EFFECT OF DIFFERENT DRYING METHODS ON ESSENTIAL OIL AND ANTIOXIDANTS ACTIVITY OF *MENTHA SPICATA* AND *ORIGANUM MARJORANA*

Elsayed,H.Hussein

Medicinal and Aromatic Plants Res.Dept.Hort.Res.Inst.A.R.C.,Alexandria, Egypt.
Div. of Prod.& Tech .of Medicinal and Aromatic Plants, Plant Production Dept.
Fac. of Agr. Saba Pasha, Alex. Univ. Alexandria, Egypt
dr.elsayed.hassan@gmail.com

ABSTRACT

Different drying methods (shade, sun and electric oven at (35° and 50 C°) were studied. Percentage and composition of volatile oils and antioxidant activity (DPPH) of marjoram and spearmint plants with using drying method in shade gave the height mean value of DPPH% for all experimental plants compared with other drying methods. Shade drying generally seemed better, especially with regard essential oil and major's compounds percentage of plants followed by drying in oven at 35°c.

ADDITIONAL INDEX WORDS: Drying methods, Marjoram and Spearmint,TCP,DPPH%, cis-Sabinene hydrate and Carvon.

INTRODUCTION

Aromatic plants represent a renewable source of flavoring substances, which can be used in the food,perfumery and pharmaceutical industries.Medicinal, aromatic and herb speices from family Lamiaceae such as spearmint (*Mentha spicata*), and Marjoram (*Origanum marjorana*), are widely distributed in Egypt. These plants are used as stomachic, spasmolytic, carminative, and expectorant agents in folk medicine and in official medicine. Ethereal oils extracted from Lamiaceae family plants can contribute the quality of food with better odor and flavor what is consider as very important quality parameter in food manufacturing (Kovaaevi 2001). The health benefits of marjoram essential oil can be attributed to its properties like analgesic, anti spasmodic, an aphrodisiac, anti septic, anti viral, bactericidal, carminative, cephalic, cordial, diaphoretic, digestive, diuretic, emenagogue, expectorant, fungicidal, hypotensive, laxative, nervine, sedative, stomachic, vasodilator and vulnerary (Konstantia *et. al.* 1998).

Dry herbs have a great importance, not only for the culinary purposes, but also for the medicinal uses (Hedrick, 1972). This the most critical process in the production of dried herbs and speices. The aim of drying is to reduce the moisture content of the product from actively growing in the field to a level that prevents deterioration of the product and allows storage in a stable condition. Drying is a two stage

process: firstly the transfer of heat to the moist product to vaporize the water in the product and secondly mass transfer of moisture from the interior to the product surface where it evaporates. The most important and immediate management concern is to ensure the harvested herbs will not rot or become grossly invaded with yeasts, bacteria and mould (producing aflatoxins) or become contaminated by pests. This is the start of the preservation process, which for most spice crops requires drying that will enable the long-term crop storage and the opportunity for further processing. In some cases, washing prior to processing is desirable to remove field contaminants (dust, soil) using anti-microbial solutions to reduce the microbial populations to a low level prior to the drying process.

The traditional open sun drying that is widely used in developing countries has major inherent limitations when trying to preserve product quality. High crop loss and low product quality result from inadequate drying, long drying times, fungal spoilage, insect infestations, bird and rodent damage and contamination plus the effects of sunlight and the weather. Even in the most favourable climate it is often not possible to get the moisture content of the product low enough for safe storage. In the tropics the high relative humidity of the air prevents drying of harvested crop products during the wet season

The traditional medicine still plays an important role in the primary health care in Egypt and most Arab countries. Many herbs and spices have been shown to contain high levels of polyphenolic compounds with potent antioxidant properties.

Free radicals are generated by a process known as redox cycling and they are catalysed by transition metals, to cause DNA and RNA damage, thiol oxidation and lipid peroxidation (Halliwell and Guttridge, 1999; Halliwell 1994). The great potential of free radicals to react with various compounds by electron transfer, proton transfer, H-atom abstraction or addition reaction may involved in the pathological of various diseases (Halliwell., 1993; Havsteen., 1983). Many plant compounds can scavenge reactive oxygen species (ROS) and thereby directly reduce-oxidative stress (Walgren *et al.*, 2000). Among these, flavonoids seem to be potent candidates because they show broad pharmacological activities and widely distributed in many edible plants (Rice-Evans *et al.*, 1996). The beneficial effect of flavonoids is mainly associated with the different various antioxidative mechanisms which act as enzyme inhibitor, reducing agents, trapping free radical and by acting as iron-chelating (Bravo., 1998; Hollman., 2001).

Phenolic compounds are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers (Elena, *et.al.*2009 and Proestos, *et.al.*2006). Consumption of fruits and vegetables with high content of antioxidative phytochemicals such as phenolic compounds may reduce the risk of cancer, cardiovascular disease and many other diseases (Robbins, and Bean 2004).Therefore, the interest in naturally occurring antioxidants has increased considerably for use in food and pharmaceutical products (Djeridane, *et.al.*2006). In recent years, there is a wide interest in finding natural compounds that could replace synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) because of its possible toxicity and due to a suspected action as promoters of carcinogenesis (Namiki,1990).

In this respect, since up to the present, there is no study on the antioxidative activity of some aromatic plants, the aim of this study was to investigate the essential oil percentage, chemical composition of oil and the antioxidative activity of different drying methods of the aromatic plants (marjoram and spearmint) extracts as new potential source of natural antioxidants.

MATERIALS AND METHODS

Plant material

The samples of aromatics plants (marjoram and spearmint) were collected from Sabhia Horticulture Researches Station, Alexandria in July.

Different methods of drying- traditional shade drying, open-sun drying and oven drying at 35 and 50 °C to establish the effect of drying techniques on oil content and antioxidative activity in (marjoram and spearmint) were selected because they are most export herbs in Egypt.

Shade drying

One kilogram (fresh weight) of each sample was used for the experiment. The samples were evenly spread on a tray and left to dry in the shade until constant weight(until the herbs were brittle and considered to be dry).

Sun Drying

One kilogram (fresh weight) of each sample was used for the experiment. The samples were evenly spread on a tray and left to dry

in the sunshine until constant weight (until the herbs were brittle and considered to be dry). .

Oven drying

Four hundred grams (fresh weight) of each sample were packed in envelopes which were punched with holes to allow for moisture escape. The envelopes were placed in the oven at 35 and 50 °C and left to dry until constant weight (until the herbs were brittle and considered to be dry).

Dry weight percentage

This may be done by taking a sample of the fresh herb prior to its harvesting, then promptly and accurately weighing it before carefully drying. A scientific method of establishing the dry weight is to take the weight of the sample and continuing the drying process until no further weight is lost. The herb can be assumed to be dry and the percentage of water loss and thus dry weight be attained. A simpler method is by feel and testing when any pieces in the sample break sharply. To calculate the dry weight percentage: Dry weight of herb / wet weight of herb x 100.

Determination of radical scavenging activity (DPPH) in drying herbs

The ability to scavenge the stable free radical (1, 1-diphenyl-2-picrylhydrazyl) (DPPH) was determined based on the method of Ohinishi *et al.*, (1994). with minor modifications. A solution of 0.2 mM DPPH in methanol was prepared and 1 ml of this solution was mixed with 1 ml of extract in methanol (5 to 150 [micro]g/ml). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. A control sample containing the same volume of solvent in place of extract was used to measure the maximum DPPH absorbance. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid and quercetin were used as references. Results were expressed as percentage of inhibition of the DPPH radical according to the following equation:

% Inhibition of DPPH = (Absorbance of control - Absorbance of sample) x 100/Absorbance of control

Determination of total phenolic content in drying herbs

Total phenolic content (TPC) of essential oils was determined according to the method described by Makkar *et al.* (1997). Four hundred µl of sample was taken in the test tube; then, 1.0 ml of Folin–Ciocalteu reagent (diluted 10-fold with distilled water) and 0.8 ml of 7.5 % sodium carbonate were added. After vortexing the reaction mixture,

the tube was placed in a dark place for 40 min and the absorption at 765 nm was measured against a blank. The total phenolic content was expressed as gallic acid equivalents in mg/g of methanol extract.

Essential oil analysis:

The percentage of volatile oil were extracted separately by the hydro distillation method utilizing apparatus similar to European Pharmacopoeia (EP).The essential oils were diluted in diethyl ether (20 ml in 1 ml) and analyzed with GC-MS (HP 8644) with flame ionization detector (FID) on a fused silica 132 capillary column DB-5, 25 m in length, 0.32 mm i.d., and 0.5 mm film thickness.133 Helium was used as the carrier gas with a flow rate of 1.6 ml/min; the detector 134 temperature was 260 °C, the oven temperature was programmed to increase from 130 to 260 °C at a rate of 4 °C/min. The split injector was heated at 250 °C, the split 136 ratio was 15:1. Data were processed on a DP 800 integrator. The percentage of . major constituents were estimated by measuring the peak area of the different compounds of the chromatogram according to Heftman (1967) and Gunther and Joseph (1978).Sources of the principal components of volatile oils which used as reference for determined essential oil of marjoram and Spearmint by GC were: Ciba Gigi, NY, USA.

There were three replications for each specimen; all the results obtained were statistically analyzed. The layout of the experiment was randomized complete design (Snedecor and Cochran, 1974).

RESULTS AND DISCUTION

Generally, data represented in Table (1and 2) and Fig.(1 and 2) showed that using drying methods in shade and oven at 35 C° gave the highest mean value of dry weight percentage for all experimental plants compared with other drying method. But all drying methods gave the allowable percentage of dry weight percentage of herbs according to Food Standards Agency (UK). Drying methods in shade and oven at 35 C° gave the highest mean value of the total phenolic content Also, using drying method in shade gave the same trend (highest mean value) of DPPH%. On the other hand the decline in the percentage of dry weight by drying methods in sun and oven at 50 C° led to a decrease in the total phenolic content and low percentage of DPPH. The increase of total phenolic content led to increasing in DPPH % , this result led to increase of anti-oxidant activity in the dry herbs (Madsen, and Bertelsen, (1995) and Molyneux, 2004) Comparison of essential oils percentage of marjoram and Spearmint

by the different methods (shade drying, sun drying and oven drying (35 and 50 C°) showed that, shade drying generally seemed better, especially with regard essential oil percentage of plants experiment followed by oven at 35 C° drying. This suggests that the highest in essential oils percentage of, among others, the drying conditions as observed in earlier studies (Osman, 2000, and Muller and Heindl 2006).

The chromatographic fractionation of essential oils showed that the main compounds of marjoram oil is cis-sabinene hydrate (21.05 – 23.21 %) , p-cymene (16.84 – 18.27%) and γ-terpinene (8.95 –10.33 %). Spearmint oil is carvone (55.04-58.21%) , limonene (11.93 -10.33 %) and 1,8-cineole (1.79 - 2.11%).The shade drying gave the highest percentage at most main compounds of essential oils. On the other hand the results indicated that using two drying methods (shade and oven at **35 C°**) gave the highest percentage of DPPH, essential oil and most of main compounds. This can be explained by the fact that the increases of percentage of main compounds for most essential oils lead to an increase the percentage of DPPH (Rice-Evans *et al.*, 1996).

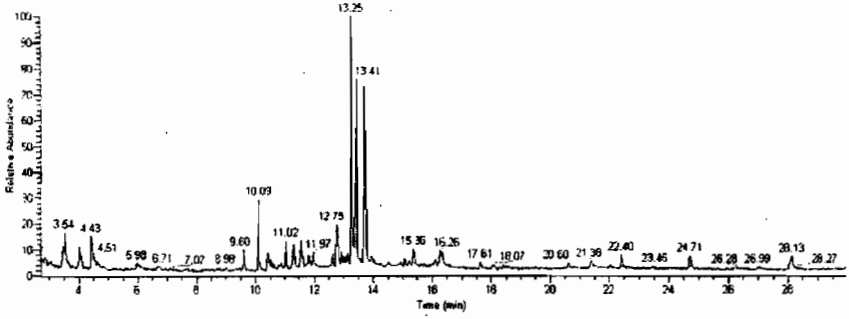

Fig.1 : Typical GC - MS Chromatogram of Marjoram oil

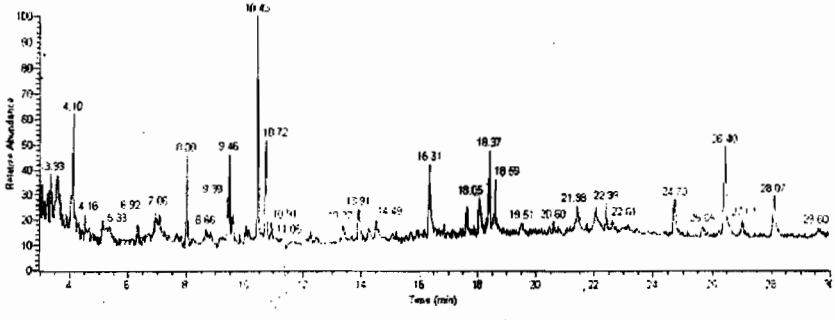

Fig. 2 : Typical GC - MS Chromatogram of Spearmint oil

Table (1): Effect of different drying methods on TCP, DPPH, and major compounds % of marjoram oil.

Drying methods	Marjoram								
	Dry Weight %	TCP (mg GAE/gmDW)**	DPPH %	Oil %	major compounds %				
					cis-Sabinene hydrate	α-Myrcene	p-Cymene	β -Caryophyllene	γ-Terpinene
Shade	12.02	23.5	38.5	1.02	23.15	1.98	18.27	3.65	10.33
Sun	10.22	19.7	32.5	0.85	21.05	1.07	16.91	3.61	9.03
35 C°	11.95	22.3	37.4	0.99	23.21	2.01	17.90	3.71	10.02
50 C°	10.06	20.1	31.2	0.86	21.06	1.06	16.84	3.59	8.95
$LCD_{0.05}$	0.96	1.96	3.70	0.04	1.24	0.55	0.19	0.03	0.07

** TCP Data expressed as mg of gallic acid equivalents per g dry weight (DW).

Table (2): Effect of different drying methods on TCP, DPPH, and major compounds % of spearmint oil

Dry methods	Spearmint								
	Dry Weight %	TCP (mg GAE/gmDW)**	DPPH %	Oil %	major's compounds %				
					Carvone	1,8-cineole	Limonene	Menthone	Myrcene
Shade	13.02		44.7	0.91	58.21	2.11	13.28	0.24	1.44
Sun	11.48		32.5	0.71	55.04	1.82	12.01	0.18	1.11
35 C°	12.95		40.9	0.89	56.37	1.99	12.98	0.26	1.41
50 C°	11.34		38.1	0.74	55.15	1.79	11.93	0.17	1.09
$LCD_{0.05}$	1.08		3.02	0.05	1.24	0.06	0.08	0.02	0.04

** TCP Data expressed as mg of gallic acid equivalents per g dry weight (DW).

REFFERENCES

Bravo, L., (199)8. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev.,56: 317-333.

Djeridane,A., M.Yousfi ,B. Nadjemi, D. Boutassouna, P .Stocker, and N. Vidal, (2006) .Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem. 95: 654-709.

Elena PC, Rocio J, Julio EP, Manuel A,Javier V. (2009). Antioxidant activity of seed polyphenols in fifteen wild Lathyrus species from South pain. LWT – Food Sci. Technol.42: 705 – 709.

Gunther, Z., and S.Joseph, (1978). Hand Book Series in Chromatography. CRC press, Inc.

Halliwell,B., (1993). The role of oxygen radicals in human disease, with particular reference to the vascular System. Haemostasis., 23: 118-126.

Halliwell, B., (1994). Free radicals, antioxidants and human disease: curiosity, cause, or consequence Lancet,344: 721-724.

Halliwell, B. and J. Guttridge, (1999). The Free radicals in biology and medicine, Oxford University Press, Oxford

Havsteen, B. (1983). Flavonoids, a class of natural products of high pharmacological potency. Biochem.Pharmacol., 32: 1141-1148.

Hedrick, U. (1972). Sturtevant's Edible Plants of the World. Dover, New York, pp. 388 - 89.

Heftman, E. (1967). Chromatography. Reinhold Pub. Crop. New York.

Hollman, P., (2001). Evidence for health benefits of plant phenols: local or systemic effects. J. Sci. Food Agric., 81: 842-852.

Konstantia,A.,A.Sivropoulou,S.Kokkini,T. Lanaras, and M. Arsenakis, (1998) .Antifungal Activities of *Origanum vulgare* subsp. hirtum, *Mentha spicata, Lavandula angustifolia*, and *Salvia fruticosa* Essential Oils against Human Pathogenic Fungi". Journal of Agricultural and Food Chemistry, 46, (5) : 1739–1745

Kovaaevia, N., (2001). Folk Medicine (first edition) Faculty of Pharmacy University of Belgrade

Madsen, H. L., and G. Bertelsen, (1995). Spices and antioxidants. Trends in Food Science and Technoogy, 6: 271–277.

Makkar,H;K.Becker;K,Abel and H,Pawelzik (1997). Nutrient contents, rumen protein degradability and antinutritional factors in some colour- and white-flowering cultivars of *Vicia faba* beans. J. Sci. Food Agric. 75: 511 - 20.

Molyneux, P. (2004). The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J. Sci. Technol., 26, 211–219.

Muller, J.,A. Heindl, (2006). Drying of medicinal plants, in Bogers, R. J. (ed.). Medicinal and aromatic plants. agricultural, commercial, ecological, legal, pharmacological and social aspects, The Netherlands, 237-252.

Namiki, S. (1990). Antioxidants/antimutagens in food, Crit. Rev. Food Sci. Nutr. 29: 273 - 90.

Ohinishi, M., H. Morishita, H. Iwahashi, T. Shizuo, S. Yoshiaki, M. Kimura, and R. Kido, (1994). Inhibitory effects of chlorogenic acids on linoleic acid peroxidation and haemolysis. Phytochemistry., 36: 579–583.

Osman, Y. (2000). The possibility of production of coriander (*Coriandrum sativum, L.*) under Sinai conditions. Ph. D. Thesis, Fac. Agric., Cairo University, Egypt.

Proestos C, I.Boziaris, G.Nychas,and M.Komaitis, (2006). Analysis of flavonoids and phenolic acids in Greek aromatic plants:investigation of their antioxidant capacity and antimicrobial activity. Food Chem. 95:664 – 671.

Rice-Evans, C.,N. Miller,and G. Paganga, (1996). Structure antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med., 20: 933-956

Robbins R, and S. Bean, (2004). Development of a quantitative highperformance liquid chromatography–photodiode array detection measurement system for phenolic acids. J.Chromatogr. 1038: 97 – 105.4.

Snedecor, G., and W. Cochran. (1974). Statistical Methods. Sixth Edition, Iowa State College Press, Ames, Iowa, USA.

Walgren, R.,J. Lin, R.Kinne,and T.Walle, (2000 b). Cellular uptake of dietary falconoid quercetin 4'-betaglucoside by sodium-dependent glucose transporter SGLT1. J. Pharmacol. Exp. Ther., 294: 837-843.

الملخص العربي

تأثير طرق تجفيف مختلفة علي الزيت العطري ونشاط مضادات الأكسدة للنعناع البلدى والبردقوش

السيد حسن حسين شعبان

قسم بحوث النباتات الطبية و العطرية - معهد بحوث البساتين - مركز البحوث الزراعية - مصر
شعبة انتاج وتكنولوجيا النباتات الطبية و العطرية قسم الانتاج النباتي - كلية الزراعة - سابا باشا- جامعة الاسكندرية - مصر

درست طرق مختلفة للتجفيف (في الظل و الشمس والفرن الكهربائي عند درجتي حرارة ٣٥ و ٥٠ درجة منوية)،تأثير تلك الطرق علي النسبة المنوية ومكونات الزيت العطري ونشاط مضادات الأكسدة لكل من البردقوش والنعناع البلدي، استخدام طريقة التجفيف في الظل أعطت أعلي متوسط لنشاط مضادات الأكسدة لكل نباتات التجربة مقارنة بطرق التجفيف الأخري . بصفه عامة طريقة التجفيف في الظل بدت أفضل الطرق وبخاصة لنسبة الزيت العطري والنسبة المنوية للمكونات الرئيسية لنباتات التجربة وتليها طريقة تجفيف الفرن الكهربائي عند درجة ٣٥ منوية.