

Effect of Drying Method on the Phenolic Content and Antioxidant Capacity of Spearmint

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Abstract

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The changes in total phenolics, hydroxycinnamic acid derivatives, and antioxidant properties of spearmint after five drying treatments (convection oven drying, freeze-drying, microwave drying, and air drying with the sun exposure and without the sun exposure) were investigated. Phenolic composition of dried spearmint was analysed by spectrophotometric assays, while DPPH radical scavenging activity and Ferric reducing/Antioxidant power (FRAP) assay was used to measure the antioxidant properties. The results showed that freeze drying produced dried spearmint that had the highest total phenolics (34.6 ± 1.9 mg/g) content and the most potent antioxidant capacity (126.2 ± 0.4 mg/g for FRAP and 88.1 ± 5.9 mg/g for DPPH, respectively). On the other hand, spearmint that was dried by convection oven and microwave drying presented the lowest amount of phenolic compounds (12.0 ± 0.5 mg/g) and antioxidant potency (49.3 ± 0.7 mg/g for FRAP and 26.9 ± 1.6 mg/g for DPPH, respectively). This might be attributed to the fact that heat-sensitive phenolics were degraded or biotransformed at high temperatures. The loss of phenolic compounds and antioxidant activity reached up to 60% compared to freeze drying.

Keywords: air-drying; freeze-drying; hydroxycinnamic acid; *Mentha viridis*; microwave drying; phytochemical

Herbs have attracted the scientific interest of the biotechnology, cosmetics, pharmaceutical and food industry since they have been used for many purposes as medicinal, flavouring, beverages, dyeing, fragrances, cosmetics, smoking and other industrial uses. Herbs are an excellent source of phytochemicals with potent antioxidative effects (PAPAGEORGIOU *et al.* 2008). *Mentha* belongs to *Lamiaceae* family and includes about 25 species. Leaves, flowers, and the stem of *Mentha* spp. are frequently used as herbal teas or as additives in foods to offer aroma and flavour or to protect against food-pathogens bacteria (GIBRIEL *et al.* 2011). *Mentha viridis* is usually consumed in Cyprus as a dried product for herbal tea or as an additive in Cypriot traditional cheese called halloumi.

Drying is considered as a critical factor for the postharvest management and the merchantability of herbs. Fresh *Lamiaceae* herbs as spearmint usually

contain 75–80% water, and the water levels need to be lowered to less than 15% for their successful preservation (DÌAZ-MAROTO *et al.* 2002). The drying of herbs inhibits microbial growth and forestalls biochemical changes but, at the same time, it can give rise to other changes that affect the herb quality. The changes in appearance and aroma are caused by losses in volatiles or the formation of new volatiles as a result of oxidation or esterification reactions. In addition, the drying of herbs is often accompanied with the loss of bioactive compounds, which may possess antioxidant activity and other health-promoting properties (HOSSAIN *et al.* 2010).

Air-drying is a traditional, low cost technique that is used to lower the water content of herbs at low temperatures. The drying at low temperatures protects against the degradation of the active constituents, but it is slow and metabolic processes may continue longer, which may lead to quality

loss of the aromatic plants and subsequently of the produced added value products (KEINÄNEN & JULKUNEN-TIITTO 1996). Many drying methods such as convection oven drying, freeze-drying, microwave drying etc. are also used to preserve medicinal herbs (HARBOURNE *et al.* 2009). To date, the studies have been mainly focused on the influence of the drying methods on the essential oil or volatile fractions in order to optimise the drying (VENSKUTONIS 1997; DÍAZ-MAROTO *et al.* 2003; FIGIEL *et al.* 2010).

The objective of the present study was to compare different methods for the drying of spearmint. Five common drying methods i.e. convection oven drying (CD), freeze-drying (FD), microwave drying (MD), and air drying with the sun exposure (SUD) and without it (SHD) were evaluated in terms of phenolic content and antioxidant capacity.

MATERIAL AND METHODS

Reagents and samples. Standards of gallic acid, caffeic acid, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St Louis, USA). 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), 4,6-tripyridyls-triazine (TPTZ), iron^(III) chloride, and all other common reagents and solvents were obtained from Sigma-Aldrich (St Louis, USA). Folin-Ciocalteu reagent and ethanol (analytical grade) were provided by Scharlau (Barcelona, Spain).

Fresh spearmint (*Mentha viridis*) was provided from local organic producer (Lemesos District, Cyprus). The leaves were separated from the stems and subsequently dried with different methods.

Drying methods. Five drying methods were applied to remove moisture content from spearmint until constant weight. The initial moisture of the leaves was $87.42 \pm 0.40\%$. Table 1 summarises the equipment used and the duration of the drying process. Dried material was stored in a desiccator until phytochemical analysis.

Extraction of phenolic compounds. An ultrasound assisted extraction was used to extract phenolic compounds from the dried spearmint. In particular, approximately 0.167 g of the dried spearmint was extracted with 10 ml EtOH. The ultrasound irradiation was applied for 10 min at a power of 6 W by an ultrasound probe (Microson; Misonix Inc., Farmingdale, USA). The extraction was performed at 25°C using water bath. The ex-

tracts were filtered through vacuum equipment, diluted to 20 ml, and were kept at 4°C until analysis.

Determination of total phenolics by Folin-Ciocalteu method. The reaction mixture consisted of 0.2 ml of the diluted extract, 4.8 ml of distilled water, and 0.5 ml of the Folin-Ciocalteu reagent. After 3 min, 1 ml of saturated sodium carbonate solution was added and the mixture was made up to 10 ml with distilled water. The mixture was thoroughly mixed, allowed to stand for 1 h in the dark at the room temperature, and the absorbance was measured at 765 nm (Jenway 6505 UV-Vis-spectrophotometer, 160A; Keison, Essex, England). Total phenolic content was expressed as mg gallic acid equivalents (GAE) per 100 g of the dried spearmint (d.s.) (GOULAS & MANGANARIS 2011).

Determination of total phenolics and hydroxycinnamic acid derivatives. Briefly, 1 ml of each extract was mixed with 1 ml 0.1% HCl-ethanol solution and 8 ml 2% HCl-ethanol solution in a 10 ml volumetric flask. The absorbance was measured at 280 and 320 nm in order to evaluate total phenolic content and hydroxycinnamic acid derivatives, respectively. The corresponding standard curves to the above determinations were prepared using ethanolic solutions of gallic acid and caffeic acid and the results were expressed as mg GAE/100 g d.s. and mg caffeic acid equivalents (CAE)/100 g d.s. (OBIED *et al.* 2005).

Determination of DPPH radical scavenging activity. Two ml of each extract were mixed with 1 ml solution of DPPH[•] (0.3 mmol/l). The absorbance of the mixture was measured after 30 min incubation time in the dark at 517 nm. The radical scavenging activity was calculated using a standard curve of Trolox and expressed as mg Trolox/100 g d.s. (GOULAS & MANGANARIS 2011).

Determination of total antioxidant activity by FRAP assay. A sample containing 3 ml of freshly prepared Ferric reducing/Antioxidant power (FRAP) solution (0.3 mol/l acetate buffer (pH 3.6) containing 10 mmol/l TPTZ and 40 mmol/l FeCl₃·10H₂O) and 100 µl of the extract was incubated at 37°C for 4 min and the absorbance was measured at 593 nm. The absorbance change was converted into the FRAP value, by relating the change of absorbance at 593 nm of the test sample to that of the standard solution of Trolox, and the results were expressed as mg trolox/100 g d.s. (GOULAS & MANGANARIS, 2011).

Statistical analysis. Statistical analysis was carried out using the software package SPSS Version 20.0 (SPSS Inc., Chicago, USA) and the comparison of

Table 1. Equipment used, drying time and % final moisture content of spearmint for drying methods

Drying method	Equipment	Time	Final moisture (%)
CD	convection oven (Venticell 111, MMM Group, Germany)	7.5 h	10.6 ± 0.2
FD	laboratory-scale freeze dryer (Christ Alpha 1-4 LD plus, SciQuip Ltd., UK)	48 h	10.5 ± 1.2
MD	microwave oven 800 W (DKL Model W-8811)	80 s	8.0 ± 1.3
SUD	–	312 h	13.4 ± 0.4
SHD	–	312 h	13.9 ± 1.0

CD – convection oven drying; FD – freeze-drying; MD – microwave drying; SUD – air drying with the sun exposure; SHD – air drying without the sun exposure

averages of each treatment was based on the analysis of variance (one-way ANOVA) according to Duncan's multiple range test at the significance level 5% ($P \leq 0.05$). The results were presented as average ± standard error of three replications per sample.

RESULTS AND DISCUSSION

The results showed that all drying methods tested reduce the moisture content below 15%, which is the critical factor for the postharvest management of herbs (Table 1). Particularly, the moisture content ranged between $7.96 \pm 1.34\%$ to $10.64 \pm 0.18\%$ for the spearmint dried by instrumental drying methods, while SUD and SHD lowered the water content of spearmint to 13.35 ± 0.37 and $13.92 \pm 0.99\%$, respectively. The results revealed the superiority of the instrumental drying methods against the traditional methods. Furthermore, the main benefit of the instrumental drying methods is correlated with the dramatic decrease of the drying time (Table 1).

Total phenolic content of the dried spearmint was determined by two spectrophotometric assays that are based on different mechanisms. Fo-

lin-Ciocalteu method is based on the transfer of electrons in alkaline solution from the phenolic compounds to phosphomolybdic/phosphotungstic acid complexes, while the second method is based on maximum wavelength of phenolic compounds at 280 nm (MEDINA 2011). The results indicated the superiority of the FD method against the tested methods as the dried spearmint had the highest amount of phenolic compounds, regardless of the analytical method used (Figure 1). The drying of spearmint using convection oven and microwave oven gave the lowest phenolic content. Phenolic content was 1891 ± 20 mg GAE/100 g d.s. for MD and 1976 ± 61 mg GAE/100 g d.s. for CD according to Obied protocol. The traditional drying methods produced dried spearmint with higher phenolic content (2489 ± 183 and 2829 ± 62) compared to CD and MD, but they were inferior compared to FD.

Since rosmarinic acid and related compounds have been mainly found in spearmint (WANG *et al.* 2004), the hydroxycinnamic acid derivatives of dried spearmint were also evaluated. The results showed a similar trend with total phenolics highlighting the advantage of FD as a drying method for spearmint (Figure 2). Hydroxycinnamic acid content of freeze-dried spearmint was 2.2 fold higher than the corresponding content of microwave-dried spearmint.

Then, the antioxidant activity of the dried spearmint was determined since a close correlation between phenolic compounds and antioxidant activity of herbs belonging to Lamiaceae family has been demonstrated before (STAGOS *et al.* 2012). FRAP and DPPH assays were used to determine the antioxidant properties of the dried spearmint since a single assay can give only a reductive suggestion of the antioxidant potency and must be interpreted with some caution. The chemical complexity of spearmint could lead to scattered results, depending on the test employed (GOULAS & MANGANARIS 2011). The antioxidant assays used are based on

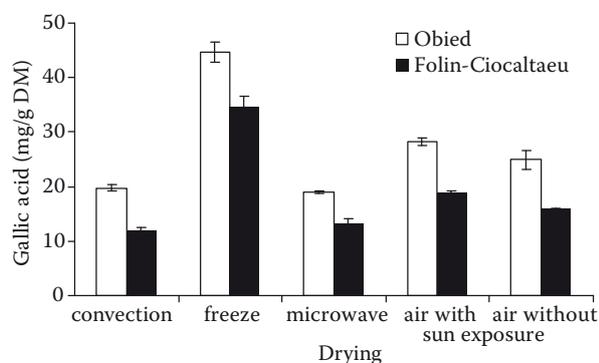


Figure 1. The effect of drying methods on phenolic content of dried spearmint (phenolic content was determined by Folin-Ciocalteu method and Obied protocol)

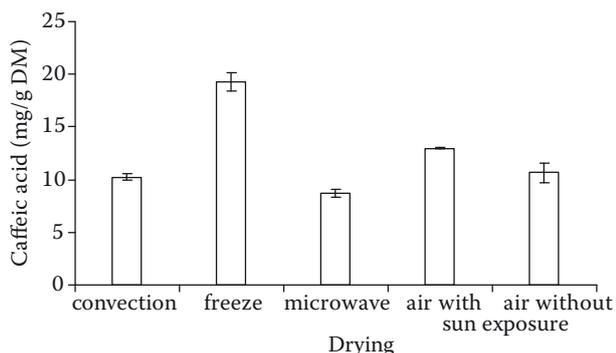


Figure 2. The effect of drying methods on hydroxycinnamic acid derivatives of dried spearmint

different mechanisms that measure the antioxidative effect of extracts. In particular, DPPH assay is based on the ability of antioxidants to act as radical scavengers and FRAP assay measures the ability of antioxidants to perform as reducing agents. Both antioxidant assays showed that FD was the most effective drying method (Figure 3). FRAP assay classified the drying method in the order FD > SUD > SHD > MD = CD, while the classification, based on DPPH assay, was slightly changed. Drying with convection oven produced dried spearmint with antioxidant activity resembling that obtained by drying with traditional methods.

Taking into consideration the results of phenolic composition and antioxidant properties, the evaluation of the drying methods showed that FD dominated against other methods used. Previous studies have also demonstrated the superiority of FD as compared to common drying methods for diverse plant materials (HARBOURNE *et al.* 2009; RIOS & GUTIÉRREZ-ROSALES 2010; GOULAS & MANGANARIS 2012). The benefits of FD can be attributed to the fact that this technique minimises the degradation of heat-sensitive spearmint compounds such as

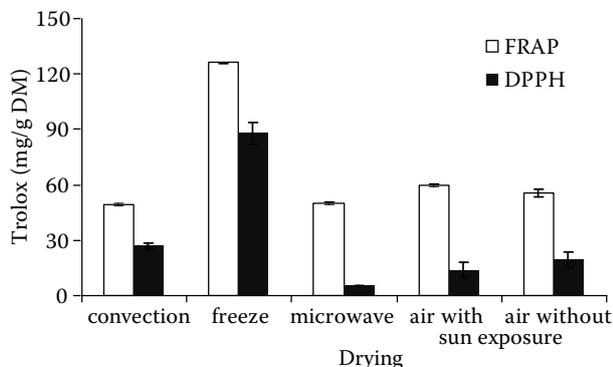


Figure 3. The influence of drying methods on antioxidant properties of dried spearmint as they were determined by FRAP and DPPH assays

antioxidant phenols because the dehydration of the plant material is performed at low temperatures (CHOU & CHUA 2001). In addition, the FD enhances the extractability of phenolic compounds from the dried spearmint since ice crystals formed within the sample matrix can rupture the cell structure, which allows the exit of cellular components and the access of solvent (GOULAS & MANGANARIS 2012).

The drying of spearmint with traditional methods induced a significant decrease of phenolic content and the antioxidant activity of in the dried spearmint compared to the freeze-dried spearmint. In particular, the phenolic content decreased by 36.6 and 44.3% for SUD and SHD, respectively. The loss of phenolic compounds with traditional drying methods may have been caused by enzymatic processes that occurred during drying. These drying methods could not inactivate the degradative enzymes such as polyphenol oxidases; therefore they are able to degrade phenolic compounds during long-time drying procedures (LIM & MURTIJAYA 2007). The lower loss of phenolic compounds in SUD compared to SHD can be attributed to the fact that the former drying method dehydrated spearmint at a higher temperature which resulted in faster inactivation of enzymes.

The loss of phenolic content in spearmint reached up to 55.7 and 57.6 % for CD and MD, respectively. A similar reduction of the antioxidant activity was also monitored. The latter decreased approximately to 60% with both drying methods. The application of heating inactivates enzymes rapidly but they may simultaneously degrade heat-sensitive phenolic compounds (LIM & MURTIJAYA 2007). Recent works also demonstrated that the temperature affects the stability of phenolic compounds in herbal infusions (RIEHLE *et al.* 2013). Furthermore, the biotransformation of hydroxycinnamic acids in aqueous solution with the application of microwave irradiation or heating has been described previously (DAWIDOWICZ & TYPEK 2010).

CONCLUSION

Drying is a very useful technique to extend the shelf-life of spearmint and to produce dried spearmint with a high phenolic content and a potent antioxidant activity. Significant effects of drying methods on phenolic composition and antioxidant properties of the dried material were found. Among the drying methods tested, FD produced the dried

spearmint of highest quality in terms of phenolic content and antioxidant potency. On the other hand, drying methods that use high temperatures to dehydrate spearmint cause a dramatic loss of phenolic compounds and antioxidant activity, which reached up to 60%. The evaluation of other drying methods that remove moisture content at low temperatures with the use of irradiation or vacuum stands a challenging prospective.

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