Influence Of Different Drying Parameters On The Composition Of Volatile Compounds Of Thyme and Rosemary Cultivated In Sardinia

Piga A.[1]*, Usai M.[2], Marchetti M.[3], Foddai M.[2], Del Caro A.[1], Meier H.P.[4], Onorati V.[4], Vinci F.[4]

[1]Dipartimento di Scienze Ambientali Agrarie e Biotecnologie Agro-Alimentari, Università degli Studi di Sassari, Viale Italia 39/A, 07100 Sassari – Phone and fax n. 079 229273, e-mail: pigaa@uniss.it

[2]Dipartimento di Scienze del Farmaco, Università degli Studi di Sassari, Via Muroni 23, 07100 Sassari

[3]CNR - Istituto di Chimica Biomolecolare sede di Sassari, Traversa la Crucca 3, 07040 Sassari

[4]Consorzio di Produttori Sardi di Piante Officinali e loro derivati, Viale Trieste 124, 09123 Cagliari

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Abstract. *The shelf life of spices is traditionally extended by drying. Fresh herbs, due to their high water content, undergo microorganism growth and adverse biochemical reactions. On the other hand drying may result in a lot of physical and chemical alterations. Air and oven-dehydration are the main methods used to stabilize spices. During oven drying, in general, losses of volatile compounds are directly dependent on the temperature and time used.*

This paper deals with the effect of different drying temperatures and air fluxes on the volatiles in rosemary (Rosmarinus officinalis L) and thyme (Thymus officinalis L.) cultivated in Sardinia. Fresh leaves were collected and soon divided in two batches, which were subjected to hydro distillation and GC-MS analysis, the first batch as fresh, the second one after drying in a laboratory pilot dryer. Three drying temperatures were used, 30, 38 and 45°C, and for each one two airflow rates were set.

The fresh and dried plant material were hydro distilled for 4 hours using a Clevenger-type apparatus (Italian Official Pharmacopeias X). The oils (liquid and light yellow) were recovered directly from above the distillate without adding any solvent and stored at –20°C before analyses, which were carried out on two replicates of each sample by gas chromatography, using a flame ionization detector. The diluted samples were injected using a split/splitless automatic injector (using 2,6-dimethylphenol as internal standard). Qualitative analysis was done by GC/Mass and mass units were monitored from 10 to 450 at 70 eV.

Results of the influence of the different drying conditions on volatile compounds of the two herbs will be reported.

Keywords. *Rosmarinus officinalis* L., *Thymus officinalis* L., essential oils, drying, storage.

Introduction

Aromatic herbs and spices are becoming increasingly requested from the consumers. Apart from the flavoring use, in fact, people are interested also in medicinal and anti-inflammatory properties (Rish, 1997). The use of plants is as old as the mankind. Natural products are cheap and claimed to be safe. They are also suitable raw material for production of new synthetic agents.

Most of herbs and spices are marketed dried, because, due to the high water content in the fresh state, they undergo severe deterioration after microbial growth and biochemical reactions.

Water removal by dehydration, in fact, stabilizes microbiologically herbs and spices by lowering the water activity (a_w) values below the threshold for microbial growth, that is 0.6.

In general, hot air drying is the most used method, anyway, it can led to thermal damage and can severely alters the volatile composition of herbs as well as the color. In fact, some compounds can evaporate during air drying, while other are in part retained (Jerkovic, Matelic & Milos, 2001). Some oxidation products can also appear during drying (Luning, Ebbenhorstseller, Derijk & Rozen, 1995). In general, losses are correlated to temperature and time of drying (Raghavan, Abraham, Shankaranarayana & Koller, 1994; Venskutonis, Poll & Larsen, 1996). In fact, ambient temperatures and temperatures below 50°C are the best to retain volatile compounds (Park, Vohnikova & Brod, 2002; Ulseth, 1996; Soysal & Oztekin, 2001). Changes, anyway, are not only process dependent, but can be attributed to the specific compound and species.

The scientific research has shown that the thyme (*Thymus officinalis* L.) has a so strong antiseptic effect to be able to kill the bacteria in 40 second. The ancient Egyptians knew it and they used it to embalm their dead persons. The ancient Greek burned it as incense aromatic, from which derives the name of the Greek word to burn thymon'.

The Romans associated it to the strength and the courage. The soldiers took a bath of thyme before entering war. This superstition has had long life and in the Middle Ages the noblewomen embroidered the thyme on the emblems of their knights. The thyme is also considered exciting and invigorating substance, recommended in case of respiratory problems, bad circulation of the blood or bad digestion. An infusion gives relief to the headache, nervousness, cough, influence and it helps against the acne from the inside.

Rosemary (*Rosmarinus officinalis* Linn.) is a common household plant grown in many parts of the world. It is used for flavouring food, a beverage drink, as well as in cosmetics; in folk medicine it is used as an antispasmodic in renal colic and dysmenorrhoea, in relieving respiratory disorders and to stimulate growth of hair. Extract of rosemary relaxes smooth muscles of trachea and intestine, and has choleretic, hepatoprotective and antitumorigenic activity. The most important constituents of rosemary are caffeic acid and its derivatives, such as rosmarinic acid. These compounds have antioxidant effect. The phenolic compound, rosmarinic acid, gains one of its phenolic rings from phenylalanine via caffeic acid and the other from tyrosine via dihydroxyphenyl-lactic acid. Relatively large-scale production of rosmarinic acid can be obtained from the cell culture of *Coleus blumei* Benth when supplied exogenously with phenylalanine and tyrosine. Rosmarinic acid is well absorbed from gastrointestinal tract and from the skin. It increases the production of prostaglandin E2 and reduces the production of leukotriene B4 in human polymorphonuclear leucocytes, and inhibits the complement system. Thus, that rosemary and its constituents especially caffeic acid derivatives such as rosmarinic acid have a therapeutic potential in treatment or prevention of bronchial asthma, spasmogenic

disorders, peptic ulcer, inflammatory diseases, hepatotoxicity, atherosclerosis, ischaemic heart disease, cataract, cancer and poor sperm motility.

Thyme and rosemary grow both wild in the Mediterranean basin and, as told before, they are very much appreciated for their aromatic, antimicrobial and antioxidant properties (Dorman & Deans, 2000; Nguyen, Takascova, Jakubik & Dang, 2000; Manou, Bouillard, Devleeschouwer & Barel, 1998; Schwarz & Ternes, 1992). The effect of mechanical air drying on the volatiles of *T.officinalis* and *R. officinalis* have been extensively reported (Blanco, Ming, Marques & Bovi, 2002; Deans & Svoboda, 1992; Di Cesare, Viscardi, Fusari, & Nani, 2001; Fadel & El-Massry, 2000; Jaganmohan-Rao, Meenakshi-Singh, Raghavan & Abraham, 1998; Koller & Raghavan, 1995; Raghavan, Abraham & Koller, 1995; Venskutonis, 1995, 1997; Venskutonis, Poll & Larsen, 1996). However, we did not find any reference on the effect of air drying on volatile composition of these two species cultivated in Sardinia.

Consequently, the aim of this work was to assess the best drying conditions, using temperatures up to 45°C, to minimize volatile loss or degradation of essential oil extracted from *T. officinalis* and *R. officinalis* cultivated in Sardinia, in order to decrease the seasonality dependence of the essential oil market and toward increasing the value of the products.

Safety Emphasis

Sun drying is also used for drying herbs, requiring low capital, simple equipment and low energy input. Nonetheless, mechanical air dehydration has gained importance because it has many advantages over sun drying, such as the following:

a) the process is carried out under better sanitary conditions as a result of reduced contamination by dust and other foreign matter;

b) drying parameters can be accurately set, controlled and changed throughout the process, thus a more uniform product can be achieved with less quality degradation;

c) dehydration is not conditioned by rain or weather changes;

d) labor costs are lower. We also have to remember that *Aspergillus* spp. fungi growth may be dramatically increased when the whole process is too slow, due to the reasons specified above. Aflatoxin contamination has been reported, in fact, in spices (Selim, Popendorf, Ibtrahim, El Sharkawi, & El Kashory, 1996).

Thus, mechanical air drying of herbs can surely be a safer technology for drying herbs.

Materials and Methods

Plant material

Plant material was furnished by “Consorzio Produttori Sardi di Piante Officinali e loro Derivati”. Thyme and rosemary samples were collected during January and February in the south-west (Serdiana) and south-east (Muravera) of Sardinia, respectively, and transported within two hours to our laboratory, were they were immediately processed. In particular, both leaves and stems were used. The samples were divided in two batches, one was immediately used for the extraction of the essential oil, the other one was subjected to drying. Dried samples were immediately sent to essential oil extraction.

Drying equipment and process parameters

Herbs were dried in a laboratory pilot dryer. The air drier was a tangential air-flow cabinet (a modified model of "Scirocco", Società Italiana Essiccatoi, Milan, Italy), equipped with automatic temperature and air moisture control devices. Air flows tangentially to the shelves carrying the herbs, while a particular air recycling system allows mixing exhaust air with fresh air and then reheating and redirecting to the product, in order to achieve the desired air moisture. The particular construction of the drier allows a continuous airflow on the herbs, avoiding turbulence, and consequently it is particularly suited to calculate drying kinetics (Figures 1 and 2). Herbs were placed on steel shelves (product load from 0.6 to 0.7 kg/m^2) using three shelves per treatment (the drier holds ten shelves). Herbs were removed when an estimated 10% water content (based on weight loss calculations) was obtained. Processing parameters were as follows:

- Air temperature at ambient conditions = 20 °C
- Drying air temperature = 30, 38 and 45 °C
- Relative humidity of air at entrance <40%
- Volumetric flow rate = 300 (low) -1250 (high) m^3/h
- Air recycling to keep relative humidity below 40%.

Isolation and analysis of the essential oils

Oil distillation and yield

Fresh and dried plant materials were separately steam distilled for 4 h in a Clevenger-type apparatus according with Italian Official Pharmacopoeias X (1999); the reached yield is reported in Figure 3.

All the obtained oils were liquid and light yellow. Three replicate samples were distilled simultaneously. The essential oil was recovered directly from above the distillate without adding any solvent. The oils were stored at –20°C (under nitrogen atmosphere) until they were analyzed.

Oil analyses: Gas-cromatography analysis

Two replicates of each sample (three for every station) were analyzed by using a Hewlett-Packard Model 5890A gas cromatograph (GC) equipped with a flame ionization detector (F.I.D.) and fitted with a 60 m x 0.25 mm, thickness 0.25μm AT-5 fused silica capillary column (Alltech). Injection port and detector temperature were 280°C. The column temperature was programmed from 50°C to 135°C at 5°C/min (1 min), 5°C/min up 225°C (5 min), 5°C/min up 260°C and held for 10 min. The samples, diluted 1/10, (using 2,6-dimethylphenol as internal standard) were injected using a split/splitless automatic injector HP 7673 and using helium as carrier gas. Measurements of peak areas were performed with a HP workstation; the threshold was set at 0, peak width at 0.02. The quantization of each compound was expressed as absolute weight percentage using internal standard and response factors. The detector response factors (RFs) were determined for key components relative to 2,6-dimethylphenol and assigned to other components on the basis of functional group and/or structural similarity, since oxygenated

compounds have lower detectability by F.I.D. than hydrocarbons (Dugo, Licandro, Cotroneo, & Dugo, 1983) The standards were > 95% also, and actual purity was checked by GC. Several response factor solutions were prepared and consisted of only four or five components (plus 2,6-dimethylphenol) to prevent interference from trace impurities.

Gas-Cromatography/Mass-Spectrum analysis

Gas-Cromatography/Mass-Spectrum (GC/MS) analyses were carried out with a Hewlett Packard G1800B-GCD System using the same conditions and column described above. The column was connected with the ion source of the mass spectrometer. Mass units were monitored from 10 to 450 at 70 eV. The identification of constituents was based on comparison of the retention times (R_t) values and mass spectra with those obtained from authentic samples and/or the NIST and Wiley library spectra (NIST98; Adams, 2001) or on the interpretation of the EI-fragmentation of the molecules.

Other assessments

Fresh and dried samples were inspected for water and dry matter content (%), water activity and colour. Drying kinetics were calculated by plotting the water content measured at regular intervals during processing versus process times, while drying rates were computed from water loss and process times. In particular, water content was determined in a vacuum oven for 12 h at 70°C until constant weight. Water activity was assessed by an electronic hygrometer (model Aw-Win, Rotronic, equipped with a Karl-Fast probe), calibrated in the range 0.1-0.95 with solutions of LiCl of known activity.

Results and Discussion

Drying kinetics

Figures 1-2 show dehydration kinetics of the two herbs. The process was stopped when samples reached an average water content of 0.11 kg of water per kg of dry matter, which is considered a safe value from the microbiological standpoint and which is suggested by the European Spice Association (2004). Drying times and rates were affected mainly by process parameters. In our experimental conditions, the time to reach the estimated water content ranged from 8 h for the combination 45°C and high fair flux to 25 hours for the combination 30°C and low air flux. Drying times did not differ significantly between species or same processing conditions.

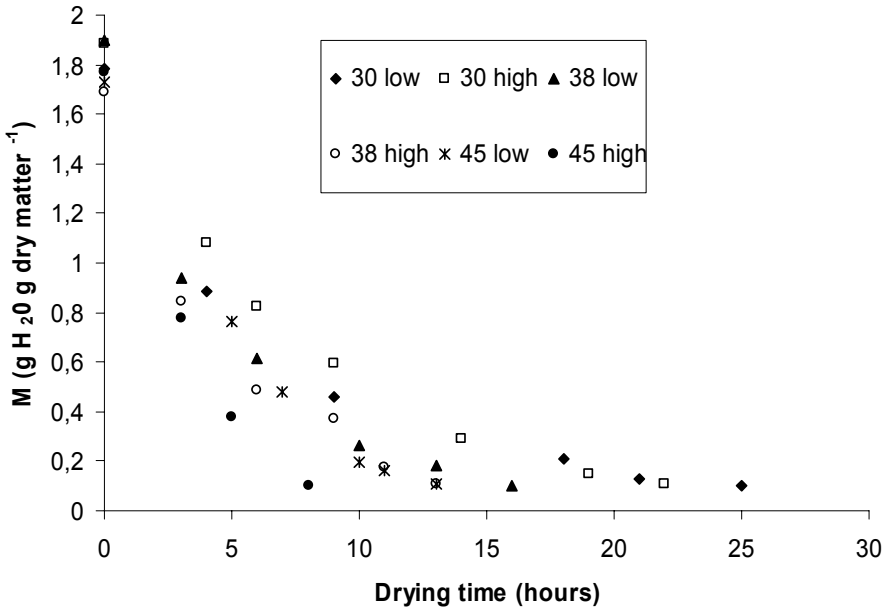


Figure 1 - Drying kinetics of Thymus officinalis samples as M (g H_2O g dry matter^{-1}) versus drying time (hours). Data are the mean of three determinations.

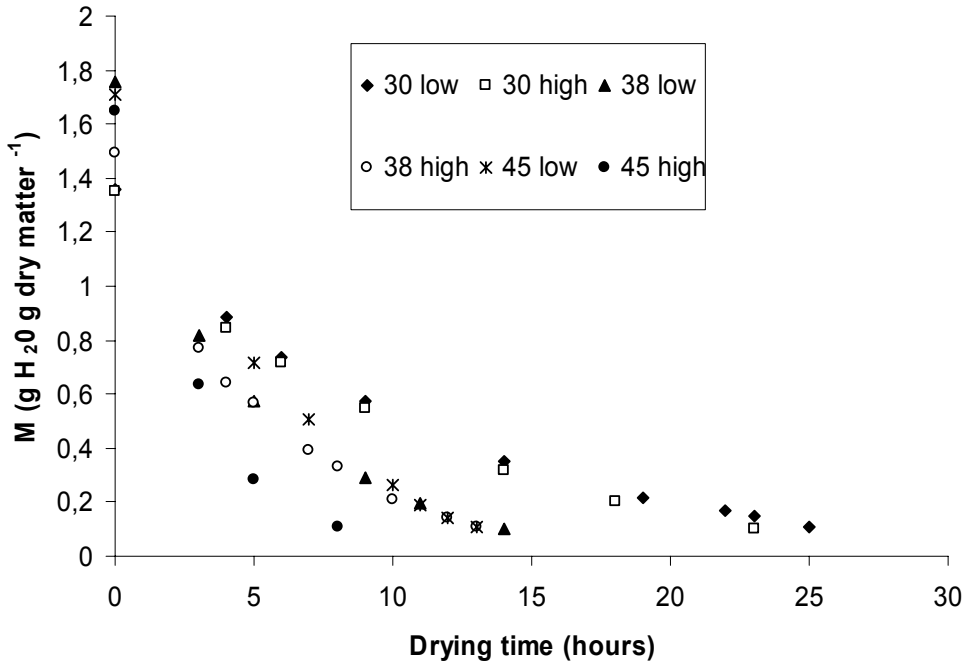
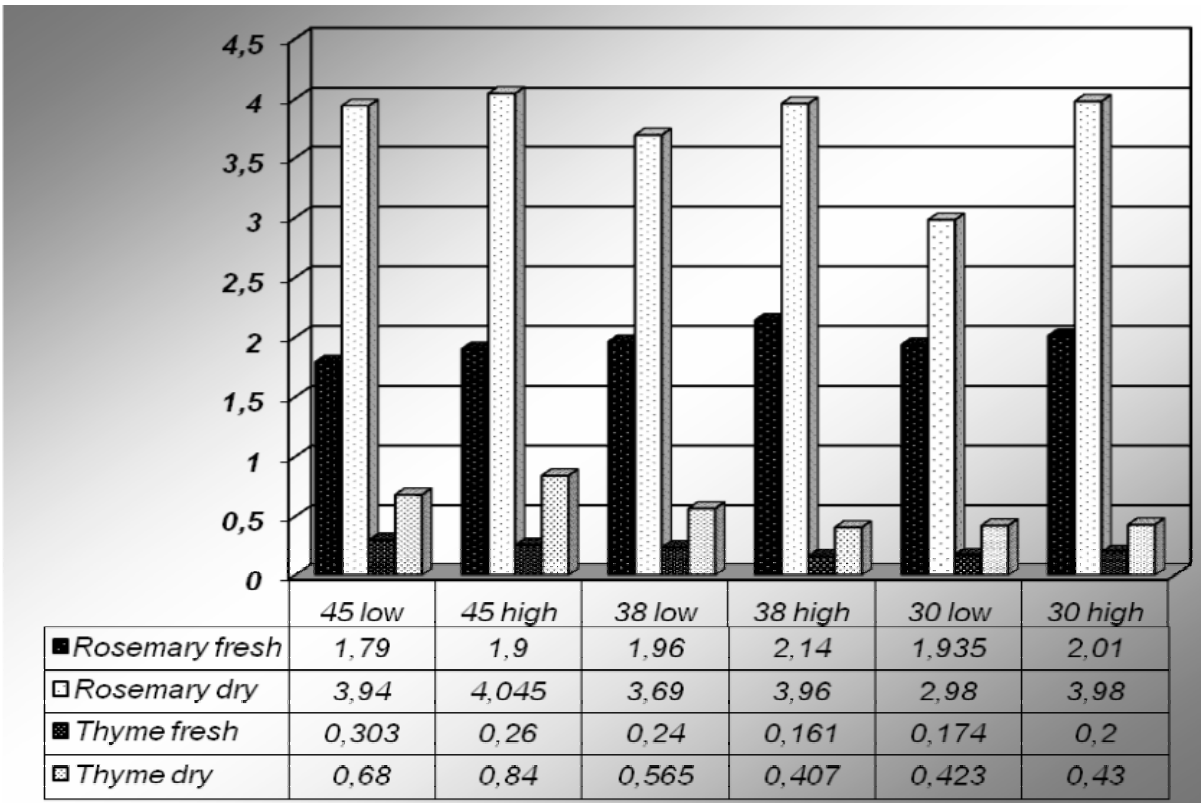


Figure 2 - Drying kinetics of Rosmarinus officinalis samples as M (g H_2O g dry matter^{-1}) versus drying time (hours). Data are the mean of three determinations.

It is to highlight that only a falling drying period was observed. Times for drying agree with those reported by other authors (Venskutonis *et al*., 1996; Jaganmohan-Rao *et al*., 1998). The a_w values ranged from 0.56 to 0.59, thus sample were surely microbiologically stable.

All the obtained samples were submitted to quantitative and qualitative essential oils analyses. The first parameter evaluated was the essential oil yields in different drying processes. In all cases, after data normalization, no significant differences were recorded, and this is in accordance with data reported by Deans, Svoboda & Bartlett (1991). In Figure 3 are reported all raw experimental data referred to the different drying procedure.



	45 low	45 high	38 low	38 high	30 low	30 high
Rosemary fresh	1,79	1,9	1,96	2,14	1,935	2,01
Rosemary dry	3,94	4,045	3,69	3,96	2,98	3,98
Thyme fresh	0,303	0,26	0,24	0,161	0,174	0,2
Thyme dry	0,68	0,84	0,565	0,407	0,423	0,43

Figure 3 – Raw data (expressed in percentage) deriving from essential oil extraction of fresh and dried herbs.

At first we consider the thyme (Figure 4): from the observation of the plot reported in figure, it is possible to note the α-thujene strongly decreased when drying at 45°C and low air flux were used; this behavior is common to all volatile components of the essential oil, in fact under this experimental conditions all non-volatile constituents of the essential oil increased significantly (e.g. thymol and carvacrol), whereas all volatile hydrocarbons were lost in significant amount. On the contrary, drying at 45°C and high air flow allowed a relatively small drying time, and this resulted in a keeping of the volatile compounds in the oil glandular hair, therefore, the composition of the essential oil deriving from dry plant is similar to that distilled from fresh herbs. This behavior has been noted in all drying trials, while, when lower temperatures were used

(38°C and 30°C), the loss of volatile compounds was not high. It is noteworthy to observe some phenomena of isomerization and/or oxidation that take place during long time drying operations. In particular, using low air flux resulted, for example, in an increase of carvacrol and in some cases thymol, and this changed the peculiar characteristics of essential oil.

The above observation induced us to consider 45°C and high air flux as the best drying conditions from the essential oil production point of view. In fact, no significant changes were recorded when comparing fresh herbs and the corresponding dried sample at the above cited process parameters (Figure 5). The results obtained are in part in accordance with that reported by Raghavan *et al*., (1995), Venskutonis *et al*. (1996) and Venskutonis (1995), even if a right comparison can not be made because we do not know exactly all the characteristics of the air-water mixture used by the authors. However, contrary to what reported by the above cited authors, the lowest temperature gave the worst results.

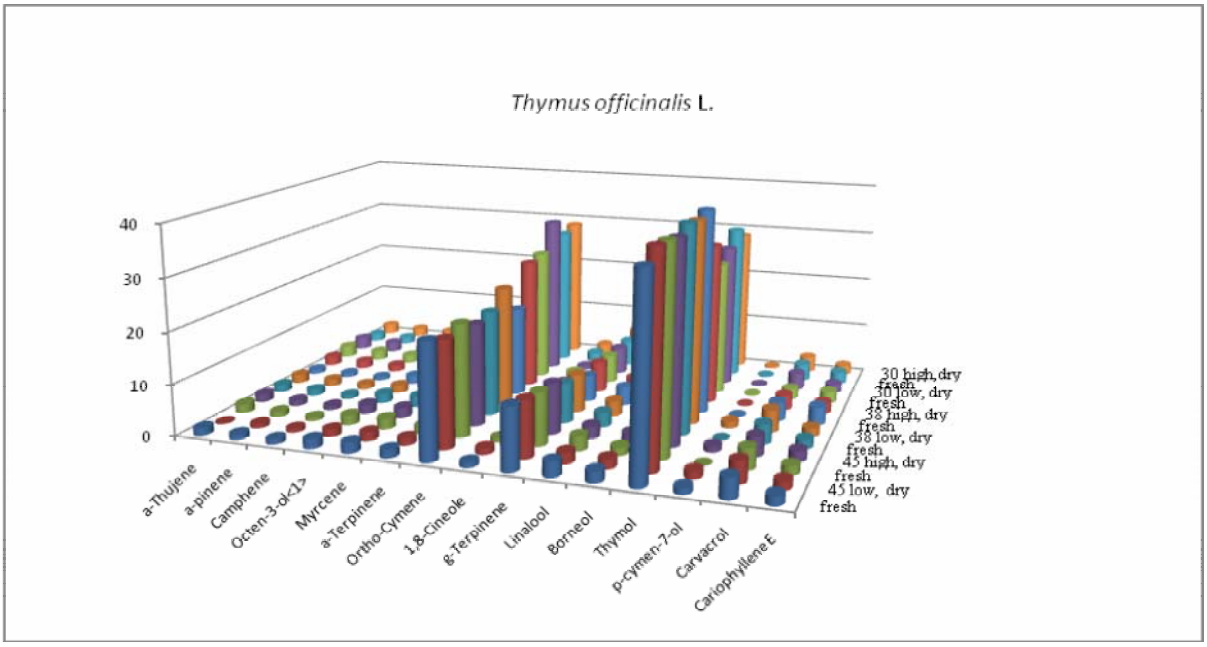


Figure 4 – Variation of main components (as RFs) of essential oil of thyme extracted from fresh and dry plants obtained under different drying conditions.

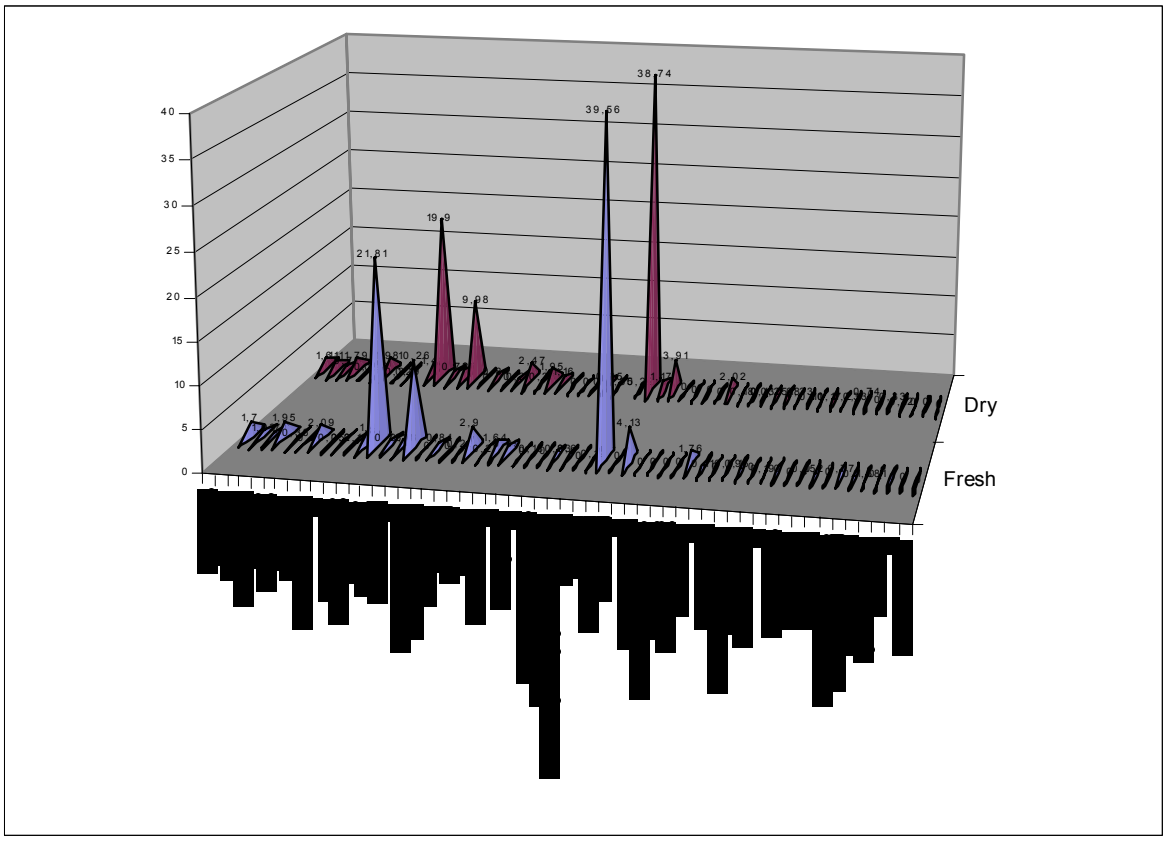


Figure 5 – Comparison of whole essential oil (as RFs) of fresh and dry thyme using the best parameters of drying.

The same criteria above described have been also used to establish the best drying parameters for rosemary plants. In this case, the percentage of main and more significant constituents of the essential oil (verbenone and α-pinene) remain practically the same when 38°C and low air flux is used (Figures 6 and 7). It is very interesting to note that if low temperature and high air flux during the drying are employed, some oxidation phenomena occur dramatically, for example limonene is almost completely converted into 1,8-cineol, when the herb is dried at 30°C using a high air flux. Our results partly agree with those reported by some other authors (Jaganmohan *et al*., 1998; Fadel & El-Massry, 2000; Blanco *et al*., 2002).

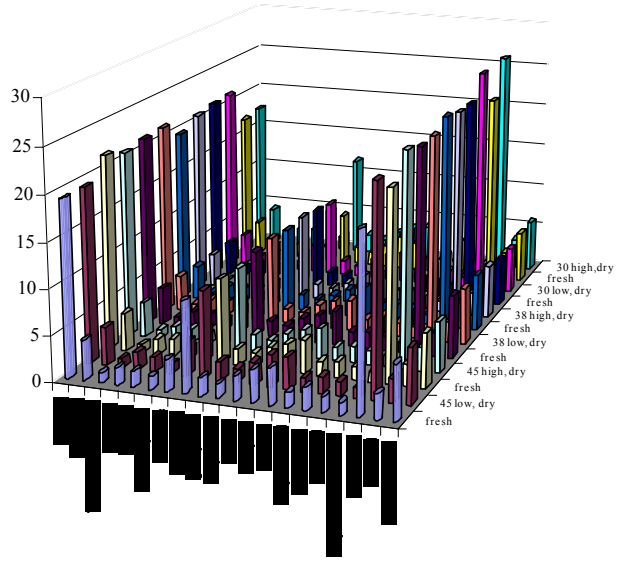


Figure 6 - Variation of main components (as RFs) of essential oil of rosemary deriving from fresh and dry plants obtained under different drying conditions.

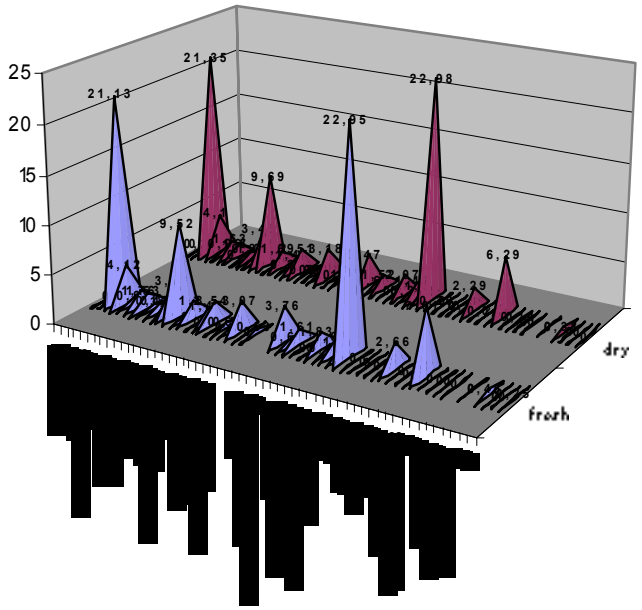


Figure 7 - Comparison of whole essential oil (as RFs) of fresh and dry rosemary using the best parameters of drying.

Conclusions

Drying rosemary and thyme cultivated in Sardinia in a cabinet flow dryer gave different and interesting results. The best process parameters combination were 45°C-high air flux and 38°C-low air flux for thyme and rosemary, respectively. These conditions prevented the decreasing of volatile constituents of essential oils, in particular of the compounds making the greatest contribution to the peculiarity of the oil.

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Nomenclature

a_w	activity of water in the product
°C	degree Celsius
eV	elettronvolt
Ei	Electron Impact Ionization
F.I.D.	Flame Ionization Detector
g	gram
GC	gas cromatograph
GC/MS	gas-cromatography/mass spectrum analyses
h	hour
kg	kilogram
m	meter
m^2	square meter
m^3	cubic meter
min	minute
%	percent
RFs	Detector response factor
R_t	Retention time

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