

Effect of drying method and storage conditions on the essential oil yield and composition of *Eugenia uniflora* L. leaves

Efecto del método de secado y condiciones de almacenamiento en el rendimiento y composición de aceite esencial en hojas de *Eugenia uniflora* L.



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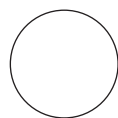
Eugenia uniflora (detail: leaves)

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ABSTRACT

The essential oil produced by *Eugenia uniflora* leaves is intensively used by the cosmetic, pharmaceutical and perfumery industries, mainly because its antioxidant effect and antimicrobial and antihelminthic activities. Drying and storage of aromatic plant leaves can affect yield and quality of essential oils. This study aimed to evaluate the effect of drying method and storage conditions on the essential oil yield and composition in *Eugenia uniflora* leaves. The essential oil was extracted from dried leaves at ambient temperature and at 45°C for six drying periods (0, 6, 24, 48, 72 and 96 hours). The essential oil yield was also analyzed after 0, 60, 120, and 180 days of storage in three types of packaging. The oil was extracted with hydrodistillation, and the composition was determined with gas chromatography-mass spectrometry. The results showed that drying leaves at ambient temperature and 45°C for 48 and 24 hours, respectively, increased the essential oil yield. At 45°C, the monoterpene and sesquiterpene compounds increased, with curzerene+viridiflorene identified as the major compounds. The packaging did not affect the essential oil yield but increasing the storage period decreased it.

Additional keywords: post-harvest; aromatic plants; sesquiterpene compounds; curzerene+viridiflorene; Surinam cherry.



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RESUMEN

El aceite esencial producido por las hojas de *Eugenia uniflora* es utilizado intensamente por las industrias cosmética, farmacéutica y de perfumería principalmente debido a su efecto antioxidante y sus actividades antimicrobianas y antihelmínticas. El secado y el almacenamiento de las hojas de plantas aromáticas pueden afectar el rendimiento y la calidad de su aceite esencial. Este estudio tuvo como objetivo evaluar el efecto del método de secado y las condiciones de almacenamiento sobre el rendimiento y la composición del aceite esencial en las hojas de *Eugenia uniflora*. El aceite esencial se extrajo de hojas secas a temperatura ambiente y a 45°C durante seis períodos de secado (0, 6, 24, 48, 72 y 96 horas). El rendimiento del aceite esencial también se analizó después de 0, 60, 120 y 180 días de almacenamiento en tres tipos de envases. El aceite se extrajo por hidrodestilación y la composición se determinó por cromatografía de gases - espectrometría de masas. Los resultados mostraron que el secado de las hojas a temperatura ambiente y 45°C durante 48 y 24 horas, respectivamente, aumentó el rendimiento del aceite esencial. A 45°C, los compuestos de monoterpeneo y sesquiterpeneo aumentaron, con curzereno + viridifloreño identificado como los compuestos principales. Los tipos de paquetes no afectaron el rendimiento del aceite esencial, pero el aumento del período de almacenamiento lo disminuyó.

Palabras clave adicionales: poscosecha; plantas aromáticas; compuestos sesquiterpenos; curzereno+viridifloreño; Pitanga.

Received for publication: 06-06-2019 Accepted for publication: 11-06-2020

INTRODUCTION

Surinam cherry, Brazilian cherry or Cayenne cherry (*Eugenia uniflora* L.), belongs to the Myrtaceae family and is a native plant species of the tropical east coast of South America, with dispersion from Suriname to southern Brazil (Lorenzi and Matos, 2008). The essential oil produced in the leaves is suggested as an important raw material for the cosmetic, pharmaceutical and perfumery industries because of its antioxidant effect and antimicrobial and antihelmintic activities (Amorim *et al.*, 2009; Gallucci *et al.*, 2010; Victoria *et al.*, 2012; Rodrigues *et al.*, 2013; Santos *et al.*, 2018).

According to Melo *et al.* (2007), the major constituents in Surinam cherry essential oil are furanodiene and its rearrangement products, furanoelemene (or curzerene, 50.2%), β -elemene (5.9%) and α -cadinol (4.7%). However, the essential oil composition of this species is reported as greatly varied in the literature, associated with phenotypic characteristics (Costa *et al.*, 2010) and edaphoclimatic conditions (Costa *et al.*, 2009; Rodrigues *et al.*, 2013; Santos *et al.*, 2018; Silva *et al.*, 2018).

Because of the high variability found in the essential oil of Surinam cherries during the different seasons of the year and also because it is a semi-deciduous species, the development of drying techniques allows

Surinam cherry leaves to be stored and marketed throughout the year. The drying of aromatic plants can be natural, which is generally carried out under shade and at ambient temperature, or done with artificial driers, with or without ventilation (Rocha *et al.*, 2011). The drying process preserves a plant's active components by reducing the oxidation process and microbial growth (Harbourne *et al.*, 2009; Young *et al.*, 2015).

The drying method can also affect the essential oil yield of aromatic plants, as demonstrated by Amaral *et al.* (2018, 2019) in native Brazilian species of the Myrtaceae and Asteraceae families. Choosing the drying method is particularly important for aromatic plants since it can affect essential oil yield and composition according to the plant species and the secretory structure where the oil is accumulated (Rahimmalek and Goli, 2013).

Some plant species rely on the drying method to reduce moisture and break the secretory structures containing the essential oil. Studies have indicated that high drying temperatures facilitate lysigenous cell disruption within the parenchyma, resulting in increased essential oil yield (Hannaa *et al.*, 2012; Pirbalouti *et al.*, 2013).

In addition to the drying process, storage conditions also affect essential oil yield (Lisboa *et al.*, 2018). However, studies on these procedures in *Eugenia uniflora* are scarce. The optimum storage conditions for Surinam cherry leaves is needed to improve essential oil production.

This study aimed to characterize the essential oil yield and composition of *Eugenia uniflora* leaves dried at different temperatures for different periods of time and after storage in different types of package materials.

MATERIALS AND METHODS

The plant material was collected in Parana State (1,040 m altitude, 25°02'34" S and 51°31'47" W), Brazil, in June (2012).

The leaves of ten Surinam cherry trees, with dark red fruits, were selected for diameter at breast height, ranging from 20 to 32 cm. Exsiccates of fresh branches were deposited in the "Integrated Spiritist College Herbarium", Curitiba, Parana (register HFIE 9.127).

Drying experiment

The drying experiment was carried out in a completely randomised design, comparing the effect of temperature (room temperature of 22°C and dryer temperature of 45°C) for 6, 24, 48, 72 and 96 h, as compared to non-dried leaves, with three replications. The fresh leaf samples were homogenised and separated into sub-samples (approximately 4 kg) for each replication. The leaves were air-dried at room temperature and relative humidity (69%) in the absence of direct sunlight incidence. The controlled temperature drying was done at 45°C with forced ventilation (Nova Ética®).

The essential oil was isolated with hydrodistillation in a Clevenger graduated apparatus using 100 g of leaves in 1 L of distilled water, for 4 h, according to the Brazilian Pharmacopoeia recommendations (ANVISA, 2020). The essential oil components were subsequently identified and quantified with gas chromatography-mass spectrometry (60-240°C, 3°C min⁻¹ ramp) in an Agilent 7890A device with a DB-5 (30 m × 0.25 mm × 0.25 μm) capillary column, operating in a 1:5 flow divider mode. The injector and detector temperatures were 250 and 280°C, respectively.

Helium was used as the carrier gas at a flow of 1 m min⁻¹. The essential oil was diluted in hexane to a concentration of 1%, and an aliquot of this solution (1 μL) of each sample was injected. The mass spectra were obtained with electron ionisation at 70 eV with an acquisition rate of 3.15 scans/s, in the range of 40 to 500 Da. The essential oil components were identified by comparing the retention indices of the components with those of a homologous series of n-alkanes (C11-C24) injected into the same column and were calculated according to the Van den Dool and Kratz (1963) equation. The mass spectra were compared with data from the Wiley/NBS Spectrotech, and the linear retention indices were verified according to the literature data (Adams, 2017).

The essential oil yield was determined by calculating the density and the corrected values for dried mass (DM), and the subsample moisture percentage was determined with 10 g of leaves per repetition.

Storage experiment

To evaluate the influence of storage type and time on the yield of the essential oil, 200 g of fresh leaves were dried to 11% moisture with the Ecodryer method with a controlled air speed and drying temperature (70°C on the leaves). The experiment design was completely randomized in a 3 × 4 factorial, comparing the package types and four storage periods (60, 120, 180 d and with no storage), with five replications. The package types included polyethylene raffia bags (70 g m⁻²), polyethylene transparent bags (10 μm) and double kraft bags (200 g m⁻²), and wrapped in a polyethylene transparent bags (10 μm). The packaged leaves were kept under a wooden "pallet" measuring 1 × 1.2 m, simulating the storage system used in the medicinal and aromatic plant industries.

Prior to storage, 15 essential oil extractions were performed on fresh leaves (controls) containing 53% moisture. A further 15 essential oil extractions were performed on dried leaves, containing 11% moisture. These extractions provided a statistical comparison related to the storage treatments.

Essential oil yield was determined by calculating the density and the corrected values for dried mass (DM). The subsample moisture percentage was determined with 10 g of leaves per repetition.

The treatment variances were tested for homogeneity with Bartlett's test. The averages were compared

with Tukey's test at 5% probability, using the statistical program ASSISTAT version 7.7 (Silva and Azevedo, 2016).

RESULTS AND DISCUSSION

Drying experiment

Essential oil yield

The drying of Surinam cherry leaves resulted in a significant increase in essential oil yield. The highest average was obtained when the leaves were dried at 45°C for 24 h (3.7 $\mu\text{L g}^{-1}$ DM) and at ambient temperature for 48 h (3.5 $\mu\text{L g}^{-1}$ DM) (Tab. 1).

Table 1. Average essential oil yield ($\mu\text{L g}^{-1}$ DM) of Surinam cherry leaves prepared by different drying treatments.

Time (h)	Drying temperature	
	Ambient (22°C)	45°C
0	2.5 aB	2.0 bB
6	2.2 aBC	2.0 aB
24	2.1 bBC	3.7 aA
48	3.5 aA	1.8 bB
72	1.6 aC	2.0 aB
96	1.7 aC	1.5 aB
CV (%)	11.3	

CV (%) = coefficient of variation; DM = dried mass; Averages with the same lowercase letters in the rows and capital letters in the columns are not significantly different according to Tukey's test ($P < 0.05$).

Previous results for other aromatic plant species are similar to this study, where a high essential oil yield was found under specific drying temperatures. In *Lippia citriodora*, the highest essential oil yield was obtained at 30°C, with a significant decrease at higher temperatures (Shahhoseini *et al.*, 2013). Pirbalouti *et al.* (2013), who compared different drying methods for *Satureja bachtiarica*, found the highest oil yields at 45°C for 48 h.

The changes in essential oil yield of aromatic plants after drying can be related to the anatomy of the storage structures. According to Fiuza *et al.* (2008), the essential oil in *Eugenia uniflora* leaves is located in the palisade parenchyma, within secretory cavities or oil

channels. Increasing the drying temperature and consequent water removal from the cells may contribute to degradation of the leaf tissue and release of the essential oil from the secretory cavities of the palisade parenchyma.

However, in other species, especially those from the Lamiaceae family, which mainly accumulate essential oil in glandular trichomes, an increase in drying temperature may reduce the essential oil yield, as observed in *Thymus vulgaris* (Sározi *et al.*, 2013), *Melissa officinalis* (Argyropoulos and Müller, 2014) and *Hysosopus officinalis* (Venditti *et al.*, 2015). In these species, an increase in temperature during the drying process drastically reduces the yield of essential oils because of the high volatilization (Abdelmageed *et al.*, 2011).

The volatilization process can also decrease the essential oil yield when subjected to prolonged drying times (Sellami *et al.*, 2011; Rahimmalek and Goli, 2013). In this experiment, drying over 48 h at room temperature or 24 h at 45°C reduced the essential oil yield at similar levels from those obtained with fresh leaves. According to Argyropoulos and Müller (2014), the decrease in the essential oil yield may be associated with moisture losses in plant material during the drying process. Prolonged drying times in species such as *Eugenia uniflora* have a high moisture loss, with a consequent increase in the rupture of the storage structures and volatilization reducing essential oil yield.

Essential oil composition

The essential oil samples of leaves dried at room temperature and 45°C had 12 compounds that were identified, with averages of 60 and 67.1% identification, respectively. Sesquiterpenes were the most representative compounds in the analyzed samples (Tab. 2), which agrees with other studies carried out on the species (Gallucci *et al.*, 2010; Rodrigues *et al.*, 2013). According to Stefanello *et al.* (2011), this class of compounds is responsible for the biological action of essential oils from species of the Myrtaceae family.

The major constituents found in the essential oil of the Surinam cherry leaves were viridiflorene+curzerene (15.5% at 19°C and 14.6% at 45°C), globulol (7.7% at 19°C and 8.8% at 45°C) and α -cadinol (7.4% at 19°C and 8.6% at 45°C) (Tab. 2). According to Pripdeevech and Chukeatirote (2010), these constituents have antifungal and antioxidant properties.

Table 2. Average essential oil composition (%) of Surinam cherry leaves with different drying temperatures.

Compound*	Rle	Rlt	Drying temperature	
			Ambient (22°C)	45°C
(E)- β -Ocimene	1052	1044	-	2.1
γ -Elemene	1433	1434	1.4	1.8
Allo-Aromadendrene	1460	1458	1.1	1.3
Germacrene D	1481	1484	3.7	4.9
Viridiflorene + Curzerene	1498	1496/ 1499	15.5	14.6
Germacrene B	1557	1559	3.9	5.9
Palustrol + ni	1569	1567	2.4	2.1
Globulol	1588	1590	7.7	8.8
Viridiflorol + Cubeban-11-ol	1595	1592/ 1595	5.8	5.0
Epi- α -Muurolol	1647	1640	5.3	7.5
α -Cadinol	1661	1652	7.4	8.6
Germacrone + ni	1694	1693	5.8	4.5
Monoterpene hydrocarbons			-	2.1
Sesquiterpene hydrocarbons			10.1	13.9
Oxygenated sesquiterpenes			49.9	51.1
Total identified			60.0	67.1

ni = not identified; Rle = retention index calculated from the compounds retention times with that of a homologous n-alkane series using a DB-5 column; Rlt = retention index Adams (2017); * Identified compounds with an average content above 1%.

The characteristic scent of *Eugenia uniflora* essential oil used in the cosmetics industry is associated with nine compounds (globulol, α -cadinol, viridiflorol, espatulenol, β -elemene, γ -elemene, germacrone, furanodiene+curzerene, atractylone). The presence of furanodiene+curzerene, γ -elemene and germacrone provides a spicy and woody aroma similar to the fruit, as verified through an olfactory comparative analysis (Melo *et al.*, 2007).

The analysis of the Surinam cherry essential oil composition in this study indicated that the genetic material presented six olfactory components (curzerene, globulol, α -cadinol, germacrene, viridiflorol and γ -elemene), which contributed to the characteristic scent of Surinam cherry, as reported by Melo *et al.* (2007). According to the Brazilian Pharmacopeia (ANVISA, 2020), curzerene is one the main Surinam cherry essential oil components, which, in this study, co-eluted with viridiflorene under the chromatographic conditions.

In *Eugenia uniflora*, the curzerene levels may be associated with the genetic diversity and wide geographic distribution. Costa *et al.* (2010) correlated the

differences in essential oil composition of Surinam cherry leaves with the fruit color and identified three chemotypes with broad variation in the curzerene levels. According to the geographic location of *Eugenia uniflora*, curzerene levels of 19.7% (Ogunwande *et al.*, 2015), 22.4% (Santos *et al.*, 2015) and 47.3% (Rodrigues *et al.*, 2013) have been found in the essential oil of its leaves.

The results (Tab. 2) demonstrated that drying leaves at 45°C increased the monoterpene and sesquiterpene compounds in the Surinam cherry essential oil. A total of 67% of the identified compounds had an increase in the concentrations, with an increase of 11.83% in sesquiterpenes. Similarly, Corrêa *et al.* (2006) dried different medicinal and aromatic plants and concluded that the ideal drying temperature was determined by the volatile properties of the essential oil of the plant material.

Changes in the essential oil composition of Surinam cherry were also reported by Sellami *et al.* (2011) in results from drying and temperature conditions and the consequent oxidative process and chemical rearrangements. In the present study, the decrease and/

or increase of certain compounds between different drying temperatures (ambient and 45°C) was related to volatilization ratio and/or chemical changes.

Storage experiment

Essential oil yield

The results showed that the essential oil yield was not significantly affected by the material type in the storage packages for the *Eugenia uniflora* leaves. On the other hand, there was a significant effect from the storage period on the essential oil yield of the dried Surinam cherry leaves (Tab. 3 and Fig. 1). The reduction in the essential oil yield was 32% after 60 d of storage, 41% after 120 d and 76% after 180 d, as compared to the yield of fresh leaves (Tab. 3).

Table 3. Relationship between average essential oil yield from dried Surinam cherry leaves during storage and from fresh (53.0% moisture) and dried (11.0% moisture) leaves without storage.

Storage (days)	Relationship between EO yield of fresh and dried stored leaves	Relationship between EO yield of 11.0% moisture dried leaves and stored dried leaves
0	1.0 a	-
60	0.7 b	0.6 a
120	0.6 b	0.5 a
180	0.2 c	0.2 b
CV (%)	23.8	31.6

CV (%) = coefficient of variation; EO = essential oil. Means with the same lowercase letters in columns are not significantly different according to Tukey's test ($P < 0.05$).

A reduction in essential oil yield was also observed by other authors, which agree with the data in this study, comparing the effect of storage time of the plant material. Verma *et al.* (2011) found a reduction of up to 59% in the yield of essential oil extracted from *Rosmarinus officinalis* after 270 d. According to Dušková *et al.* (2016), the increase in the storage period of *Lavandula angustifolia* flowers also decreased the essential oil yield when compared to flowers before storage. The reduction in essential oil yield during storage of plant material could be related to the high volatility of the essential oil components (Zuzarte and Salgueiro, 2015), which have an intrinsic characteristic. The loss in essential oil yield during storage could also be a result of the quality of the plant

material. Drying plant material affects the integrity of the structures where the essential oils of *Eugenia uniflora* leaves are stored because it increases the volatilization of the essential oil components.

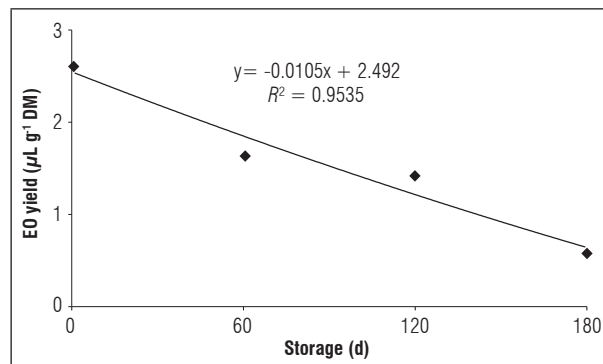


Figure 1. Essential oil (EO) yield of dried Surinam cherry leaves (11.0% moisture) during storage. Storage conditions: relative humidity 59.0-72.0%, temperature 19-29°C; DM = dried mass.

CONCLUSION

Drying the Surinam cherry (*Eugenia uniflora*) leaves increased the essential oil yield after 24 and 48 h at 45°C and at ambient temperature. The levels of monoterpenes and sesquiterpenes also increased when the leaves were dried at 45°C. The major compounds in the essential oil of the evaluated genetic material were curzerene and viridiflorene, which co-eluted under the chromatographic conditions used in this study. The essential oil yield of the Surinam cherry leaves was not influenced by the packaging type during storage. However, storage reduced the essential oil yield of the dried *Eugenia uniflora* leaves.

ACKNOWLEDGMENTS

This research was supported by funding from the Agronomy Graduate Program of the University of Parana. The authors are grateful for the funding provided by CAPES – Brazilian Agency Foundation for Research, Brazil – who sponsored the senior author's graduate studies.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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