

## Essential oil chemotypes of turmeric (*Curcuma longa* L.) cultivated in four different farmers in Southwest of Paraná State - Brazil

Vaneza Paula Poplawski Carneiro<sup>1</sup>, Mariana Moraes Pinc<sup>1</sup>, Rayane Monique Sete da Cruz<sup>2</sup>,  
Mariane Pavani Gummy<sup>1</sup>, Bruna Freitas Biezes<sup>3</sup>, Odair Alberton<sup>1\*</sup>

<sup>1</sup>Paranaense University, Umuarama, Brazil

<sup>2</sup>State University of Maringá, Maringá, Brazil

<sup>3</sup>Paranaense University, Francisco Beltrão, Brazil

\*Corresponding author, e-mail: [odair@prof.unipar.br](mailto:odair@prof.unipar.br)

### Abstract

Turmeric (*Curcuma longa* L.) has economic value for its medicinal and nutritional properties. This study aimed to evaluate the yield and phytochemical profile of essential oil (EO) from turmeric rhizomes produced by family farmers in rural communities in Francisco Beltrão – PR, and soil microbiological quality from those farmers. Four turmeric farmers from the communities of São Braz, Rio Tuna, Rio Saudade and Nova Sessão were selected. Soil samples and rhizomes of turmeric were collected and subsequently evaluated for soil microbial biomass carbon, soil basal respiration and soil metabolic quotient and EO yield of rhizomes by hydrodistillation. After, EO yield (%) was calculated and the chemical identification by GC/MS. The Nova Sessão community had better soil microbiological quality, whereas the EO yield (3.02%) was higher for the Rio Tuna community. The four rural communities showed good results for the EO yield (ranging from 1.68 to 3.02% and compared to the literature, which ranged from 0.2 to 5.27%), with the São Braz community, showing a significant reduction and the Rio Tuna community had the highest EO yield. Also was observed variation in the major EO chemical compounds, e.g.: ar-turmerone (44.27 to 64.21%), curlone (12.27 to 20.83%), a-zingiberene (1.30 to 15.97%) and β-sesquiphellandrene (1.24 to 10.07%). It is concluded that among the four communities studied, there was significant variation in the EO yield and differences in EO quality of turmeric, especially in the major components ar-turmerone and a-zingiberene.

**Keywords:** bioactives, family farming, soil quality, zingiberaceae

### Introduction

Currently, it is believed that the main objective of using natural products is not to replace commercialized and already registered medicines, but to increase the therapeutic option in the pharmaceutical market, using products with therapeutic indications that are complementary to existing medications, stimulating popular traditions and providing indigenous substrates and products for the development of a local trade (Ayati et al., 2019). Medical plants can represent a drug that is easily accepted by communities, as it belongs to the culturally constructed traditional therapeutic usages (Ayati et al., 2019).

Turmeric (*Curcuma longa* L.), plant species belonging to the Zingiberaceae family, popularly known as turmeric, is characterized as an herbaceous and aromatic plant. Its leaves are big and long. Its flowers are yellowish or white, arranged in long spikes. The rhizome is

ovoid and can reach up to 10 cm in length and uses for plant propagation and when cut, it has a reddish-yellow color and the aroma is pleasant and its flavour is spicy (Ferrari et al., 2020 a, b; Pal et al., 2020). Its a species with wide traditional use and due to its importance for popular medicine in Brazil, it was included in the National List of Medicinal Plants of Interest to the SUS (RENISUS), as part of the Ministry's National Policy on Medicinal Plants and Herbal Medicines in health and its monograph is included in the 5th edition of the Brazilian Pharmacopoeia (Brasil, 2015).

Turmeric has economic value for its medicinal and nutritional properties, being used in traditional medicine for various purposes, as it is rich in phenolic compounds, essential oils (EO) and curcuminoids as its main chemical component (Ayati et al., 2019), with antioxidant, antitumor, anti-inflammatory, antimicrobial properties (Ferrari et al., 2020b), decreased lipid

peroxidation, treatment of osteoarthritis, dyspepsia, flatulence, treatment of dermatological disorders, wound healing and analgesics (Brasil, 2021).

Turmeric is widely cultivated around the world. It is a plant native to Southeast Asia, more precisely on the slopes of hills of the tropical forests of India and is distributed in tropical and subtropical regions. In Brazil, the largest turmeric producer is the Mara Rosa region – Goiás State, belonging to the micro-region of Porangatu, located in the north of Goiás (Quemel et al., 2021). Nowadays, India concentrates the largest production and consumption of turmeric, followed by China, the Caribbean and Latin America, with India being responsible for approximately 80% of the world production of turmeric and 60% of world exports (Das, 2016).

It is an easy-to-cultivate species and has the advantage of not requiring special agronomic treatments. The quality and quantity of secondary metabolites of turmeric EO maybe associated with its planting region, soil and climate (Sandeep et al., 2016; 2018; Akbar et al., 2018; Mekonnen & Garedew, 2019; Aarathi et al., 2020; Pal et al., 2020; Quemel et al., 2021), in addition to genetic and morphological factors (Aarathi et al., 2020; Ferrari et al., 2020b). In this way, the present study aimed to analyse the microbiological soil quality, determinate the EO yield and the phytochemical profile of turmeric rhizomes produced by the local family farmers in four rural communities in Francisco Beltrão, Southwest of Paraná State, Brazil.

## Material and Methods

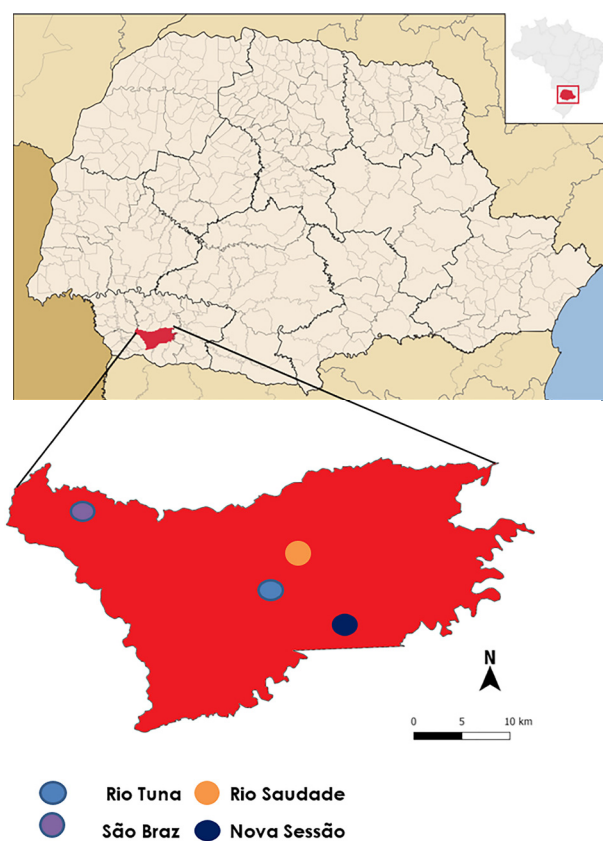
### Local area characterization

The municipality of Francisco Beltrão is located in the Southwest region of Paraná State, with a territorial area of 735.111 km<sup>2</sup>. Its geographic coordinates Latitude 26°04'52"S and Longitude 53°03'18"W, average of 570 m of altitude, cfa koppen climate classification with -5 °C to 38 °C and 20 °C of average temperature, the anual rainfall is 1400 to 1800 mm. It has an estimated population of 92,216 people (IBGE 2020) and 11,494 individuals live in the rural area of the municipality, distributed in 2,621 rural properties (Censo Agropecuário, 2017). The relief of the Southwestern mesoregion is marked by a morphological homogeneity resulting from the predominance of flat and wavy features. It is located in the hydrographic basin of the Chopim River, a tributary of the Iguaçu River, which is made up of an extensive fluvial network, the river's floodplain areas occupy a good portion of its territory.

The soils of Francisco Beltrão, Paraná State, in the Western and Southern areas, are of the oxisol type and it is noticed the presence of structured soil type,

which is located in the Northern and Eastern parts of the municipality. Due to its geographical position, Francisco Beltrão has a temperate climate for most of the year, with frost in winter and high temperatures in summer (Prefeitura Municipal de Francisco Beltrão, 2017).

The first property is located in the community (C1) of São Braz (Latitude 26°01'00. 87" S and Longitude 53°15'28.46" O), located to the West of the municipality. The second property is located in the community (C2) of Rio Tuna (Latitude 25°58'20.11" S and Longitude 53°04'50.92" O), in the North of the municipality. The third Property is located in the Rio Saudade community (C3) (Latitude 26°02'21.22" S and Longitude 53°06'55.91" O), in the North of the municipality. Property fourth is located in the Nova Sessão community (C4) (Latitude 26°03'40.28" S and Longitude 53°02'00.92" O), located to the East of the municipality (Figure 1).



**Figure 1.** Municipality location and the selected communities.

The selection of the areas studied was done by indication, and the criterion for inclusion of rural properties in the study was the sale of turmeric rhizomes to the local community of Francisco Beltrão.

### Plant material

Four representative samples of turmeric rhizomes were collected from each property. The turmeric

rhizomes were planted in May of 2019 and was added around 20 to 25 ton ha<sup>-1</sup> of organic fertilizer (mixed of gattle and chicken manure) All sample collections were carried out in the morning, in the second half of October 2019. The samples were placed in coolers with ice for phytochemical analysis at the Laboratory of Pre-Clinical Research of Natural Products at Universidade Paranaense.

#### Determination of microbial biomass carbon, basal respiration, and soil metabolic quotient

Approximately 200 g of soil samples were collected, in a 10 cm layer, in four different points of the same place where the turmeric rhizomes were collected from each rural property.

The determination of microbial biomass C (MB-C) in the soil was modified according to the fumigation-extraction method proposed by Vance et al. (1987) and Tate et al. (1988) using 10-g soil sample (Ferrari et al., 2020a). Soil basal respiration (SBR) was determined according to Jenkinson & Powlson (1976), using a 30-g soil sample. The soil metabolic quotient ( $qCO_2$ ) is the ratio between the SBR per unit of the soil MB-C (Ferrari et al., 2020a).

#### Essential oil yield

The technique used to extract the essential oil was hydrodistillation, using a modified Clevenger type extractor, according to Cruz et al. (2020). Then, the yield (%) of EO, fresh mass versus essential oil mass (m/m %) was calculated.

#### Chemical Identification of essential oil

The chemical identification of the EO was done by chromatography-mass spectrometry in a GC-MS QP 2010 SE (Shimadzu) equipment. For each analysis, 10  $\mu$ L of the sample was diluted in 1000  $\mu$ L of dichloromethane (Anhydrol) before being injected into a SH-RTx-5MS column (Shimadzu, 5% phenylmethylsiloxane, 30 m x 0.25 mm id, 0.25  $\mu$ m) using an automatic injector (Shimadzu AOC-20j). The column temperature was initially set at 40

°C, heating to 6 °C min<sup>-1</sup> to reach a final temperature of 300 °C. Injector and GC-MS interface temperatures were kept at 250 °C. Helium was used as carrier gas at a flow rate of 1 mL min<sup>-1</sup>, 2:1 split and 1  $\mu$ L injection. Afterward, the library and software "GC-MS Postrun Analysis" were used to identify the components of the OE (Cruz et al., 2020).

#### Statistical analysis

Data were subjected to analysis of variance (ANOVA). Means were compared by Duncan's test ( $p \leq 0.05$ ) using the statistical program SPSS version 22 for Windows (SPSS Inc., Chicago, IL, USA). Cluster (CA) and Principal Component (PCA) analyzes were performed to discriminate the composition of EO based on the different communities were analyzed using "Statistica v. 13.3" software (Statsoft, 2017).

#### Results and Discussion

The soil microbiological analysis showed a significant increase of MB-C in the C4 Nova Sessão property, indicating better soil quality, as the MB-C is directly related to the greater absorption and storage of carbon, nutrients, and minerals (Table 1). These compounds are fundamental for plant growth and for the yield and chemical composition of EO (Mekonnen & Garedew, 2019; Ferrari et al., 2020 a, b). The property at C4 uses domestic organic compost, made from food husks, dried leaves and poultry manure. The property cultivates turmeric in the organic system.

However, SBR and  $qCO_2$  were not influenced by different communities (Table 1). The SBR indicates the level of activity of microorganisms and can be influenced by humidity, temperature, availability of nutrients, carbon/nitrogen ratio, and presence of organic residues, among others. Furthermore, the microorganisms present in the soil collaborate with the cycling of nutrients (Balota et al., 2012). Kaschuk et al. (2010) state that the higher the  $qCO_2$  ratio decreases the soil quality.

**Table 1.** Microbial biomass carbon (MB-C) (mg C kg<sup>-1</sup> soil), soil basal respiration (SBR) (mg C-CO<sub>2</sub> kg soil<sup>-1</sup> hour<sup>-1</sup>) and soil metabolic quotient ( $qCO_2$ ) (mg C-CO<sub>2</sub> g<sup>-1</sup> CBM h<sup>-1</sup>) of the soil cultivated with turmeric in Francisco Beltrão.

Property	MB-C	SBR	$qCO_2$
C1	180.22±6.21b	0.86±0.04a	4.77±0.11a
C2	180.08±5.43b	0.93±0.09a	5.21±0.64a
C3	198.80±16.97ab	1.15±0.15a	5.67±0.28a
C4	235.48±18.86a	1.02±0.08a	4.44±0.66a
Significance	0.047	0.301	0.371

\*Means ( $\pm$  standard error, n = 3). Means followed by the same letter in the column do not differ significantly from each other by Duncan's test ( $p \leq 0.05$ ). Property C1 community of São Braz; Property C2 community of Rio Tuna; Property C3 Rio Saudade community; Property C4 community Nova Sessão.

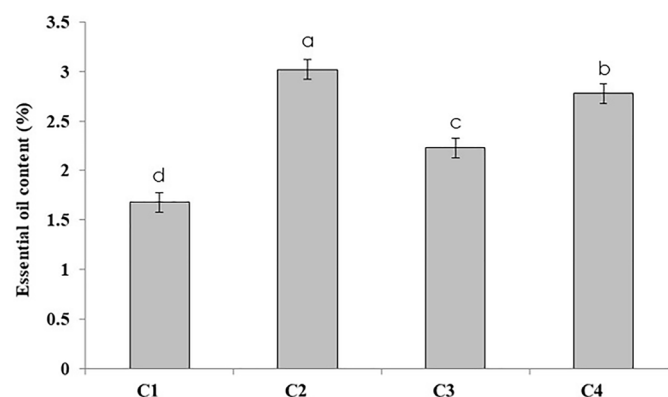
The adequate soil for the cultivation of most medicinal species requires high levels of MB-C and low  $qCO_2$  as is the case with turmeric. Thus, it indicates that carbon is being sequestered in the soil and is not being lost to the atmosphere, providing a sustainable environment (Kaschuk et al., 2010).

Based on the results, the Nova Sessão community presented the best soil microbiological quality; whereas the EO yield (3.02%) was higher in the Rio Tuna community. Among the four rural communities, all showed good results for the yield of turmeric EO (ranging from 1.68 to 3.02% and literature ranging from 0.2 to 5.27%), with the São Braz community showing a significant reduction and the Rio Tuna community showing the highest EO yield. (Figure 2). In the researched scientific literature, the values found for the EO yield of turmeric rhizomes range from 0.2% (Setzer et al., 2021) to 5.27% (Zhang et al., 2017).

The results obtained in the present study point out the interference of environmental factors, observed at the sampling site, as small changes in the environment can cause major changes in the EO yield, corroborating with Sandeep et al. (2016) and Akbar et al. (2018) in which concluded that the variations in EO yield were coupled with altitude, environmental variables and soil nutritional factors. The main factors described in the literature that influence the variation of EO yield based on plant mass

are related to the climatic conditions of the time and region, due to the effects of temperature, humidity, and light, also related to the effects of seasonality and the time of sampling for each plant (Sandeep et al., 2016; 2018; Ayati et al., 2019; Aarathi et al., 2020).

The GC-MS analysis detected 25 chemical compounds (Table 2). The major compounds were, ar-turmerone (44.27 to 64.21%), curlone (12.27 to 20.83%),  $\alpha$ -zingiberene (1.30 to 15.97%) and  $\beta$ -sesquiphellandrene (1.24 to 10.07%) (Figure 2).



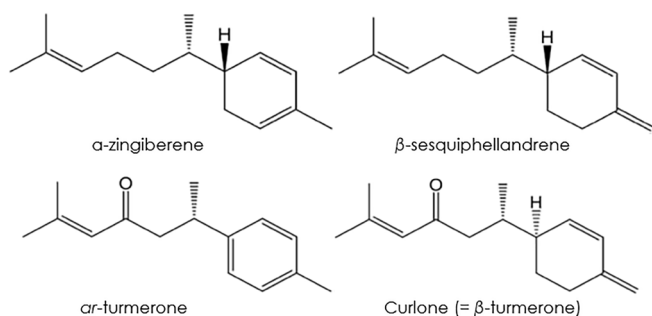
**Figure 2.** Essential oil yield (%) of turmeric in four properties in Francisco Beltrão. Bars followed by the same letter do not differ significantly from each other by Duncan's test ( $p \leq 0.05$ ). Bars = standard error. Property C1 community of São Braz; Property C2 community of Rio Tuna; Property C3 Rio Saudade community; Property C4 community Nova Sessão.

**Table 2.** Chemical composition of turmeric essential oil (%) from four different properties in Francisco Beltrão – PR in 2019.

Pick	Compounds	RT	C1	C2	C3	C4
1	$\alpha$ -pellandrene	10.260	†	†	0.36	1.26
2	o-cymene	10.982	†	†	†	0.35
3	Eucalyptol	11.172	†	0.37	0.62	0.61
4	p-menth-4(8)-ene	13.224	†	0.41	†	†
5	Caryophyllene	24.617	†	0.42	†	†
6	$\beta$ -farnesene	25.691	†	0.49	†	†
7	$\alpha$ -curcumene	26.570	0.82	3.38	2.39	1.38
8	$\alpha$ -zingiberene	26.962	1.30	15.97	14.23	2.60
9	$\beta$ -bisabolene	27.341	†	1.99	1.64	0.38
10	$\beta$ -sesquiphellandrene	27.832	1.24	10.07	8.15	2.23
11	Cis-nuciferol	29.565	1.27	0.61	0.57	1.00
12	Trans-sesquisabinene hydrate	29.849	†	0.76	†	†
13	Z- $\alpha$ -trans bergamotol	30.256	0.88	0.86	0.89	0.93
14	Epi- $\beta$ -santalol	30.390	†	0.50	0.64	†
15	$\alpha$ -santalol	30.392	0.99	†	†	0.92
16	Zingiberenol	30.522	1.87	2.32	1.05	1.63
17	Pregna-3,5-dien-9-ol-20-one	31.169	1.62	0.48	0.61	1.33
18	Ar-turmerone	32.200	64.21	44.27	49.34	62.19
19	Germacrene	32.900	†	1.96	1.48	†
20	Curlone (= $\beta$ -turmerone)	33.059	20.83	12.27	14.34	19.48
21	(6R,7R)-bisabolone	34.190	0.76	1.26	0.96	0.60
22	Artemisia ketone	34.689	1.64	0.45	0.75	1.04
23	Pentadecane, 2-methyl-2-phenyl-	34.832	0.89	†	†	0.52
24	(E)-atlantone	34.945	†	†	†	0.27
25	Cyclohexene, 1-pentyl-4-(4-propylcyclohexyl)	35.264	†	0.63	0.40	†
Total			98.32	99.47	98.42	98.72

RT - Retention time. †: trace. Identification was based on a comparison of mass spectra on the library and GC-MS Postrun Analysis program (Cruz et al., 2020).

Property C1 community of São Braz; Property C2 community of Rio Tuna; Property C3 Rio Saudade community; Property C4 community Nova Sessão.



**Figure 3.** The chemical major constituents of turmeric essential oil.

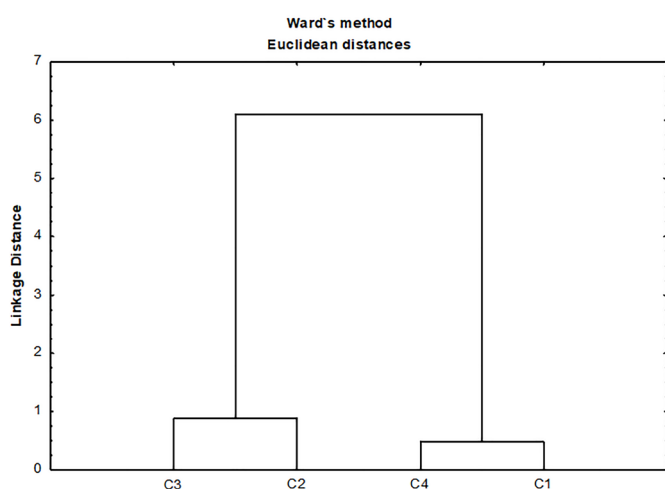
Data were submitted to hierarchical clustering analysis to verify the chemical variability of the principal

components of turmeric EO collected among different communities. The samples were grouped into two main groups (Figure 3) and displayed in a dendrogram obtained by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The first cluster includes the plants collected in C2 and C3 while the second cluster includes C1 and C4 communities. The grouping result showed the order of the clusters according to the chemical composition of EO. Ar-turmerone was the major component in all communities, however ar-turmerone reduced in C2 and C3 and  $\alpha$ -zingiberene increased (Table 3).

**Table 3.** The major components of the turmeric essential oil (%) were collected from four different communities in Francisco Beltrão – PR in 2019.

Treatments	$\alpha$ -zingiberene	$\beta$ -sesquiphellandrene	ar-turmerone	Curlone	Yield
C1	1.30	1.24	64.21	20.83	1.68
C2	15.97	10.07	44.27	12.27	3.02
C3	14.34	8.15	49.34	14.34	2.23
C4	2.60	2.23	62.19	19.48	2.78
Literature, data from 32 articles					
Mean	4.63	4.09	45.53	14.03	2.04
SE	1.41	0.31	0.98	0.49	0.17
Amplitude	0.19-25.05	0.01-14.88	0.92-63.69	0.03-37.75	0.2-5.27
n	100	108	137	134	92

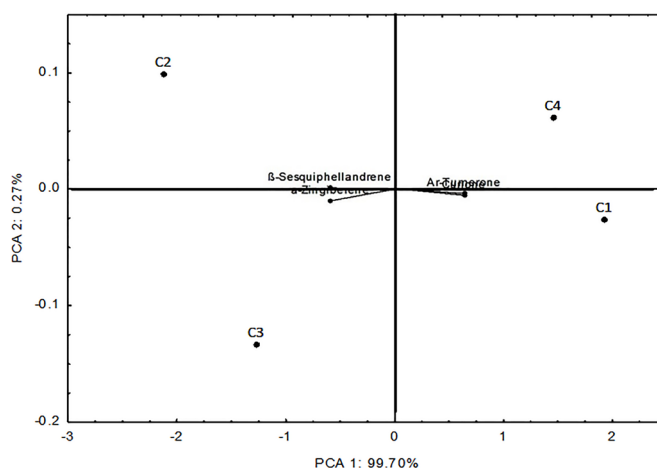
Property C1 community of São Braz; Property C2 community of Rio Tuna; Property C3 Rio Saudade community; Property C4 community Nova Sessão. SE: standard error; Amplitude: the difference between the highest and the lowest values and n: number of observations.



**Figure 4.** Hierarchical clustering dendrogram based on the major chemical compounds of turmeric essential oils of plants collected from four different communities in Francisco Beltrão – PR in 2019. Property C1 community of São Braz; Property C2 community of Rio Tuna; Property C3 Rio Saudade community; Property C4 community Nova Sessão.

We performed principal component analysis (PCA) based on EO data from the four communities (Turmeric producers). The first two components (PC1 and PC2) explained 99.70 % and 0.27% of the variability across the chemical EO constituents (Table 3). The PCA factor loading plot (Figure 4) of the first two PCs of EO constituents clearly separated the communities C2 and C3 from the others communities C1 and C4, where the

communities C2 and C3 were clustered together and are separated from communities C1 and C4, which corroborates the grouping observed in the dendrogram (Figure 5).



**Figure 5.** Biplot representation of a PCA (Principal Component Analysis) performed based on major chemical compounds of turmeric essential oils of plants collected from four different communities in Francisco Beltrão – PR in 2019. Property C1 community of São Braz; Property C2 community of Rio Tuna; Property C3 Rio Saudade community; Property C4 community Nova Sessão.

PCA factor loading revealed that *ar*-turmerone and curlone were highly correlated with PC1; while *a*-zingiberene and  $\beta$ -sesquiphellandrene were highly correlated with PC2. Through the projection of concentrations on the chemical composition of the EO, principal component analysis demonstrates that the community C3 is positioned toward vector 1 and *a*-zingiberene and  $\beta$ -sesquiphellandrene; while the community C2 is positioned towards vector 2 and with *a*-zingiberene and  $\beta$ -sesquiphellandrene. Community C4 is positioned toward vector 3 and community C1 is positioned toward vector 4 and with *ar*-turmerone and curlone. Therefore, PCA mainly highlights the differences in communities related to EO composition (Figure 5).

The EO yield, as well as the chemical composition obtained, was similar to that described in the literature, where turmerone is generally the main component of the EO (Table 3). In the study done by Teles et al. (2019), the turmeric EO yield was 3.4% and found 17 chemical compounds, the major compounds were,  $\gamma$ -turmerone (6.96%),  $\beta$ -turmerone (12.02%), and *ar*-turmerone (55.43%).

Avanço et al. (2017) found the composition of the turmeric EO the major compounds were, *ar*-turmerone (12.9%),  $\beta$ -turmerone (16.0%), and *a*-turmerone (42.6%) sesquiterpenes, and monoterpenes 1,8-cineole (3.2%) and *a*- phellandrene (6.5%). These compounds were responsible for the antioxidant, antifungal, and antimycotoxigenic activities against *Fusarium verticillioides* (Sacc.) Nirenberg, which are involved in the deterioration of agricultural products, suggesting that turmeric EO has a protective function for postharvest food preservation (Avanço et al., 2017).

Chagas et al. (2016) demonstrated the ability of the compound turmerone against ticks, having potential use for parasite control. Oyemitan et al. (2017) evaluating the neuropharmacological activity of turmeric EO in mice, detected sedative, anxiolytic, anticonvulsant, and hypothermic properties. In the same study, turmerone (35.9%) was the main component of the EO, which indicates the importance of this compound.

Quemel et al. (2021) evaluated chemotypes of turmeric EO from four different states of Brazil and demonstrated the formation of three distinct groups based on the analysis of turmeric EO chemical composition, even if they are accesses of the same species and under the same cultivation conditions, there is chemical variability of turmeric EO. In this present study, we find two distinct groups, also based on the analysis of turmeric EO chemical composition, even in the same State and municipality.

Environmental factors and soil nutrients play a critical role in the plant's productivity. There are many reports regarding the effect of altitude, temperature, light, rainfall and soil nutrients on plant secondary metabolite production (Sandeep et al., 2016; 2018; Zhang et al., 2017; Akbar et al., 2018; Aarathi et al., 2020; Pal et al., 2020; Quemel et al., 2021; Setzer et al., 2021). Analysis of variation in EO yield and quality of turmeric at different locals will not only expand the knowledge towards the role of environment and soil nutrients but also develop alternative strategies to enhance its yield and quality, as was shown in the present study (Sandeep et al., 2016; 2018).

## Conclusions

Nova Sessão community showed better soil quality, with a higher soil MB-C, which is directly related to greater absorption and storage of carbon, nutrients, and minerals.

The communities São Braz and Nova Sessão presented higher levels of *ar*-turmerone in the chemical composition of the EO turmeric, however a lower yield of the EO compared to the Rio Tuna community. The major compounds found were *ar*-turmerone (44.27 to 64.21%), curlone (12.27 to 20.83%), *a*-zingiberene (1.30 to 15.97%), and  $\beta$ -sesquiphellandrene (1.24 to 10.07%).

It is possible to find in the local commerce of Francisco Beltrão different phytochemical profiles of turmeric and taking into account the EO yield and composition, the turmeric produced in the Nova Sessão community presented EO yield (2.78%) and composition *ar*-turmerone (62.19%) being the better community to grown turmeric for industries interest.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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