Revista ÁRVORE
ISSN 1806 - 9088

Scientific Article

INFLUENCE OF DIFFERENT DRYING METHODS ON YIELD, DENSITY, COLOR, AND CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *Ocotea lancifolia* (SCHOTT) Mez LEAVES

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1 Received on 30.08.2024 accepted for publication on 26.03.2025. Editors: Iara Fontes Demuner and João Gilberto Meza Ucella Filho.
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ABSTRACT

The compounds in essential oils (EOs) are directly linked to their potential use. Drying methods are used to process large amounts of plant material while maintaining or even maximizing quality. This study observed the influence of different drying methods on the yield, density, organoleptic properties, and chemical components of EOs from *Ocotea lancifolia* leaves, comparing them with oils from fresh leaves. Fresh plant material (F) was subjected to hydrodistillation, a method also used for leaves after air drying (AIR), freeze-drying (FL), microwaving (MW), and oven drying at 45 °C (OD 45) or 60 °C (OD 60). Drying and extraction were performed in triplicate, using 350 g of fresh leaves and 250 g for the other samples. Yield was calculated as a percentage. Chemical compositions were analyzed by gas chromatography (GC). EO yields were: 0.4863% (F); 0.7400% (AIR); 1.050% (FL); 1.1167% (MW); 0.5867% (OD 45) and 0.7487% (OD 60). Microwaving and freeze-drying provided the highest yields. No differences in densities were observed. A drastic color change was noted in the EOs from leaves dried in an oven at 45 °C and 60 °C. The major compound identified was caryophyllene oxide, with percentages varying among treatments. Other components showed significant differences in structure and/or percentage. Drying time and temperature influenced modifications and degradation of some compounds, sometimes resulting in changes in EOs color and composition.

Keywords: Natural compound; Plant extractive; Caryophyllene oxide

How to cite:
Batista, B. F., Bianchini, N. H., Pavlack, A. S., Andrade, G. E., Muniz, M. F. B., & Heinzmann, B. M. (2025). Influence of different drying methods on yield, density, color, and chemical composition of the essential oil of *Ocotea lancifolia* (Schott) Mez leaves. *Revista Árvore, 49*(1). https://doi.org/10.53661/1806-9088202549263844

Revista ÁRVORE
ISSN 1806 - 9088

INFLUÊNCIA DE DIFERENTES MÉTODOS DE SECAGEM NO RENDIMENTO, DENSIDADE, COR E COMPOSIÇÃO QUÍMICA DO ÓLEO ESSENCIAL DAS FOLHAS DE *Ocotea lancifolia* (Schott) Mez

RESUMO - Os compostos dos óleos essenciais (OEs) estão diretamente ligados ao seu potencial de uso. Métodos de secagem são utilizados para processar grandes quantidades de material vegetal, mantendo ou até maximizando a qualidade. Este estudo observou a influência de diferentes métodos de secagem no rendimento, densidade, propriedades organolépticas e componentes químicos dos OEs de folhas de *Ocotea lancifolia*, comparando-os com óleos de folhas frescas. O material vegetal fresco (F) foi submetido à hidrodestilação, método também usado para folhas após secagem ao ar (AIR), liofilização (FL), micro-ondas (MW) e estufa a 45 (OD 45) ou 60 ºC (OD 60). A secagem e extração foram feitas em triplicata, utilizando 350 g de folhas frescas e 250 g para as demais amostras. O rendimento foi calculado em porcentagem. As composições químicas foram analisadas por cromatografia gasosa (CG). Os rendimentos de OE foram: 0,4863% (F); 0,7400% (AIR); 1.050% (FL); 1,1167% (MW); 0,5867% (OD 45) e 0,7487% (OD 60). Micro-ondas e liofilização proporcionaram os maiores rendimentos. Não houve diferença nas densidades. Observou-se mudança drástica na cor dos OEs de folhas secas em estufa a 45 e 60 °C. O composto majoritário identificado foi o óxido de cariofileno, com porcentagens variando entre os tratamentos. Outros componentes mostraram diferenças significativas em estrutura e/ou porcentagem. O tempo de secagem e a temperatura influenciaram as modificações e degradação de alguns compostos, resultando, às vezes, em mudanças na cor e composição dos OEs.

Palavras-Chave: Composto natural; Extrativo vegetal; Óxido de cariofileno

1. INTRODUCTION

Plant materials considered waste from logging activities in forests are also recognized as an important source of value-added products such as plant extracts. Particularly leaves, although often overlooked, represent a promising and underutilized source from which phytochemicals can be extracted (Devappa et al., 2015). Species with potential for use as spices, medicinal purposes and obtaining extracts are developed for commercialization and can reach high market values. Therefore, they must meet several quality requirements, evaluated through their sensorial and organoleptic characteristics, chemical composition, content of bioactive compounds, microbial load, chemical contamination with heavy metals and pesticides, and water content, among others (Chua et al., 2019; Sallew and Ahmad, 2017). Its stability is also of fundamental importance for the preservation of these products. Drying plant material is considered a post-harvest process for maintaining desirable characteristics (Orphanides et al., 2015; Turek and Stintzing, 2013; Celia et al., 2023).

Essential oils are consumed all over the world. Brazil stands out in this market in terms of production and exports, mainly citrus essential oil (EO) (Choo et al., 2022). These extracts are formed by a complex set of compounds, such as straight and branched chain hydrocarbons, mainly monoterpenoids and sesquiterpenoids, andphenylpropanoids (Silva et al., 2017). EO components are products of secondary plant metabolism, occurring in fruit peels, flowers, leaves, bark, wood, seeds, roots and rhizomes (Silva et al., 2018; Turek and Stintzing, 2013; Belwal et al., 2022). The components of the EO are generally linked to its action and purposes of use, therefore the analysis of the chemical composition of the EO becomes essential to ensure consistency in the chemical composition and, therefore, bioactivity between different batches (Chau et al., 2019; Silva et al., 2015).

Species of the genus *Ocotea* are

significant representatives of the Lauraceae family, growing in tropical and subtropical climates. These have great economic importance due to their use in furniture, food, perfumery and medicines (Sallew and Ahmad, 2017). *Ocotea lancifolia* (Schott) Mez is native to Rio Grande do Sul and other Brazilian states, and Paraguay (Silva et al., 2018). For this species, previous studies have described antioxidant, antifungal, sedative and anesthetic activity in fish, as well as antiparasitic activity (Sallew and Ahmad, 2017; Silva et al., 2017; Silva et al., 2018).

To maintain and maximize quality requirements, studies on drying methods are carried out to ensure the biochemical and microbiological stability of raw materials, especially in the industrial processing of large quantities of plant material (Orphanides et al., 2015; Omidbaigi et al. , 2004). This study aimed to evaluate the influence of different drying methods for plant material on the productivity, density, color and chemical composition of EO from *O. lancifolia* leaves.

2. MATERIAL AND METHODS

2.1 Obtaining the plant material

Ocotea lancifolia leaves were collected from a native population in the district of Santo Antão, in the municipality of Santa Maria, Rio Grande do Sul, Brazil, under the coordinates 29º 37' South and 53º 52' West. Two collections were performed in December 2018, during the spring, at less than a week, with the plant material collected in the morning. A voucher specimen was filed in the Herbarium of the Department of Forest Sciences, Universidade Federal de Santa Maria (HDCF/UFSM) under number 6399.

2.2 Drying methods

The collected leaves were divided into six lots to compare and analyze the EOs. A batch of fresh leaves (F) was extracted shortly after collection, and another five were submitted in triplicate to the following drying methods: air-drying at room temperature (AIR), lyophilization (FL), microwave-drying (MW), and oven-drying at 45 °C (OD 45) and 60 °C (OD 60). The drying conditions and methods used were selected considering the studies by Sellami et al. (2011) and Rahimmalek and Goli (2013) with adaptations. The drying time was determined considering the constant weight of the leaves, since the different drying methods required different periods until the leaves reached the point of dry. Air-dried leaves required 14 days until weight stabilization, leaves dried by lyophilization required 120 hours, leaves dried in a microwave at 500W power required 2 minutes, leaves dried in an oven at 45 °C required seven days, and leaves dried in an oven at 60°C required five days (Table 1).

2.3 Drying equipments

For air-drying, the leaves were exposed to an acclimatized environment at 22 ± 2 ºC in a closed room, with fluorescent light for approximately 8 hours light/16 hours dark.

Table 1. Drying time of leaves of *Ocotea lancifolia* under different methods
Tabela 1. Tempo de secagem de folhas de *Ocotea lancifolia* sob diferentes métodos

Drying Method	Drying Time
Air drying	14 days
Lyophilization	120 hours
Microwave (500W)	2 minutes
Ovendrying (45 °C)	7 days
Ovendrying (60 °C)	5 days

Revista ÁRVORE
ISSN 1806 - 9088

For drying by lyophilization, first, the leaf samples were frozen at -30 ºC for 48 hours, then they were transferred to a benchtop Lyophilizer model L101 (Liobras) for 120 hours at -45 ºC and pressure of 200 µHg. Microwave drying was performed in a domestic digital oven (Panasonic NN-ST369WRUK) with the following technical characteristics: 220 V-60 Hz, time adjustment with the help of the ovendigital clock, and at 500 W. Oven drying was carried out in a forced air circulation oven (J Prolab) with a digital thermometer at 45 ºC and 60 ºC.

2.4 Extraction and analysis of the essential oil color and chemical composition

350 g was used for each extraction of fresh leaves, and 250 g of dried leaves was obtained by different drying methods. The leaves were ground in a domestic multiprocessor model All-in-one Citrus (Philco). Subsequently, the batches of the different treatments were submitted to hydrodistillation in a Clevenger-type apparatus for 3 h (European Pharmacopoeia 2010) in triplicate. Later, the EO of each treatment was collected, observing and recording its color. To determine the color of the essential oil, the visually perceived aspect was considered, as this is one of the organoleptic characteristics and the sensory analysis is one of the well-established and used quality control methods for medicinal plants and their exudates In this case, the essential oil from fresh leaves was used as a reference sample for comparison (Brasil, 2024; Melo et al., 2007). Subsequently, the EO yield was calculated in relation to the dry weight of extracted plant material (% $m.m^{-1}$), and the EO density was obtained ($g.mL^{-1}$).

The analyses of the EO chemical compositions were carried out quantitatively through gas chromatography (GC) in a 7890A gas chromatograph (Agilent) equipped with a flame ionization detector (FID). For the analysis, an HP-5 silica capillary column (5% phenyl, 95% methylsiloxane; 30 m x 0.25 mm x 0.25 µm of phase thickness) was used, using Helium (1 mL/min) as carrier gas and a sample injection volume of 2.0 µL (2:1000 in hexane; v/v). The initial oven temperature was 40 °C, held isothermally for 4 min, and gradually increased to 320 °C at 4 °C/min. The total run time of each analysis was 76 min, where the injector and detector were operated at 300 °C (Amaral et al., 2015).

The EO samples qualitative analyses were carried out in a 7890A gas chromatograph coupled to an Agilent 5975C mass spectrometry detector, with electron impact ionization at 70 eV, using an HP5-MS capillary column (5% phenyl, 95% methylsiloxane; 30 m x 0.25 mm x 0.25 µm phase thickness). Injector, interface, ion source, and detector temperatures were maintained at 250, 280, 230, and 150 °C, respectively. The other parameters were the same as described above. The EO constituents were identified by comparing the Kovats retention indices (RI), calculated through a curve obtained with a standard mixture of n-alkanes (C8-C32), injected under the same conditions as the samples and by the fragmentation pattern of the mass spectra with data bank (Adams, 2009; Nist, 2009). Analyzes were performed using MS Chemstation Data Analysis software (Agilent Technologies, version 2.0).

2.5 Statistical analysis

The yield and density data were tested to verify the normality and homogeneity of variances, to which they did not fit. Therefore, a non-parametric Kruskal-Wallis test was used. The results are presented relative to the mean ± standard deviation, with a significant difference at $P<0.05$. Analyzes were performed using the GraphPad Prism software. The relationships between the EO chemical compositions and the drying methods were performed by Hierarchical Cluster Analysis (HCA) using the Ward method with Euclidean distance as a dissimilarity measure. The results obtained from HCA were complemented by Principal Component Analysis (PCA). Thus, two data matrices were constructed using average

values of the peak integrations obtained from the GC-FID analysis, where the constituents with ≥ 1.0% in at least one sample (46 compounds) were considered independent variables, and drying methods were considered cases. For multivariate analysis, data were submitted to the PAST-Paleontological Statistics software, Version 3.25.

To analyze the distribution of chemical compounds among the different drying methods, a Venn diagram was used, allowing the visualization of the exclusivity and overlap of compounds present in the essential oils (EOs) obtained from leaves subjected to different treatments. The compounds identified in each treatment were organized into three main sets, according to the groupings observed in hierarchical cluster analysis (HCA) and principal component analysis (PCA). The first group included the EOs from leaves dried by microwave (MW) and in an oven at 45 ºC (OD-45), the second group comprised the EOs from leaves dried in an oven at 60 ºC (OD-60) and by air drying (AIR), while the third group consisted of the EOs from fresh (F) and freeze-dried (FL) leaves. The diagram was constructed using the matplotlib venn library in Python 3.11. The sets were defined based on the presence or absence of compounds in each treatment, as identified in chromatographic analyses. Compounds common to two or more methods were positioned in the intersections of the sets, while exclusive compounds were individually allocated to each group.

3. RESULTS

3.1 Essential oil yield and density

The yield result obtained for the EO of fresh leaves was 0.4863%. For leaves submitted to five different drying methods, the highest yield was observed for the EO extracted from microwave-dried leaves, which was 1.1167%, followed by leaves dried by lyophilization, 1.050%. Oven drying at 45 ºC and 60 ºC provided yields of 0.5867 and 0.7487%, respectively, and air-dried leaves yielded 0.7400%. The statistical analysis of the yield results showed that the drying methods by lyophilization and in the microwave provided higher extractive yields than fresh leaves and other drying treatments (Figure 1).

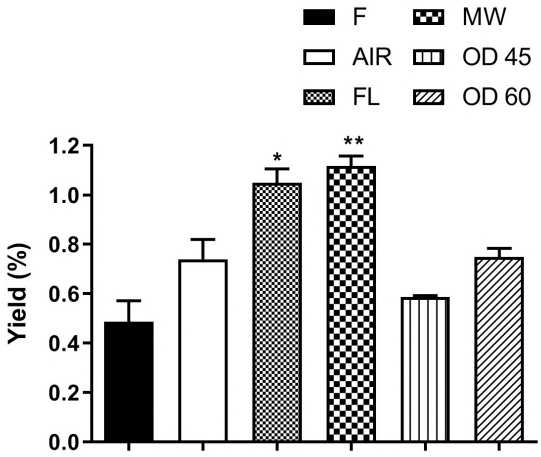

Figure 1. Yield of the essential oil of *Ocotea lancifolia* leaves comparing fresh leaves (F) with plant material subjected to drying methods. AIR: air-dried leaves, FL: leaves dried by lyophilization, MW: leaves dried in microwaves, OD 45: oven-dried leaves at 45 °C and OD 60: oven-dried leaves at 60 °C

Figura 1. Rendimento do óleo essencial das folhas de *Ocotea lancifolia* comparando folhas frescas (F) com material vegetal submetido a métodos de secagem. AIR: folhas secas ao ar, FL: folhas secas por liofilização, MW: folhas secas em micro-ondas, OD 45: folhas secas em estufa a 45 °C e OD 60: folhas secas em estufa a 60 °C

The density of the EOs showed no statistically significant difference between all extractives obtained, being 1.0738 g/mL for fresh leaves, 0.8755 g/mL for air-dried leaves, 0.9009 g/mL for freeze-dried leaves, 0.9559 g/mL for microwave-dried leaves, 0.9209 g/mL for oven-dried leaves at 45 °C, and 0.9571 g/mL for oven-dried leaves at 60 °C.

3.2 Essential oil composition

Forty-six chemical constituents were identified by analyzing the EO samples, considering those with a percentage greater than 1.0% in at least one of the chemical profiles analyzed. Nine peaks were identified for the EO of fresh leaves, making up 92.95% of the total EO. The analysis indicated caryophyllene oxide (57.43%), elixene (11.44%), cedranene (5.34%), and nootkatone (4.72%) as major components. The EO of air-dried leaves presented caryophyllene oxide (37.41%) as the major component. Twelve peaks were observed and identified in this extractive, representing 91.31% of the total. In the EO of leaves dried by lyophilization, the presence of the major compounds caryophyllene oxide (41.52%), longifolol (18.53%), 8-cedren-13-ol (8.98%), and γ-gurjunene (7.14%) were detected, in a total of 13 identified peaks, totaling 97.8% of the composition (Table 2).

The oil from microwave-dried leaves was characterized by the presence of caryophyllene oxide, representing 43.81% of the extractive, followed by 2Z,6E-farnesol, with 11.38%, γ-gurjunene, with 8.74%, and 8-cedren-13-ol, with 7.59%. Ten peaks were identified, corresponding to 87.93% of its chemical composition. Leaves dried in an oven at 45 ºC presented caryophyllene oxide (31.87%), 8-cedren-13-ol (12.51%), 2Z,6E-farnesol (9.00%), and cedr-8(15)-en-10-ol (8.17%) as major compounds. Thirteen peaks and 92.78% of the EO composition were identified. On the other hand, leaves submitted to oven drying at 60 ºC provided EO containing caryophyllene oxide (47.31%), Z-α-E-bergamotol (14.57%), E-nerolidyl acetate (11.48%), and β-costol (7.54%), with tenpeaks totaling 93.29% of the identified composition. The EO of *O. lancifolia* was mainly composed of oxygenated sesquiterpenoids, providing more than 70% of the chemical composition in all treatments, followed by sesquiterpene hydrocarbons and monoterpene hydrocarbons (Table 2).

The chemical composition differed significantly when we analyzed the components of the EO from leaves of *O. lancifolia* submitted to different drying

Table 2. Chemical composition of essential oils obtained from *Ocotea lancifolia* leaves, fresh and submitted to different drying methods

Tabela 2. Composição química dos óleos essenciais obtidos das folhas frescas de *Ocotea lancifolia* e folhas submetidas a diferentes métodos de secagem

Peak	Constituent	Class	IK calc.	IK lit.	F	AIR	FL	MW	OD 45	OD 60
1	β-E-Ocymene	MH	1040	1043[n]	3.02	1.43	-	1.85	1.36	-
2	β-Z-Ocymene	MH	1047	1047[n]	-	-	1.76	-	-	-
3	Aromadendrene	SH	1425	1429[n]	3.25	1.95	-	-	-	-
4	α-Guayene	SH	1425	1424[n]	-	-	3.40	3.88	5.13	-
5	γ-Gurjunene	SH	1475	1475[n]	-	-	7.14	8.74	-	2.34
6	γ-Elemene	SH	1484	1482[n]	-	4.61	-	-	-	-
7	Elixene	HS	1485	1482[n]	11.44	-	-	-	-	-
8	Z-α-Bisabolene	SH	1485	1495[n]	-	-	-	-	6.16	-
9	Caryophyllene oxide	OS	1534	1541[n]	57.43	37.41	41.52	43.81	31.87	47.31
10	α-Selinene	OS	1536	1530[n]	-	-	-	-	-	2.40
11	β-Nerolidol	OS	1565	1565[n]	-	1.28	-	-	-	-
12	Spathulenol	OS	1565	1569[n]	-	-	-	-	4.05	-

cont...

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Peak	Constituent	Class	IK calc.	IK lit.	F	AIR	FL	MW	OD 45	OD 60
13	6,8,8-Trimethyl-tricyclo-[5.2.2.0(1,6)]-undecan-3-ol, 2-methylene	OS	1600	1599[n]	-	-	-	-	2.01	-
14	β-Atlantol	OS	1610	1608a	-	-	1.01	-	-	-
15	Z-Asarone	PP	1612	1617a	-	-	-	-	1.24	-
16	Cedranene	OS	1613	1600[n]	5.34	-	-	-	-	-
17	E-cadinol	OS	1627	1628[n]	-	-	-	2.06	-	-
18	Eremoligenol	OS	1630	1630[n]	-	1.46	-	-	-	-
19	τ-Cadinol	OS	1636	1636[n]	-	-	1.75	-	-	-
20	8-Cedren-13-ol	OS	1644	1657[n]	3.70	-	8.98	7.59	12.51	1.89
21	Z-α-E-Bergamotol	OS	1644	1654[n]	-	9.66	-	-	-	14.57
22	Alohimachalol	OS	1655	1662[a]	-	-	-	5.26	-	-
23	Gimnomitrol	OS	1656	1660[a]	-	6.68	-	-	-	-
24	E-Bisabol-11-ol	OS	1657	1667[a]	2.58	-	5.42	-	-	-
25	Cedr-8(15)-en-10-ol	OS	1657	1652[a]	-	-	-	-	8.17	-
26	E-10,11-Di-hydroatlantone	OS	1666	1669[n]	-	5.29	-	-	-	-
27	Z-nerolidylacetate	OS	1677	1677[n]	-	-	4.03	-	-	-
28	(2Z,6E)-Farnesol	OS	1702	1701a	-	-	-	11.38	9.00	-
29	Geranyltiglate	OS	1703	1703[n]	-	16.27	-	-	-	-
30	Longifolol	OS	1714	1714[a]	-	-	18.53	-	-	-
31	E-nerolidylacetate	OS	1719	1717[a]	-	-	-	-	-	11.48
32	Cryptomerione	OS	1729	1724[a]	-	-	-	-	-	3.13
33	β-Costol	OS	1767	1767[a]	-	-	-	-	-	7.54
34	Nootkatone	OS	1771	1775[n]	4.72	-	-	-	-	-
35	α-Costol	OS	1772	1774[a]	-	3.71	-	-	1.56	-
36	14-Hydroxy-δ-cadinene	OS	1772	1780[a]	-	-	-	1.49	-	-
37	γ-eudesmylacetate	OS	1784	1784[a]	-	-	1.36	-	-	-
38	a-Muurolen-14-ol	OS	1788	1780[a]	-	-	-	1.87	-	-
39	2-(4a,8-Dimethyl-6-oxo-1,2,3,4,4a,5,6,8a-octahydro-naphtalene-2-yl)-propionaldehyde	OS	1799	1803[n]	-	-	-	-	6.5	-
40	β-bisabolenol	OS	1800	1790[a]	1.48	-	-	-	-	-
41	E-Isovalencenol	OS	1800	1793[n]	-	2.08	-	-	-	-
42	Vativenicacid	OS	1812	1811[a]	-	-	1.5	-	-	-
43	α-Vetivone	OS	1839	1843[a]	-	-	-	-	-	1.03
44	Cubitene	DH	1871	1878[a]	-	-	-	-	-	1.6
45	Kaur-16-ene	DH	2029	2032[n]	-	-	-	-	3.22	-
46	Kaurene	DH	2042	2043[a]	-	-	1.4	-	-	-
Monoterpenehydrocarbons					3.02	1.43	1.76	1.85	1.36	-
Sesquiterpenehydrocarbons					14.69	6.56	10.54	12.62	11.29	2.34
Oxygenatedsesquiterpenoids					75.25	83.84	84.1	73.46	75.67	89.35
Phenylpropanoids					-	-	-	-	1.24	-
Diterpenehydrocarbons					-	-	1.4	-	3.22	1.6
TOTAL IDENTIFIED					**92.95**	**91.83**	**97.80**	**87.93**	**92.78**	**93.29**

IK cal.: Calculed Kovats Index; IK lit.: Literature Kovats Index. n NIST, 2009. a Adams, 2009. Where: F: fresh leaves; AIR: air-dried leaves; FL: freeze-dried leaves; MW: microwave-dried leaves; OD 45: leaves dried in an oven at 45 ºC; OD 60: leaves dried in an oven at 60 ºC; MH = Monoterpene Hydrocarbons; OM = Oxygenated monoterpenoids; SH = Sesquiterpene hydrocarbons; OS = Oxygenated sesquiterpenoids. PP = Phenylpropanoids; DH: Diterpenehydrocarbons.

IK cal.: Índice de Kovats calculado; IK lit.: Índice de Kovats da literatura. n NIST, 2009. a Adams, 2009. Onde: F: folhas frescas; AIR: folhas secas ao ar; FL: folhas liofilizadas; MW: folhas secas em micro-ondas; OD 45: folhas secas em estufa a 45 ºC; OD 60: folhas secas em estufa a 60 ºC; MH = Hidrocarbonetos monoterpênicos; OM = Monoterpenoides oxigenados; SH = Hidrocarbonetos sesquiterpênicos; OS = Sesquiterpenoides oxigenados. PP = Fenilpropanoides; DH: Hidrocarbonetosditerpênicos.

methods. This was evidenced when we analyzed their chemical compositions using HCA and PCA. The application of HCA resulted in two large distinct groups (Figure 2), which, after cutting (dashed line) at the Euclidean distance of 25 units, gave rise to four groups, with a clear separation of samples of fresh (F) and dried leaves by lyophilization (FL), with few similar compounds according to the CG analyses. In the group formed by the EOs obtained from leaves subjected to microwave drying (MW) and drying in an oven at 45 ºC (OD 45), the compounds that are probably involved in this grouping are caryophyllene oxide (mean value 37.84%), 2Z,6E-farnesol (mean value 10.19%), 8-cedren-13-ol (mean value 10.05%), α-guayene (mean value 4.51%), and β-ocymene (mean value 1.60%). The group formed by EO obtained from leaves submitted to oven drying at 60 ºC (OD 60) and air drying (AIR) may be related to the presence of caryophyllene oxide (mean value 42.36%) and Z-α-E-bergamotol (mean value 12.12%).

The relationship between these

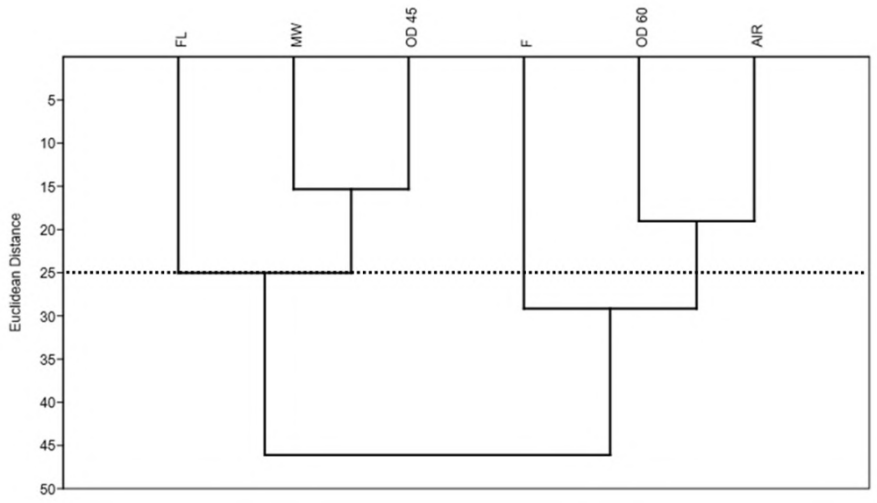

Figure 2. Hierarchical Cluster Analysis of the chemical composition of essential oils from *Ocotea lancifolia* fresh leaves using Ward's method and submitted to different drying methods. Where: F, fresh leaves; AIR, air-dried leaves; FL, freeze-dried leaves; MW, microwave-dried leaves; OD 45, leaves dried in an oven at 45 ºC; OD 60, leaves dried in an oven at 60 ºC

Figura 2. Composição química dos óleos essenciais obtidos das folhas frescas de *Ocotea lancifolia* e folhas submetidas a diferentes métodos de secagem Onde: F, folhas frescas; AIR, folhas secas ao ar; FL, folhas liofilizadas; MW, folhas secas em micro-ondas; OD 45, folhas secas em estufa a 45 ºC; OD 60, folhas secas em estufa a 60 ºC

compounds in the different treatments is best visualized in the Venn Diagram (Figure 3), which illustrates the exclusive and shared compounds among the groups. It is observed that caryophyllene oxide is present in both groups, suggesting a central role in the chemical similarity of the EOs independently on the drying method applied

The PCA showed that the first two components represented about 60% of the total variance, CP1 32.67% and CP2 27.62%, in which the most involved compounds corresponding to the numbers 20 and 9 (see Table 1), 8-cedren-13-ol and caryophyllene oxide, respectively (Figure 4). It is possible to observe that compounds 20 and 28 are located between the MW and OD 45 treatments, corresponding to the substances 8-cedren-13-ol and 2Z,6E-farnesol,common to both EOs compositions, and the proximity of compound 21 to the grouped methods OD 60 and AIR, corresponding to Z-α-E-

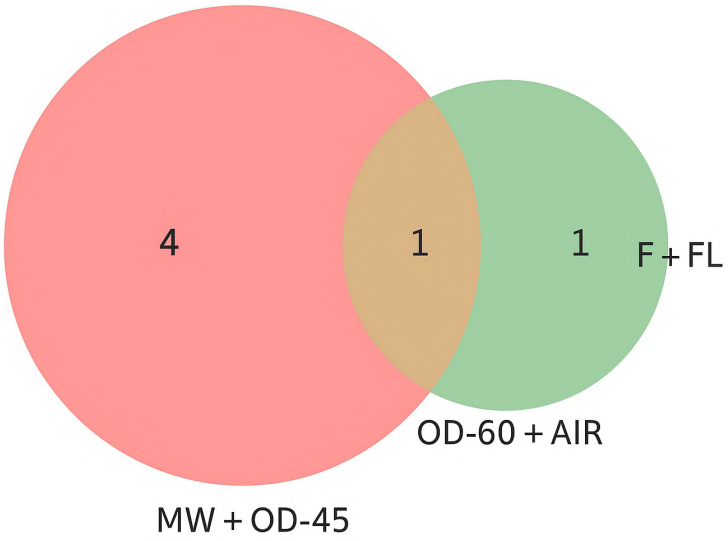

Figure 3. Venn Diagram representing the distribution of chemical compounds in the essential oils (EOs) of *O. lancifolia* obtained after different drying methods of plant material. The groups considered were: leaves dried by microwave and in an oven at 45 ºC (MW + OD-45), leaves dried in an oven at 60 ºC and air-dried (OD-60 + AIR), and fresh/freeze-dried leaves (F + FL). Where: F, fresh leaves; AIR, air-dried leaves; FL, freeze-dried leaves; MW, microwave-dried leaves; OD 45, leaves dried in an oven at 45 ºC; OD 60, leaves dried in an oven at 60 ºC

Figura 3. Diagrama de Venn representando a distribuição dos compostos químicos nos óleos essenciais (EOs) de *O. lancifolia* obtidos após diferentes métodos de secagem do material vegetal. Os grupos considerados foram: folhas secas por micro-ondas e estufa a 45 ºC (MW + OD-45), folhas secas por estufa a 60 ºC e secagem ao ar (OD-60 + AIR) e folhas frescas/liofilizadas (F + FL). Onde: F, folhas frescas; AIR, folhas secas ao ar; FL, folhas liofilizadas; MW, folhas secas em micro-ondas; OD 45, folhas secas em estufa a 45 ºC; OD 60, folhas secas em estufa a 60 ºC

bergamotol. Therefore, PCA confirmed the HCA findings and demonstrated that EO compositions were mainly influenced by the presence of certain chemical compounds, which were determinants for the separation of *O. lancifolia* EOs into different groups.

3.3 Changes in essential oil color

In this study, the most significant change in the color of EOs was observed after drying in an oven at 45 ºC and 60 ºC. The EO obtained from plant material submitted to 45 ºC presented the highest color intensification, greenish, darkerthan that from leaves dried at 60 °C. The EOs extracted after the other drying methods and the one obtained from fresh leaves had a slightly yellowish color. Therefore, the observed changes were more subtle and thus did not characterize an essential change in their visual characteristics (Figure 5).

4. DISCUSSION

4.1 Essential oil yield and density

The methods that showed the highest yield in this study are considered innovative drying technologies for post-harvest processing of plants used as condiments and to obtain extractives (Ng et al., 2020; Abbapour-Gilandeh et al. 2019). Microwave drying, compared to traditional drying methods, has additional advantages, such as the short processing time and consequent rapid dehydration of the material (Abbapour-Gilandeh et al. 2019; Choo et al., 2022). Drying methods and their enhancement, particularly, in industrial processes, shape research trends. Key objectives include enhancing product quality, reducing pollution, increasing capacity, improving process control, achieving economic efficiency, and shortening drying time (Belwal et al., 2022).

Revista ÁRVORE
ISSN 1806 - 9088

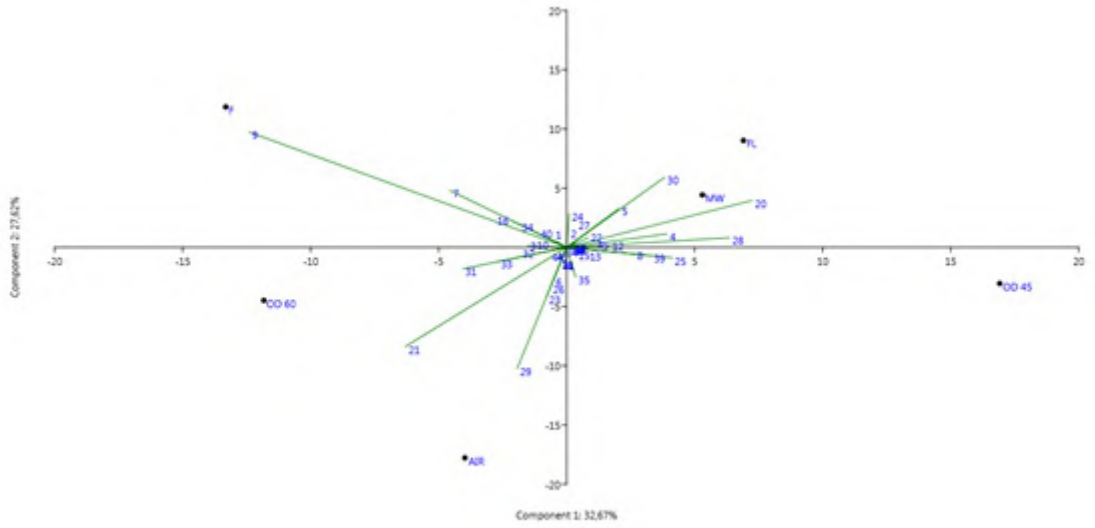

Figure 4. Principal Components dispersion graph considering the major components of the essential oils of *Ocotea lancifolia* leaves and their correlation between different treatments. Where: Table 1 lists the numbers of the compounds, highlighting: 9 = Caryophyllene oxide, 20= 8-Cedren-13-ol, 21= Z-α-E-Bergamotol, and 28= 2Z,6E-Farnesol. F: fresh leaves; AIR: air-dried leaves; FL: freeze-dried leaves; MW: microwave-dried leaves; OD 45: leaves dried in an oven at 45 ºC; OD 60: leaves dried in an oven at 60 ºC

Figura 4. Gráfico de dispersão dos componentes principais considerando os componentes químicos dos óleos essenciais das folhas de *Ocotea lancifolia* e sua correlação entre os diferentes tratamentos. Onde: Tabela 1 lista os números dos compostos, destacando: 9 = Óxido de cariofileno, 20 = 8-Cedren-13-ol, 21 = Z-α-E-Bergamotol e 28 = 2Z,6E-Farnesol. F: folhas frescas; AIR: folhas secas ao ar; FL: folhas liofilizadas; MW: folhas secas em micro-ondas; OD 45: folhas secas em estufa a 45 ºC; OD 60: folhas secas em estufa a 60 ºC

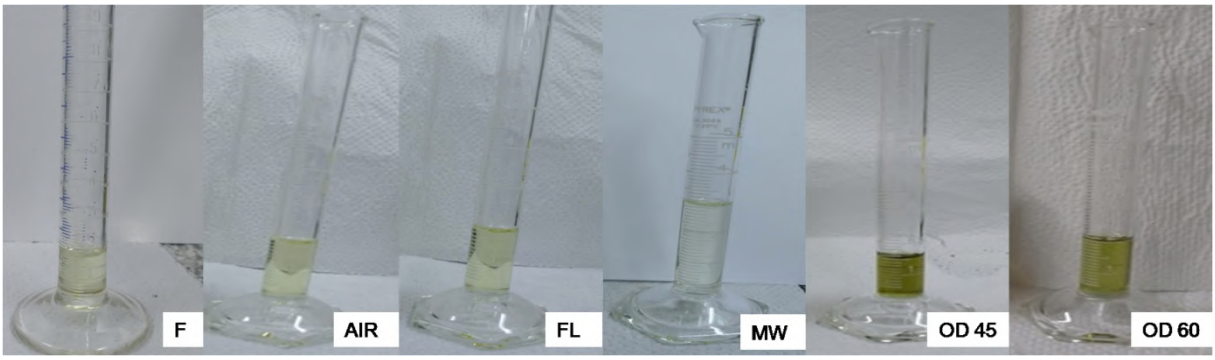

Figure 5. Color of the essential oils of *Ocotea lancifolia* leaves obtained after different post-harvest processes. Where: F, fresh leaves; AIR, air-dried leaves; FL, freeze-dried leaves; MW, microwave-dried leaves; OD 45, leaves dried in an oven at 45 ºC; OD 60, leaves dried in an oven at 60 ºC

Figura 5. Cor dos óleos essenciais das folhas de *Ocotea lancifolia* obtidos após diferentes processos pós-colheita. Onde: F, folhas frescas; AIR, folhas secas ao ar; FL, folhas liofilizadas; MW, folhas secas em micro-ondas; OD 45, folhas secas em estufa a 45 ºC; OD 60, folhas secas em estufa a 60 ºC

Silva et al. (2018) found higher EO levels in *O. lancifolia* collected during spring and summer, averaging 1.03% and 0.96%, respectively. Following their recommendation, we gathered leaves during spring, though the period had milder temperatures than usual. December in the region experiences higher temperatures and intense sunlight, potentially impacting extractive yields (Silva et al., 2015; Amaral

et al., 2015). Climatic conditions may have thus negatively affected EO yield from fresh leaves.

Some authors propose that drying plant material before hydrodistillation can increase extractive yield. In drying some aromatic plants, the movement of moisture diffusion on the surface of the leaves can carry EO compounds toward the outside, which may be related to a great extractive yield when compared to fresh leaves (Chua et al., 2019; Sellami et al., 2011). The EO content in dried leaves may be influenced by moisture removal and the location of secretory structures on the plant. In Lauraceae species, secretory structures are primarily inside leaf idioblasts, promoting lower volatilization (Silva et al., 2015; Gonçalves et al., 2018).

In a study on the influence of drying methods on the content and chemical composition of *Chamaemelum nobile* (L.) All. EO, popularly known as Roman chamomile, widely used as a medicinal plant, the drying methods affected the number of chemical compounds and reflected significantly in their proportion (Omidbaigi et al., 2004). In this study, when verifying the percentage sum of the compounds identified in the oils from leaves submitted to each drying treatment concerning the EO composition of fresh leaves, for microwave drying, 34.68% of the total composition differed, for drying by lyophilization, 41.88% of the chemical composition of the EO was different, drying in an oven at 60 ºC provided 44.09% of different chemical composition, followed by drying in an oven at 45 ºC, with 47.04% of different composition identified and air drying, with 51.03% of the total composition distinct from the EO of fresh leaves.

4.2 Essential oil composition

Twenty-six substances were identified in a previous study conducted with EO of fresh leaves of the same species, collected from the same population and in the same season, where caryophyllene oxide was also the major component, followed by bicyclogermacrene, bulnesol, calarene epoxide, and E-nerolidyl acetate. The study also revealed differences in the contents and chemical composition of EOs from leaves, fruits, and inflorescences (Silva et al., 2018). Comparing the EO composition of fresh leaves in this study, with that analyzed by Silva et al. (2018) both contained the same major compound.

Numerous chemical reactions occur in the biosynthesis of secondary metabolites in plants, which can result in chemical rearrangements originating different compounds. After auto-oxidation, cyclization, and epoxidation, sesquiterpenoids, such as β-caryophyllene, form β-caryophyllene oxide (Silva et al., 2010). Alteration in chemical structures and the appearance of some compounds can influence the biological activities related to the extractives and their organoleptic properties (Chua et al., 2019; Sallew and Ahmad, 2017). According to Saad, Muller, and Lobstein (2013), oxygenated molecules, like the major constituent of the EO of *O. lancifolia* leaves, caryophyllene oxide, have a more significant antifungal action than hydrocarbons as its precursor, β-caryophyllene.

These results align with the high concentrations of representatives of these chemical classes, also observed in other *Ocotea* species (Brustulim et al., 2020; Mallmann et al., 2020). The formation of artifacts is possible in preparing plant material to obtain EOs, as during the drying processes, compounds present in the fresh plant material may change their structure, becoming derivatives through oxidation, isomerization, cyclization, or dehydrogenation reactions. The processes may be correlated with factors such as drying time and temperatures, even natural chemical rearrangements, causing differences in chemical compositions observed through the appearance or absence of compounds from the extractive of the same species and plant organ (Santini and Haselein, 2002; Sellami et al. 2011; Turek and Stintzing, 2013; Celia et al., 2023).

Revista ÁRVORE
ISSN 1806 - 9088

Among the analyses of the EO compositions, extracted from fresh leaves and dried leaves, the only common constituent in all extracted EOs was caryophyllene oxide, which was also the major constituent in all analyzed extractives. Studies on the activities of extracts containing caryophyllene oxide among the major components have demonstrated a high potential forcytotoxic activity on cancer cells (Prinsloo et al., 2018). Caryophyllene oxide is also used as a food preservative and in the cosmetic and drug industry (Chavan et al., 2010; Silva et al., 2010). In addition, 8-cedren-13-ol was identified in five of the six analyzed EOs and was not present only in the EO obtained from air-dried leaves. This absence may be linked to the longer air drying period compared to the other drying methods, combined with the volatile characteristics of this compound, since some compounds may be lost after 14 days of drying, being carried away due to their affinity with leaf moisture (Sellami et al., 2011; Mokhtarikhah et al., 2020).

The percentage of eugenol varied according to the applied temperature in a study conducted by Celia et al. (2023) on drying cinnamon leaves (*Cinnamomum zeylanicum* Blume) at different temperatures using a dryer with trays of metallic layers and an oven with forced air circulation. The authors identified 23 compounds in the study, detecting trace components below 1% regardless of temperature, including caryophyllene oxide.

4.3 Changes in essential oil color

EOs obtained by hydrodistillation of *Ocotea* species leaves are generally light to yellowish transparent (Brustulim et al., 2020). The color of products of natural origin after harvesting and processing is a crucial organoleptic characteristic directly linked to the acceptance of these products in the market. Color change is generally attributed to the possible degradation of compounds by imposing thermal treatments using heat (Rahimmalek and Goli, 2013). In microwave drying, the vegetation water present in the plant material is heated by microwaves, providing a more uniform distribution of energy and heat throughout the leaves tissues. This accelerates the drying process, as it generates internal pressure caused by the opening of capillaries on the leaf surface, promoting the movement of water and steam during drying (Orphanides et al., 2015). The rapid and uniform heating process also causes protein denaturation, which includes loss of enzymatic activity in plant tissues (Pratama et al., 2022). Removing moisture and enzymatic inactivation by MW before hydrodistillation of the leaves can generate less intense changes in the EO, giving leaves biochemical stability compared to fresh ones (Celia et al., 2023; Choo et al., 2022; Pratama et al., 2022).

Because fresh leaves have not been dried, we can also consider hydrodistillation as a possible factor involved in color changes, as hydrodistillation also imposes heat and the movement of particles in the plant material by dragging water vapor, which can still change the EO. Previous studies on different drying methods noted sensory alterations in extracts from plant material dried in an oven at temperatures between 50 ºC to 80 ºC (Rahimmalek and Goli, 2013).

The exclusive presence of certain compounds can be linked to the color differentiation of the EO since these may result from the degradation of genuine compounds. Relationships and stability assessments are generally carried out considering mainly different compounds with higher percentages (Celia et al., 2023). However, in this study, of the compounds detected exclusively in the EOs obtained from leaves dried in an oven at 45 ºC and 60 ºC, to which the color variation could be attributed, none was common to the two compositions. Thus, it is impossible to attribute the color change observed in the two samples to a single compound, suggesting the possible degradation of the set of substances.

Once the presence of some compounds in extracts is related to their bioactivities, the drying method may influence the possibility of using these products. Thus, increasing or decreasing/transforming some compounds can reinforce or decrease desirable or undesirable biological activities, reinforcing the purpose or discouraging their use (Chua et al., 2019; Turek and Stintzing, 2013). In this context, the compound Z-Asarone, present in the EO obtained after drying in an oven at 45 ºC, has been identified in other species of Lauraceae. Biological activities, such as insecticide, have been reported for this component. However, it has also been related to carcinogenic action, demonstrating that its presence in the EO is not desirable for its use in humans/animals, as it confers toxicity to the EO (Sallew and Admad, 2017; Brustulim et al., 2020; Prinsloo et al., 2018). Therefore, this drying method may not be recommended for *O. lancifolia* leaves.

5. CONCLUSION

This study demonstrated that the selection of the drying method for *O. lancifolia* leaves can influence the yield and chemical composition of the essential oil. Thus, it is necessary to observe a series of variables, including the drying method, time, temperature, stability of chemical components, and organoleptic characteristics of the obtained extractive. Microwave drying and lyophilization methods stood out due to the higher yield and the reduced drying time. Regardless of the drying method used, the major compound in the EO of *O. lancifolia* leaves was caryophyllene oxide, the same detected for the oil of fresh leaves. Of the evaluated drying methods, the most appropriate is microwave drying, which resulted in a higher yield of essential oil (230% greater than that of fresh leaves), additionally presenting a greater similarity of its chemical composition with that of the essential oil of fresh leaves. However, this work is relevant and can be used as a basis for other studies and development of optimized essential oil extraction protocols.

6. ACKNOWLEDGEMENTS

To CNPq, CAPES and FAPERGS for the scholarships granted (Financial code 001).

AUTHOR CONTRIBUTIONS

BFB: Investigation, Data curation, Methodology, Validation, Writing – original draft, Writing – Review and Editing. NHB: Investigation, Methodology, Validation. ASP: Methodology, Data curation. GEA: Methodology, Data curation. MFBM: Conceptualization, Formal analysis, Funding acquisition, Supervision. BMH: Conceptualization, Investigation, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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