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Evaluation of the storage and drying processes of *Melissa officinalis* L. leaves

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***Melissa officinalis* L. (Lamiaceae), medicinal plant used as sedative commercialized in natura and as dry plant in Brazil. The aim of this study was to determine the postharvest life and drying processes of *Melissa* leaves in function of essential oil contents. Leaves (10 g) were stored at room temperature (RT=19.6°C) and refrigeration temperature (10°C, AR), measured daily loss of fresh mass. In the evaluation, the drying processes used were microwave equipment (MW), thin-layer drying (TLD) and conventional oven (CO). The essential oil was obtained by the Clevenger apparatus. All treatments were done with four replicates and the data compared at 5% significance. The efficiency of the storage process was more effective in RT ($\hat{y}=9.5222x^2-51.271x+98.981$; $R^2=0.99$) with shelf-life of three days. AR had chilling causing a loss of essential oil and making commercialization impossible. The ideal wet mass was estimated to be between 2.70 and 2.83 g (ideal theoretical drying point) and the best dryings occurred in CO and TLD. The essential oil contents decreased in function of inadequate drying (MW), in relation to TLD and CO. The most suitable drying was in conventional oven (CO) and the major shelf-life time at room temperature (RT) and both processes had the best biomarker preservation.**

Key words: Postharvest, shelf-life, medicinal plant, drying process, quality control, bio-actives.

INTRODUCTION

Melissa officinalis L. (Lamiaceae) is popularly known in Brazil as lemon balm herb, true lemon balm herb, crawling lemon, melissa, cidrilha and meliteia. It is a European plant introduced in the Brazil, widely used in infusions, compresses and tisanes (Brant et al., 2009; Martins et al., 2003). It is a perennial plant with the quadrangular stem and the leaves are opposite, oval, bright green and with toothed margins (Figure 1). It has a lemon odor and the leaves are used to attract bees. Its main constituents, already isolated, are polyphenolic

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Figure 1. *Melissa officinalis* L. (A) Fresh stems, (B) Stems 1 day after storage, (C) Storage after three days at RT, (D) Storage after three days in AR, detail of injury, € Drying in microwave apparatus (MW), (F and G) Obtaining essential oil.
Source: Ouro Preto, Brazil (2017).

compounds (rosmarinic acid and caffeic acid), essential oils (citral), monoterpenoid aldehydes, sesquiterpenes, flavonoids (luteolin) and tannins (EMA, 2013; Shakeri et al., 2016; Lorenzi and Matos, 2002; Meira et al., 2011; Martins et al., 2003). The chemical marker for plant drugs is rosmarinic acid higher than 1% (PH EUR, 2018).

The essential oils of Lamiaceae family plants are composed primarily of mono and sesquiterpenes (Lewinsohn et al., 2000). The main components of volatile oil (0.02 to 0.8%) are geranial (citral a), neral (citral b), citronellal (major compound 40 to 75%), linalool, geraniol, geranyl acetate, methyl citronellate, α-octen-4-ol, 6-methyl-5-hepten-2-on, β-caryophyllene, cariofilen epoxide, germacren-D, and eugenol (ESCOP, 2013; PH. EUR., 2018, Gruenwald et al., 2007).

The main uses are in nervous crisis, agitation, tachycardia, melancholia, hysteria and anxiety (Haber et al., 2005; Meira et al., 2012; Lorenzi and Matos, 2002) or as a calming, digestive, carminative, antispasmodic and anti-neuralgic. The leaves are used in the treatment of insomnia, nervous problems, wounds and acts as a hypotensive agent. The leaves are used as flavouring add in foods king and in liqueurs (Martins et al., 2003; Lorenzi and Matos, 2002). In Europe, it is traditionally used in the symptomatic treatment of digestive disorders, such as epigastric distension, slow digestion, flatulence, among others (PH EURO, 2018; EMA, 2013).

Due to its ethnopharmacological relevance, the use of Melissa leaves was validated as a soothing and sedative, the species was selected as a therapeutic option in Green Pharmacy Program (phytopharmacy) in the state of Minas Gerais, Brazil. This program aims at the insertion and supply of phytotherapy to the users of the Single Health System (SUS-BRAZIL) through certified medicinal plants produced in an agroecological way and cultivated by the agricultural family. It is important to remember that commercial cultivation by the agricultural family is an option for income generation and family attachment in the countryside. According to Martins et al. (2003) the commercial plantation can produce about 1.800 kg of dry leaves ha^{-1} $year^{-1}$.

The use of dry leaves is called plant drugs. The drug *M. officinalis folium* consists of fresh and/or dried leaves of *M. officinalis* L. and other preparations in effective dosage. The leaves contain at least 0.05% (v/w) of essential oil, based on the dried herb (PH EUR, 2018).

Although drying is the best form of commercialization of medicinal plants, data referring to acceptance of use refer to the acquisition of fresh plants by the population. It is believed that the option to purchase the fresh plant refers to the vitality of the product, the healing properties as well as adequate storage.

In the chain of production of medicinal plants, there is little technical information that correlates the drying processes to the production of the plant drug, the preservation of bioactive materials and the shelf life of the *Melissa* leaves. In this sense, this study had as objective to evaluate packaging for the commercialization of fresh leaves of *M. officinalis* L. in function of the preservation of essential oil contents and shelf life. The effectiveness of the drying process and preservation of the biomarkers was also studied.

MATERIALS AND METHODS

Obtaining treatments

The seeds propagation was done by sowing in organic substrate.

After the development of branches already in secondary growth, that is, fully expanded, these were picked from a single access, at Ouro Preto, Brazil, in December. The transport was made in refrigerated boxes to the Laboratory of Medicinal and Phytotherapeutic Plants at Federal University of Ouro Preto. The leaves were removed from the stems, separating the intact from the non-perfect.

Evaluation of storage in ecological packaging

For packaging, to evaluate storage, commercial type of 25 g was chosen, consisting of a neutral, white, heat-resistant, multi-polypropylene cellulose (paper), packaging that did not allow the passage of light or moisture, being absorbent but not excessively porous. The rules for storing and marketing products were observed.

Leaves (10 g) were stored at room temperature (RT, average temperature = 19.60°C, RH = 80.30%) and refrigeration temperature (AR, 10°C), disposed on multifolium paper and measured daily loss of fresh mass and wilting.

The sealing of the packaging was manual, by enveloping, with folding and manual creasing. Metallized staples or any other form of closure were not used as adhesive tapes.

Evaluation of drying processes

The determination of the initial water content in fresh leaves of *M. officinalis* L. was previously determined by gravimetry. Approximately, 10 g of fresh leaves were weighed in a porcelain crucible, pre-weighed and dried for 30 min at 105°C. Fresh leaf weights were conditioned in a preheated oven at 105°C for 5 h. After this period, the crucibles were removed, cooled in desiccator for 1 h at room temperature and again weighed. The moisture content was calculated as a function of initial mass and desiccation loss (Brazil, 2010).

In the evaluation of the drying processes, the artificial and natural drying methods were used. Microwave equipment (MW, BRASTEMP BMG45AR) and conventional oven (TECNAL, TE394) were used for the artificial process, while thin-layer drying (TLD) was used as the natural process.

Fresh leaves (10 g) were packed in neutral, white, multifolium paper, commercial type packaging for 25 g, previously weighed, identified and destined to the treatments MW, CO and TLD.

Individually, 10 g of leaves, duly pre-weighed (paper and sheets) were placed in a microwave oven (WO) at an average temperature of 80°C, being removed every 30 s and cooled in the desiccator for 5 min, being weighed to constant weight (total of 5 weighings), with total time of 150 s.

Leaves (10 g) of duly conditioned and pre-weighed leaves were placed in a conventional oven (CO) at a temperature of 40°C, up to constant weight. The moment of interruption of the drying process was previously determined when the final mass was equivalent to the water content of 8 to 14% of initial water (wet basis, wb) (Barbosa et al., 2006).

In the natural drying process, TLD, 10 g of leaves were packed in neutral, duly identified paper, arranged at room temperature, under direct light. The internal, external temperature and relative humidity of the air were monitored with the aid of a digital thermohygrometer (HOBO U14-001).

TLD and CO treatments were weighed daily, until constant weight was obtained. In the drying, the mass loss and the residual moisture were evaluated as a function of the time spent, in hours for the conclusion of the process. The calculations of moisture determination were expressed according to the Equations 1 to 5:

$$1.\ Mf = Mi \times \frac{(100 - Wi)}{(100 - Wf)}$$

$$2.\ \%WB = \frac{Mw}{Mi} \times 100$$

$$3.\ \%DB = \frac{Mw}{Ms} \times 100$$

$$4.\ \%WB = \frac{WB}{100 + WB}$$

$$5.\ Mi = Md + Mw$$

where Mf = final mass (g); Mi = initial mass of fresh stems (g); Wi = initial water content of fresh stems (% d.b.); Wf = final water content (% d.b.); Mw = mass of water; Md = dry mass; WB = moisture, on wet basis (wb); and Db = moisture on dry basis (Barbosa et al., 2006).

Obtaining essential oil

From the drying procedures, the essential oil was obtained by hydrodistillation in a Clevenger apparatus (Vidrolabor) for 4 h according to the methodology of The European Pharmacopeia 8th (2018), the volume obtained was quantified in microliters and the yield was estimated.

Experimental design

The experimental design was in a completely randomized block (5×4) where the five treatments (AR, RT, TLD, MW, CO) and their four replicates (per treatment) were evaluated over time, compared to each other at 5% significance.

RESULTS AND DISCUSSION

In the marketing of products of plant origin, whether *in natura* or fresh plant or as plant drug or dry plant, some aspects must be observed. Perhaps the most important is the consumer profile. The consumer attributes the effectiveness of medicinal plants as a function of the visual and affective memory of the organoleptic aspects (color, odor and taste). Therefore, the fresh plant is always better accepted, being more acquired and used, than the vegetal drug. To these factors are added the primary conditions of hygiene processing, storage and commercialization of the dried plants, besides origin that in most of the times is unknown.

In Brazil, medicinal plants must come from organic or agroecological cultivation, not being allowed the use of herbicides or chemical fertilizers or any treatment coming from conventional agriculture. Today, the best option for medicinal plants is associated with crops consorted with vegetables made by the agricultural family.

The agroecological concepts for production and commercialization range from the selection of the species, with certified seeds and seedlings, to the type of manure, dealing with integrated management, harvesting, postharvest treatments, storage in ecological packages that are effective in preserving shelf life.

The search for packaging that does not cost (low cost) the producer/consumer, allow greater shelf life and are ecologically is fundamental. When one chooses not to market vegetables in plastic packaging, such as

polyethylene terephthalate (pet) or styrofoam associated with plastic films, it breaks with the paradigm of better hygiene of the final product and longer shelf life, however, it promotes the appreciation of the agroecological culture, allowing the optimization of green concepts of sustainability. The option for paper packaging, in this experiment, sought to associate all these variables.

The purpose of packaging is the preservation of the product in the physical, chemical and microbiological aspects for a relatively long time, minimizing the physiological changes during transportation and storage (Smith et al., 2004). For the consumer, the packaging must awaken the desire to buy, transmit information, communication, and be support for promotional actions. Currently, these factors are added to the question of bioactive packaging, which signals to the consumer the state of the product, whether in terms of validity or functional maturity. The return of sustainable, organic packaging is tied to another aspect that is the sustainability of the planet (Landim et al., 2016).

The choice of white multifoil paper packaging was due to the ease of acquisition (low cost, availability) and because it is an option to the craft paper (brown). The white color, popularly, refers to hygienic characters and allows the visualization of spots or residual moisture.

Packaging and materials that come into direct contact with food are intended to contain them, from their manufacture to their delivery to the consumer, to protect them from external agents, changes and contaminations, as well as from adulterations (Brasil, 2010).

The enveloping (form of closure of the packages) allowed complete sealing of the experiment. The classification of the type of packaging used was in primary despite being in double envelopment. By primary packaging, it understood the one that maintains direct contact with the vegetal drug (Brasil, 2009).

In this experiment, the efficiency of the storage process was more effective in RT ($\hat{y} = 9.5222x^2 - 51.271x + 98.981$; $R^2 = 0.99$) with a maximum time of three days (72 h). In AR ($\hat{y} = 10.444x^2-54.248x + 98.366$; $R^2 = 0.98$), there was a cold injury, which made commercialization unfeasible and caused loss of essential oil (Figure 1D). This loss of quality and commercial value, in vegetables, occurs due to intense respiratory activity and great water loss (Mota et al., 2003).

In coriander (*Coriandrum sativum* L., Lamiaceae), spice and medicinal species, the shelf life was equivalent to 48 h, undergoing a great influence of the hydrocooling (72 h) and the reduction of the shelf life of the coriander was wilted and yellowing of the leaves (Oliveira, 2012). The wilting and/or wrinkling occurs due to the loss of water and altering the organoleptic characteristics (color, odor, flavor), resulting in loss of external quality, consequently the final appearance of the products for commercialization and bioactivity (Chitarra and Chitarra, 2005).

In the determination of moisture by gravimetry, the water loss was 78%. In drying, the ideal wet mass estimated was between 2.70 and 2.83 g, considered the ideal theoretical drying point (10 to 14%), with TLD = 2.60 g; MW = 2.52 g and CO = 2.98 g. According to Martins et al. (2003), the drying air temperature of medicinal plants generally ranges from 20 to 40°C for leaves and flowers. In drying, another priority factor is the speed with which the water is withdrawn, that is, the drying rate, because a very fast process can degrade the active principles (Melo et al., 2004). On the other hand, it should not be too slow, as it may lead to the appearance of undesirable microorganisms (Silva and Casali, 2000). In the analyzed treatments, the drying temperatures were for CO 40°C, MW 80°C and TLD 41°C, and relative humidity of 37%.

In the drying processes, in the production of the plant drug, the comparison of methods is linked to energy expenditure, drying temperature, dry mass yield and preservation of active compounds. In addition to the drying air temperature, which affects the relative humidity, the drying rate is influenced by the air velocity passing through the product (Melo et al., 2004). Although MW is the fastest process, the final dry mass values were lower than the values estimated in obtaining the final drying point (constant mass).

When evaluating such processes (three treatments) over time, the following equations were obtained in MW where $\hat{y} = -67.522x + 100$, $R^2 = 1$; CO where $\hat{y} = -80.785x + 100$, $R^2 = 1$, and TLD where $\hat{y} = -11.801x^2 - 59.113x + 98.973$, $R^2 = 0.99$.

Regarding the preservation of color and odor, it can be observed that in MW although the color was preserved (Figure 1), the odor was very discrete. In TLD and CO, the staining was well preserved, with better CO scent. According to Martins (2002), in medicinal plants, the relative humidity of the air during drying directly influences the composition of the essential oil. It was observed that drying air influenced the citral volatilization process, stating that the lower the moisture, the stronger the volatilization of monoterpenes.

During the drying process, the loss of color is one of the indicative changes in the material and consequently of the vegetable drug. It is common to see in specialized markets, dry plants with brownish appearance, which reflects ineffective processes of drying, with excessive loss of water and compounds (thermolabile). One can often observe leaf burning due to temperature excess, dehydration and black spots. It is emphasized that the parts of the plant after drying preserve the color, that is, if these parts (leaves, for example) are green they remain green, if yellow, like flowers, they will be yellow. In quality control of plant drugs, the visual aspects are easy to be observed even in sealed packaging. Regarding the packages for commercialization of dried plants, the ideal is the secondary type, with double envelopment and small display for identification of the species and visual contact of the consumer. They can be totally recyclable (cellulosic and with biofilms) where the vegetal drug will

be protected from light and heat and with an ecological presentation.

Considering the results to evaluate the drying processes of *M. officinalis* it can be affirmed that the best drying process occurred in conventional stove CO, followed by thin layer TLD. In MW, mean values of essential oil were 106.6981 ± 25.3975 mgg^{-1} dry mass (approximately 0.35% yield relative to the initial dry mass). The proportion of essential oil varies between 0.1 and 0.45% of the fresh mass based on climatic, soil and crop differences (Guimaraes et al., 2015). Thus, a yield of 478.620 ± 50.1483 mgg^{-1} of fresh mass (0.45%) and 106.36 ± 11.1440 mgg^{-1} of fresh mass (0.1%) was expected. The average oil content corresponds to 0.1% of the initial fresh mass (0.1003%). The essential oil dosages in the treatments during storage were at RT of 113.4159 ± 18.60992 mgg^{-1} and AR of 106.3394 ± 17.15054 mgg^{-1} of post-storage mass, demonstrating the superiority of storage at room temperature.

In the drying processes, the oil contents were small in MW relative to TLD and CO. The yields were, respectively 108.553 ± 40.1677 mgg^{-1} in TLD, 126.3952 ± 7.385318 mgg^{-1} in CO, being greater than MW 78.7867 ± 19.5749 mgg^{-1}, values estimated in dry mass.

The process of drying and yield of essential oil is linked to the time and date of harvesting of leaves of aromatic plants (seasonal variations). Several studies have shown that the contents fluctuate according to the maturity of the leaves (postharvest stages and extractive methods).

According to Rosado et al. (2011), the type of drying and the processing of leaves of *Ocimum basilicum* (basil) influenced in the content and chemical composition of the essential oil where greater percentage of linalool was obtained post-drying, being that drying in greenhouse conserved the aroma and coloration leaves, preserving the original characteristics of the cultivar.

The impact of hot air drying at temperatures of 30 to 90°C with constant specific humidity of 10 g kg^{-1} of dry air and uniform air flow of 0.2 ms^{-1} on the essential oil contents in leaves of lemon balm (*M. officinalis* L.) were evaluated and most of the oil loss was observed at the beginning of the drying process and was proportional to the drying temperature. Pronounced changes occurred at 60°C where the main components of the neral, geranial and citronellal essential oil were decreased, while citronellol showed an increasing tendency. The authors conclude that in addition to component temperature sensitivity, the loss of essential oil can also be attributed to structural changes caused by drying (Argyropoulos and Muller, 2014).

Leaves of *Ocimum gratissimum* L. (Lamiaceae) submitted to the different drying methods (greenhouse, dehumidification and air drying) did not alter the essential oil contents or caused damage to the trichomes, but dried leaves in a forced ventilation oven at 60°C had the trichomes damaged with the reduction of essential oil contents and all drying methods had a reduction in fungal contamination (Santana et al., 2014).

In the leaves of *Plectranthus barbatus* (Brazilian Boldus) and *Plectranthus ornatus* (Brazilian mulled leaf boldus), when the method of natural (thin layer) and artificial drying (forced ventilation oven and microwave) was compared, the best drying method was forced ventilation stove, for causing less damage to the leaves and for having less influence on the degradation the secondary principles (Rodrigues et al., 2011).

The drying process did not interfere with the oil yield in *Piper hispidinervum* (Piperaceae) (Negreiros et al., 2015). In *Varronia curassavica* Jaqc. (Borraginaceae), the essential oil content was not influenced by the harvesting schedule, although the picking time influenced the chemical composition of the *Varronia* essential oil (Queiroz et al., 2016). Thus, in native plants, physiologically by foliar, structural and morphological resistance, the drying processes did not alter the essential oil contents, contrary to the one demonstrated in Lamiaceae.

Conclusion

The most suitable drying was in a conventional oven and longer time of commercialization at room temperature and both processes had better preservation of biomarkers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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