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Effect of drying temperature on the yield and phytochemical quality of the essential oil of mint (*Mentha* x *villosa* Huds.)

Efeito da temperatura de secagem no rendimento e na qualidade fitoquímica do óleo essencial de hortelã (*Mentha* x *villosa* Huds.)

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Maira Christina Marques Fonseca
Doutora em Fitotecnia pela Universidade Federal de Viçosa (UFV)
Pesquisadora - Empresa de Pesquisa Agropecuária de Minas Gerais
Vila Gianeti, casa 46. Campus da UFV, Viçosa, MG, Brasil. CEP: 36570-900
E-mail: mairacmf@yahoo.com.br

Cláudia Lúcia de Oliveira Pinto
Doutora em Microbiologia Agrícola pela Universidade Federal de Viçosa (UFV)
Pesquisadora aposentada - Empresa de Pesquisa Agropecuária de Minas Gerais
Rua dos Estudantes, 120, apto 202, Centro, Viçosa, MG, Brasil. CEP: 36570-081
E-mail: caudia.epamig@gmail.com

Maria Aparecida N. Sediyama
Doutora em Fitotecnia pela Universidade Federal de Viçosa (UFV)
Pesquisadora aposentada da Empresa de Pesquisa Agropecuária de Minas
Rua Vinícius de Moraes, 157, Bairro de Fátima, Viçosa, MG, Brasil. CEP: 36572-164
E-mail: mariasediyama@gmail.com

Adilson Sartoratto
Doutor em Química pela Universidade Estadual de Campinas (Unicamp)
Pesquisador do Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas da Unicamp
Rua Alexandre Cazelatto, 999 - Vila Betel, Paulínia, SP, Brasil. CEP: 13148-218
E-mail: adilson@cpqba.unicamp.br

Túlio I. Machado
Mestrando em Microbiologia pela Universidade Federal de Viçosa (UFV)
Av. Peter Henry Rolfs, s/n - Campus Universitário, Viçosa, MG, Brasil. CEP: 36570-900
E-mail: tulio.iglesias@ufv.br

Sérgio Maurício L. Donzeles
Doutor em Engenharia Agrícola pela Universidade Federal de Viçosa.
Pesquisador da Empresa de Pesquisa Agropecuária de Minas Gerais
Vila Gianetti, Casa 46, Campus da UFV, Viçosa, MG, Brasil. CEP: 36570-900
E-mail: slopes@epamig.br

Estermary P. Bitencourt
Farmacêutica pela Univiçosa – Centro Universitário e Viçosa
Av. Maria de Paula Santana, 3815 - Silvestre, Viçosa, MG, Brasil, CEP: 36576-340
E-mail: estermarypb@hotmail.com

Maria Regina de M. Souza
Doutora em Fitotecnia pela Universidade Federal de Viçosa (UFV)
Pesquisadora da Empresa de Pesquisa Agropecuária de Minas
Vila Gianeti, casa 46. Campus da UFV, Viçosa, MG, Brasil. CEP: 36570-900
E-mail: reginas.epamig@gmail.com

Andréia F. Silva
Mestre em Botânica pela Universidade Federal de Viçosa.
Pesquisadora da Empresa de Pesquisa Agropecuária de Minas
Av. José Cândido da Silveira, 1.647, União, Belo Horizonte, MG, Brasil. CEP: 31170-495
E-mail: andreiasilva@epamig.br

Yonara P. Neves
Doutora em Fitotecnia pela Universidade Federal de Viçosa (UFV)
Pesquisadora da Empresa de Pesquisa Agropecuária de Minas Gerais
Vila Gianeti, casa 46. Campus da UFV, Viçosa, MG, Brasil. CEP: 36570-900
E-mail: yonarapoltronieri@hotmail.com

RESUMO

A hortelã (*Mentha x villosa* Huds.) consta na Relação Nacional de Plantas Medicinais de Interesse ao SUS (RENISUS) devido ao reconhecimento de suas propriedades medicinais, dentre elas a atividade antiparasitária. Dentre as etapas do processamento pós-colheita de plantas medicinais, destaca-se a secagem como etapa crítica e essencial para a conservação dos compostos bioativos vegetais. Desta forma, objetivou-se avaliar o efeito da temperatura de secagem sobre o rendimento e a qualidade fitoquímica do óleo essencial extraído de folhas de hortelã. Realizou-se o cultivo em sistema orgânico em Oratórios, Minas Gerais, Brasil. Os ramos de hortelã foram colhidos e as folhas destacadas e submetidas à secagem em estufa de circulação forçada de ar a 40, 50 e 60 °C. A extração do óleo essencial foi feita por hidrodestilação em aparelho Clevenger, e para identificação dos constituintes químicos utilizou-se a cromatografia gasosa acoplada ao espectrofotômetro de massas (CG-MS). Constatou-se redução ($P<0,001$) do rendimento do óleo essencail com o aumento da temperatura de secagem das folhas. Não houve alteração do teor de óxido de piperitona (OP) (78 %), composto majoritário ao qual se atribui a atividade antiparasitária, nas temperaturas de secagem testadas. Entretato, observou-se maior diversidade de constituintes químicos identificados no óleo essencial obtido de folhas submetidas a secagem a 50 ºC. Os resultados contribuem para orientação de produtores no prcessamento pós-colheita das folhas de *Mentha x villosa* Huds visando a qualidade fitoquímica do óleo essencial e seu uso terapêutico como antiparasitário.

Palavras-chave: constituintes químicos, plantas medicinais, pós-colheita.

ABSTRACT

Mint (*Mentha* x *villosa* Huds.) is included in the National List of Medicinal Plants of Interest to SUS (RENISUS) due its medicinal properties recognition, including antiparasitic activity. Among the stages of medicinal plants post-harvest processing, drying stands out as a critical and essential step for bioactive compounds conservation and activity. This work aimed to evaluate the drying temperature effect on the yield and phytochemical quality of essential oil extracted from mint leaves. Cultivation was carried out in an organic system in Oratorios, Minas Gerais, Brazil. The mint branches were harvested and the leaves detached and submitted to drying in a forced air circulation oven at 40, 50 and 60 °C. The essential oils were extracted from the leaves by hydrodistillation in Clevenger apparatus and the chemical constituents were identified by gas chromatography coupled to the mass spectrophotometer (CG-MS). There was a reduction ($P < 0.001$) in the essential oil with drying temperature increase. There was no change in piperitone oxide (OP) (78%) content, the majoritary compound to which antiparasitic activity is attributed, at all drying temperatures tested. However, there was a major diversity of chemical constituents identified in the essential oil when leaves were submitted to drying at 50 ºC. These results contribute to guiding producers in the post-harvest processing of *Mentha* x *villosa* leaves aiming at the essential oil phytochemical quality and its therapeutic use as an antiparasitic.

Keywords: chemical constituents, medicinal plants, postharvest.

1 INTRODUCTION

Mint (*Mentha x villosa* Huds) is a medicinal species of the Lamiaceae family, originally from Europe and it is cultivated in several countries (LORENZI & MATOS, 2008). In Brazil, it is known as common mint (MARTINS et al., 2002). Its medicinal use against *Entamoeba*, *Giardia* and *Trichomonas* has been validated (LORENZI & MATOS, 2008) and is related to its chemical compound, the piperitenone oxide (OP) (SILVA, 2014). Regarding to its medicinal relevance, *M. x villosa* was included in the National List of Medicinal Plants of Interest to the Unified Health System (BRASIL, 2009) and selected for antiparasitic use within the scope of the State Green Component Program of the Minas Pharmacy Network, to be made available in the Unified Health System of Minas Gerais (SUS / MG).

The use of essential oils from species of the genus *Mentha* spp. is growing, considering its applications in the agriculture, food industry as well as in the production of medicines, cosmetics and perfumes (DESCHAMPS et al., 2013). Its antioxidant and antimicrobial effect is attributed to the chemical compounds present in its essential oils, such as menthol and eucalyptol (SINGH, SHUSHNI, & BELKHEIR, 2015) In the food industry, *Mentha* oil is widely used as a flavoring agent (DE SOUSA GUEDES et al., 2016). Among the factors influencing the composition and content of the chemical compound of these oils, we can mention: chemotypes, genotypes, plant development stage, solar radiation, water and nutrient availability (PEGORARO et al., 2010), genetic factors (DESCHAMPS et al., 2013), season (DESCHAMPS et al., 2008), harvest time, soil, drying temperature and storage conditions (INNECCO et al., 2003; MELO et al., 2004; ROCHA et al., 2011). The essential oils of *Mentha* spp. is produced and stored in specialized structures called glandular trichomes, which are located, in general, on the abaxial face of the leaves, but also occurs also on its the adaxial face, in lower density (GASPARIN et al. 2014). The essential oil extracted from *Mentha* spp. leaves has a great medicinal interest and its composition can vary with the chemotype and abiotic factors, being the most commonly found chemotypes: linalool; piperitenone oxide; carvonadihydrocarvone and pulegone-mentone-isomentone (DESCHAMPS et al., 2006). Piperitenone oxide (OP) is an effective active ingredient against the etiologic agents that cause amebiasis and giardiasis, used in drug formulations such as Giamebil (SILVA, 2014). Matos et al. (2002) reported the efficiency of antiparasitic action of 90% in the control of amebiasis and 70% for giardiasis in humans. Thus, among the known medicinal species, *Mentha* spp. it is one of the most used for this type of treatment. Its essential oil can be used in the pharmaceutical industry for the production of cosmetics and medicines. In the food industry, its oil and dried plants can be used in the manufacture of condiments, teas, among others (DIAS et al., 2012).

It is noteworthy there are still barriers in the productive chain of medicinal plants, among them are the dependence on extractivism for some species, the irregularity in chain supply, safety and quality of the plant raw material (BARATA, 2005; VENTURA et al., 2018). In these last two cases, obtaining plant material contaminated by microorganisms, such as bacteria and fungi, or by chemical contaminants, such as pesticides and heavy metals, contributes to the low phytochemical quality of essential oil and its safety to human health. These problems are mainly related to incorrect agronomic practices in soil management and post-harvest treatment of plant material, such as transportation, packaging, and the adequate time for drying processing (MARTINAZZO, 2006). In this scenario, the development and application of suitable technologies for its cultivation and post-harvest is essential for the right production methods and conservation of its active ingredients, since they are extremely important to ensure therapeutic efficacy and drug safety. The drying temperature is one of the most critical control points of the post-harvest processing of medicinal plants, considering that it can significantly affect the yield and phytochemical quality of essential oils extracted from medicinal plants. Thus, our objectives were evaluate the effect of drying temperature on the yield and phytochemical quality of essential oil extracted from mint leaves (*Mentha x villosa* Huds) as a way to contribute to the adequacy of post-harvest technologies for drying and conservation of chemical constituents of therapeutic interest present mint oil.

2 MATERIAL AND METHODS

2.1 GEOGRAPHIC COORDINATES, SOIL CHARACTERISTICS, FERTILIZATION AND IRRIGATION

The research was carried out at the Vale do Piranga Experimental Field of the Minas Gerais Research and Agriculture Company (EPAMIG), in Oratórios-MG. The geographical coordinates are: latitude 20 ° 30 ” S, 43 ° 00 ” and altitude 500 m, with the following meteorological variables: average annual maximum temperature of 21.8 °C and annual minimum of 19.5 °C and precipitation average annual rainfall of 1,250 mm. The predominant climatic type in the place is the Cwa, humid tropical, the Aw, semi-humid of hot summers, being the original vegetation constituted of subcaducifolia tropical forest, according to Köppen's classification (CUNHA et al., 2000). The loam-clay-sandy soil presented the following chemical characteristics: pH (water 1: 2.5) = 6.2; P (Mehlich) = 49.8 mg dm^{-3}; P (remainder) = 40 mg L^{-1}; K = 220 mg dm^{-3}; Ca = 2.6 cmol dm^{-3}; Mg = 1.3 cmol dm^{-3}; Al = 0 cmol dm^{-3}; H + Al = 2.8 cmol dm^{-3}; SB = 4.5 cmol dm^{-3}; t = 4.5 cmol dm^{-3}; m = 0%; T = 7.3 cmol dm^{-3}; V = 61%; Organic matter = 2.63 dag kg^{-1}. Fertilization was carried out using tanned bovine manure, with the following chemical characteristics (%): N (1.4), P (0.39), K (0.88), Ca (1.54), Mg (0, 27), S (0.23), CO (10.45); C/N (7.46). The drip irrigation system was used and, for the control of spontaneous plants, manual weeding was performed when necessary.

2.2 BOTANICAL IDENTIFICATION

Samples of species were sent to EPAMIG's PAMG Herbarium, where the botanical identification was confirmed. Exsiccates were assembled (herborization), according to usual botanical standards and incorporated into the collection of the PAMG / EPAMIG Herbarium*:* *Mentha* x *villosa* Huds. (Lamiaceae), PAMG 58227.

2.3 SEEDLING PRODUCTION, PLANTING, CULTIVATION AND HARVESTING

Seedlings of *M. x villosa* Huds were purchased in Viçosa, Minas Gerais (MG). The cultivation was carried out in an organic system at the Vale do Piranga Experimental Field of the Minas Gerais Agricultural Research Company, in Oratórios – MG, in an area of 27 m^2, using a 0.6 x 0.4 m spacing. The harvesting of branches from 60 useful plants in the central part of the production area in order to obtain homogeneous experimental material was carried out in December 2019 (spring), in the morning (between 8:00 and 10:00 am).

2.4 DRYING OF LEAVES

The drying was carried out at the Laboratory of Medicinal Plants of the Agricultural Research Corporation of Minas Gerais (EPAMIG), Southeast Unit, located on the Campus of the Federal University of Viçosa.

A completely randomized design was used. Three drying treatments were evaluated: air heated to 40, 50 and 60 ºC, each performed in ten repetitions.

The mint leaves packed in Kraft paper bags were dried at 40, 50 and 60 ºC, in an oven with forced air circulation until reaching constant weight. The air temperature was monitored during the drying period of the leaves. Subsequently, the samples were separated and packed in polyethylene bags, sealed and stored in an air-conditioned room temperature until the moment of essential oil extraction.

2.5 EXTRACTION OF ESSENTIAL OIL AND STORAGE

The extraction of essential oils was done in a Clevenger apparatus, adapted to a round-bottomed flask with a capacity of 1000 mL. Distilled water (500 mL) and 50 g of dry leaves of the medicinal species were added to the flask, beginning the hydrodistillation process. The extraction was carried out by dragging the essential oil through water vapor during 2h. Afterwards, the percentage of oil yield was calculated. The oil samples were stored in an amber glass bottle with a screw cap, under refrigeration (4 °C) for further chromatographic analysis.

2.6 IDENTIFICATION OF VOLATILE CONSTITUENTS

The analysis of volatile constituents was performed in an Agilent gas chromatograph, model HP-6890, equipped with an Agilent mass selective detector, model HP-5975 and an HP-5MS capillary column (30 m x 0.25 mm x 0.25 µm). The analysis was performed in splitless injection mode, with the following temperature conditions: injector at 220 °C, column at 60 °C, with heating ramp of 3 °C min^{-1} and final temperature of 240 °C and detector at 250 °C. Helium was used as carrier gas at a flow rate of 1 mL . min^{-1}. A sample of essential oil was dissolved in ethyl acetate (20 mg · mL^{-1}) for analysis. The identification of the chemical constituents of essential oils was carried out by comparing the retention indices (IR) obtained by the injection of hydrocarbon standards (C-8 to C-24), through the equipment database (NIST-11 library) and with literature data (ADAMS, 2007).

2.7 STATISTICAL ANALYSIS

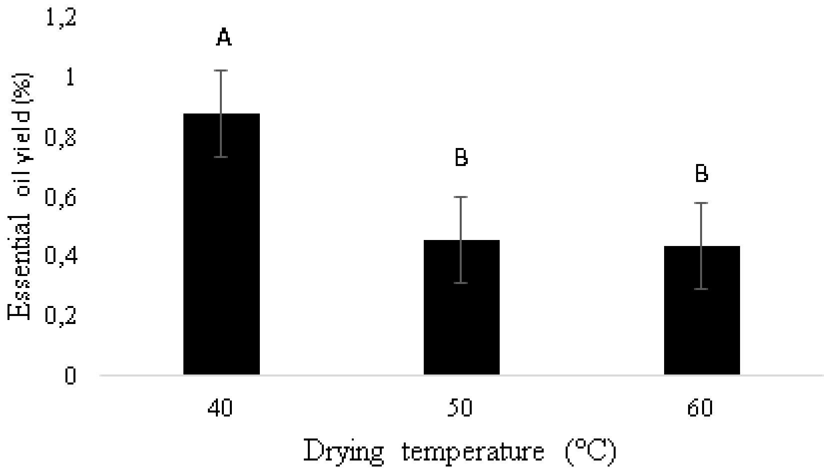
For the oil yield analysis, the Tukey test was used to compare the averages at 1% probability, with the aid of the SAEG statistical analysis program (RIBEIRO JR., 2001) and R Core Team (2020).

3 RESULTS AND DISCUSSION

3.1 YIELD OF ESSENTIAL OIL EXTRACTED FROM

There was a significant difference ($P < 0.01$) between the yields of essential oils extracted from mint leaves submitted to drying at 40, 50 and 60 °C (Figure 1). The essential oil yield was higher ($P < 0.001$) in extractions made from leaves subjected to drying at 40 ºC. However, there was no difference ($P > 0.001$) in essential oil yield between leaves subjected to drying at 50 ºC and 60 ºC (Figure 1). Our results differ from the results of Radünz et al. (2006), who found a higher yield (0.8733%), at 50 ºC, a value similar to what we found in leaves subjected to drying at 40 ºC (0.876%). They also differ from the results of Gasparin et al. (2014) who, when evaluating the drying of *Mentha x piperita* L. at 30, 40, 50, 60 and 70 °C, however, in a fixed bed dryer, found the best drying temperature 50 °C, with an air speed between 0.3 to 0.5 ms^{-1} to obtain maximum essential oil yield and minimal color degradation. This difference is probably due to the anatomical differences of the species of the genus *Mentha* and the use of different drying techniques (Dias, 2012).

Figure 1. Yields of essential oils extracted from leaves of *Mentha x villosa* Huds. submitted to drying in an oven with air circulation at 40, 50 and 60 ºC.



3.2 IDENTIFICATION OF CHEMICAL CONSTITUENTS IN *M x villosa* Huds. ESSENTIAL OILS

We quantified 16 compounds in the essential oil of *M. x villosa* Huds, extracted from leaves subjected to drying at 40, 50 and 60 ºC. Piperitenone oxide, the major compound to which antiparasitic activity is attributed, remained constant (78%), regardless of the drying temperature used (Table 1). Our result was similar to those found by Radünz et al. (2004) and Teles (2013). The chemical composition of the essential oil of the leaves varied with the drying temperature (Table 1), and this can occur due to the change of chemical constituents by the action of the drying temperature (FONSECA et al., 2020).

Table 1. Identified analytes and their content in essential oils extracted from *M. x villosa* leaves submitted to drying in an oven with forced air circulation at 40, 50 and 60 ºC.

R (min)	IR	Identification	Drying temperatures (°C)		
			40	50	60
5,45	932	alfa-pinene	*	0,31	*
6,48	971	sabinene	*	0,24	*
6,58	975	beta-pinene	*	0,47	*
6,64	977	1-octen-3-ol	0,57	0,58	*
6,95	989	beta-mircene	*	0,30	*
8,17	1027	limonene	0,46	0,72	*
8,46	1035	cis-beta-ocimene	*	0,36	*
11,18	1111	1-octen-3-ol- acetate	*	0,49	*
22,05	1372	**piperitenone oxide**	**78,54**	**78,70**	**78,00**
23,48	1406	alpha-gurjunene		0,53	*
26,40	1478	germacrene D		0,92	*
27,71	1511	gama-cadinene	*	*	*
28,06	1520	trans-calamenene		1,39	*
32,62	1640	epi-alpha-cadinol		0,64	1,05
33,13	1653	alpha-cadinol		1,00	2,25
34,40	1688	shyobunol		0,88	*

* not found

A greater number of constituents identified in the essential oil was found in leaves subjected to drying at 50 ºC (Table 1), and this result can be associated with the change in the constituents of essential oils at this temperature (GUIMARÃES et al., 2008). Similar results were found by Innecco et al. (2003).

Essential oils are, in general, made up of terpenes, and the prevalence of one or more components in the essential oil determines its chemotype. The determination of the chemotype of a

Mentha access results in an indication of its use and importance for culinary, medicinal use as well as in the perfumery industry. In an investigation of *M. spicata* chemotypes, the predominance of carvone and 2 dihydrocarvone was observed in commercial accesses, and in native accessions the greatest variation with the occurrence of the four other chemotypes: linalool; piperitone oxide-piperitenone oxide; carvonadihydrocarvone and pulegone-mentone-isomentone (KOKKINI & VOKOU 1989; DESCHAMPS et al., 2006).

4 CONCLUSIONS

To obtain a higher yield of essential oil extracted from leaves of *Mentha x villosa* Huds, it is recommended to dry it at 40 ºC. However, if the interest is to obtain greater diversity of chemical constituents, the temperature of 50 ºC is recommended. The choice of drying temperature for medicinal species must be made according to the chemical constituents of interest. For therapeutic use as an antiparasitic, any of the tested temperatures can be used, since piperitenone oxide remained constant.

Our results contribute to post-harvest guidance in maintaing the bioactive compounds of interest present in the essential oil extracted from *M. villosa Huds* leaves, in other words, its phytochemical quality.

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