Postharvest management affects spearmint and calamint essential oils

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Postharvest management affects spearmint and calamint essential oils.

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Running title: dehumidification affects essential oil in mints

Abstract

BACKGROUND: The objectives of the work were to evaluate the phytomass yield, essential oil content and essential oil yield of *Mentha spicata* L. var. *rubra*, *M. spicata* L. var. *viridis*, and *Calamintha nepeta* Savi in Piedmont (Italy), and to study how postharvest management (hydrodistillation of essential oil from fresh, dehumidified or oven-dried herbs) can affect the essential oil content and profile of the three species.

RESULTS: *M. spicata* L. var. *rubra* gave the greatest phytomass yield (1997 g m^{-2}), which was statistically different from *M. spicata* L. var. *viridis* and *C. nepeta*. The highest EO yield was obtained from *C. nepeta* (3.75 g m^{-2}), which was significantly different from the *Mentha* genus. Postharvest management significantly affected both the EO content and the EO profile of each species, with the dehumidifying process leading to a significantly higher EO content than the oven-drying process. The EO profile was different not only from species to species but also because of the postharvest management.

CONCLUSION: The dehumidifying process is a relatively new postharvest technology that has shown positive results, in terms of EO yield, and it can be applied to species which have a high EO value, after evaluation of the resulting EO profile.

Keywords: *Mentha spicata* L.; *Calamintha nepeta* (L.) Savi; dehumidifying process; carvone; limonene; pulegone

INTRODUCTION

Lamiaceae, together with *Asteraceae* and *Rosaceae*, are the botanical families that are most commonly used as herbs (also known as Medicinal and Aromatic Plants = MAPs). This could be due to a possible cultural heritage, which is favoured by the botanical and phytochemical characteristics of the family, with plants easily recognizable because of their striking flowers, intense aromas and particular flavours.[1] The importance of this group of plants in Italy is underlined by the presence of farms specifically dedicated to MAP production. Nevertheless, this production is only a niche sector, in which the farmers and operators are scattered throughout the country. Moreover, there is a lack of association of producer organizations and an adequate extension service.[2] This results in a lack of information regarding the real situation, in terms of cultivation areas, marketing issues, and logistics. According to the latest national agricultural census, the MAP sector in Italy is only represented by farms that grow annual crop herbs. This list includes 1932 farms,[3] which represent 0.2% of the total number of farms that grow annual crops at a national level. These MAPs are cultivated over 3509.17 ha, which represent 0.05% of the total surface dedicated to annual crops in Italy. More than 8% of the national farms can be found in the Piedmont Region, North Italy, with 735.79 ha (Elaborated data).[3] However, no official data on pluriannual MAP crops can be found in literature.

The Piedmont Region is famous for the production of the *Lamiaceae* genera, which accounts for ca. 64% of all the cultivated MAPs.[2] The main local species (ca. 54%) is black peppermint (*Mentha* × *piperita* L. var. *officinalis* forma *rubescens* Camus), known as the Italo-Mitcham hybrid.[2] A decrease in price of *M.* × *piperita* EO (Ferrero, 2008, personal communication) induced growers to look for

other mint alternatives in order to expand the mint sector offer and extend the harvesting season. Growers in the area have started to cultivate red spearmint (*Mentha spicata* L. var. *rubra*) (hereafter: *M. spicata* var. *rubra*) and a type of mint imported from Morocco by a local grower, which has thus been nicknamed "Casablanca", a variety classified as *Mentha spicata* L. var. *viridis* (syn. *M. viridis* Auct.) (hereafter: *M. spicata* var. *viridis*), according to Pignatti.[4] Both varieties contain limonene and (–)-carvone, which are characteristic monoterpenes of spearmint EO.[5] Another *Lamiaceae* species, which is known in Italy as "mentuccia", is calamint (*Calamintha nepeta* (L.) Savi) (hereafter: *C. nepeta*), a hardy and endemic species found in Southern and Northern Italy.[4] It is used as a food spice and for medicinal purposes in Italy, especially in the Southern Regions of Basilicata,[6] Sicily[7] and Apulia.[8] The antimicrobial property of its EO has already been studied *in vitro*,[9] and for this reason calamint could be introduced as a cultivation, along with spearmint, in the Northern area.

The crop cycle in the Piedmont Region usually has two main harvesting seasons: at the flowering stage in summer, for the extraction of EO, and at the vegetative stage in autumn for the dried herb market. EO can be extracted from both fresh and dried herbs, and its yield and quality depend on the species and drying method that is adopted.[10-12]

Herbs have a high moisture content in the flowers, leaves and roots,[13] and are prone to microbial proliferation, if not processed immediately, usually through alcoholic infusion or hydrodistillation. Herbs can also be dried and processed after storage, when processing is not possible straight after harvesting. The drying process reduces the water content and limits microbial and enzyme activities; however, physical and chemical changes can occur in the raw material. Convective oven-drying is the most popular method used to reduce the moisture content of fruit, vegetables, and herbs[11] and to obtain a product that lasts at room temperature. However, the method suffers from several disadvantages and limitations, such as the relatively long times and high temperatures that are necessary to be effective.[11] The contact of dried material with hot air causes a degradation of the phytochemicals, as well as colour alterations and volatile compound losses, as has been reported for thyme (*Thymus vulgaris* L.),[12] sage (*Salvia officinalis* L.),[12] and clary sage (*Salvia sclarea* L.).[14]

Innovative drying systems based mainly on the microwave principle,[11] or the freeze-drying method,[10] have been studied to reduce volatile compound losses, . Szumny *et al.*[11] evaluated the effects of the convective drying method, the vacuum-microwave method (VM) and a combination of both on the volatile compounds of *Rosmarinus officinalis* L. (a mixture of leaves, branches and stems). The authors found that convection and VM-dried samples contained 64.6% and 54.1% of the fresh rosemary volatile content (135 g kg^{-1}), respectively. Samples dried using a combination of convection drying to a moisture content of 0.44 kg kg^{-1} and VM-drying at 360 W presented the lowest loss, and contained 74.1% of the fresh rosemary volatile content. They recommended this combined process for rosemary, because reductions in both the volatile content and sensory quality occur when VM-drying is used on its own. Grayer *et al.*[10] studied the EO profile of fresh and freeze-dried leaves of 16 basil (*Ocimum basilicum* L.) accessions. They found that many small and some larger changes take place during freeze-drying, with a decrease in the ratios between methyl chavicol to linalool and eugenol to linanool.

Microwave and freeze-drying systems are rarely implemented in small Italian farms because of the high costs and high technology required. An alternative to traditional oven-drying at high temperatures and to innovative drying systems could be the dehumidification process, which involves removing water from plant cells at temperatures close to room temperature. We have shown that the dehumidifying process affects the EO content and profile of Greek oregano (*Origanum vulgare* L. ssp. *Hirtum* (Link) Ietswaart) in a similar way to the oven-drying process.[15] However, there is still a lack of studies in literature on this system extensively applied to several herbs. Thus, the objectives of the work were 1) to evaluate the EO of mint species recently introduced into the Piedmont Region, and 2) to study how postharvest management can affect the EO content and the EO quality of these mints.

EXPERIMENTAL

Plant growth and raw material production

The work was conducted in 2009 and involved two studies. The first study consisted of an instrumental analysis that compared three mint species (*M. spicata* var. *rubra*; *M. spicata* var. *viridis*; *C. nepeta*) for the EO profiles extracted from fresh herbs. The second study consisted in testing the effect of three postharvest management systems on the EO content and profile of the three species. The postharvest management systems were 1) EO extracted from fresh herbs; 2) EO extracted from 25-°C dehumidified herbs, and 3) EO extracted from 60-°C oven-dried herbs. Another specific aim was to compare the content of EO extracted from fresh and processed herbs. The processed herbs were obtained either through a dehumidifying (DH) process or an oven-drying (OD) process, both of which were conducted in commercial drying structures at the experimental centre at the Faculty of Agriculture. Samples of 2 kg per species were arranged in a 0.10-m layer on perforated steel trays. For the DH process, the trays were placed in a temperature-controlled cell (25 °C ± 2 °C), equipped with a dehumidifier (KT-38/S- Tecno.Klima s.r.l., Granarolo Emilia, BO, Italy) for 48 h. The DH process takes place through the use of a heat pump-assisted dehumidified air drying system, as described by Phoungchandang (2009)[16] and by Adapa and Schoenau (2005).[17] For the OD process, the trays were placed in a conventional oven-drying cell set at 60 °C for 4 days.

The studied species were from a second year crop, planted in the field in spring 2008. Rooty turiones were used for *M. spicata* var. *rubra* and *M. spicata* var. *viridis*, while transplants obtained from seeds (Jelitto Staudensamen GmbH, Schwarmstedt, Germany) were used for *C. nepeta*. The species were grown in the same environment (Lombriasco, TO – Italy; 44°50'35.15"N; 7°38'17.36"E - 254 m a.s.l.) and soil (sandy-loam, according to the USDA criteria, with 10.9% of skeletal material). Mature vaccine manure was applied to the soil at a 4.0 kg m^2 dosage. Nitrophoska® Gold® (COMPO Agricoltura Spa, Cesano Maderno, MI, Italy), a slow-release complex fertilizer with 6.8% nitrate nitrogen, 8.2% ammonia nitrogen, 9.0% neutral ammonium citrate and water-soluble phosphate, 15.0% water-soluble potassium, 2.0% magnesium, 16.0% sulphate, and micronutrients, was applied at transplanting to the soil at a 100 g m^{-2} dosage. The plant density for each species was ca. 23.28

plant/m^2. Flowering was scalar for all the species. Harvesting took place at full bloom on 2 July 2009 for *M. spicata* var. *rubra*, on 8 July 2009 for *C. nepeta*, and on 21 July 2009 for *M. spicata* var. *viridis*. The phytomass (flowers, leaves and stems) yields, EO contents, and EO yields were recorded at harvesting, and the EO profile extracted from fresh, dehumidified, and oven-dried herbs was then analysed.

Hydrodistillation

An aliquot of ca. 300 g of fresh, DH, and OD material was steam-distilled using glass distillation equipment, assembled by Exacta+Optech (San Prospero, MO, Italy). This equipment was composed of an electrical heating mantle (500 Watts - Thermo Scientific Electro Thermal, Waltham, MA, USA), a 2-L Pyrex glass balloon filled with 1.5 L of deionised water with a 4-L modified Pyrex glass balloon filled with the herb material above it, a cooling column operating in co-current and a graded burette. Each distillation cycle lasted ca. 105 min, with 45 min of steaming produced by the boiling water in the glass balloon. The 45 min distillation time started when the first drop of liquid, condensed in the cooling column, dripped into the graded burette. The EO content was calculated as the ratio in weight ($^{w}/_{w}$) between the cold oil collected from the burette and the original weight of the material.

The EO was analyzed in a laboratory (Alchim di Roberto Masante, Chieri, TO, Italy) that used an Agilent 5973 N GC-MS system (Agilent Technologies Inc., Palo Alto, CA, USA) with an Rtx5 capillary column (30 m × 0.25 mm i.d.). The column was set for a 60 °C hold for 3 min, then warmed at 5 °C min^{-1} to 250 °C. The auto injection volume was 0.5 μL, neat, with split injection (80:1). Peak identification was made according to an external standard (Supelco, Bellefonte, PA, USA) and single compound standards (Sigma-Aldrich Corp. St.Louis, MO, USA).

Statistical design and analyses

The statistical experimental design was a randomized complete block design (RCBD). Until harvesting, 3 treatments (3 species: *M. spicata* var. *rubra*; *M. spicata* var. *viridis*; *C. nepeta*), with 3

replications, were used for the phytomass yield, EO content and EO yield analyses. After harvest, 9 treatments (3 species × 3 herb postharvest managements), with 3 replications, were used for the EO content analysis. Due to the particular EO composition, the EO profile was assessed separately for each species, with 3 replications, after the postharvest managements.

The data were submitted to ANOVA, using the Statistical Package for Social Science (SPSS Version 17.0, SPSS Inc., Chicago, IL, USA). When ANOVA was significant, Tukey's multiple range test was used for the species effect on the phytomass yield, the total EO content of fresh herbs and the EO yield and for the postharvest management effect on the EO compounds of the three species. Pre-planned orthogonal contrasts were set for these three species, to test the EO content from either the fresh herbs against the processed herbs, or from the DH herbs against the OD herbs.

RESULTS AND DISCUSSION

Raw material and EO content from fresh phytomass

At harvest, the species significantly affected the phytomass yield, the total EO content, and the EO yield extracted from the fresh phytomass (Table 1). *M. spicata* var. *rubra* produced the greatest phytomass yield and differed substantially from the other two species (*M. spicata* var. *viridis* and *C. nepeta*), which had an average phytomass yield of 1421 g m^{-2}. However, as far as the EO content and yield are concerned, the tested species can be considered in two groups, with respect to the two genera. The highest EO content and yield were obtained from *C. nepeta*, which was significantly different from the *Mentha* genus group. The former had a 48% higher content and a 47% greater yield than the mean value of the latter group.

The phytomass yield values found for the *Mentha* species were lower than the ca. 2.3 kg m^{-2} values that have been reported by other authors for menthol mint (*M. arvensis* L.).[18-19] No specific data are known for *C. nepeta*, even though it has been cultivated for the herb in Latium Region and for EO in Moldova since the beginning of the '90s.[20]

The EO content of all the studied species was between 0.1% and 0.3% of the fresh phytomass, and was comparable with the usual values of 0.2-0.5% found for *Mentha* species by other authors who extracted EO from fresh phytomass.[21-24] No other information related to the EO content of the fresh phytomass has been found. The EO content can vary according to the chemotype and the habitat in which the *Mentha* genus grows.[23,25] Generally, the EO content, referring to dry weight (DW), is considered to be lower than 2%,[25] and this corresponds to approximately 0.5% on a fresh weight basis (our elaboration). Higher contents (max 3.9% $^{v}/_{w}$ DW) can only be obtained in plants grown in areas with little rain during the summer, high temperatures, and a high amount of total sunshine, such as in the Island of Crete.[25] In relation to the studied mints, *M. spicata* var. *rubra* and *M. spicata* var. *viridis* contained 65% and 36% less EO content, respectively, than the average content extracted from spearmint grown in similar climate conditions in North Indiana, the USA.[26] This difference is probably due to the anhydrous ammonia fertilization levels used to increase the production of the spearmint grown in America.

Very few data are available in literature on the EO content of *C. nepeta*, and we therefore attempted to compare our results with those obtained by Riela *et al*.,[27] although the authors distilled the fresh phytomass using *n*-pentane. The authors obtained 0.36% $^{w}/_{w}$ EO (data elaborated), while we obtained 0.24% EO. This difference could be due to the solvent, since *n*-pentane has a higher extracting power than the deionised water used in the present research.

EO profiles

The EOs extracted from the fresh mints resulted in different terpene profiles (Table 2). The EO of *M. spicata* var. *rubra* was mainly composed of carvone, limonene, β-caryophyllene and 1,8-cineole. This EO profile is not comparable with the data reported by Kokkini and Voukou,[28] who described the EO of four *M. spicata* chemotypes grown wild in Greece, the place of origin of the species. The most similar chemotype to ours is what the authors called "chemotype II", the most commercially cultivated chemotype for EO extraction, which was rich in carvone (35.2-49.7% EO) and

dihydrocarvone (5.4-21.5% EO), although our *M. spicata* var. *rubra* contained higher carvone (66.0% EO) and lower dihydrocarvone (1.4% EO). It is possible to speculate that these differences could be due to the intraspecific factors of *M. spicata*, which are perhaps due to a further hybridation between the cultivated species and *M. longifolia* L., as already reported by Kokkini and Voukou[28] for North American spearmint hybrids.

The EO of *M. spicata* var. *viridis* was mainly composed of carvone, limonene, 1,8-cineole, and β-caryophyllene (Table 2). Its EO profile is comparable with that of the Egyptian chemotype of *Mentha spicata* var. *viridis*, which has been evaluated by Edris *et al.*,[29] thanks to the particularly high limonene and lower carvone contents than the other *M. spicata* chemotypes. Large amounts of 1,8-cineolele in EO of both *M. spicata* varieties have been confirmed by Orio et al. (2012),[30] after either HD or microwave-generated hydrodistillation.

The EO of *C. nepeta* was mainly composed of pulegone, which amounted to 73.6% of the EO (Table 2). The EO profile of the species differed from the other studied mints, and it had a surprising similar pulegone content to *M. pulegium* L. (73.4% EO).[31]

The menthol-related compounds that were found are involved in the terpene synthesis from the plastidial methyl erythritol phosphate (MEP) pathway, as shown for *M.* × *piperita.*[32] The authors[32] also reported that no message was found for the mevalonate pathway in the oil glands of *M.* × *piperita*, while Humphrey and Beale[33] explained that the mevalonate pathway in higher plant cells is responsible for synthesizing the sesquiterpenes in the cytosol. All these compounds, expect β-caryophyllene, are present in smaller amounts in the EOs of all the studied species.

Effect of postharvest management on the EO content and profile

Postharvest management significantly affected both the content and profile of EO in each of the studied species. The interaction between species and postharvest management significantly influenced the EO content of the tested species, which was calculated on a fresh weight basis (*P=0.003*). All the EO contents of *Mentha* spp. and *C. nepeta* were affected by processes and showed

different responses (Table 3). The EO content from processed *M. spicata* var. *rubra*, *M. spicata* var. *viridis* and *C. nepeta* was 50.4% lower, 13.4%, higher and 24.4% lower, respectively, than the EO content from the corresponding fresh herbs. The OD process reduced the EO content in all the tested species, compared to the DH process, by 88.1% in *M. spicata* var. *rubra*, 39.4% in *M. spicata* var. *viridis*, and 61.9% in *C. nepeta*.

Of the 24 compounds detected in the EO, postharvest management significantly influenced 21 compounds in *M. spicata* var. *rubra* (Table 4). The percentage of carvone and limonene, the predominant EO compounds, did not change statistically in the fresh or DH samples, while it decreased by 17.5% and 50%, respectively, in the OD samples, which were significantly different from the fresh and DH samples. After carvone and limonene, the other main monoterpene was 1,8-cineole, which was not influenced significantly by postharvest management. The contents of sesquiterpenes, β-caryophyllene, β-bourbonene, germacrene D, and β-elemene, were the highest in the OD samples and significantly different from those in the fresh and DH samples (approximately 3 fold), whose values were not statistically different.

Of the 28 compounds detected in the EO, postharvest management significantly influenced 21 compounds in *M. spicata* var. *viridis* (Table 5). The main EO compound, carvone, was 33.6% of the EO in the fresh samples and significantly different from the content in both the DH and OD samples, in which it was 36.6% and 27.8% higher, respectively. Conversely, limonene, the other main monoterpene, was higher in the fresh samples and differed significantly from both the DH and OD samples, in which the content was ca. 30% lower in both. The content of the third main compound, 1,8-cineole, did not change statistically in either the fresh or OD samples, which were both significantly different from the DH samples, in which 34.2% less 1,8-cineole was reported. The β-caryophyllene content was the highest in the OD samples and differed statistically by 32.3% from both the fresh and DH samples.

Of the 32 compounds detected in the EO, postharvest management influenced 10 compounds in *C. nepeta* (Table 6). The predominant compound, pulegone, which on average represented 69.9% of the

EO, was not influenced by the treatments. Limonene was the first monoterpene to be significantly influenced by postharvest management, and the OD samples had 36.2% less limonene than the fresh samples, while no statistical differences were found between the DH samples and the other treatments. The sesquiterpenes, germacrene D and β-caryophyllene, were higher in content when *C. nepeta* was OD. Their contents in the OD samples were significantly different from those of the fresh and DH samples, which were not statistically different from each other. The germacrene D and β-caryophyllene values from the OD samples were 86.5% and 157.3% higher than the fresh samples, respectively.

Of the compounds detected in the EO of the species evaluated in this work, the most relevant were pulegone, carvone, limonene, menthol, 1,8-cineole, β-caryophyllene, germacrene D, and β-bourbonene.

Pulegone was the most abundant and the main compound in the *C. nepeta* EO (more than 70%), while it was present in a lower amount in both *M. spicata* var. *rubra* and *M. spicata* var. *viridis*. It was only affected by postharvest management in the latter species, a result that is in contrast with what has been reported by Asekun *et al.*[34] for *M. longifolia* var. *capensis*, another spearmint. The authors found that only the OD process led to a significant composition modification of pulegone, and resulted in the virtual disappearance of pulegone, as well as of menthone and 1,8-cineole, compared to fresh, air- and sun-dried samples. They suggested a compound evaporation or a chemical conversion during the OD process. Although the herbs in our experiment were oven-dried at higher temperatures than the herbs tested by Asekun *et al.*[34] (60 °C vs. 40 °C), the pulegone concentration in *M. spicata* var. *viridis* increased more than 3 fold and it is possible to hypothesize that a higher reactivity of other compounds in this species led the pulegone, an oxygenated terpene, to increase its relative presence in the oil. Venskutonis,[12] studying thyme constituents, found a similar tendency for oxygenated terpenes, and hypothesised that the oxidation of some non-oxygenated compounds occurs during drying. Furthermore, it is possible to hypothesize that the reason why pulegone is present in a low quantity in the EO of mint species is that this monoterpene is an intermediate compound in the

menthol compound group and it is synthesised from limonene,[35] as it is driven by the regioselectivity of the hydroxilase enzymes, depending on the species, which can co-act to produce pulegone-menthol in *M.* × *piperita* and the carvone compound group in *M. spicata.*[33] Thus, it is possible to assume that the postharvest management systems blocked the enzyme activities and other compounds.

Carvone was the most abundant compound in spearmint EO. It reached approximately 66% of *M. spicata* var. *rubra* EO, and 34% of *M. spicata* var. *viridis* EO, while it was completely undetected in *C. nepeta*. Although *M. spicata* var. *rubra* and *M. spicata* var. *viridis* belong to the same species, a different response occurred during the postharvest management. The carvone remained stable in *M. spicata* var. *rubra* during the DH process and decreased during the OD process. The same trend was observed for its precursor **limonene**. In the *M. spicata* var. *viridis* EO, which had less carvone and more limonene than the *M. spicata* var. *rubra* EO, the former compound increased during both the DH and the OD processes, while the latter decreased in both processes. These results have confirmed that carvone is biogenetically related to and formed by limonene,[33] and that limonene is susceptible to OD processes as has also been reported for lavandin (*Lavandula* × *intermedia* Emeric ex Loisel.).[36] However, the contemporaneous decrease in carvone and limonene in *M. spicata* var. *rubra* is not clear and has not been supported by other findings for the related species.

1,8-Cineole was another main monoterpene that was found, but its response did not result to be clear. It did not change on the basis of the postharvest management in *M. spicata* var. *rubra*; it statistically decreased in the DH samples, which were significantly different from the fresh and OD samples in *M. spicata* var. *viridis*; it increased in the OD samples, and these were significantly different from the fresh and DH samples in *C. nepeta*. All these data have not been supported by literature data, which indicate that 1,8-cineole decreases with an increase in the drying temperature.[12,37] Di Cesare *et al.*[37] found a 44% retention of 1,8-cineole in air-dried basil at 50 °C, compared to fresh raw basil. No other references have been found and further studies are therefore needed since it seems that an intra-specific variability exists that is influenced by postharvest management.

Finally, **β-caryophyllene**, **germacrene D** and **β-bourbonene** were the main sesquiterpene compounds, and they were all statistically influenced by postharvest management in the *Mentha* genus, while only the first two compounds were statistically influenced in *C. nepeta*. These compounds had a similar response; they were higher in content when the herbs were OD and also increased slightly in the DH samples. Similar results on sesquiterpenes were obtained in our previous works on other *Lamiaceae* species[14] and in studies by other authors.[38]

Postharvest management had a significant effect on the concentrations of some other minor compounds, although their presence could be considered irrelevant compared to the total EO composition.

CONCLUSION

The species studied have produced different phytomass yields, total EO contents per fresh mass and EO yields per phytomass obtained in the field. Although the greatest amount of phytomass was produced by *M. spicata* var. *rubra*, the EO content per fresh weight and EO yield per square meter of phytomass produced were higher in *C. nepeta* than in both the *M. spicata* varieties. Significant differences were expected in the EO profile, between the two species. However, significant intra-specific differences in *M. spicata* were also found, especially due to the postharvest management that was adopted. Postharvest management has been confirmed to affect the EO content, leading to a higher yield when the dehumidifying process is used instead of the oven-drying process. The DH process is a relatively new postharvest technology that has shown positive results in terms of EO yield, and it can be applied to species which have a high EO value; however, further studies are necessary to investigate the different responses, in terms of EO profile, of different species. Using low temperatures, combined with the DH technique, could in fact limit the duration of the process, compared to the widespread convective OD method (2 vs. 6 days), which would allow the drying cycle to be increased, and would thus lead to a decrease in the costs for postharvest processing and a decrease in time, which in turn would enhance factory efficiency.

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The authors have contributed equally to the research and to the manuscript.

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References

1. González-Tejero MR, Casares-Porcel M, Sánchez-Rojas CP, Ramiro-Gutiérrez JM, Molero-Mesa J, Pieroni A, Giusti ME, Censorii E, de Pasquale C, Della A, Paraskeva-Hadijchambi D, Hadjichambis A, Houmani Z, El-Demerdash M, El-Zayat M, Hmamouchi M and ElJohrig S, Medicinal plants in the Mediterranean area: Synthesis of the results of the project Rubia. *J Ethnopharmacol* **116**: 341-357 (2008).
2. Nicola S, Hoeberecht J, Fontana E and Saglietti D, Medicinal and aromatic plant in Italy: situation and perspective for the Piedmont Region. *Acta Hortic* **629**: 375-382 (2004).
3. ISTAT, *Struttura e produzioni delle aziende agricole 2005*. http://www.istat.it/dati/dataset/20050421_00/tav04aep.htm [18 November 2010] and http://www.istat.it/dati/dataset/20050421_00/tav04sep.htm [18 November 2010].
4. Pignatti S, 655. *Mentha*, L. - Menta, in *Flora d'Italia*, ed. by Pignatti S. Il sole 24 ore - Ed. Agricole, Bologna, pp. 494-499 (2002).
5. Crozier A, Yokota T, Jaganath IB, Marks SC, Saltmarsh M and Clifford MN, Chapter 7: Secondary metabolites in fruits, vegetables, beverages and other plant-based dietary components, in *Plant secondary metabolites. Occurrence, structure and role in the human diet*, ed. by Crozier A, Clifford MN and Ashihara H. Blackwell Publishing, Oxford, UK, pp. 208-302 (2007).
6. Guarrera PM, Salerno G and Caneva G, Folk phytotherapeutical plants from Maratea area (Basilicata, Italy). *J Ethnopharmacol* **99**: 367-378 (2005).

7. Lentini F and Venza F, Wild food plants of popular use in Sicily. *J Ethnobiol Ethnomed* **3**: 15 http://www.ethnobiomed.com/content/3/1/15, doi:10.1186/1746-4269-3-15 (2007).
8. Perrino P, Laghetti G and Terzi M, Modern concept for sustainable use of plant genetic resources in the Mediterranean natural protected areas: the case study of the Alta Murgia Park (Italy). *Genet Resour Crop Evol* **53**: 695-710 (2006).
9. Sarac N and Ugur A, The *in vitro* antimicrobial activities of the essential oils of some *Lamiaceae* species from Turkey. *J Med Food* **12**(4): 902-907 (2009).
10. Grayer RJ, Kite GC, Goldstone FJ, Bryan SE, Paton A and Putievsky E, Infraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum. Phytochem* **43**(5): 1033-1039 (1996).
11. Szumny A, Figiel A, Gutiérrez-Ortíz A and Carbonell-Barrachina ÁA, Composition of rosemary essential oil (*Rosmarinus officinalis*) as affected by drying method. *J Food Eng* **97**: 253-260 (2010).
12. Venskutonis PR, Effect of drying on the volatile constituents of thyme (*Thymus vulgaris* L.) and sage (*Salvia officinalis* L.). *Food Chem* **59**(2): 219-227 (1997).
13. Müller J and Heindl A, Drying of medicinal plants, in *Medicinal and aromatic plant,* ed. by Bogers, RJ, Craker LE and Lange D. Springer, The Netherlands, pp. 237-252 (2006).
14. Tibaldi G, Fontana E and Nicola S, Cultivation practices do not change the *Salvia sclarea* L. essential oil but drying process does. *J Food Agric Environ* **8**(3&4): 790-794 (2010).
15. Tibaldi G, Fontana E and Nicola S, Growing conditions and postharvest management can affect the essential oil of *Origanum vulgare* L. ssp. *Hirtum* (Link) Ietswaart. *Ind Crops Prod* **34**: 1516-1522 (2011).
16. Phoungchandang S, Simulation Model for Heat Pump-Assisted Dehumidified Air Drying for Some Herbs. *World J Agric Sci* **5**(2): 138-142 (2009).
17. Adapa PK and Schoenau GJ, Re-circulating heat pump assisted continuous bed drying and energy analysis. *Int J Energy Res* **29**(11): 961-972 (2005).

18. Ram D, Ram M and Singh R, Optimization of water and nitrogen application to menthol mint (*Mentha arvensis* L.) through sugarcane trash mulch in a sandy loam soil of semi-arid subtropical climate. *Bioresour Technol* **97**: 886-893 (2006).
19. Singh MK and Saini SS, Planting date, mulch, and herbicide rate effects on the growth, yield, and physiochemical properties of menthol mint (*Mentha arvensis*). *Weed Technol* **22**: 691-698 (2008).
20. Hammer K, Laghetti G and Pistrick K, *Calamintha nepeta* (L.) Savi and *Micromeria thymifolia* (Scop.) Fritsch cultivated in Italy. *Genet Resour Crop Evol* **52**: 215-220 (2004).
21. Zheljazkov V and Topalov V, Effect of planting time and density on yields from rooted mint cuttings. *J Herbs Spices Med Plants* **4**(3): 15-24 (1997).
22. Zheljazkov VD, Cantrell CL, Astatkie T and Ebelhar MW, Productivity, oil content, and composition of two spearmint species in Mississippi. *Agron J* **102**: 129-133 (2010).
23. Telci I, Sahbaz N, Yilmaz G and Tugay ME, Agronomical and chemical characterization of spearmint (*Mentha spicata* L.) originating in Turkey. *Econ Bot* **58**(4): 721-728 (2004).
24. Zheljazkov VD, Cantrell CL and Astatkie T, Study on Japanese cornmint in Mississippi. *Agron J* **102**: 697-703 (2010).
25. Kokkini S, Karousou R and Lanaras T, Essential oils of spearmint (carvone-rich) plants from the Island of Crete (Greece). *Biochem Syst Ecol* **23**(4): 425-430 (1995).
26. Alkire BH and Simon JE, Response of Midwestern peppermint (*Mentha* × *piperita* L.) and native spearmint (*M. spicata* L.) to rate and form of nitrogen fertilizer. *Acta Hortic* **426**: 537-549 (1996).
27. Riela S, Bruno M, Formisano C, Rigano D, Rosselli S, Saladino ML and Senatore F, Effects of solvent-free microwave extraction on the chemical composition of essential oil of *Calamintha nepeta* (L.) Savi compared with the conventional production method. *J Sep Sci* **31**: 1110-1117 (2008).
28. Kokkini S and Vokou D, *Mentha spicata* (*Lamiaceae*) chemotypes growing wild in Greece. *Econ Bot* **43**(2): 192-202 (1989).

29. Edris AE, Shalaby AS, Fadel HM and Abdel-Wahab MA, Evaluation of a chemotype of spearmint (*Mentha spicata* L.) grown in Siwa Oasis, Egypt. *Eur Food Res Technol* **218**: 74-78 (2003).

30. Orio L, Cravotto G, Binello A, Pignata G, Nicola S and Chemat F, Hydrodistillation and *in situ* microwave-generated hydrodistillation of fresh and dried mint leaves: a comparison study. *J Sci Food Agric* **92**: xxx-xxx. DOI 10.1002/jfsa.5730.

31. Lorenzo D, Paz D, Dellacassa E, Davies P, Vila R and Cañigueral S, Essential oil of *Mentha pulegium* and *Mentha rotundifolia* from Uruguay. *Braz Arch Biol Technol* **45**(4): 519-524 (2002).

32. Croteau RB, Davis EM, Ringer KL and Wildung MR, (–)-Menthol biosynthesis and molecular genetics. *Naturwissenschaften* **92**: 562-577 (2005).

33. Humphrey A and Beale MH, Chapter 3: Terpenes, in *Plant secondary metabolites. Occurrence, structure and role in the human diet*, ed. by Crozier A, Clifford MN and Ashihara H. Blackwell Publishing, Oxford, UK. pp. 47-101 (2007).

34. Asekun OT, Grierson DS and Afolayan AJ, Effects of drying methods on the quality and quantity of the essential oil of *Mentha longifolia* L. subsp. *capensis*. *Food Chem* **101**: 995-998 (2007).

35. Alonso WR, Rajaonarivony JIM, Gershenzon J and Croteau R, Purification of 4S-limonene synthase, a monoterpene cyclase from the glandular trichomes of peppermint (*Mentha* x *piperita*) and spearmint (*Mentha spicata*). *J Biolog Chem* **267**(11): 7582-7587 (1992).

36. Baydar H and Erbaş S, Effects of harvest time and drying on essential oil properties in lavandin (*Lavandula* × *intermedia* Emeric ex Loisel). *Acta Hortic* **826**: 377-382 (2009).

37. Di Cesare LF, Froni E, Viscardi D and Nani RC, Changes in the chemical composition of basil caused by different drying procedures. *J Agric Food Chem* **51**: 3575-3581 (2003).

38. Jerković I, Mastelić J and Miloš M, The impact of both the season of collection and drying on the volatile constituents of *Origanum vulgare* L. ssp. *hirtum* grown wild in Croatia. *Int J Food Sci Technol* **36**: 649-654 (2001).

Tables

Table 1.

Effect of the species on the phytomass yield, the total essential oil (EO) content on fresh weight and the EO yield of fresh phytomass.

Species	Phytomass yield ($g\ m^{-2}$)	Total EO content ($\%\ ^{w}/_{w}$)	EO yield ($g\ m^{-2}$)
M. spicata var. *rubra*	1996.5a*	0.133b	2.61b
M. spicata var. *viridis*	1272.2b	0.194b	2.50b
C. nepeta	1570.2b	0.242a	3.75a
Significance (P=)	*<0.001*	*0.001*	*0.008*

* Values followed by the same letter, within the same column, are not significantly different according to Tukey's multiple range test P≤0.05.

Table 2.

Average composition of the essential oil (EO) distilled from fresh *M. spicata* var. *rubra*, *M. spicata* var. *viridis*, and *C. nepeta* grown in Northern Italy.

RT*	**EO Compound**	*M. spicata* var. *rubra* (%EO)	*M. spicata* var. *viridis* (%EO)	*C. nepeta* (%EO)
6.69	α-pinene	0.55	1.29	0.69
7.70	sabinene	0.51	1.24	0.46
7.83	β-pinene	0.92	2.08	1.12
8.10	myrcene	1.52	1.45	0.72
8.22	3-octanol	0.29	1.15	0.41
8.22	β-phellandrene	--	--	--
8.86	α-terpinene	0.15	0.58	--
9.21	limonene	12.67	26.92	3.54
9.31	1,8-cineole	3.01	14.49	0.23
9.68	β-ocimene	0.27	0.31	--
10.06	γ-terpinene	0.29	0.91	0.11
10.37	sabinene hydrate	0.44	0.68	0.28
10.93	α-terpinolene	0.13	0.27	--

12.86	menthone	--	--	0.25
13.17	menthofuran	--	--	--
13.17	isomenthone	--	--	2.39
13.41	menthol	--	--	0.41
13.51	isopulegone	--	--	1.89
13.75	neo menthol	--	--	0.44
13.95	4 terpineol	0.55	1.21	0.20
14.12	dihydrocarvone	1.37	0.57	--
14.75	carveol	0.29	0.12	0.13
15.41	pulegone	0.30	0.29	73.65
15.48	carvone	65.96	33.60	--
15.79	*trans*-piperitone	0.36	--	0.33
16.75	menthyl acetate	--	--	0.17
18.17	*cis*-piperitone	--	--	4.56
18.81	*cis*-piperitone oxide	--	--	2.48
19.35	β-bourbonene	1.89	2.00	0.12
19.44	β-elemene	0.71	0.75	0.11
20.29	β-caryophyllene	3.13	3.09	0.95
21.15	α humulene	0.28	0.11	0.11
21.83	germacrene D	0.74	1.86	2.35
	TOTAL	96.33^	94.94^	99.11^

* RT= retention time expressed in min; ^= the total % considers also random compounds not calculated in the single compound average.

Table 3

Orthogonal comparisons of the EO content of each species (*M. spicata* var. *rubra*, *M. spicata* var. *viridis*, *C. nepeta*).

	Species		
	M. spicata var. *rubra*	*M. spicata* var. *viridis*	*C. nepeta*
Postharvest management	EO content (% $^{w}/_{w}$)		
Fresh herbs	0.133	0.194	0.242
Processed herbs*	0.066	0.220	0.183
Significance (P=)	*<0.001*	*<0.001*	*<0.001*

DH herbs	0.118	0.274	0.265
OD herbs	0.014	0.166	0.101
Significance (P=)	*<0.001*	*<0.001*	*<0.001*

* Processed herbs= Dehumidified herbs + Oven-dried herb pooled treatments.

Table 4

Effect of postharvest management on the essential oil (EO) composition of *M. spicata* var. *rubra* grown in Northern Italy.

M. spicata* var. *rubra	Postharvest management			
	Fresh herbs	DH herbs	OD herbs	
Compound	(%EO)	(%EO)	(%EO)	Significance (P=)
carvone	65.96a*	62.35a	54.44b	*<0.001*
limonene	12.67a	13.57a	6.84b	*<0.001*
β-caryophyllene	3.13b	3.36b	9.95a	*<0.001*
1,8-cineole	3.01	3.36	3.25	0.622
β-bourbonene	1.89b	2.03b	5.54a	*<0.001*
myrcene	1.52a	1.80a	0.65b	*<0.001*
dihydrocarvone	1.37a	1.28a	0.81b	*<0.001*
β-pinene	0.92	1.17	0.83	0.137
germacrene D	0.74b	0.83b	2.10a	*<0.001*
β-elemene	0.71b	0.71b	2.35a	*<0.001*
α-pinene	0.55ab	0.79a	0.47b	*0.037*
4 terpineol	0.55a	0.33b	0.54a	*<0.001*
sabinene	0.51ab	0.68a	0.35b	*0.018*
sabinene hydrate	0.44b	0.61a	0.23c	*<0.001*
trans-piperitone	0.36a	0.27b	0.30ab	*0.034*
3-octanol	0.29a	0.23b	0.14c	*<0.001*
γ-terpinene	0.29b	0.32b	0.63a	*<0.001*
carveol	0.29ab	0.23b	0.33a	*0.011*
pulegone	0.30	0.17	0.94	0.061
α humulene	0.28b	0.30b	0.77a	*<0.001*
β-ocimene	0.27a	0.26a	0.07b	*0.005*
α-terpinene	0.15b	0.19b	0.37a	*<0.001*
α-terpinolene	0.13b	0.16b	0.23a	*<0.001*

β-phellandrene	--	0.11b	0.19a	*0.011*

* Values followed by the same letter, within the same row, are not significantly different according to Tukey's multiple range test P≤0.05.

Table 5

Effect of postharvest management on the essential oil (EO) composition of *M. spicata* var. *viridis* grown in Northern Italy.

M. spicata* var. *viridis	Postharvest management			
	Fresh herbs	DH herbs	OD herbs	
Compound	(%EO)	(%EO)	(%EO)	Significance (P=)
carvone	33.60b*	45.92a	42.95a	*<0.001*
limonene	26.92a	18.56b	19.00b	*<0.001*
1,8-cineole	14.49a	9.54b	13.31a	*<0.001*
β-caryophyllene	3.09b	3.39b	4.09a	*<0.001*
β-pinene	2.08a	1.55b	1.90a	*0.003*
β-bourbonene	2.00b	1.77b	2.50a	*<0.001*
germacrene D	1.86b	2.46a	2.58a	*<0.001*
myrcene	1.45a	1.00b	0.97b	*<0.001*
α-pinene	1.29	1.07	1.26	0.050
sabinene	1.24a	1.05b	1.06b	*0.022*
4 terpineol	1.21a	0.65b	0.82b	*<0.001*
3-octanol	1.15a	0.80b	0.82b	*<0.001*
γ-terpinene	0.91a	0.54b	0.93a	*<0.001*
β-elemene	0.75b	0.74b	1.12a	*<0.001*
sabinene hydrate	0.68b	1.50a	0.93b	*<0.001*
α-terpinene	0.58a	0.35b	0.67a	*<0.001*
dihydrocarvone	0.57	0.42	0.64	0.062
β-ocimene	0.31a	0.22b	0.16c	*<0.001*
pulegone	0.29b	0.26b	0.96a	*0.001*
α-terpinolene	0.27a	0.18b	0.27a	*<0.001*
carveol	0.12b	0.14a	--	*<0.001*
α humulene	0.11	0.17	0.29	0.313
β-phellandrene	--	0.11b	0.19a	*0.045*
trans-piperitone	--	0.03b	0.18a	*0.002*

neo menthol	--	--	0.63	--
menthone	--	--	0.34	---
menthofuran	--	--	0.35	---
isomenthone	--	--	0.32	---

* Values followed by the same letter, within the same row, are not significantly different according to Tukey's multiple range test P≤0.05.

Table 6

Effect of postharvest management on the essential oil (EO) composition of *C. nepeta* grown in Northern Italy.

C. nepeta	Postharvest management			
	Fresh herbs	DH herbs	OD herbs	
Compound	(%EO)	(%EO)	(%EO)	Significance (P=)
pulegone	73.65	72.03	64.00	0.279
cis-piperitone	4.56	4.80	4.78	0.953
limonene	3.54a*	2.47ab	2.26b	*0.035*
cis-piperitone oxide	2.48	3.90	4.22	0.243
isomenthone	2.39b	3.44a	--	*0.003*
germacrene D	2.35b	2.63b	4.38a	*0.013*
isopulegone	1.89	1.61	2.38	0.274
β-pinene	1.12	0.86	1.04	0.490
β-caryophyllene	0.95b	1.06b	2.44a	*0.016*
myrcene	0.72	0.60	0.52	0.244
α-pinene	0.69	0.58	0.60	0.677
sabinene	0.46	0.43	0.35	0.067
neo menthol	0.44	--	0.98	0.146
menthol	0.41	0.11	--	0.717
3-octanol	0.41	0.34	0.37	0.706
trans-piperitone	0.33	0.27	0.41	0.367
sabinene hydrate	0.28b	0.53a	0.31b	*<0.001*
menthone	0.25b	0.34b	5.38a	*<0.001*
1,8-cineole	0.23b	0.15b	0.49a	*<0.001*
4 terpineol	0.20b	0.47a	--	*0.002*
menthyl acetate	0.17b	0.63a	0.24b	*0.015*

carveol	0.13	--	--	---
β-bourbonene	0.12	0.48	0.35	0.542
β-ocimene	--	0.05b	0.21a	*<0.001*
α humulene	0.11	0.16	0.34	0.188
γ-terpinene	0.11	0.10	0.41	0.098
β-elemene	0.11	0.10	0.25	0.051
dihydrocarvone	--	0.17	0.28	0.066
α-terpinolene	--	0.07b	0.25a	*0.022*
α-terpinene	--	0.06	0.24	0.256
β-phellandrene	--	0.04b	0.07a	*0.017*
menthofuran	--	0.01	--	---

* Values followed by the same letter, within the same row, are not significantly different according to Tukey's multiple range test P≤0.05.