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Original Research

Influence of Seasonal Variation on the Chromatographic Profile and Physicochemical Properties of Basil (*Ocimum basilicum*) Essential Oil

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Abstract: Basil (*Ocimum basilicum*) is important in the Brazilian economy and its essential oil is used in cooking, industry and perfumery, and has antimicrobial properties. The chemical composition of essential oils can be altered by the action of various factors, whether internal or external to the plant, such as genetics, temperature, water availability and seasonality. The aim of this research was to assess whether seasonality interferes with the chromatographic profile and physicochemical characteristics of basil essential oil. The study was carried out by investigating the technical reports of the Haje Insumos company on the chemical composition using gas chromatography, physical-chemical analysis (density, refractive index and humidity) and organoleptic analysis (appearance, color and odor). Due to the presence of the linalool component in greater quantity, the oil analyzed is characterized as being of the linalool chemotype. The product showed no alterations to its organoleptic characteristics and all the results were in accordance with its specification. In addition, one of the batches had a higher moisture content, which may be related to the environmental conditions at the time of year, which requires attention as water can trigger a hydrolysis reaction in fat-soluble molecules. It is understood that seasonality may be related to changes in the essential oil, and it was possible to contribute to the recognition of this plant's product.

Keywords: Basil; Essential oil; Seasonality; Chromatography.

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1. Introduction

Essential oils or volatile oils are secondary plant metabolites made up mainly of terpenic compounds such as monoterpenes, sesquiterpenes and phenylpropanoids. They give off an aroma, are volatile and can be extracted from different parts of plants, such as flowers, leaves, roots and fruit. In plants, they protect against pathogens, attract or repel insects and confer resistance to stress factors [1, 2]. The way in which essential oils are extracted is adapted according to the part of the plant and the purpose. One of the most widely used methods is steam distillation, which is a process that occurs when the plant drug is subjected to water vapor, causing its volatile substances to be dragged along with the vapor to a condenser, making them liquid again so that they can later be separated by the difference in density [3].

Basil (*Ocimum basilicum L.*), also known as basil, originated in tropical Asia, prefers warm to mild climates and is part of the *Lamiaceae* family. Among aromatic plants, it is of

great importance to the Brazilian economy, obtaining essential oil for use in cooking and in the cosmetics and perfumery industry, with antimicrobial and repellent properties [4]. Essential oils have many substances in their composition, forming a synergy between them, so among the approximately 20 components of *Ocimum basilicum L* oil are linalool, citral, geraniol, camphor and thymol. These give the oil its properties and have been increasingly studied over the years with the aim of gaining a scientific understanding of the use of medicinal plants [5].

The chemical composition of essential oils can vary depending on various factors, such as biotic and abiotic factors. Biotic factors are related to the plant itself, such as genetic factors. Abiotic factors refer to external aspects such as harvest time, temperature, light, water availability and nutrition. These factors, together with seasonality, affect the growth and development of the plant and the composition of its secondary metabolites [6]. In relation to seasonality, changes in temperature, rainfall and intensity of solar radiation throughout the year interfere with the production of essential oil, which can affect the yield, its physicochemical characteristics and composition, and can directly interfere with the products of this input [7].

The aim of this study was to assess whether seasonality interferes with the chromatographic profile and physicochemical characteristics of basil essential oil (*Ocimum basilicum L*) in 2021 and 2022.

2. Material and Methods

The study was based on an analysis of the data contained in the technical reports of the Haje Insumos company, referring to chemical composition analyses, through the results of gas chromatography with mass spectrometry and the industry's internal quality control, presenting physical-chemical and organoleptic analyses. The chemical constituents of basil essential oil were identified using gas chromatography coupled with mass spectrometry (GC-MS). The analysis was carried out by the company Bio Assets Biotecnologia, and the methodology used was based on the fourth edition of the Adams guide [8].

Density was determined using a pycnometer (RBR, Mod. Laboratorial) at an ambient temperature of 20 °C. The mass of the pycnometer was determined on an analytical balance, after it had been cleaned and dried, then 1 mL of the essential oil was added, and the mass determined again. The density was determined using the equation: D (g/mL) = [(pycnometer mass + sample) - (pycnometer mass)] (g)/Oil volume (mL). To measure the refractive index (nD), a refractometer was used to observe the refraction of light by the sample. Moisture was analyzed using the Marte Moisture Determinator, which measures moisture content quickly using infrared, allowing quick responses for quality control.

The plant of choice was basil - *Ocimum basilicum L*. It was grown in the region of Anápolis - GO, which is in the central-western region of Brazil, with a tropical climate, where temperatures vary between 15 °C and 30 °C throughout the year. The months of December and January have the highest rainfall, while June and July have the lowest rates [9, 10].

3. Results

The chemical composition of the essential oil of various plant species can be influenced by seasonality, affecting the biosynthesis of secondary metabolites in different periods, whether rainy, dry, hot or cold [11]. Table 1 below shows the results of gas chromatography to identify the compounds in basil essential oil in different batches; for the study, the five components with the highest concentration were selected. It can be seen that there was a change in the concentration of each compound according to the time of year in which it was extracted. The most significant change observed in table 1 is for eugenol, which had a more than fourfold increase in its concentration between April/2022 and December/2021, which may be influenced by temperature and precipitation rate.

It can be seen that each compound peaked in a different month, and when comparing November 2021 with the same month in 2022, the values are irregular, which can be explained by the fact that their function in the plant is different from one another, which corroborates the study by Gobbo-Neto and Lopes [12], who presented seasonality, circadian rhythm and plant development as influential factors in the production of secondary metabolites. Table 1 highlights the linalool component, which appeared as the majority in all seasons, a fact that is like the results of Hussain et al. [13] who also analyzed the chemical composition of *Ocimum basilicum* in seasonal variations. Thus, this volatile oil can be characterized as a linalool chemotype; chemotypes are due to cultivars having different genetic characteristics and producing different sets of compounds [14].

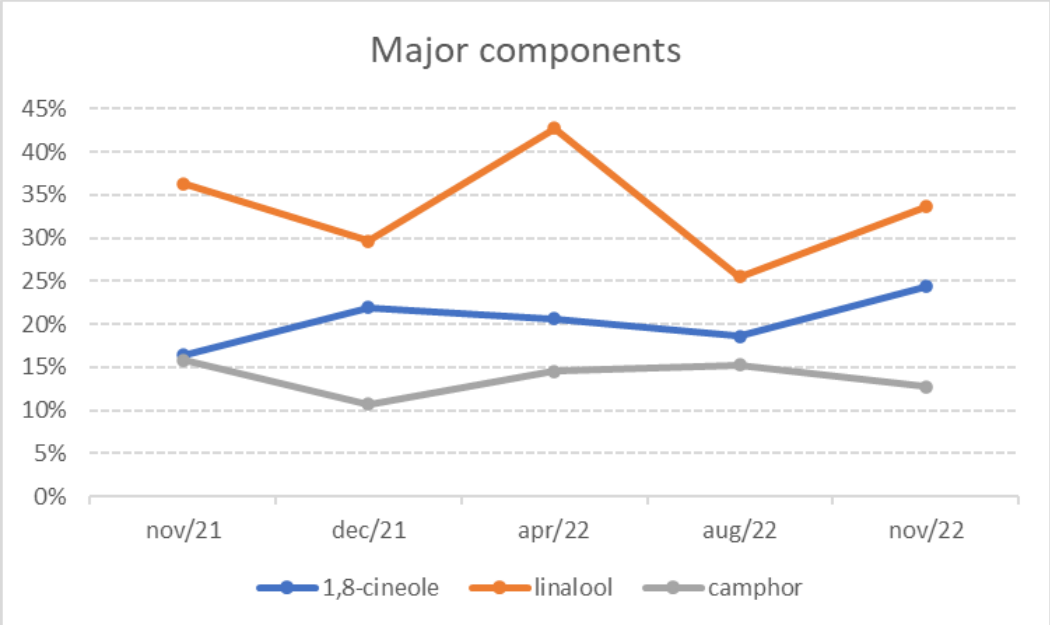
Table 1: Chemical constituents in greatest quantity.

	nov/21	**dez/21**	**abr/22**	**ago/22**	**nov/22**
1,8-cineol	16,37%	21,92%	20,60%	18,58%	24,35%
linalool	36,32%	29,66%	42,70%	25,46%	33,66%
camphor	15,82%	10,66%	14,50%	15,27%	12,70%
α-terpineol	3,90%	2,23%	3,10%	3,54%	2,41%
eugenol	4,25%	5,76%	1,40%	2,27%	2,07%

Source: Official chromatogram, CG-MS Bio Assets Biotecnologia report.

Linalool is a monoterpene alcohol, which has therapeutic properties such as anxiolytic and antidepressant, and can help in cases of anxiety, insomnia and depression [15]. In addition, it is an important substance in the synthesis of fragrances, due to its unique aroma, in industry it is used in perfumes, deodorants and pharmaceutical products [16]. Essential oils form synergies between their components and often feature more than one of them prominently, as can be seen in figure 1 below, the representation of linalool, 1,8-cineole and camphor and their variations in concentration for each batch.

Figure 1: Graphical representation of the 3 components in greatest quantity.



Source: Authors, using data obtained from the CG-MS Bio Assets Biotecnologia report.

As for the organoleptic characteristics, table 2 shows that all the batches meet the specifications for the product and there were no recorded differences in appearance, color or smell. The same circumstance was observed by Sellem et al. [17] when they analyzed

different harvest times for obtaining essential oil from *Inula graveolens (L.)* in Tunisia. In all the batches evaluated, the essential oil was described in terms of its physicochemical characteristics of density, refractive index and humidity, as shown in Table 3. About the refractive index, the values analyzed are within the range described as standard for the oil; this index is always greater than or equal to 1.0 and can be determined from the angles of incidence and refraction of light [18].

Table 2: Organoleptic characteristics of basil essential oil.

	Specification	**Jun/21**	**Nov/21**	**Dec/21**	**Apr/22**	**Aug/22**
Aspect	Clear liquid	In accord	In accord	In accord	In accord	In accord
Color	Pale yellow to almost colorless	Transparent	In accord	In accord	In accord	In accord
Odor	Aromatic, slightly spicy, herbaceous	Herbal, fresh, soft	In accord	In accord	In accord	In accord

Source: Data obtained from the Haje Insumos company's certificates of analysis.

When analyzing the humidity results for the different batches of essential oil, it can be seen that all are within specification, however, what is striking is the batch of Dec/2021, which has a considerably higher percentage of humidity than the average of the batches (1.28), this sample was collected in the month of December 2021, which according to INMET [19] recorded a more significant accumulation of rainfall in the region. The humidity present in essential oils can be related to changes in the chemical profile, as observed by Cruz and collaborators [20] in their study, the relative humidity and temperature of the environment could correlate with the yield and composition of the essential oil analyzed. On the other hand, the presence of water in contact with the essential oil, although they are not miscible, can trigger a hydrolysis reaction of the fat-soluble molecules [21].

When looking at the density parameter, it can be seen that there was no significant change, only a small change between the batches, which may be due to the variation in composition, correlating with table 1, the variation in the concentration of compounds, such as monoterpenes and sesquiterpenes is capable of altering the physical properties of the oil, including the total density of the sample [22].

4. Conclusion

In accordance with the research objective, it is suggested that the time of year and climatic variations may be related to changes in the physicochemical profile and concentrations of the chemical constituents of *Ocimum basilicum L.* essential oil. It is understood that the secondary metabolites of plants undergo quantitative changes associated with seasonality. The data from this study suggests the importance of recognizing the behavior of each species in relation to abiotic factors such as temperature, sunlight, humidity and rainfall.

This research contributes to the recognition of *Ocimum basilicum* essential oil, which is already used in medicine and industry. Knowledge of the variability in chemical composition makes it possible to choose the appropriate chemical profile for each product to be developed, with quality and a guarantee of achieving the objectives. It was possible to identify significant differences between the results of the chromatographic profile. However, further research is recommended to clarify more specific questions about the plant and its compounds, as well as to obtain more data that can prove the hypotheses raised. One of the limitations of this research was the limited number of results available for analysis.

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