INFLUENCE OF DRYING AIR TEMPERATURE ON THE CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF MELALEUCA

Diana Rodrigues Herinque Lemos[1], Evandro de Castro Melo[2], Ronicely Pereira Rocha[3], Luiz Cláudio de Almeida Barbosa[4], Antonio Lelis Pinheiro[5]

ABSTRACT

This study was done to evaluate the influence of drying air temperature on the chemical composition of the essential oil of Melaleuca alternifólia Cheel. Three samples were taken at random from each treatment which were analyzed still fresh, determining the moisture content and chemical composition of the essential oil.The plants were chopped and placed in a fixed-bed dryer and dried with air temperature of 40, 50, 60, 70 and 80 °C. The identification of compounds was done using gas chromatography coupled to mass spectrometry. The major components of essential oil of melaleuca subjected to drying air temperatures of 40, 50, 60, 70 and 80 ° C were within the ISO Standard 4730, which classifies plant as medicinal or not.There was an increase in the levels of terpinen-4-ol and α-terpineol and decrease of α-pinene compared to the control.

Keywords: active substances, chromatography, medicinal plant, tea tree.

RESUMO

INFLUÊNCIA DA TEMPERATURA DO AR DE SECAGEM SOBRE A COMPOSIÇÃO QUÍMICA DO ÓLEO ESSENCIAL DE *MELALEUCA*

Este trabalho visou avaliar a influência da temperatura do ar de secagem sobre a composição química do óleo essencial de *Melaleuca alternifólia* Cheel. Foram tomadas aleatoriamente três amostras de cada tratamento que foram analisadas ainda frescas, avaliando o teor de água e os constituintes químicos. Para a realização dos testes de secagem foram utilizadas amostras da planta triturada, utilizando um secador de leito fixo. As temperaturas do ar de secagem foram 40, 50, 60, 70 e 80°C. A identificação dos compostos foi feita empregando-se cromatografia em fase gasosa acoplada à espectrometria de massas. Os componentes majoritários do óleo essencial de melaleuca submetidos às temperaturas do ar de secagem de 40, 50, 60, 70 e 80 °C atendem dentro Padrão ISO 4730, que classifica a planta como medicinal ou não. Foi observado também o aumento no teor de terpinen-4-ol e α-terpineol, e redução do teor de α-pinene comparado com a testemunha.

Palavras-chave: substâncias ativas, cromatografia, planta medicinal, árvore do chá.

Recebido para publicação em 11/06/2010. Aprovado em 25/10/2011.

1- Agricultural Engineer, Master student in Agricultural Engineering, UFV, Viçosa – Brazil.
2- Agricultural Engineer, Dr. Professor in Agricultural Engineering, UFV, Viçosa – Brazil. e-mail: evandro@ufv.br
3- Agronomist Engineer, Doctorate student in Agronomist Engineering, UFV, Viçosa - Brazil. e-mail: ronicely.rocha@ufv.br
4- Chemical engineer, PhD in Chemistry, UFV, Viçosa – Brazil.
5- Forestry Engineer, Dr. Professor in Forestry Engineering, UFV, Viçosa - Brazil.

INTRODUCTION

Melaleuca alternifolia Cheel, known as "tea tree", belong to the family Myrtaceae, is a small tree, up to 7 m height. It has a thin skin and leaves tapered, approximately 2 cm long. The leaves are small, sessile, alternate and are arranged spirally around the rachis and blooms in summer. It is native from the southeastern coast of Australia, New South Wales region, and grows in swampy areas or near rivers (VIEIRA, 2002). The essential oil of tea tree presents a complex mixture of 97 compounds and the main constituents are tepinen-4-ol, 1,8-cineol, α-terpinene, γ-terpinene, α-pinene, β-pinene, α-terpineol and p-cymene, representing approximately 90% of the essential oil (BROPHY et al., 1989).

The agronomic practices related to aromatic plant species are often managed in order to propitiate the abundant and homogenous vegetative material of the best final quality. The growing demand for medicinal species indicates the emergence of a market with high potential for consumption, requiring a consistent and readily available supply of high quality raw material. The post-harvesting process of medicinal plants has great importance in the production chain, because of its direct influence on the quality and quantity of the active principles in the product sold (SILVA & CASALI, 2000).

The yield and chemical composition of essential oils that originated from aromatic plants are related to a variety of internal and external factors, e.g., the drying process (RADÜNZ *et al.*, 2002; BRAGA *et al*., 2005; BORSATO et al., 2009; SZUMNY *et al*., 2010).

According to Lorenzi and Matos (2002), drying of medicinal species is a preparation process, carried out to meet the needs of the pharmaceutical industry. For this reason, adequate dryers are needed, using temperature, velocity and humidity values for drying air that provides a rapid reduction in the moisture content without affecting the quality of the active principles of medicinal plants (CALIXTO, 2000). The essential oils are the most sensitive constituents in the drying process of medicinal species. The limits of drying air temperature are determined according to the sensitivity of these chemicals substances and from their storage structures, because the product temperature is increased during the drying process (MARTINS, 2000; VENSKUTONIS, 1997).

This study aimed to evaluate the influence of the drying air temperature on the chemical composition of *Melaleuca alternifólia* essential oil.

MATERIAL AND METHODS

Fresh tea tree plants (*M. alternifolia* Cheel) were grown in São Miguel do Anta, Minas Gerais, Brazil. The plants were harvested at 9:00 am, because at this time there is the highest concentration of active principles (SILVA, 2001). After harvest the tea tree samples were transported to Agricultural Engineering Department at the Federal University of Viçosa for drying.

Three samples of 0.20 kg were taken randomly from each treatment which was analyzed fresh, evaluating the moisture content and the essential oil chemical composition. The moisture content of the samples was determined using the gravimetric method recommended by ASAE Standards (2000) for forage and similar plants. This was done by placing 25 g of the product in an oven with forced air circulation at 103 ± 2 °C for 24 h, each done in triplicate.

The drying treatments were arranged in randomized blocks with three repetitions. Five drying temperatures were evaluated: air heated at 40, 50, 60, 70 and 80 °C. For each temperature, 500 g from chopped plants (leaves and stems) were used, making a layer of 5 cm thick in the drying chamber. A fixed-bed dryer was used with upward air flow, equipped with a liquefied petroleum gas burner, used to heat the drying air (Figures 1 and 2), as described by Radünz (2004). The samples were drying until reached moisture content of 0.11 d.b.

The control of drying air temperature was performed with an automatic controller, with a variation of ± 2 °C, as described by Jesuz et al. (2001). Values of temperature and relative humidity of drying air was taken with thermocouples, previously calibrated and placed in pre-set points of the dryer and coupled to an automatic data acquisition system (ADAS) that registered their values in a microcomputer. The drying air velocity was tracked by an anemometer.

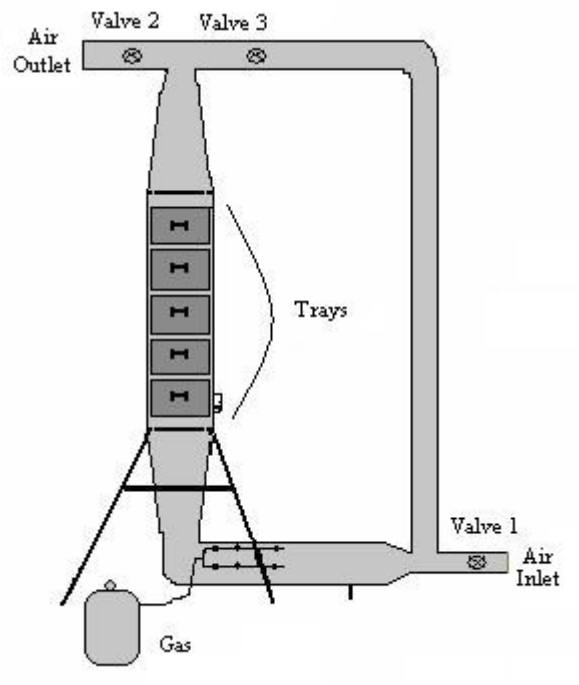

Figure 1. Dryer front view

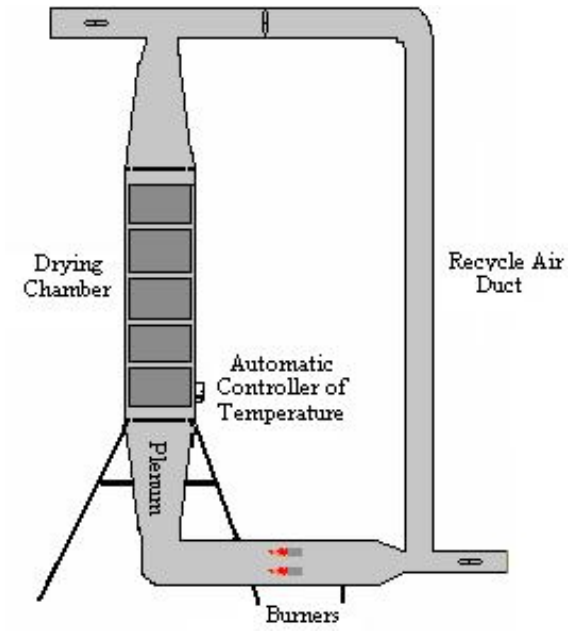

Figure 2. Dryer front cut

After drying the samples were brought to Chemistry Department at the Federal University of Viçosa for extraction and chromatography. Hydrodistillation with clevenger apparatus was used for the extraction of the volatile compounds of tea tree (fresh and dried). A suspension of 50 g of tea tree leaves was placed in a 2000 mL round flask together with 1000 mL of ultrapure distilled water. Sample flask was heated for 3 h, after the boiling point was reached (SILVA et al., 2002). After 3 h of extraction samples (mixture of water and oil) were collected. The oil was separated with pentane (3x30 mL) in a 500 mL separation funnel. The organic fraction (pentane and essential oil) obtained was transferred to a 125 mL Erlenmeyer flask and treated anhydrous magnesium sulfate (5 g). The mixture was filtered directly to a flask of 125 mL, concentrated in a rotary evaporator at 38 °C and the oil obtained was transferred into a 5 mL vial and kept at 4 °C until GC–FID analyses were performed. Analyses were run in triplicate.

The isolation, identification, and quantification of the volatile compounds were performed on a gas chromatograph associated to flames ionization detection (GC-FID). The equipment model used was the gas chromatographer Shimadzu GC-17A coupled to the flames ionization detector, using a Supelco ® brand column SPB-5, with 30 m in length, internal diameter of 0.25 mm, film intern of 0.25 μm thickness, and nitrogen as the carrier gas. The equipment operation conditions were: column internal pressure of 100 kPa, split ratio of 1:10, gas flow in the column of 1.74 mL min^{-1}, gas linear velocity of 37.72 cm s^{-1} and temperatures in the injector and detector of 220 °C to 240 °C, respectively. The column temperature was programmed to begin at 45 °C for 2 minutes and then increase 3 °C per minute until 240 °C was reached, giving a total operation time of 87 minutes. The sample volume injected was 1 μL at a 10.000 ppm concentration and using hexane as a solvent.

Most of the compounds were identified by using three different analytical methods: (1) Kovats indices (KI), (2) GC–MS retention indices (authentic chemicals), and (3) mass spectra (authentic chemicals and spectral library collection).

Results were analyzed using the SAEG, version 9.1 (SAEG, 2007). A completely randomized block design was used, with three replications of treatments, applying the simple regressions on a significance level of $P<0.05$, relating drying temperature with essential oil composition. The averages of essential oil composition, obtained from the dried and fresh leaves (control) of chopped tea tree were compared with test Dunnett, 5% probability.

RESULTS AND DISCUSSION

Table 1 shows the average values of moisture content of fresh plant and dried samples. Also reported in the table are the average of relative humidity (ambient and plenum) and total drying time for the drying tests of 40, 50, 60, 70 and 80°C.

The main essential oil components found after being submitted to drying air temperatures and compared with the ISO 4730 standard as described in Table 2.

Table 2 shows that the main essential oil components of tea tree submitted to drying air temperatures of 40, 50, 60, 70 and 80 °C were within the ISO 4730 Standard, in other words, the drying process did not affect the samples medical characteristics studied, keeping the essential oil quality compared to the fresh plant. The chemical composition of the tea tree essential oil containing high levels of terpinon-4-ol was also observed in others studies (HAUSEN et al., 1999; KIM et al., 2004; SILVA, 2007).

Table 3 shows the percentage of main chemical constituents of essential oil extracted from chopped leaves of tea tree, submitted to drying at 40, 50, 60, 70 and 80 °C.

Fresh plant (control) of tea tree presents α-pinene level on average 20% higher than the dried plant at temperatures of 40, 50, 70 and 80 °C ($P < 0.05$) (Table 3). However there was no significant difference between the fresh and dried plant at 60 °C. This decrease can be attributed to the oxidation of α-pinene during the drying process, converting it into p-cymene, which may also explain the increased of p-cymene level although no significant differences between the drying treatments when they were compared to fresh plant. The oxidation of terpenes (α-pinene, α-terpinene, γ-terpinene, terpinolene) to p-cymene was also observed by Hausen (1999) and Shabir, (2005).

Table 1. Parameters evaluated during the drying process of tea tree

Drying air temperature (°C)	Parameters evaluated				
	Moisture content fresh plant (d.b)	Moisture content dried plant (d.b)	Relative humidity ambient (%)	Relative humidity plenum (%)	Drying time (min)
40	1.04	0.11	59 ± 9.18	31 ± 8.37	135
50	1.04	0.12	60 ± 5.91	17 ± 7.68	80
60	1.04	0.12	66 ± 2.73	12 ± 11.57	55
70	1.04	0.11	62 ± 4.44	7 ± 1.47	42
80	1.04	0.11	64 ± 12.22	5 ± 1.12	30

Table 2. Main components of the essential oil found after being submitted to drying air temperatures and compared with the ISO 4730 standard

Composition	Composition (%)	
	Standard ISO 4730	Range found after drying (40 to 80 °C)
α-pinene	1 – 6	1.6 a 1.9
α-terpinene	5 – 13	5.8 a 8.0
p-cymene	0,5 – 12	3.0 a 6.2
1,8 -cineol	≤ 15[a]	3.1 a 3.2
γ-terpinene	10 – 28	15.6 a 18.6
terpinolene	1,5 – 5	2.6 a 3.0
terpinon-4-ol	≥ 30[b]	46.3 a 47.7
α-terpineol	1,5 – 8	3.4 a 3.5

[a] Limit minimum and [b] No upper limit is fixed

Table 3. Percentage of the main chemical constituents of essential oil extracted from tea tree leaves, submitted to different drying air temperatures

Treatments	Oil composition (%)							
	α -pinene	α -terpinene	p-cymene	1,8-cineol	γ -terpinene	terpinolene	terpinon-4-ol	α -terpineol
Control	2.13	8.63	2.87	4.27	19.68	3.17	43.31	3.12
40ºC	1.65*	5.82^{ns}	6.17^{ns}	3.23^{ns}	15.55^{ns}	2.63^{ns}	46.84*	3.48*
50ºC	1.76*	7.33^{ns}	3.69^{ns}	3.13^{ns}	17.53^{ns}	2.88^{ns}	47.72*	3.46*
60ºC	1.88^{ns}	8.04^{ns}	3.05^{ns}	3.23^{ns}	18.61^{ns}	3.00^{ns}	46.34*	3.40*
70ºC	1.75*	6.91^{ns}	4.55^{ns}	3.07^{ns}	16.98^{ns}	2.82^{ns}	46.92*	3.43*
80ºC	1.68*	6.45^{ns}	5.46^{ns}	3.25^{ns}	16.57^{ns}	2.79^{ns}	47.19*	3.46*
CV	7.86	16.51	41.03	4.63	9.99	7.51	1.79	1.86

^{ns} Non significant; *Significant compared to control (chopped fresh plant) by the Dunnet test at 5% probability.

No significant difference (P> 0.05) between fresh and dried plant at different temperatures for the constituents α–terpinene, *p*–cymene, 1.8–cineole, γ-terpinene and terpinolene, was observed. Fresh plant showed decrease of constituents terpinen-4-ol (7.87%) and α-terpineol (9,57%) compared to the dried plant at different temperatures (P <0.05). The increase of the constituents terpinen-4-ol and α-terpineol may be related to volatilization in higher quantities of the others constituents present in the plant. In drying studies of several medicinal species, carried out by other authors, were also observed changes in the essential oil composition (RADÜNZ et al. 2002; BLANK et al., 2005, SOARES et al., 2007, BORSATO et al., 2009; SZUMNY et al., 2010).

CONCLUSIONS

- It could be concluded that the major essential oil components of *Melaleuca alternifólia* were within ISO 4730 quality standards and that drying can be recommended with air heated up to 80 ºC; and
- The drying process caused a significant increase in the content of terpinen-4-ol and α-terpineol and significant decrease in α-pinene level compared to the fresh plant.

ACKNOWLEDGMENTS

The authors express their thanks to FAPEMIG, CNPq and CAPES for the financing support essential to conduct the project.

REFERENCES

ASAE Standards. **Standards Engineering Practices Data:** Moisture measurement-forages, ASAE S358.2 DEC99. Adopted and published by: American Society of Agricultural Engineers, 2000, p.565-572.

BLANK, A.F.; FONTES, S.M.; CARVALHO FILHO, J.L.S.; ALVES, P.B.; SILVA-MANN, R.; MENDONÇA, M.C.; ARRIGONI-BLANK, M.F.; RODRIGUES, M.O. Influência do horário de colheita e secagem de folhas no óleo essencial de melissa (*Melissa officinalis* L.) cultivada em dois ambientes. **Revista Brasileira de Plantas Medicinais**, v.8, n.1, p.73-78, 2005.

BORSATO, A.V.; DONI-FILHO, L.; RAKOCEVIC, M.; CÔCCO, L.C.; PAGLIA, E. C. Chamomile essential oils extracted from flower heads and recovered water during drying process. **Journal of Food Processing and Preservation**, v.33, p.500–512, 2009.

BRAGA, N.P., CREMASCO, M.A., AND VALLE, R.C.C.R. The effects of fixed-bed drying on the yield and composition of essential oil from long pepper (Piper hispidinervium c. dc) leaves. **Brazilian Journal of Chemical Engineer,** v. 22, n.2, p.257-262, 2005.

BROPHY, J.J., DAVIES, N.M., SOUTHWELL, I.A., STIFF, I.A. e WILLIAMS, L.R. Gas chromatography quality control for oil of

melaleuca terpin-4-ol type (Australian tea tree). **Journal Agricultural Food Chemistry**, v.37, n.5, p.1330-1335, 1989.

CALIXTO, J.B. Efficacy, safety, quality control, market and regulatory guidelines for herbal medicines (phytotherapeutic agents). **Brazilian Journal of Medical and Biological Research**, v.33, n.2, p.179-189, 2000.

HAUSEN, B.A.; REICHLING, J.; HARKENTHAL, M. Degradation products of monoterpenes are the sensitizing agents in tea tree Oil. **American Journal of Contact Dermatitis**, v.10, n.2, p.68-77, 1999.

JESUZ, J.C.; RADUNZ, L.L.; MELO, E.C.,; SOUZA, J.A.R.; ROCHA, R.P.; BERBERT, P.A. Sistema de Controle Automático da Temperatura do ar de Secagem em Secador de Plantas Medicinais. **Revista Brasileira de Produtos Agroindustriais**, v.3, n.1, p.43-46. 2001.

KIM, H.J.; CHEN, F.; WU, C. WANG, X. CHUNG, H.Y.; JIN, Z. Evaluation of antioxidant activity of Australian tea tree (*Melaleuca alternifolia*) oil and its components. **Journal of Agricultural and Food Chemistry.** v.2, p.2849-2854, 2004.

LORENZI, H.; MATOS, F.J.A. **Plantas medicinais no Brasil: nativas e exóticas.** Nova Odessa: Instituto Plantarum, 2002.

MARTINS, P.M. **Influência da temperatura e da velocidade do ar de secagem no teor e na composição química do óleo essencial de capim-limão** (Cymbopogon citratus (D.C.) Stapf). 2000. 77 f. Dissertação (Doutorado em Engenharia Agrícola) - Universidade Federal de Viçosa, Viçosa, 2000.

RADÜNZ, L.L.; MELO, E.C.; BERBERT, P.A.; BARBOSA, L.C.A.; ROCHA, R.P.; DE GRANDI, A.,M. Efeitos da temperatura do ar de secagem sobre a qualidade do óleo essencial de alecrim pimenta (*Lippia sidoides* Cham). **Revista Brasileira de Armazenamento**, v.27, n.2, p.09-13, 2002.

RADÜNZ, L.L. **Secagem de alecrim pimenta, guaco e hortelã comum sobre diferentes temperaturas e sua influência na quantidade e qualidade dos princípios ativos**. 2004. 90 f. Dissertação (Doutorado em Engenharia Agrícola) - Universidade Federal de Viçosa, Viçosa, 2004.

SAEG - **Sistema para Análises Estatísticas**, Versão 9.1: Fundação Arthur Bernardes - UFV - Viçosa, 2007.

SHABIR, G.A. Method development and validation for the GC-FID assay of p-cymene in tea tree oil formulation. **Journal of Pharmaceutical and Biomedical Analysis**. v.39, p. 681-684, 2005.

SILVA, C.J. **Morfoanatomia foliar e composição química dos óleos essenciais de sete espécies de Melaleuca L. (Myrtaceae).** 2007. 81 f. Dissertação (Mestrado em Botânica) - Universidade Federal de Viçosa, Viçosa, 2007.

SILVA, F.; CASALI, V.W.D. **Plantas Medicinais e aromáticas: Pós-Colheita e Óleos Essenciais.** Viçosa-MG: UFV, DFT, 2000. 135p.

SILVA, S.R.S. **Composição química, avaliação da atividade antimicrobiana do óleo essencial e deficiência de Melaleuca alternifolia Cheel crescida no Brasil.** 2001. 60 f. Dissertação (Mestrado em Agroquímica) - Universidade Federal de Viçosa, Viçosa, 2001.

SILVA, S.R S.; DEMUNER, A.J.; BARBOSA, L.C. DE A.; CASALI, V. W. D.; NASCIMENTO, E.A.; PINHEIRO, A.L. Efeito do estresse hídrico sobre característica de crescimento e a produção de óleo essencial de *Melaleuca alternifolia* Cheel. **Acta Scientiarum.** Maringá, v. 24, n. 5, p. 1363-1368, 2002.

SOARES, R.D.; CHAVES, M.A.; SILVA, A.A.L.; SILVA, M.V.; SOUZA, B.S. Influência

da temperatura e velocidade do ar na secagem de manjericão (*Ocimum basilicum* L.) com relação aos teores de óleos essenciais e de linalol. **Ciência e Agrotecnologia**, Lavras-MG. v.31, n.4, p. 1108-1113, 2007.

SZUMNY, A.; FIGIEL, A.; GUTIERREZ-ORTIZ, A.; CARBONELL-BARRACHINA, A. A. Composition of rosemary essential oil (*Rosmarinus officinalis*) as affected by drying method. **Journal of Food Engineering**, v.97, p.253–260, 2010.

VENSKUTONIS, P.R. Effect of drying on the volatile constituents of thyme (*Thymus vulgaris*) and sage (*Salvia officinalis* L.). **Food Chemistry**, v. 59, p.219-227, 1997.

VIEIRA, T. R. **Anatomia foliar de espécies do gênero Melaleuca L. e caracterização da composição química de Melaleuca alternifolia Cheel (Myrtaceae).** 2002. 122 f. Dissertação (Mestrado em Botânica) - Universidade Federal de Viçosa, Viçosa, 2002.