Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
16(1): 24-30, Jan./Mar. 2006
Received 03/31/05. Accepted 01/23/06

Artigo

Influence of the harvesting time, temperature and drying period on basil (*Ocimum basilicum* L.) essential oil

José Luiz S. Carvalho Filho[1], Arie F. Blank[1*], Péricles B. Alves[2], Polyana A.D. Ehlert[1], Alberto S. Melo[1], Sócrates C.H. Cavalcanti[3], Maria de Fátima Arrigoni-Blank[1], Renata Silva-Mann[1]

[1]Departamento de Engenharia Agronômica, Universidade Federal de Sergipe, Av. Marechal Rondon s/n, Jardim Rosa Elze, 49100-000, São Cristóvão, Sergipe, Brasil,
[2]Departamento de Química, Universidade Federal de Sergipe, Av. Marechal Rondon s/n, Jardim Rosa Elze, 49100-000, São Cristóvão, Sergipe, Brasil,
[3]Departamento de Fisiologia, Universidade Federal de Sergipe, Av. Marechal Rondon s/n, Jardim Rosa Elze, 49100-000, São Cristóvão, Sergipe, Brasil

RESUMO: "**Influência do horário de colheita, temperatura e tempo de secagem no óleo essencial de manjericão (*Ocimum basilicum* L.)"**. Óleo essencial de *Ocimum basilicum* L. com alta concentração de linalol é valorizado no mercado internacional e amplamente usado na indústria de condimentos e cosméticos. Para garantir excelente qualidade e rendimento de óleo essencial é crucial a determinação do efeito dos fatores ambientais e de processamento na sua composição. O objetivo do presente trabalho foi avaliar o efeito do horário de colheita, da temperatura e do tempo de secagem no teor e na composição química do óleo essencial de *O. basilicum*. Colheitas foram realizadas aos 40 e 93 dias após transplantio das mudas. Colheitas realizadas às 8:00 h e 12:00 h proporcionaram os maiores rendimentos de óleo essencial. Ao quinto dia de secagem o teor de linalol no óleo essencial subiu de 45,18% para 86,80%. *O. basilicum* deve ser colhido pela manhã e a biomassa deve ser seca a 40 ºC por um período de cinco dias para obter óleo essencial rico em linalol.

Unitermos: *Ocimum basilicum*, manjericão, colheita, pós-colheita, óleo essencial, linalol.

ABSTRACT: *Ocimum basilicum* L. essential oil with high concentration of linalool is valuable in international business. *O. basilicum* essential oil is widely used as seasoning and in cosmetic industry. To assure proper essential oil yield and quality, it is crucial to determine which environmental and processing factors are affecting its composition. The goal of our work is to evaluate the effects of harvesting time, temperature, and drying period on the yield and chemical composition of *O. basilicum* essential oil. Harvestings were performed 40 and 93 days after seedling transplantation. Harvesting performed at 8:00 h and 12:00 h provided higher essential oil yield. After five days drying, the concentration of linalool raised from 45.18% to 86.80%. *O. basilicum* should be harvested during morning and the biomass dried at 40ºC for five days to obtain linalool rich essential oil.

Keywords: *Ocimum basilicum*, basil, harvest, post-harvesting, essential oil, linalool.

INTRODUCTION

Basil (*Ocimum basilicum* L., Lamiaceae) is an annual or perennial plant from southeast Asia and central Africa introduced in Brazil by the Italians. This plant is widely used for its therapeutic properties, as well as, for aromatic and culinary purposes (Morales; Simon, 1997; Bara; Vanetti, 1997/1998). The essential oil may be used directly in food or at the cosmetics industry for the production of shampoos, soaps, and perfumes. It is also used on folk medicine to relieve respiratory problems, as antiseptic, digestive (Lorenzi; Matos, 2002), and against intestinal parasites.

O. basilicum essential oil is widely used in the cosmetic industry, becoming its principal raw material. According to Lawrence (1993), 43 tons of *O. basilicum* essential oil was produced worldwide in 1992, which is equivalent to 2.8 million dollars.

Harvesting and post-harvesting steps of medicinal and aromatic plants are essential to obtain higher essential oil content and better quality. The main factors to be accounted on harvesting aromatic plants are

* E-mail: afblank@ufs.br, Tel. + 55-79-32126929

ISSN 0102-695X

the harvesting time, drying temperature, and period of drying.

The oil composition is altered during harvesting and post-harvesting processes. This alteration occurs due to spontaneous conversions occurring continuously, which changes the essential oil composition. Based on these facts, commercialization of such unstable material turns into a dilemma, since the oil should have a pre-established composition as a market demand. The essential oil of *O. basilicum* is a case of spontaneous conversions. Because of its instability in composition, the market valorizes *Ocimum basilicum* from Europe (Simon, 1990), which is rich in linalool (40.5 to 48.2%) and methyl-chavicol (estragol, 28.9 to 31.6%) (Fleisher, 1981; Charles; Simon, 1990).

In view of these facts it was of our interest to evaluate the influence of harvesting time, drying temperature, and drying period on content and chemical composition of the essential oil of basil (*Ocimum basilicum* L.) cultivar *Fino Verde*.

MATERIAL AND METHODS

Our study was separated in two experiments performed in our research station *Campus Rural* of The Federal University of Sergipe (UFS), São Cristóvão city, Sergipe State, from December 03, 2002 to April 28, 2003. The drying and extraction operations were performed in the Plant Technology Laboratory of the Agronomy Department of The Federal University of Sergipe.

Cultivation

Seedlings were produced from commercial seeds of *O. basilicum* cultivar *Fino Verde* (obtained from Johnny's Selected Seeds Company) in expanded polystyrene seedbeds with 72 cells (120 cm^3/cell). Those were seeded at an approximate profundity of 0.3 cm from the substrate surface. As substrate we used a mixture of washed coconut dust, bovine manure, and carbonized rice husk (1:1:1).

After 30 days, seedlings were transplanted to a plant bed, spaced by 30 cm from each other and 25 cm between lines. The cultivation was performed in a greenhouse, using black mulch to cover plant beds. To each plant bed (1.2 m width by 9.6 m length by 0.2 m height) we added 60 m^3/ha of bovine manure homogeneously mixed to soil. We also used a drip irrigation system with 10 cm spacing between each drip emitter.

First harvesting

The first harvesting (Experiment 1) was performed 40 days after seedlings transplantation during full bloom on 03/06/2003. Harvesting was performed cutting plants at 20 cm height from the soil. The collected material was transported to our laboratory and processed, which consisted on separating leaves and inflorescences from the stalk. In the first experiment we only used leaves in the analysis.

Randomized block design in a 3x4 factorial scheme with three replications was used. Each plot was composed of five plants. Treatments were: three harvesting periods (8:00; 12:00, and 16:00 h) combined with three drying temperatures (40, 50, and 60 ºC) and fresh leaves. The analyzed variables were: content, yield, and concentrations of compounds in essential oil of *Ocimum basilicum* cultivar *Fino Verde*.

Drying occurred in ovens with air renewal and circulation (Marconi model MA-037/5) until constant weight.

Samples of 100 g for fresh leaves and 50 g for fresh inflorescences were used in each treatment. To calculate the content of essential oil in fresh samples, the theoretical dry weight was obtained by the determination of humidity. In order to obtain a theoretical yield of essential oil from fresh leaves we multiply the essential oil content with the estimated dry matter production per ha. Content, yield and chemical composition of essential oils were determined.

Second harvesting

To perform the second harvesting (Experiment 2) we collected the regrowth of plants used in Experiment 1. Plants were harvested fifty three days after the first harvesting (on 04/28/2003) at 8:00 h using the same procedures as the first one; however both leaves and

Table 1. Average yield (L/ha) of essential oil from *Ocimum basilicum* cultivar *Fino Verde*, as a function of three harvesting times, drying temperatures, as well as, fresh leaves.

Drying temperature	Harvesting time		
	8:00 h	12:00 h	16:00 h
	Essential oil yield (L/ha)		
Fresh leaves	12.02^{a}	10.03^{a}	10.92^{a}
Dry leaves (40ºC)	9.55^{ab}	7.21^{ab}	6.63^{b}
Dry leaves (50ºC)	9.91^{ab}	8.70^{ab}	6.89^{ab}
Dry leaves (60ºC)	7.41^{b}	5.50^{b}	5.32^{b}

Tukey test ($p<0,05$); column values with the same letter are statistically equal.

Table 2. Composition of essential oil from leaves of *Ocimum basilicum* cultivar *Fino Verde* (CG-MS analysis - Experiment 1). Relative percentages as a function of drying temperatures and harvesting times.

Compounds	Fresh leaves			Dry leaves (40ºC)			Dry leaves (50ºC)			Dry leaves (60ºC)		
	8:00 h	12:00 h	16:00 h	8:00 h	12:00 h	16:00 h	8:00 h	12:00 h	16:00 h	8:00 h	12:00 h	16:00 h
	Relative percentage of chemical constituents											
Myrcene	0.73	--	--	0.87	--	--	--	--	--	--	--	--
1,8 -Cineol	6.50	1.84	--	4.03	4.23	3.55	4.97	2.09	5.73	3.47	3.38	2.14
β-(Z)-Ocimene	2.58	2.78	1.95	1.18	0.73	--	--	--	--	--	--	--
Linalool	49.72	43.00	45.18	64.32	60.09	69.33	62.72	61.74	60.51	63.11	59.63	45.28
Camphor	--	--	--	1.00	0.97	--	--	0.74	0.74	1.08	1.24	--
Borneol	--	--	--	--	0.73	--	--	0.72	0.74	0.94	0.91	1.29
Terpin-4-ol	2.09	1.01	1.78	2.35	5.35	1.17	2.78	4.71	2.17	5.01	2.48	2.97
α-Terpineol	0.96	--	--	--	0.95	--	1.10	0.67	0.95	0.92	0.89	--
Geraniol	0.85	1.37	1.29	1.29	2.12	1.31	1.48	1.78	1.61	2.22	2.35	1.99
Bornyl Acetate	--	--	--	--	--	--	--	--	--	0.74	0.63	1.30
Eugenol	29.41	39.44	41.20	14.41	13.75	10.85	13.98	14.24	15.06	9.88	10.35	9.49
β-Elemene	--	--	--	--	--	--	--	--	--	--	0.88	1.38
α–*trans*-Bergamotene	2.59	4.33	3.88	4.80	4.70	7.75	6.33	6.31	6.36	6.66	6.59	11.16
Germacrene-D	0.79	1.02	1.11	--	--	--	1.08	0.78	--	--	--	--
α- Bulnesene	--	0.81	--	0.83	0.78	1.07	--	--	0.85	--	1.07	1.99
γ-Cadinene	0.74	1.00	--	1.33	1.35	1.58	1.47	1.49	1.44	1.51	1.80	2.92
Geranyl Acetate	--	--	--	--	--	--	--	0.70	--	--	--	--
Spathulenol	--	--	--	--	--	--	--	--	--	--	0.88	1.74
Cubenol	--	--	--	--	--	--	--	--	--	--	0.89	1.63
α-Cadinol	3.03	3.40	3.61	3.61	3.38	4.01	4.09	4.04	3.85	4.45	6.02	13.06
Phytol	--	--	--	--	--	--	--	--	--	--	--	1.68

inflorescences were used in the analysis.

Randomized block design with three replications was used. Treatments were drying periods of 0, 1, 2, 3, 4, 5, 6, 7, 9, 11, 13, and 16 days for leaves and inflorescences in ovens with air renewal and circulation (Marconi model MA-037/5) at 40ºC. After harvesting we selected twelve 100 g samples from each block of fresh leaves. Since the amount of inflorescence was not enough to select larger samples we selected twelve 50 g samples from each block of inflorescence. These sampling composed the treatments described above. Content, yield and chemical composition of essential oils were determined.

Extraction of essential oils

The essential oil was extracted using hydrodistillation in a Clevenger apparatus. Each sample was placed in a 3 liter round bottomed flask containing 1.5 L of water and refluxed for 3 h. After completion, the oil volume (mL) was recorded, collected in amber flasks, and stored in freezer at -20ºC until further analysis. The essential oil of the three replications of each treatment were collected into the same flask for GC/MS analysis.

Humidity

Humidity of each treatment was obtained from three samples (100 g for leaves and 50 g for inflorescences) dried at 105ºC until constant weight.

Analysis of the essential oil by GC/MS

Essential oils were analyzed by GC/MS using a Shimadzu QP5050A equipped with a DB-5MS fused silica column (30m x 0.25 mm; film thickness 0.25 μm), in the following conditions: helium as carrier gas at 1.0 mL/min; injector split at 250ºC (split ratio 1/20); detector at 280ºC, column temperature program was 80ºC for 1.5 min, followed by 4ºC/min to 180ºC, them 10ºC/min to 300ºC, ending with a 10 min isothermal at 300ºC. The mass spectra were taken at 70 eV with scanning speed of 0.85 scan/s from 40 to 550 Da. The identification of the constituents was assigned on basis of comparison of their retention indices relative *n*-alkane homologous series obtained by co-injecting the oil sample with a linear hydrocarbon mixture as well as by computerized matching of the acquired mass spectra with those stored in NIST21 and NIST107 mass spectral libraries of the GC/MS data system and other published mass spectra (Adams, 1995).

Statistical analysis

Results were analyzed by ANOVA and comparison of means by the Tukey test ($p<0.05$). We applied regression analysis to establish equations for the drying time.

RESULTS AND DISCUSSION

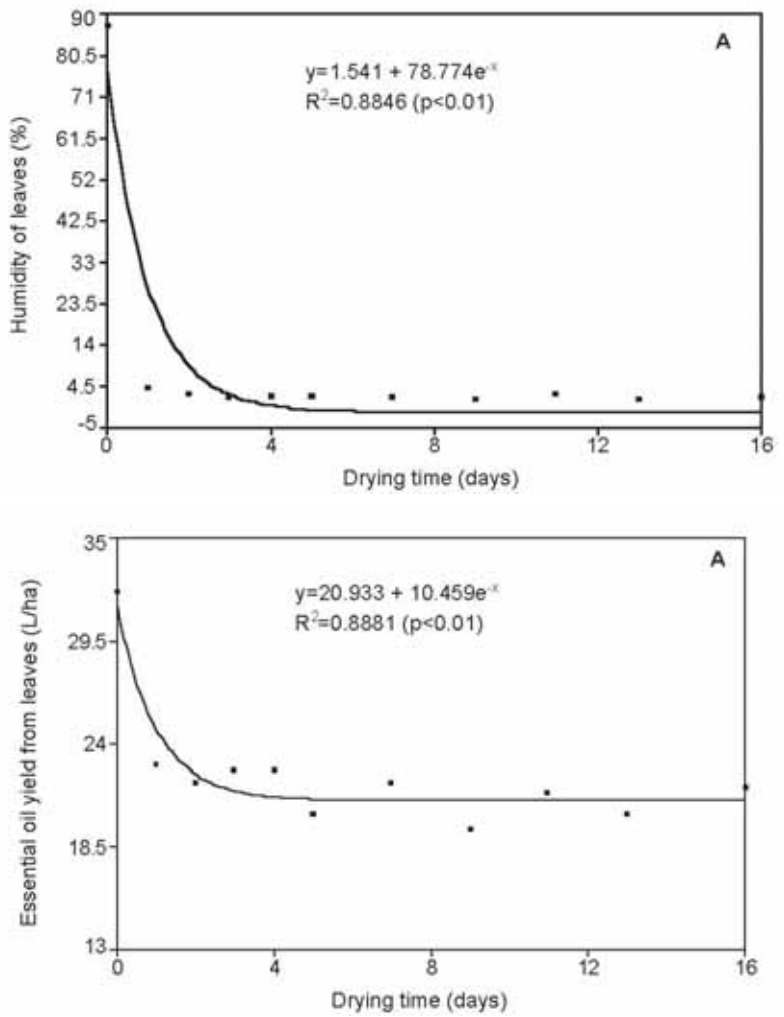

Figure 1. Humidity (%) of leaves (A) and inflorescences (B) of *Ocimum basilicum* cultivar *Fino Verde*, as a function of the drying time.

Drying temperature and harvesting time

The best average on yield of the essential oil was obtained by fresh leaves harvested at 8:00 h (12.02 L/ha). Drying leaves at 40 and 50ºC caused less loss of essential oil content than drying at 60ºC; major essential oil contents of dried leaves were obtained by harvesting at 8:00 h (Table 1).

Linalool and eugenol are the major compounds in leaves (Table 2). The concentration of linalool (major compound) in the essential oil changed according to treatments (Table 2). An increase on linalool concentration was observed during the drying process. *O. basilicum* harvested at 16:00 h and dried at 40ºC showed the highest linalool concentration (69.33%), however for fresh leaves and leaves dried at 50 and 60ºC, the highest concentration of linalool was always obtained by harvesting at 8:00 h. In *Lippia alba* citral-limonene chemotype highest citral and limonene content also was observed when harvesting was realized in the afternoon (15:00 h) (Nagao et al., 2004).

Similar to linalool, concentrations of geraniol in fresh leaves were lower than in dried ones; however the concentrations of geraniol were directly proportional to the drying temperature.

Eugenol was the second major compound in the essential oil composition. Higher concentrations of eugenol in both leaves and inflorescences were obtained from fresh biomass (Table 2). This compound decreased its concentration from 41.20% (fresh leaves) to 9.49% (leaves dried at 60ºC) during the drying process (harvesting performed at 16:00 h, Table 2).

Drying period

The regression curve for leaves and inflorescences humidity as a drying period function had an exponential behavior (Figure 1). It was also found, after the first drying day, that nearly all humidity in leaves and inflorescences had been removed. An exponential behavior was also found for yield of the essential oil from leaves and inflorescences, as a function of the drying time

Table 3. Composition of essential oil from leaves of *Ocimum basilicum* cultivar *Fino Verde* (CG-MS analysis - Experiment 2). Relative percentages as a function of drying times.

Compounds	Drying time (days)										
	0	1	2	3	4	5	7	9	11	13	16
Myrcene	--	0.82	0.67	0.57	--	--	0.68	--	--	--	0.59
1,8 -Cineol	--	7.24	3.40	3.58	5.08	7.62	5.82	4.19	6.18	4.62	4.69
β-(Z)–Ocimene	1.95	1.59	1.10	0.78	--	--	--	--	--	--	--
Linalool	45.18	65.60	68.86	59.61	70.93	86.80	72.27	67.18	73.59	74.93	68.89
Camphor	--	0.72	0.76	0.69	--	--	0.80	0.80	0.81	--	0.77
Borneol	--	--	--	0.46	--	--	--	--	--	--	--
Terpin-4-ol	1.78	2.79	2.78	3.29	3.02	1.50	3.58	3.23	3.13	2.19	2.87
α-Terpineol	--	0.97	--	0.77	0.88	--	0.80	0.79	0.84	--	0.76
Octyl acetate	--	--	--	--	--	--	--	--	--	--	0.56
Geraniol	1.29	0.91	0.94	1.67	1.19	--	0.95	1.37	1.01	--	1.42
Bornyl Acetate	--	1.01	1.00	0.96	1.00	--	1.05	1.11	0.98	--	1.02
Eugenol	41.20	8.23	8.01	11.78	7.71	--	4.46	8.74	4.29	7.56	6.62
Geranyl Acetate	--	--	--	0.72	--		--	--	--	--	0.61
β-Elemene	--	--	--	0.46	--	--	--	--	--	--	--
α–*trans*-Bergamotene	3.88	4.44	5.03	4.97	5.03	4.07	4.73	5.44	4.62	5.92	4.79
Germacrene-D	1.11	--	--	--	--	--	--	--	--	--	--
α- Bulnesene	--	0.74	0.93	0.90	--	--	--	0.83	--	--	0.64
γ-Cadinene	--	1.11	1.32	1.36	1.14	--	1.14	1.45	1.11	1.23	1.19
Eugenol acetate	--	--	0.67	0.85	--	--	--	--	--	--	0.61
1-Epi-Cubenol	--	--		0.61	--	--	--	--	--	--	--
α-Muurolol	--	3.84	4.52	5.01	4.02	--	3.73	4.88	3.45	3.55	3.96
α-Cadinol	3.61	--	--	--	--	--	--	--	--	--	--

Table 4. Composition of essential oil from inflorescences of *Ocimum basilicum* cultivar *Fino Verde* (CG-MS analysis - Experiment 2). Relative percentages as a function of drying times.

Compounds	Drying time (days)										
	0	1	2	3	4	5	7	9	11	13	16
1,8-cineol	1.05	--	1.44	1.67	1.72	1.03	1.58	1.41	1.45	1.48	2.06
β-(Z)-Ocimene	1.31	--		0.62	0.55	--	--	--	--	--	--
Linalool	80.68	75.63	78.40	82.67	89.58	90.25	82.80	81.73	92.62	86.20	84.63
Terpin-4-ol	--	--	2.13	2.46	1.46	1.44	0.92	1.27	1.19	1.33	1.02
Geraniol	1.80	1.93	2.91	2.02	1.02	0.90	2.40	2.09	1.20	1.60	1.63
Bornyl Acetate	--	--	--	0.53	--	--	--	--	--	0.60	--
Eugenol	9.31	3.76	5.72	3.51	1.24	1.76	2.29	2.47	--	0.85	0.87
β-Elemene	1.52	2.11	--	0.70	0.67	0.56	0.80	1.16	--	0.72	0.85
α-*trans*–Bergamotene	--	1.18	1.31	0.87	0.90	0.98	1.20	1.79	--	0.98	1.19
α-Guaiene	--	1.28	--	--	--	--	--	--	--	--	0.62
Germacrene-D	1.43	2.63	1.77	0.82	0.61	0.74	1.33	1.38	0.82	--	0.90
Bicyclogermacrene	--	1.07	--	--	--	--	--	--	--	--	--
α-Bulnesene	1.85	3.51	1.96	1.27	1.02	1.04	1.51	1.67	1.10	1.44	1.49
γ-Cadinene	--	1.47	--	0.58	--	--	0.99	1.21	--	0.88	1.06
α-Muurolol	1.04	5.41	4.37	2.27	1.22	1.31	3.97	3.83	1.63	3.03	--

(Figure 3).

The essential oil content and yield were higher when leaves and inflorescences were fresh; furthermore, there was a decrease on content and yield after two days drying. After four days drying leaves (eighth day for inflorescences) there was a tendency for stabilization of the essential oil yield (Figure 2).

The relative composition of the essential oil from leaves and inflorescences were different during the drying process. The furthermost linalool concentration (major compound) from leaves essential oil was obtained after five days drying (86.80%); the concentration of linalool in essential oil distilled from fresh leaves was 45.18% (Table 3). In a similar way, the furthermost linalool concentration from inflorescences essential oil was obtained after eleven days drying (92.62%, Table 4). The lower number of compounds from leaves was obtained after five days drying.

The absence of camphor in inflorescences essential oil composition was an interesting finding, since this compound is important for *O. basilicum* aromatic classification. Camphor was only found in leaves essential oil.

Results from experiment 1 are probably associated with volatilization of the essential oil. Similar results were obtained for *Mentha piperita* L., which a

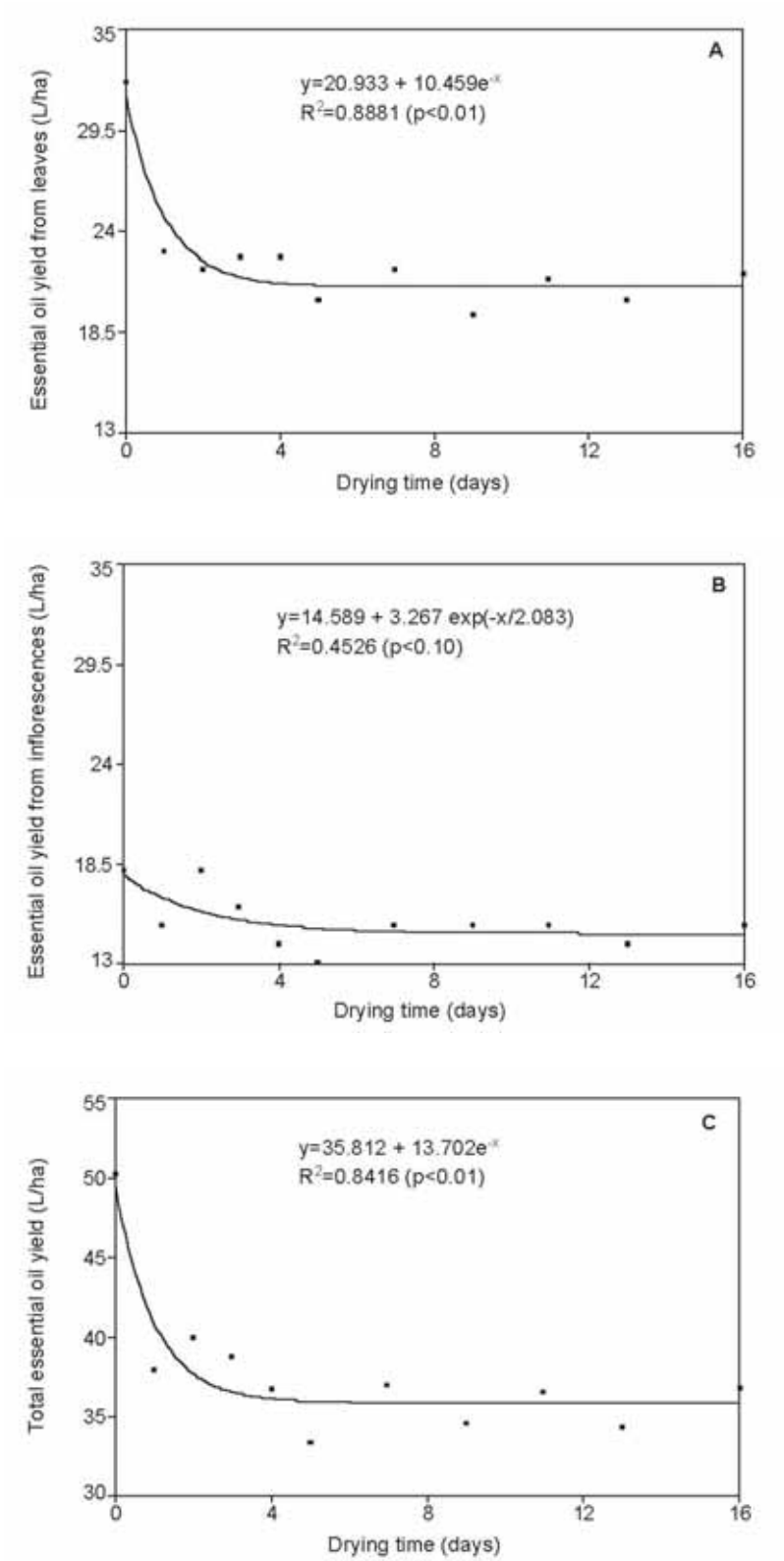

Figure 2. Essential oil yields (L/ha) from leaves (A), inflorescences (B), and total (C) of *Ocimum basilicum* cultivar *Fino Verde*, as a function of the drying time.

decrease on essential oil contents as the temperature increased was observed, with loss up to 80% when leaves were dried at 60ºC (Pinheiro et al., 2002). Similarly, fresh *Lychnophora pinaster* leaves had higher yields (0.71%) than dried ones (0.04%, 35ºC) (Blanco et al., 2000a). Conte et al. (2001) also obtained *Ocimum gratissimum* L. essential oil, by way of several treatments, such as, drying leaves in the air, frozen leaves, leaves triturated in a mill, fresh leaves, and dried in oven at 40ºC. Similarly, reduction on content was also observed after drying leaves.

Furthermore, alteration on chemical composition of essential oils may be related to the connection between variations in temperature with plants metabolic activity. A study performed with *Mentha piperita* L. showed variation on composition by varying drying temperatures (40ºC, 60ºC and 80ºC); menthol had higher concentrations after drying at 60 and 80ºC (Blanco et al., 2000b).

Blanco et al. (2000a), performed a study with *Rosmarinus officinalis* L., which showed that the essential oil composition did not change after drying at 40 and 60ºC. On the other side, our study showed significant changes in the essential oil composition at the same drying temperatures used in our work. In their work camphor concentration decreased considerably at 80ºC. In our work camphor concentration was higher at 60ºC compared to 40ºC.

Results from experiment 2 suggest that loss of essential oil at the beginning of drying may be limited to places of easy removal. After removal of this fraction, stabilization on amounts of essential oil occurs. Stabilization after the fourth drying day supports the existence of places of difficult removal, where essential oil is located, being only removed by hydrodistillation. The same tendency of essential oil constituents volatility during drying processes of plants also were observed in *Mentha piperita* L. and *Ocimum gratissimum* L. (Blanco et al., 2000b; Conte et al., 2001). Conflicting results were found for *Lippia alba* chemotype III limonene-carvone showing higher essential oil content at the fourth drying day (Santos; Innecco, 2003).

The influence of the drying period at the essential oil composition was also evaluated for *L. alba* chemotype III limonene-carvone, to which the highest concentrations of limonene and carvone (major compounds) during the rainy season was obtained at the eighth drying day (Santos; Innecco, 2003). During the dry season, the highest concentration of limonene was observed at the sixth drying day and the highest concentration of carvone was observed at the fourth drying day. The essential oil yield was high at the eighth drying day (Santos; Innecco, 2003).

All observed information entitle us to propose the execution of similar assays, testing the aromatic species in each cultivation environment.

AKNOWLEDGEMENTS

The authors wish to acknowledge ETENE/ FUNDECI/BNB and CNPq for supporting grants.

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