mass analyzer iontrap type, installed in the Mass Spectrometry Laboratory of the Department of Chemistry at UFMG. The samples were analyzed in the following attributions: injector temperature of 250°C; splitless mode injection, desorption time of 5 minutes; injector temperature of 200°C; interface temperature of 275°C. The column heating temperature was programmed: starting at 40°C and remaining for 1 minute and then with gradual heating from 10°C/minute to 100°C, maintaining the isotherm for 1 minute, from 12°C/minute to 150°C, maintaining the isotherm for 1 minute and then 15°C/minute up to 245°C, temperature at which the isotherm was maintained for 1 minute. The detector was kept in scan mode (fullscan, from 35 to 300 m/z), using the electron impact ionization (EI) technique, with energy of 70 electron-volt (eV). A capillary chromatographic column HP-5 MS (5% phenyl and 95% methylpolysiloxane) was used throughout the process, covering the following dimensions: 30m in length, 0.25mm in internal diameter and 0.25μm in film thickness (Agilent Technologies INC, Germany) (Garcia et al., 2016; Figueiredo et al., 2021).

To identify the volatile compounds found, it was based on the m/z ratio corresponding to each peak generated by the chromatogram, and compared with the mass spectra obtained by EI ionization, which used an energy of 70eV, with the range of scan from 35 to 300m/z (Garcia et al., 2019; Silva et al., 2019; Oliveira Júnior et al., 2020). Thus, the mass spectra of the analytes found were compared with the mass spectra data obtained from the NIST library (National Institute of Standards and Technology), using as an auxiliary tool to the data recorded in the literature for the confirmation of volatile compounds present in the samples of the seeds. The RSI index consists of a numerical comparison factor where, the higher its value, the closer the compound is to the finding in the NIST library literature. However, only peaks with a value above 500, relative standard intensity (RSI), and a signal-to-noise ratio (S/N) above 50 decibels were selected. The peak intensity values obtained and the S/N ratio were obtained from the Thermo Electron Corporation Xcalibur 1.4 program and transferred to Microsoft Office Excel 2013, through this programs peak selections were made according to the S/N ratio at the UFSJ/CSL Chemistry Laboratory.
Results and discussion
36 volatile organic compounds (VOCs) were identified and quantified among the pink pepper and black pepper seed samples using the DVB-PDMS fiber, of which 16 were found in black pepper, and 20 in pink pepper (Table 1).

Table 1. Volatile organic compounds found in the pink pepper and black pepper samples.

| N° | Volatile Organic Compounds | CAS     | Formula | Sample % |
|----|----------------------------|---------|---------|----------|
|    |                            |         |         |          |
| 1  | 3-Carene                   | 13466-78-9 | C₁₀H₁₆ | 3.04     | 12.49    |
| 2  | Terpinolene a, c, d, e, g, i | 586-62-9 | C₁₀H₁₆ | 1.10     | 0.90     |
| 3  | p-Mentha-1(7),8-diene a, b | 499-97-8  | C₁₀H₁₆ | 1.00     |          |
| 4  | Trans-carene               | 18968-23-5 | C₁₀H₁₈ | -        | 0.45     |
| 5  | (+)-cis-Sabinol             | 471-16-9  | C₁₀H₁₅O | -     | 0.38     |
| 6  | Bornilacetate              | 76-49-3   | C₁₀H₂₀O₂ | -   | 0.66     |
| 7  | Beyerene                   | 3564-54-3 | C₂₀H₁₂ | 30.84     |          |
| 8  | Linalyl butyrate            | 78-36-4  | C₁₆H₂₀O₂ | 0.08   |          |
| 9  | (+)-Terpinen-4-ol a, b, h, i | 562-74-3 | C₁₀H₁₆O | 0.16   |          |
| 10 | alpha-Terpineol a, b, c, k  | 98-55-5  | C₁₀H₁₆O | 0.07   |          |

| N° | Volatile Organic Compounds | CAS     | Formula | Sample % |
|----|----------------------------|---------|---------|----------|
|    |                            |         |         |          |
| 11 | 10s,11s-Himachala-3(12),4-diene | NA     | C₁₅H₂₄ | -        | 0.76     |
| 12 | α – Guaiene                | 3691-12-1 | C₁₅H₂₄ | -        | 1.13     |
| 13 | β-Guaiene                   | 88-84-6  | C₁₅H₂₄ | 5.29     | 7.11     |
| 14 | (+)-alpha-Gurjunene a, d, j, k | 489-40-7 | C₁₅H₂₄ | 6.10     | 3.11     |
| 15 | (+)-gamma-Gurjunene        | 22567-17-5 | C₁₅H₂₄ | -        | 1.66     |
| 16 | Caryofurone                | 87-44-5  | C₁₅H₂₄ | 2.29     |          |
| 17 | (+)-Cyperene                | 2387-79-2 | C₁₅H₂₄ | 5.60     |          |
| 18 | α – Muurolene a, b, c, d, e, f, g, h, i, j, k, l | 12306047 | C₁₅H₂₄ | 1.36     |          |
| 19 | β-Cadinene l, k             | 523-47-7  | C₁₅H₂₄ | -        | 5.39     |
| 20 | Epizoneare                  | 41702-63-0 | C₁₅H₂₄ | 0.53     |          |
| 21 | 1,1,4a-Trimethyl-5,6-dimethylenedecahydropaphthalene | NA | C₁₅H₂₄ | 1.15 |          |
| 22 | 1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo[5.3.1.0(4,11)]jundec-8-ene | NA | C₁₅H₂₄ | 6.10 | 12.43 |

| N° | Volatile Organic Compounds | CAS     | Formula | Sample % |
|----|----------------------------|---------|---------|----------|
|    |                            |         |         |          |
| 23 | Carnegine                  | 490-53-9 | C₁₃H₁₅NO₂ | 36.32   |          |
| 24 | Isopseudocumendiol         | 697-82-5 | C₉H₁₄O | -        | 7.26     |
| 25 | γ-Eudesmol                 | 1209-71-8 | C₁₀H₁₆O | -        | 1.02     |
| 26 | (-)-Phyllocladene          | 20070-61-5 | C₂₀H₃₂ | -        | 36.16     |
| 27 | β-Eudesmol b, k            | 473-15-4 | C₁₄H₂₈O | -        | 0.44     |

Letters indicate compounds that have been identified by other authors. *Singh et al., 2004; Kapoor et al., 2009; Melo et al., 2021; Figueiredo et al., 2021; Silva, 2017; Santos et al., 2014; Cole, 2008; Clemente, 2006; Costa et al., 2010; Dourado, 2012; Oliveira et al., 2014; Barbosa et al., 2007.

Among the 16 VOCs found in black pepper, 7 were classified as monoterpenes, 8 as sesquiterpenes, and carnegine which is a non-alkaloid. As in the work by Costa et al. (2010), a higher number of sesquiterpenes in black pepper seed was identified. Among the monoterpenes, 4 oxygenated: bornylacetate, linalyl butyrate, terpinen-4-ol, and alpha-terpineol, results that resemble those of Costa et al. (2010). However, these results differ from those found by Figueiredo et al. (2021) where the proportion of monoterpenes were higher (80%).

The VOCs found in higher concentrations in black pepper were carnegine with 36.32%, beryerene (30.84%), alpha-gurjunene (6.10%) and 1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo[5.3.1.0(4,11)]jundec-8-ene also with 6.10%. The compound carnegine is a simple tetrahydrosoquinoline, isolated from different plants around the world, such as Cactaceae, Chenopodiaceae and Boraginaceae.
Hyllocladene (36,16%), 3-carene, terpinolene and α-gurjene are sesquiterpenes, and was also found in the work made by Singh et al. (2004). However, the compound with higher concentrations found in the present study differ from those found in Melo et al. (2021), as well as in Costa et al. (2010), where they found E-Caryophyllene, Limonene, e Sabinene with the highest concentrations.

In pink pepper, of the 20 compounds found, 5 are classified as monoterpenes, 11 as sesquiterpenes, 4 belonging to different classes (phyllolcadene, isopseudocumenol, γ-eudesmol and β-eudesmol). The compounds with the highest concentrations were phyllolcadene (36,16%), 3-carene (12,49%), and 1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo [5.3.1.0(4,11)]undec-8-ene (12,43%). Of those compounds, the monoterpene 3-carene is often found in the literature regarding the chemical profile of Schinus terebinthifolius (Figueiredo et al. 2021; Silva, 2017; Cole, 2008, Clemente, 2006), however, analyzing its concentration, in the present study, 3-carene had a lower concentration (12,49%) compared to the works of Figueiredo et al. (2021) (26,81%). Silva (2017) analysing 11 seed samples, presenting a minimum concentration of 33,78%, and a maximum of 36,73%; Cole (2008) (30,37%), and Clemente (2006) with 29,22%, where all evaluated the pink pepper fruit. In the work of Santos et al. (2014), evaluating samples of pink, green and ripe pepper fruits, respectively, 3-carene was not even identified, the compound with highest concentration being limonene (70,49%) (67,15%), followed by β-phellandrene (19,24%) (18,94%). Volatile organic compounds found in pink pepper in higher concentrations, in this study, also differ in terms of the composition found in studies related to Schinus terebinthifolius, as in Figueiredo et al. (2021), tracing the comparative profile of pink pepper and aroeira, pink pepper had the highest concentrations (in addition to the 3-carene already discussed), β-guaiene (14,93%), and isopseudocumenol (14,62%) in this study was found with a concentration of 7,26%, as well as in Clemente (2006) that in addition to 3-carene, the others with higher concentrations were β-phellandrene (18,08%), and α-phellandrene (13,04%).

Among the oxygenated compounds in the pink pepper, two were found, isopseudocumenol and β-eudesmol, the same ones observed in Figueiredo et al. (2021).

In both samples, pink pepper and black pepper, 3 compounds were found in common, 3-carene, terpinolene, and β-guaiene.

The different concentrations and qualities of volatile organic compounds found in this study and compared with the literature on the subject are inherent, as is known, to the extraction method, the parts of the plants used, the place of harvest of the species, as well as the local edaphoclimatic conditions at a given time, and that exert different influences on the production and composition of secondary metabolites.

**Conclusion**

The headspace solid phase microextraction method was efficient in the extraction of volatile compounds, with 36 compounds being identified and quantified among the black pepper and pink pepper seed samples, using DVB-PDMS fiber, being identified 16 compounds in black pepper and 20 in pink pepper samples. There was a greater number of sesquiterpenes between both samples.

The compounds found in higher concentrations in black pepper were carnegine with 36,32%, beyerene (30,84%), alpha-gurjuneun (6,10%), as well as 1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo[5.3.1.0(4,11)]undec-8-ene also with 6,10%. In pink pepper, the compounds with the highest concentrations were phyllolcadene (36,16%), 3-carene (12,49%), and 1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo[5.3.1.0(4,11)]undec-8-ene (12,43%). In both samples, 3 compounds were found in common, being 3-carene, terpinolene and β-guaiene.

The extraction of volatile compounds by the HS-SPME method used in the two species in this study helps in greater knowledge of the chemical profile of these two spices, and in the prospecting of pharmacological, gastronomic and industrial potentialities.

The gas chromatography technique coupled with mass spectrometry has been shown to be very efficient and has been highlighted due to its practicality in execution, in addition to providing representative results.

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