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Effect of drying method on the antioxidant capacity of six Lamiaceae herbs

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a b s t r a c t

The present study investigated the changes in total phenols (TP), rosmarinic acid content and antioxidant capacity of six Lamiaceae herbs (rosemary, oregano, marjoram, sage, basil and thyme) after three drying treatments (air-, freeze- and vacuum oven-drying) stored for 60 days at Â20 °C and compared to fresh samples. Ferric reducing antioxidant property (FRAP) and oxygen radical absorbance capacity (ORAC) were used as markers for antioxidant capacity. Air-dried samples had significantly ($p < 0.05$) higher TP, rosmarinic acid content and antioxidant capacity than had freeze-dried and vacuum oven-dried samples throughout the storage period. Fresh samples had the lowest values for the parameters tested. Vacuum oven-drying resulted in higher TP and FRAP values in rosemary and thyme during 60 days of storage than did freeze-drying. In ORAC assay, the difference was significantly higher only in thyme. Storage did not show any effect on the dried samples for the parameters tested.

1. Introduction

The food industry is becoming increasingly interested in aromatic herbs, mainly of the Lamiaceae family, due to growing consumer demands for healthy foods of natural origin. These are used, not only for flavouring, but also for other purposes, including their medicinal and anti-inflammatory properties or their antioxidant activities (Pizzoccaro, Mascaro, Caffa, Gasparoli, & Fedeli, 1987). In addition to the food industry, Lamiaceae herbs are also of high demand in dyeing, fragrances, cosmetics, beverages and smoking industries (Papageorgiou, Mallouchos, & Komaitis, 2008). Lamiaceae herbs are very rich sources of antioxidant compounds (Hossain, Brunton, Barry-Ryan, Martin-Diana, & Wilkinson, 2008; Shan, Cai, Sun, & Corke, 2005) which could be used in food preservation against oxidative damage. Lamiaceae herbs, namely rosemary, oregano, marjoram, sage, basil and thyme, are popular aromatic herbs, growing in many regions of the world. Immediately after harvesting, these highly perishable raw materials have to be preserved against deterioration and spoilage. Fresh Lamiaceae herbs usually contain 75–80% water, and water levels need to be lowered to less than 15% for their preservation (Diaz-Maroto, Perez Coello, & Cabezudo, 2002). Drying is by far the most widely used treatment. Drying of herbs inhibits microbial growth and forestalls certain biochemical changes but, at the same time, it can give rise to other alterations that affect herb quality, such as changes in appearance and alterations in aroma caused by losses in volatiles or the formation of new volatiles as a result of oxidation reactions or esterification reactions. Certain compounds (normally present) have been observed to increase in different herbs after drying: for example, eugenol in bay leaf (Diaz-Maroto et al., 2002), thymol in thyme (Venskutonis, 1997), and some sesquiterpenes in different herbs (Baritaux, Richard, Touche, & Derbesy, 1992; Yousif, Scaman, Durance, & Girard, 1999). Most studies have reported changes in colour and volatile compounds of the aromatic herbs after drying (Di Cesare, Forni, Viscardi, & Nani, 2003; Diaz-Maroto et al., 2002). Use of freeze-drying as the drying treatment has been reported to retain features that are closer to the characteristic appearance of the fresh plant (Venskutonis, 1997). However, information on changes in total phenol, rosmarinic acid content and antioxidant capacity of herbs after drying is very limited. For this reason, the present study investigated the effects of three drying methods; freeze-drying, vacuum oven-drying and air-drying on total phenols (TP), rosmarinic acid content and antioxidant capacity of six Lamiaceae herbs, as measured by ferric reducing antioxidant activity (FRAP) and oxygen radical absorbance capacity (ORAC) assays and compared them with the fresh products.

2. Materials and methods

2.1. Samples and reagents

Six different fresh Lamiaceae herbs were procured from Flc Premium Herbs Ltd. (Israel). All samples were cultivated in the northern Negev Desert, Israel (Latitude 30 30₀ ON, Longitude 34 55₀ OE,

annual rainfall 12 inches). The herbs were transported to Ireland in premium condition, at 1–3 °C, within 3 days after harvesting. Folin–Ciocalteu reagent, gallic acid, sodium acetate (anhydrous), ferric chloride hexahydrate, 2,4,6-tri(2-pyridyl)-s-triazine, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, sodium carbonate, fluorescein, 2,2′-azo-bis(2-amidinopropane) dihydrochloride and rosmarinic acid were purchased from Sigma–Aldrich.

2.2. Drying of fresh herbs

Each herb was divided into five batches. One was immediately used for extraction to measure the antioxidant capacity of the fresh herbs. Another batch was kept at À20 °C for analyzing the effect of storage on fresh herbs every 15 days until 60 days, in comparison to the dried herbs. The remaining batches were dried using one of the following drying methods: (a) drying at ambient temperature in a dark, well-ventilated room for 3 weeks (mean temperature 14 °C; mean relative humidity 10%); (b) drying in a vacuum oven (Gallenkamp, UK) at 70 °C for 16 h in the vacuum of 600 mbar; (c) freeze-drying in a frozen in time limited freeze-drier, model no. A 6/14 at a temperature of À54 °C and a pressure of 0.064 mbar for 72 h. All the dried samples, like the fresh ones, were analysed immediately (day 0), vacuum-packed and kept at À20 °C for analysing the effect of storage after drying every 15 days until 60 days. The film (75 lm thickness) of the vacuum pack pouches (Allfo vakuumverpackungen Hans Bresele KG, Germany) were composed of a mixture of polyamide (PA) and polyethylene (PE) with an oxygen and carbon dioxide permeability rate of 60 cm_3/m_2/24 h/atom and 180 cm_3/m_2/24 h/atom, respectively (23 °C, 75% RH). The water vapour permeability of the film was 2.7 g/m_2/24 h at 23 °C and 85% RH.

2.3. Preparation of extracts from fresh herbs

The fresh herbs were chopped into small pieces of approximately 0.5 cm_2 and milled into semi-paste by a kitchen blender (Kenwood Limited, UK). The semi-paste herb (4 g) was immediately mixed with 25 ml of methanol (80%) and homogenised with an Omni-prep multisample homogeniser (Omni International, USA) at 24,000 rpm. The homogenised sample suspension was shaken overnight at 1500 rpm at room temperature. The sample suspension was then centrifuged at 3000 rpm for 15 min and immediately filtered through 0.22 lm polytetrafluoethylene (PTFE) filters. The extracts were kept in À20 °C for subsequent analysis.

2.4. Preparation of extracts from dried herbs

The dried herbs were milled directly without chopping by a kitchen blender (Kenwood Limited, UK). The powdered herbs (0.5 g) were mixed with 25 ml of 80% methanol and homogenised with an Omni-prep multisample homogeniser (Omni International, USA) at 24,000 rpm. The homogenised sample suspension was shaken overnight with a V400 Multitude Vortexer (Alpha laboratories, North York, Canada) at 1500 rpm at room temperature. The sample suspension was then centrifuged for 15 min at 2000g (MSE Mistral 3000i, Sanyo Gallenkamp, Leicestershire, UK) and filtered through 0.22 lm polytetrafluoethylene (PTFE) filters. The extract was kept at À20 °C for subsequent analysis. FRAP and ORAC values were used as indicators of antioxidant capacity of the samples.

2.5. Determination of total phenols (TP)

The total phenolic content was determined using Folin–Ciocalteu reagent (FCR), as described by Singelton, Orthofer, and Lamuela-Raventos (1999). The experiment was performed in two batches which included three replications for each sample and standard. Methanolic gallic acid solutions (10–400 mg/l) were used as standards. In each replicate, 100 ll of the appropriately diluted sample extract, 100 ll of methanol, 100 ll of FCR and finally 700 ll of Na_2CO_3 (20%) were added together and vortexed. The mixture was incubated for 20 min in the dark and room temperature. After incubation, the mixture was centrifuged at 13,000 rpm for 3 min. The absorbance of the supernatant was measured at 735 nm by spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE)/100 g dry weight (DW) of the sample.

2.6. Ferric ion reducing antioxidant power (FRAP) assay

The FRAP assay was carried out as described by Stratil, Klejdus, and Kuban (2006) with slight modifications. The FRAP reagent was prepared by mixing 38 mM sodium acetate (anhydrous) in distilled water pH 3.6, 20 mM $FeCl_3Á6H_2O$ in distilled water and 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl in proportions of 10:1:1. This reagent was freshly prepared before each experiment. To each sample, 100 ll of appropriately diluted sample extract and 900 ll of FRAP reagent were added and the mixture was incubated at 37 °C for 40 min in the dark. In the case of the blank, 100 ll of methanol were added to 900 ll of FRAP reagent. The absorbance of the resulting solution was measured at 593 nm by spectrophotometer. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (a synthetic antioxidant), at concentrations from 0.1 to 0.4 mM, was used as a reference antioxidant standard. FRAP values were expressed as g trolox/100 g DW of the sample.

2.7. Oxygen radical absorbance capacity (ORAC)

ORAC assay was conducted using fluorescein ($C_{20}H_{10}Na_2O_5$) as the fluorescent probe, according to previously described procedures (Huang, Ou, Hampsch-Woodil, Flanaghan, & Deemer, 2002). The final assay mixture (200 ll) contained 150 ll of fluorescein (10 nM), 25 ll of AAPH (2,2′-azo-bis(2-amidinopropane) dihydrochloride, 240 mM), and 25 ll of sample extracts or phosphate buffer (10 mM phosphate buffer, pH 7.4) as the blank. The fluorescences of the assay mixtures were recorded at every 30 s cycle with the automated BMG FLUOstar Omega microplate reader system. The complete run was comprised of 250 cycles. This resulted in a fluorescence decay curve due to the oxidative degradation of fluorescein by AAPH. The decay of fluorescence was faster in the blank than in the antioxidant-containing sample extracts, as the antioxidant protected fluorescein from the damaging effect of AAPH. The area between the fluorescence decay curve of the blank and the sample extract was used to calculate the ORAC values of the samples. Trolox, at different concentrations (5–20 lM), was used to obtain a standard curve and to compare ORAC values of various samples. The data were analysed with the data analysis software, MARS, linked with Omega reader control software.

2.8. HPLC analysis of the extracts

Reversed phase high performance liquid chromatography (RP-HPLC) of the filtered sample extracts was carried out according to the method of Tsao and Yang (2003). The chromatographic system (Shimadzu-model no. SPD-M10A VP, Mason Technology, Dublin 8, Ireland) consisted of a pump, a vacuum degasser, a diode-array detector, and was controlled through EZ Start 7.3 software (Shimadzu) at 37 °C. An Agilent C18 column (15 cm Â 4.6 cm, 5 lm, Agilent Technologies, USA) was utilised with a binary mobile phase of 6% acetic acid in 2 mM sodium acetate (final pH 2.55, v/v, solvent A) and acetonitrile (solvent B). Solvent A was prepared, first, by making 2 mM sodium acetate water solution, which was

then mixed with acetic acid at a ratio of 94:6 by volume. All solvents were filtered through a 0.45 lm membrane filter prior to analysis. The flow rate was kept constant at 1.0 ml/min for a total run time of 80 min. The following gradient programme was carried out: 0–15% B in 45 min, 15–30% B in 15 min, 30–50% B in 5 min, 50–100% B in 5 min and 100–0% B in 10 min. The injection volume, for all the samples, was 10 ll. All the standards for quantification purposes were dissolved in methanol. The detector was set at 320 nm for monitoring of rosmarinic acid. Identification of the compounds was achieved by comparing their retention times and UV–Vis spectra with those of authenticated rosmarinic acid standard, by using the inline DAD with a 3D feature.

3. Results and discussion

3.1. Effect of drying on total phenol content and antioxidant capacity

Total phenol content, FRAP and ORAC values showed wide variation among the samples. The total phenolic content ranged from 0.96 g GAE/100 g DW in fresh sage at day 0 to 11.2 g GAE/100 g DW in air-dried thyme at day 0. The ranges of FRAP and ORAC values were 1.51–22.9 g Trolox/100 g DW and 8.45–48.0 g Trolox/100 g DW, respectively. Fresh sage samples had the lowest FRAP values and air-dried thyme had the highest among all the samples (Figs. 1–3). The highest oxygen radical absorbance capacity was reflected in total phenol and FRAP assays in air-dried thyme at day 0; however, the lowest ORAC value was observed in fresh rosemary at day 0 instead of fresh sage samples (Table 1). Storage did not have any significant ($p < 0.05$) effect on any of the assays tested except for the fresh samples (Figs. 1–3). The possible reason for this could be that the samples were vacuum-packed immediately after treatments and stored at À20 °C. This created an oxygen-free environment and the very low temperature prevented enzymatic oxidation of antioxidants. Therefore, there was no further degradation of antioxidants during storage which helped to maintain a similar antioxidant capacity and total phenolic content throughout storage. On the other hand, fresh samples, when stored at À20 °C, suffered severe chilling injury, due to their high moisture content and losing their integrity. This resulted in release of more antioxidant compounds in the extracts on day 15 (Figs. 1–3 and Table 1). Further injury did not occur during the rest of the storage period. For this reason there was no further increase in total phenols or antioxidant capacity of fresh samples after day 15. The very woody herb, thyme, was not affected by chilling injury. This was probably the reason why fresh thyme did not show any increase of total phenolic content or antioxidant capacity after being stored at À20 °C. All three drying methods exhibited strong influences on the total phenolic contents and antioxidant capacities of the herbs tested. Efficiencies of the drying methods were very similar, as they produced similar dry weights in samples after drying (Table 2). Identical amounts of dry weight from the dried samples were taken while doing the extraction. Therefore, there was no influence of residual moisture on the antioxidant capacity or total phenol content of the samples. Total phenol content and antioxidant capacity of the samples significantly increased when fresh samples were dried (Figs. 1–3 and Table 1). The lowest values of TP and antioxidant capacity of fresh samples were not due to the dilution effect caused by their high moisture content. Antioxidant capacities and total phenolic contents of fresh samples were corrected for their moisture contents. The range of moisture content of the Lamiaceae herbs was 71.2–89.6%, with basil having the highest and thyme the lowest moisture contents. Fresh weights of each of the fresh samples were converted into dry weights, on the basis of their respective moisture contents and then the dry weight was used for calculation of the antioxidant capacity and total phenolic

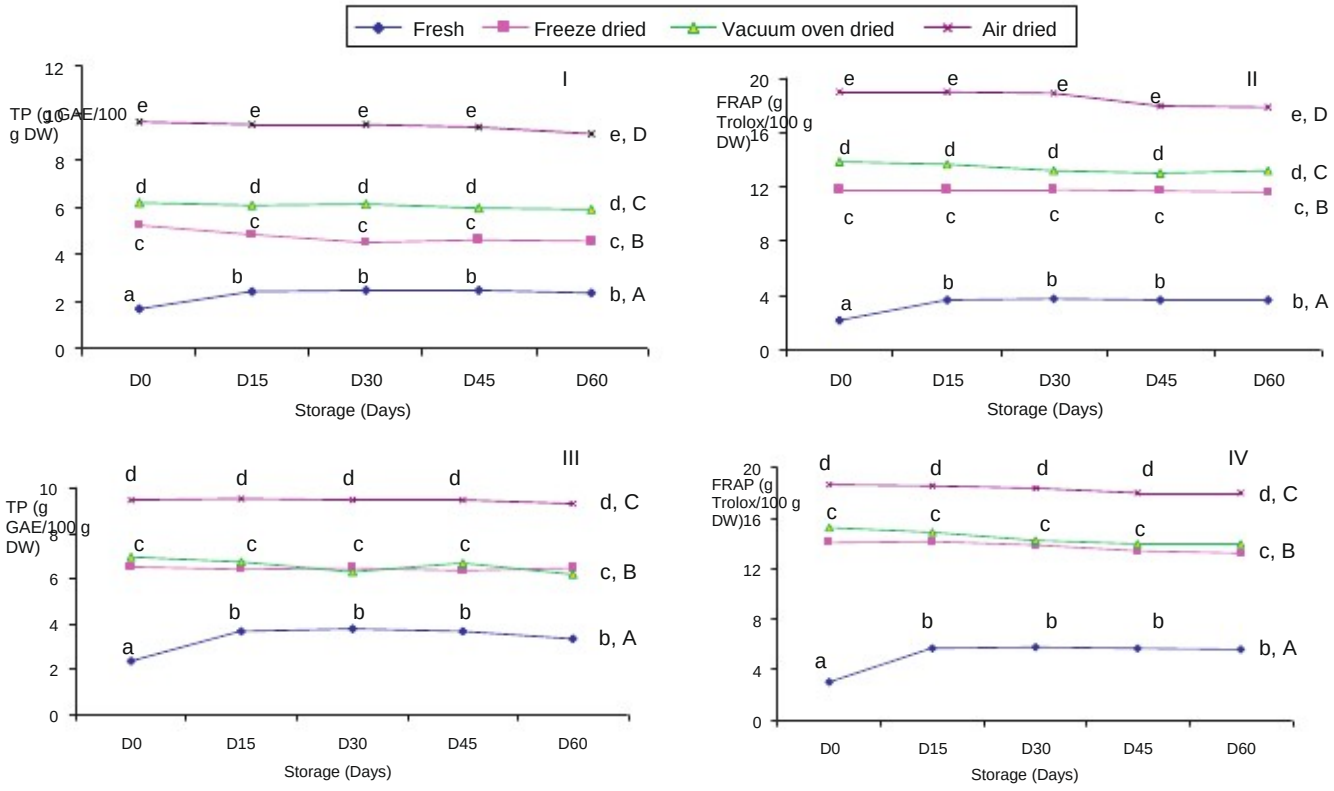

Fig. 1. Total phenol content (g GAE/100 g DW) and ferric ion reducing antioxidant power (g trolox/100 g DW) in rosemary (I for TP and II for FRAP) and oregano (III for TP and IV for FRAP) herbs during 60 days of storage.

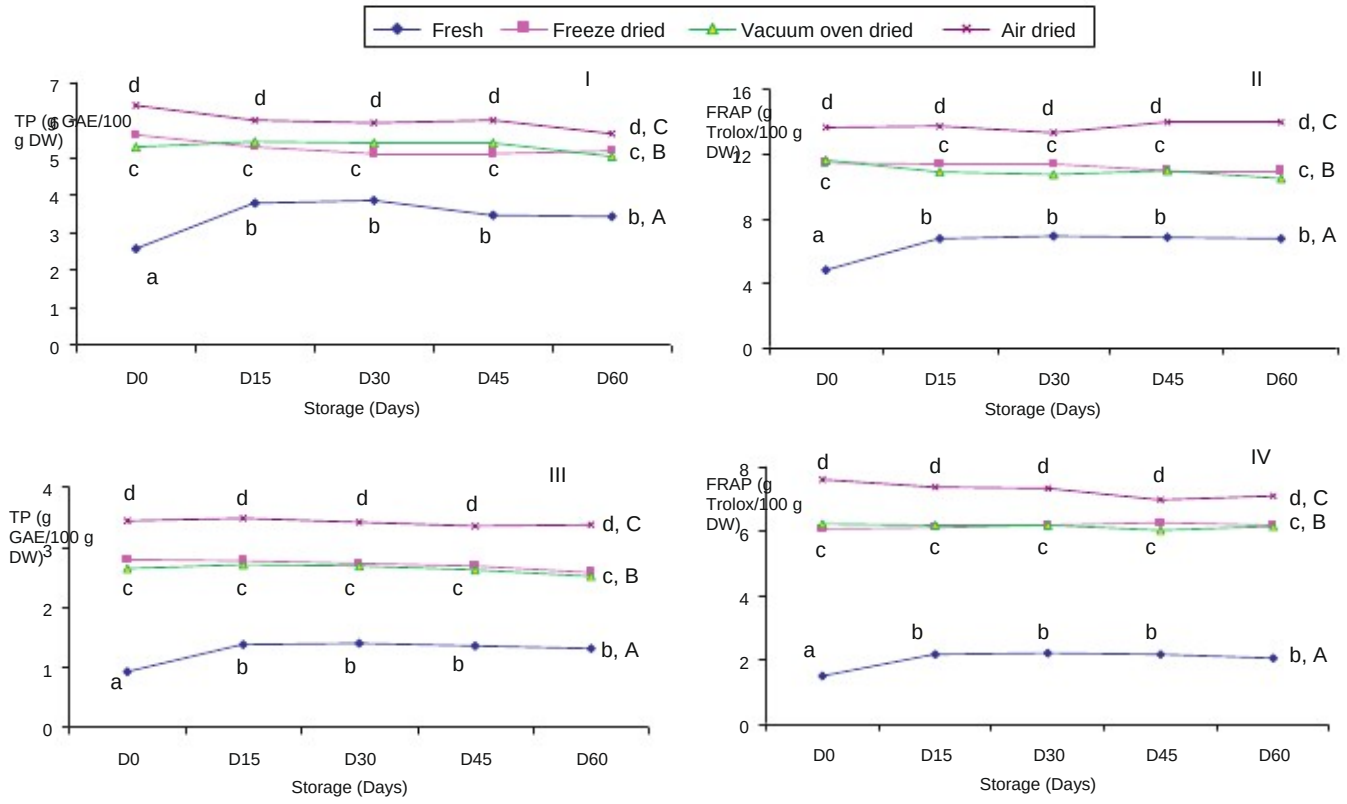

Fig. 2. Total phenol content (g GAE/100 g DW) and ferric ion reducing antioxidant power (g trolox/100 g DW) in marjoram (I for TP and II for FRAP) and sage (III for TP and IV for FRAP) during 60 days of storage.

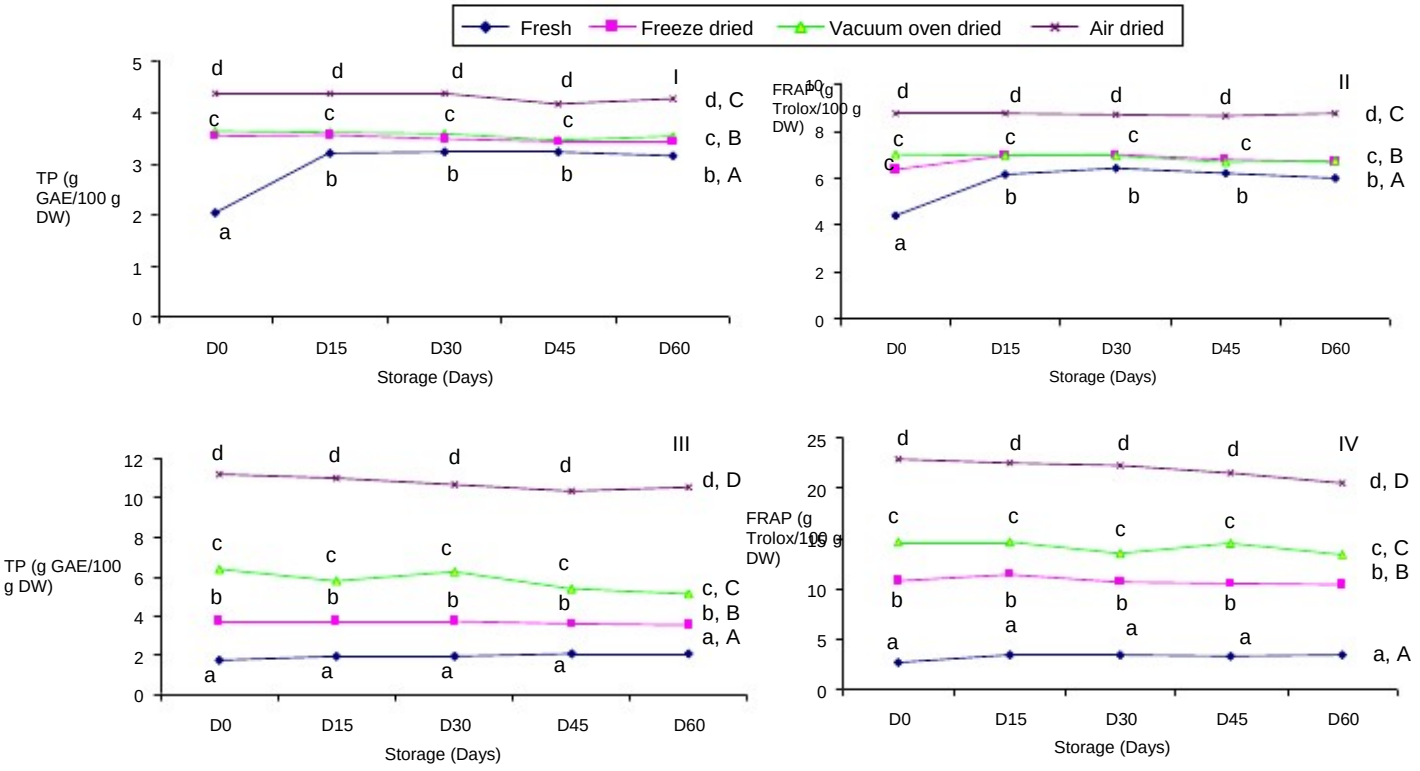

Fig. 3. Total phenol content (g GAE/100 g DW) and ferric ion reducing antioxidant power (g trolox/100 g DW) in basil (I for TP and II for FRAP) and thyme (III for TP and IV for FRAP) during 60 days of storage.

Table 1
Oxygen radical absorbance capacity (g trolox/100 g DW) of six Lamiaceae herbs (fresh and dried) during 60 days of storage.[A,B]

Name	Drying method	Significance of difference	Day 0	Day 15	Day 30	Day 45	Day 60
Rosemary	Fresh	S	8.45 [a]	13.1 [b]	12.6 [b]	13.0 [b]	12.6b
	Freeze-dried	NS	40.2c	41.7c	40.2c	39.5c	39.9c
	Vacuum oven-dried	NS	39.6c	39.1c	38.6c	39.3c	38.7c
	Air-dried	S	45.7d	45.3d	44.6d	43.8d	43.4d
Oregano	Fresh	S	11.3a	18.8b	18.0b	17.3b	16.9b
	Freeze-dried	NS	33.5c	32.8c	32.6c	31.9c	32.9c
	Vacuum oven-dried	NS	31.1c	31.5c	31.0c	30.7c	31.0c
	Air-dried	S	39.1d	38.7d	38.3d	37.2d	38.6d
Marjoram	Fresh	S	20.4a	28.4b	28.1b	27.3b	26.8b
	Freeze-dried	NS	36.5c	37.2c	36.4c	35.8c	35.2c
	Vacuum oven-dried	NS	37.7c	38.2c	37.4c	36.9c	35.5c
	Air-dried	S	43.9d	44.5d	43.8d	42.6d	42.0d
Sage	Fresh	S	9.39a	15.3b	15.0b	14.6b	14.2b
	Freeze-dried	NS	24.3c	23.3c	22.2c	23.5c	22.4c
	Vacuum oven-dried	NS	22.9c	22.1c	21.2c	21.8c	20.8c
	Air-dried	S	28.1d	29.2d	27.5d	28.1d	27.2d
Basil	Fresh	S	13.0a	20.5b	19.9b	19.7b	18.9b
	Freeze-dried	NS	22.4c	22.1c	23.5c	21.8c	21.5c
	Vacuum oven-dried	NS	23.0c	22.4c	21.3c	20.4c	21.0c
	Air-dried	S	26.1d	25.6d	24.1d	24.6d	24.7d
Thyme	Fresh	S	9.39a	11.9a	9.86a	10.6a	9.76a
	Freeze-dried	S	31.7b	32.1b	31.2b	30.5b	29.7b
	Vacuum oven-dried	S	38.6c	38.2c	36.7c	37.5c	36.4c
	Air-dried	S	48.0d	47.5d	46.0d	46.4d	44.5d

[A] S and NS for each sample denote significant ($p < 0.05$) and non-significant ($p > 0.05$) difference, respectively.
[B] For all the values of each sample, different superscripts mean significant differences ($p < 0.05$).

Table 2
Dry weight (%) of the samples after air-, freeze- and vacuum oven-drying.

Name of the herb	Dry weight (%) after air-drying	Dry weight (%) after freeze-drying	Dry weight (%) after vacuum oven-drying
Sage	16.9 ± 1.01	15.2 ± 0.86	15.8 ± 0.94
Rosemary	24.8 ± 0.63	25.8 ± 0.75	24.2 ± 0.56
Basil	11.0 ± 0.76	11.4 ± 0.67	10.4 ± 0.99
Marjoram	15.4 ± 1.10	14.8 ± 0.97	14.5 ± 0.87
Oregano	16.0 ± 0.41	15.5 ± 0.69	14.8 ± 0.36
Thyme	31.0 ± 0.81	29.2 ± 0.95	29.8 ± 0.92

content. Drying of the samples might make the tissue more brittle, which in turn results in rapid cell wall breakdown during the milling and homogenisation steps of the extraction procedure. These broken cells could release more phenolic compounds into the solvents when they are shaken overnight. Another possibility is that the fresh samples might lose antioxidant compounds due to enzymatic degradation during processing as the enzymes were still active in fresh samples. This might also be the cause of low rosmarinic acid yield in the extracts of fresh samples (Table 3). Suhaj (2006) suggested use of dried samples for extraction as some of the antioxidants might be unstable or degraded by enzymatic action in undried samples. The dried samples avoided this loss, as the enzymes were inactivated due to decreased water activity and thus retained high antioxidant capacity and total phenols in the extracts. Air-dried samples showed the highest total phenol content and antioxidant capacity of all the samples. In air-drying, the fresh herbs were kept at ambient temperature. It took more than 2 weeks to get the herbs completely dried. During this period, metabolically active plants lost moisture slowly and might have sensed the moisture loss as stress. Plants, in general, produce phenolic compounds in stress response as a defence mechanism (Rhodes, 1985). The synthesis of several phenylpropanoid compounds (flavonoids, isoflavonoids, psoralens, coumarins, phenolic acids, lignin and suberin) is induced in plants by biotic and abiotic stress factors, such as wounding, low temperature and attack of pathogens (Dixon & Paiva, 1995). This was evidenced in the rosmarinic acid content of the extracts obtained from the air-dried samples, which showed significantly ($p < 0.05$) higher amounts of rosmarinic acid (Table 3) than did other dried and fresh samples. Rosmarinic acid might have been accumulated in the sample plants as a defence reaction due to stress response during air-drying. Accumulation of rosmarinic acid in plants, as a defence compound, was also reported by Petersen and Simmonds (2003). Rosmarinic acid is one of the most important compounds in Lamiaceae herbs with high antioxidant potential (Hossain et al., 2008). The presence of four hydroxyl groups on the rosmarinic acid molecule might be related to its high antioxidant capacity (FRAP value = 406 g trolox/100 g DW). Vacuum oven-dried samples did not show significant difference ($p < 0.05$) in total phenol content and antioxidant capacity from the freeze-dried samples, except for rosemary and thyme. Rosemary showed significantly higher total phenols and ferric reducing antioxidant property in vacuum oven-drying than freeze-drying. However, in the ORAC assay, no significant difference was observed between these two methods in rosemary samples. It has been reported that freeze-drying showed a less pronounced damaging effect on the tissue structure than did other drying methods (Yousif et al., 1999). In woody herbs, such as rosemary and thyme, this effect could have been very low. Intact tissue structure might be very useful for a good appearance but, in relation to antioxidant capacity of the extract, it acts as a barrier for the release of phenolic compounds in the extracts, resulting in lower antioxidant capacity. Since freeze-dried samples were dried at very low temperature (Â54 °C), the degrading enzymes might still be activated when they come into contact with moisture of any source, e.g., probably from air. In thyme, the difference of antioxidant capacity and total phenols between vacuum oven-drying and freeze-drying was higher than in the other samples. The highly sensitive ORAC assay also showed significant difference between vacuum oven-drying and freeze-drying. During vacuum-drying leaves of the herbs are heated (70 °C) and their intercellular spaces collapse, liberating more phenolic compounds (Di Cesare et al., 2003). The significantly ($p < 0.05$) higher rosmarinic acid contents in vacuum oven-dried rosemary, oregano and thyme samples than

Table 3
Rosmarinic acid content (mg/g DW) in the extracts of six Lamiaceae herbs (fresh and dried) as measured by high performance liquid chromatography during 60 days of storage.[A,B]

Name	Drying method	Significance of difference	Day 0	Day 15	Day 30	Day 45	Day 60
Rosemary	Fresh	S	6.95 [a]	8.44 [b]	7.95 [b]	8.26 [b]	8.13b
	Freeze-dried	S	15.5c	15.7c	15.8c	16.3c	16.2c
	Vacuum oven-dried	S	18.3d	19.1d	19.5d	19.1d	18.6d
	Air-dried	S	22.7e	22.4e	22.7e	22.0e	22.2e
Oregano	Fresh	S	5.78a	7.33b	6.97b	7.54b	7.93b
	Freeze-dried	S	13.7c	13.5c	14.2c	13.9c	14.3c
	Vacuum oven-dried	S	15.7d	16.2d	15.5d	15.9d	16.2d
	Air-dried	S	18.4e	18.8e	18.4e	18.2e	19.1e
Marjoram	Fresh	S	4.3a	6.33b	5.86b	6.13b	5.94b
	Freeze-dried	NS	11.0c	11.2	10.3c	11.2c	10.2c
	Vacuum oven-dried	NS	11.7c	11.6c	11.4c	11.3c	11.2c
	Air-dried	S	13.3d	13.6d	14.8d	13.3d	13.4d
Sage	Fresh	S	3.30a	5.65b	5.54b	5.62b	5.09b
	Freeze-dried	NS	8.04c	7.51c	7.86c	8.15	7.08c
	Vacuum oven-dried	NS	8.21c	8.11c	8.35c	8.43c	8.03c
	Air-dried	S	9.74d	9.54d	9.65d	9.41d	9.31d
Basil	Fresh	S	4.71a	5.82b	5.44b	6.15b	5.74b
	Freeze-dried	NS	9.10c	8.73c	9.59c	8.44c	8.25c
	Vacuum oven-dried	NS	9.30c	9.78c	9.67c	10.0c	9.16c
	Air-dried	S	12.2d	12.5d	12.6d	12.6d	11.9d
Thyme	Fresh	S	10.9a	11.9a	9.86a	10.6a	9.76a
	Freeze-dried	S	14.3b	14.0b	14.8b	14.0b	14.1b
	Vacuum oven-dried	S	16.0c	16.7c	16.7c	15.9c	15.8c
	Air-dried	S	24.6d	24.3d	25.5d	24.4d	24.1d

[A] S and NS for each sample denote significant ($p < 0.05$) and non-significant ($p > 0.05$) difference, respectively.
[B] For all the values of each sample, different superscripts mean significant differences ($p < 0.05$).

Table 4
Correlations among the results obtained from total phenolic content (TP), ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays.

Correlation between	Linear equations of relationship	Correlation coefficient (R_2)
TP and FRAP	Y (TP) = 0.465X (FRAP) + 0.1087	0.921
TP and ORAC	Y (TP) = 0.1926X (ORAC) Â 0.861	0.672
FRAP and ORAC	Y (FRAP) = 0.4351X (ORAC) Â 2.693	0.806

in freeze-dried samples (Table 3) might have been accounted for by the reasons mentioned above. Higher amounts of phenols account for higher antioxidant capacity which has been observed in the current study in the following descending order: air-dried samples > vacuum oven-dried samples P freeze-dried samples > fresh samples. Among the plant phenolics responsible for antioxidant capacity, phenolic acids and flavonoids might play the major role (Kähkönen et al., 1999; Lagouri & Boskou, 1996).

3.2. Correlation among the data obtained from TP, FRAP and ORAC assays

The results obtained from TP, FRAP and ORAC assays showed a high degree of correlation. The highest correlation was observed between the total phenolic content (TP) and FRAP values (Table 4). This was expected, as both assays followed the same principle of electron-transfer (ET)-based anti-oxidation reactions. However, ORAC assay, being a hydrogen atom transfer (HAT)-based assay, showed relatively low but highly significant correlation with TP and FRAP assays. A number of authors (Shan et al., 2005; Zheng & Wang, 2001) reported high correlation between total phenolic content (TP) and antioxidant capacity assays. These results suggest that phenolic compounds are key contributors to the antioxidant capacity of the herb extracts. The correlations among the values of different assays have been established in linear equations presented in Table 4. Using these equations, values of one assay could be approximately calculated from another. Similar findings were reported by Shan et al. (2005), who presented the equation of correlation between total phenolic content and ABTS$_{A+}$ radical-scavenging activity in dried herb extracts.

4. Conclusion

Drying of herbs has been found to be a very useful technique for increasing the amount of phenolic compounds and antioxidant capacity of the extracts. Among the drying methods tested, air-drying was found to be the best method for all the samples. The effect of air-drying was more pronounced in woody herbs, such as thyme and rosemary. Vacuum oven-drying showed a better or similar efficiency in extracting antioxidant compounds from Lamiaceae herbs. Fresh herbs exhibited the worst performance among the treatments, revealing that they were very prone to external (oxidation by atmospheric oxygen) and internal (enzymatic) degradation. Storage did not affect the phenolic content or antioxidant capacity. The increase of total phenol and antioxidant capacity in fresh samples of rosemary, oregano, marjoram, sage and basil from day 0 to day 15 was due to severe chilling injury of the soft tissues of the samples.

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