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Influence of different stabilizing operations and storage time on the composition of essential oil of thyme (*Thymus officinalis* L.) and rosemary (*Rosmarinus officinalis* L.)

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ABSTRACT

The effect of different stabilizing techniques on the composition of essential oil of rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymus officinalis* L.) during one year of storage is reported. The study was aimed to know what is the stabilizing technique to keep at the best the original essential oil composition. The fresh samples were collected and treated as follows: air-dried in a laboratory scale pilot dryer, frozen in a forced-air freezer and freeze-dried in a laboratory freeze-dryer. The fresh sample served as control. The treated samples were packaged with appropriate packaging material and stored at 20 °C or −20 °C for 12 months. All the samples were hydrodistilled every three months and the oils composition was obtained by means of gas chromatography/mass spectrometry (GC/MS). Quantification of known compounds was done with the use of an internal standard. Freezing best maintained the composition of rosemary and thyme essential oil. Appropriate packaging of air-dried and freeze-dried herbs resulted in negligible quality loss up to one year of storage. The frozen and stored thyme samples showed the best retention of thymol, the most important compound, as well as of γ-terpinene and carvacrol.

1. Introduction

Thyme (*Thymus officinalis L.*) and rosemary (*Rosmarinus officinalis* L.) grow wild in the Mediterranean basin, where they are appreciated for their aromatic, antimicrobial and antioxidant properties (Dorman & Deans, 2000; Nguyen, Takascova, Jakubik, & Dang, 2000; Schwarz & Ternes, 1992). Most herbs and spices are usually sold dried, due to the high water content in the fresh state which causes them to undergo severe deterioration caused by microbial growth and biochemical reactions. Water removal by dehydration microbiologically stabilizes herbs and spices by lowering the water activity (a_w) values below the threshold for microbial growth (0.6). Hot air-drying is the most common commercial operation, but it can result in thermal damage and can severely alter the volatile composition and color of the herbs. Some volatile compounds evaporate during air-drying, whereas others are partially retained (Jerkovic, Mastelic, & Milos, 2001), and some oxidation products appear during drying (Luning, Ebbenhorstseller, Derijk, & Rozen, 1995). The loss of volatile compounds is generally correlated to drying air temperature and time (Raghavan, Abraham, Shankaranarayana, & Koller, 1994; Venskutonis, Poll, & Larsen, 1996). The decrease in volatiles can be minimized when drying air temperature is 50 °C or below (Park, Vohnikova, & Brod, 2002; Soysal & Oztekin, 2001). However, changes in volatiles are not only process-dependent but can also be attributed to the specific compound and species. Other drying methods have been proposed, such as freeze-drying and microwave-drying (Diaz-Maroto, Pérez-Coello, & Cabezudo, 2002; Paakkonen, Malmsten, & Hyvonen, 1990; Venskutonis, 1997; Yousif, Duranca, Scaman, & Girard, 2000); however, the best results in retaining the volatiles have been obtained by ambient or hot air-drying, with some exceptions (di Cesare, Forni, Viscardi, & Nani, 2003; Yousif et al., 2000). Stabilization by freezing has also been tested on basil (di Cesare, Viscardi, Fusari, & Nani, 2001) and bay leaf (Diaz-Maroto et al., 2002), with unsatisfactory results.

The effects of mechanical air-drying and freeze-drying on the volatiles of *T. officinalis* and *R. officinalis* have been extensively reported (Bendl, Kroyer, Washüttl, & Steiner, 1988; Blanco, Ming,

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Marques, & Bovi, 2002; Deans & Svoboda, 1992; di Cesare et al., 2001; Fadel & El-Massry, 2000; Jaganmohan-Rao, Meenakshi-Singh, Raghavan, & Abraham, 1998; Raghavan, Abraham, & Koller, 1995; Venskutonis, 1997; Venskutonis et al., 1996). However, while the effects of different stabilizing techniques on the volatile contents of thyme and rosemary have been reported, only a few papers have discussed the changes in volatiles in stabilized herbs during storage. Venskutonis et al. (1996) studied the compositional changes of thyme aroma compounds of air-dried and freeze-dried products during 10 months of storage, but they only discussed aggregated data (dried plus freeze-dried). To our knowledge, there have been no reports on the effects of freezing and subsequent storage on volatile compounds in essential oils.

The aim of the present study was to assess the changes in composition of the essential oils of *T. officinalis* and *R. officinalis* after air-drying, freeze-drying and freezing and to check for changes of stabilized samples during one year of storage.

2. Materials and methods

2.1. Plant material

The aerial parts of *T. officinalis* L. and *R. officinalis* L. plants were provided by the "Consorzio Produttori Sardi di Piante Officinali e loro derivati". Thyme and rosemary samples were organically grown and collected during January and February at Soleminis (39°35′98″N, 9°19′56″E) and Muravera (39°32′66″N, 9°60′06″E) in south-eastern Sardinia. After harvesting, they were transported within 2 h to our laboratory where they were immediately processed. The rosemary was a verbenone chemotype. The samples were divided into four batches, hydrodistilled and analyzed by GC/MS. The four batches were: fresh, dried in a pilot scale dryer, frozen in a forced-air freezer and freeze-dried in a laboratory freeze-dryer.

2.2. Stabilizing equipment, process parameters and storage conditions

The fresh herbs were dried, frozen or freeze-dried immediately after harvest.

2.2.1. Drying

The fresh herbs were air-dried in a pilot scale dryer. The air-dryer was a tangential airflow cabinet (a modified Scirocco model, Società Italiana Essiccatoi, Milan, Italy), equipped with automatic temperature and air moisture control devices. The air tangentially flows to the shelves holding the herbs, while a particular air recycling system allows mixing of the exhaust air with the fresh air and then reheating and redirecting to the product in order to achieve the desired air moisture. The particular construction of the dryer allows a continuous airflow on the herbs, avoiding turbulence; hence it is particularly suited to calculate drying kinetics. The herbs were placed on steel shelves (product load from 0.6 to 0.7 kg/m^2) using ten shelves *per* treatment. A total of 2.5 kg of fresh herbs were dried per treatment. The herbs were removed when an estimated 10 g/100 g or 12 g/100 g wet basis water content (based on weight loss calculations) was obtained for rosemary and thyme, respectively, according to European Spice Association (2005) requirements. Processing parameters were as follows:

- Air temperature at ambient conditions = 20 °C.
- Drying air temperature = 38 and 45 °C.
- Relative humidity of air at entrance <40 g/100 g.
- Volumetric flow rate = 300 (low)–1250 (high) m^3/h.
- Air recycling to keep relative humidity as low as possible.

Rosemary was dried using the combination 38 °C and low drying air flux, whereas 45 °C and high drying air flux were preferred for thyme; these sets of parameters were selected to give the best results in terms of volatile retention, as demonstrated in a previous paper (Piga et al., 2007).

2.2.2. Freezing and freeze-drying

Freezing was carried out by placing the fresh herbs in a monolayer (<5 cm) and subjecting them to cold air at −30 °C in a freezer (Artic 400, Fiocchetti, Luzzara, Italy). The time to reach −18 °C was less than 4 h, as revealed by a temperature data logger (Micropack MPIII, Mesa Laboratories, Lakewood, CO, USA) placed at the mid-layer position.

Half of the frozen samples were freeze-dried for 24 h in a freeze-dryer (Modulyo, Edwards, Tonawanda, NY, USA) at a pressure of 1.1 Pa, while the condenser and drying chamber were at −50 °C and 20 °C, respectively.

Triplicate samples were obtained both for frozen and for freeze-dried herbs. A total of 2.5 kg or 1.5 kg of fresh herbs was used for freeze-drying and freezing, respectively.

The dried samples had a wet basis water content of 8.73 g/100 g ± 0.64 and 10.86 g/100 g ± 0.72 for rosemary and thyme, respectively.

2.2.3. Storage of dried, frozen and freeze-dried herbs

The dried samples were packaged under vacuum with a 95 μm thick polyethylene film; the freeze-dried herbs were packaged in an aluminized polyethylene film under vacuum. Both samples were stored in the dark at 20 °C. The frozen herbs were sealed in polyethylene bags and stored at −20 °C. The packaged samples were stored for one year and sampled every three months for analysis. Each stabilizing trial was repeated twice.

2.3. Oil distillation and yield

The fresh, stabilized and stored plant materials were separately steam-distilled for 4 h in a Clevenger-type apparatus according to the Farmacopea Ufficiale Italiana (1999). A total of 400 g were used for each distillation for fresh and frozen samples, while 100 g were considered for dried and freeze-dried samples. Three replicate samples were simultaneously distilled. The essential oil was directly recovered from above the distillate without the addition of solvent. After each extraction the oils were stored at −20 °C (under nitrogen atmosphere) for a short period and then analyzed.

2.4. Oil analyses: GC-FID analysis

Three replicates of each sample were analyzed with a Hewlett-Packard 5890A gas chromatograph (Hewlett–Packard, Avondale, PA, USA) equipped with a flame ionization detector (FID) and fitted with an AT-5 fused silica capillary column (60 m, 0.25 mm i.d., 0.25 μm film thickness, Alltech, Deerfield, IL, USA). The injection port and detector temperature were 280 °C. The column temperature was programmed from 50 °C to 135 °C at 5 °C per min, held at 135 °C per 1 min, programmed at 5 °C per min to 225 °C, held at 225 °C per 5 min, programmed at 5 °C per min to 260 °C and held at 260 °C for 10 min. The samples were diluted 1:10 (hexane) with 2,6-dimethylphenol as internal standard (Aldrich Chemicals, Milwaukee, WI, USA). Samples were injected using a split/splitless automatic injector and helium as carrier gas. The measurement of peak areas was performed with a HP B.02.04 chemstation; the threshold was set at 0 and the peak width at 0.02. The quantification of each compound was expressed as absolute weight percentage using an internal standard and the response factors. The detector response factors (RFs) were determined for the key

components relative to 2,6-dimethylphenol and assigned to the other components on the basis of functional group and/or structural similarity, since the oxygenated compounds have lower detectability by FID than hydrocarbons (Dugo, Licandro, Cotroneo, & Dugo, 1983). The standards were >95% pure, and the actual purity was checked by GC. Several response factor solutions were prepared, consisting of only four or five components (plus 2,6-dimethylphenol) to prevent interference from trace impurities.

2.5. GC/MS analysis

GC/MS analyses were carried out with a Hewlett–Packard G1800B-GCD System (Hewlett–Packard, Avondale, PA, USA) using the same conditions and column described above. The column was connected with the ion source of the mass spectrometer. The 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), Kovats indices (Davies, 1990; Jennings & Shibamoto, 1980) and mass spectra with those obtained from authentic samples and/or the NIST and Wiley library spectra (Adams, 2001; NIST98), or on interpretation of the EI-fragmentation of the molecules.

2.6. Other assessments

The fresh, stabilized, and stored samples were inspected for water and dry matter content (g/100 g) and water activity. The water content was determined in a vacuum oven for 12 h at 70 °C until constant weight. The water activity was assessed with an electronic hygrometer equipped with a Karl-Fast Aw-Win probe (Rotronic, Bassesdorf, Switzerland), calibrated in the range 0.1–0.95 with solutions of LiCl of known activity.

2.7. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using appropriate software (Statistica for Windows, version 6), considering each experimental condition as the "group variable". The analysis was carried out between the fresh and stabilized (dried, freeze-dried and frozen) samples and between stabilized samples and stored ones. When required, differences between means were analyzed by Tukey's test (significance level $P \leq 0.01$).

3. Results and discussion

3.1. Drying kinetics

Fig. 1 shows the dehydration curves of the two herbs. The drying times and rates were mainly affected by process parameters. The estimated water content was reached after 8 h for thyme and 14 h for rosemary. Drying always followed a trend of decreasing drying rate (Barbosa-Cánovas & Vega-Mercado, 1996). Times for drying agree with those reported by other authors (Jaganmohan-Rao et al., 1998; Venskutonis et al., 1996). The a_w values ranged from 0.56 to 0.59, thus the samples were microbiologically stable.

3.2. Identification of essential oil components

We identified 28 compounds in rosemary and 40 in thyme (98% of total compounds in both species). The main compounds are shown in Table 1. All the identified compounds are known (De Mastro, Ruta, Mincione, & Poiana, 2004; Jaganmohan-Rao et al., 1998; Özcan & Chalchat, 2008; Pintore et al., 2002; Venskutonis, 1997). Since the peak area of many compounds was negligible, we will only consider the 16 principal representative compounds shown in Table 1, including the main aroma principles for both species. In particular, verbenone, α-pinene, 1,8-cineole and bornyl acetate are the main aroma compounds in rosemary, while thyme is characterized by thymol and α-pinene.

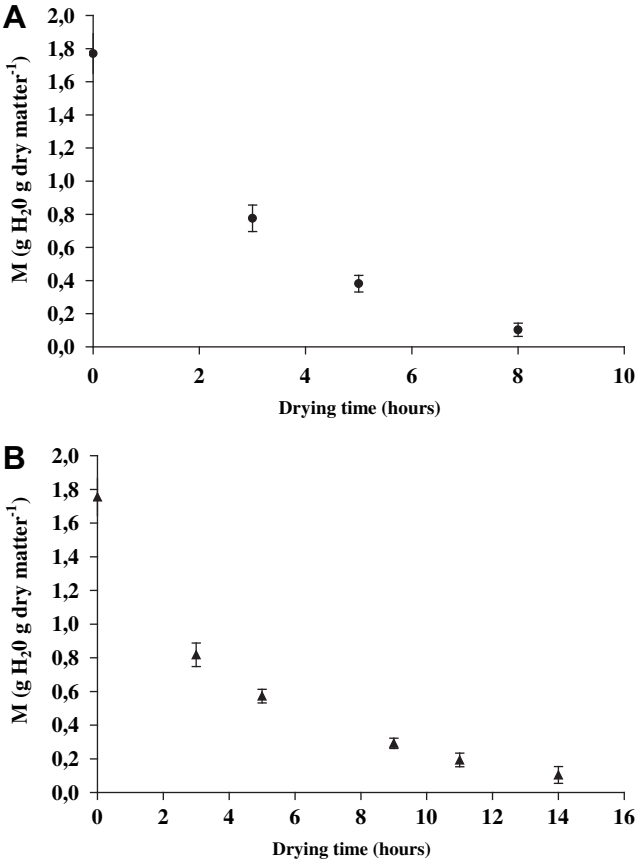

Fig. 1. Drying curve of thyme (A) and rosemary (B), shown as moisture content (M) (g H_20/g dry matter) versus drying time (hours). Rosemary was dried at 38 °C and 300 m^3/h drying air flux, whereas thyme was dried at 45 °C and 1250 m^3/h drying air flux. Data are the means of five determinations.

Table 1
Principal essential oil constituents and their relative abundance[a] in fresh thyme and rosemary.

Compounds	Thyme (%)	Compounds	Rosemary (%)
α-Thujene	1.47 ± 0.01	α-Pinene	23.06 ± 0.01
α-Pinene	1.27 ± 0.02	Camphene	4.50 ± 0.01
Camphene	1.28 ± 0.01	β-Pinene	2.16 ± 0.01
Oct- 1-en-3-ol	1.33 ± 0.01	Limonene	3.33 ± 0.05
Myrcene	1.42 ± 0.01	1,8-Cineole	8.91 ± 0.04
α-Terpinene	1.73 ± 0.01	γ-Terpinene	1.92 ± 0.04
p-Cymene	21.63 ± 0.01	Terpinolene	1.36 ± 0.02
1,8-Cineole	1.09 ± 0.01	Linalool	1.95 ± 0.03
γ-Terpinene	12.01 ± 0.01	Camphor	3.12 ± 0.04
Linalool	2.56 ± 0.01	Borneol	3.49 ± 0.05
Borneol	2.02 ± 0.01	Terpinen-4-ol	1.52 ± 0.04
Terpinen-4-ol	1.04 ± 0.01	α-Terpineol	1.70 ± 0.03
Thymol	34.87 ± 0.01	Myrtenol	1.32 ± 0.02
Carvacrol	2.69 ± 0.01	Verbenone	24.11 ± 0.02
β-caryophyllene	2.46 ± 0.01	Geraniol	1.79 ± 0.02
Caryophillene oxide	1.70 ± 0.02	Bornyl acetate	5.50 ± 0.02
Total thyme (%)	**90.57**		
Total rosemary (%)			**89.74**

[a] The quantification of each compound was expressed as absolute weight percentage using an internal standard and the response factors. Data are the mean of three replicates plus standard deviation.

3.3. Changes of the essential oils after air-drying, freezing or freeze-drying

For better quantitative evaluation of the effects of the different stabilizing methods on the main compounds, we calculated the percentage retention of each compound on the basis of the amounts before and after stabilization, as proposed by di Cesare et al. (2003).

The three stabilizing operations all allowed the preservation of volatile compounds of rosemary (Table 2). The frozen samples underwent no changes with respect to fresh herbs. Although verbenone was best preserved in the air-dried herbs, freezing seems to have given the best results, allowing the best retention of 6 important compounds (1,8-cineole, bornyl acetate, α-terpineol, terpinen-4-ol, terpinolene, myrtenol). The freeze-dried and air-dried samples retained respectively 3 (camphene, borneol and camphor) and 2 (verbenone and geraniol) of the compounds better than the frozen samples. The results for the air-dried samples are in agreement with previous findings (Blanco et al., 2002; di Cesare et al., 2001; Jaganmohan-Rao et al., 1998). To our knowledge, these are the first data on the chemical composition of essential oils extracted by hydrodistillation of freeze-dried and frozen samples.

When only the weighted means were considered, the retention of compounds in the thyme essential oil was very similar to that observed for rosemary (Table 3). Freezing and air-drying seemed to give the best results. The frozen samples showed a 20% loss of *p*-cymene but a strong increase of 1,8-cineole, whereas the air-dried samples exhibited a high increase of β-caryophyllene and caryophyllene oxide. On the other hand, the freeze-dried and frozen samples exhibited a dramatic decrease of caryophyllene oxide, whereas 1,8-cineole sharply decreased in the oils extracted from the air-dried herbs. From a quantitative point of view, the results do not agree with those reported by some authors, who found a higher retention of volatiles in freeze-dried herbs than in air-dried ones (Venskutonis, 1997; Venskutonis et al., 1996), whereas they are in accordance with the results of others (Raghavan et al., 1995). However, the freeze-dried herbs preserved thymol and carvacrol better than the air-dried samples, in accordance with literature data (Bendl et al., 1988; Venskutonis, 1997; Venskutonis et al., 1996).

3.4. Changes of the essential oils in air-dried, freeze-dried and frozen samples during storage

The retention of volatile compounds in rosemary was very high during the storage period (Table 2). The best results were with the freeze-dried samples, with an increase of 9 compounds at the end of the storage. The frozen samples displayed the worst results, with a significant decrease of 9 compounds. The air-dried samples showed the best preservation of verbenone and α-pinene, at the end of the storage. The freeze-dried samples had the significantly highest percentage retention of 7 compounds including 1,8-cyneole, bornyl acetate and linalool. The frozen samples showed a dramatic decrease of 5 of the 7 oxygenated compounds (1,8-cyneole, borneol, α-terpineol, terpinen-4-ol and myrtenol) and only two remained unchanged, while the air-dried and freeze-dried samples showed the opposite behavior. This difference may be due to the different packaging method: the freeze-dried and air-dried herbs were packaged under vacuum, whereas the frozen herbs were packaged in the presence of air and with a film permeable to oxygen. Hence, the oxygen availability may have hastened the loss of oxygenated compounds during storage. Thus the storage of rosemary at freezing temperature gave good results up to 6 months, while the air-dried and freeze-dried herbs showed a negligible quality loss throughout the storage time (Table 2). To our knowledge, these results are a novelty for volatiles extracted by hydrodistillation of stored rosemary.

For thyme, there was a high retention of volatile compounds during the storage period (Table 3). The air-dried and freeze-dried samples gave the best results, with an increase of respectively 7 and 8 compounds after one year of storage. The frozen samples gave the worst result, with a significant loss of 9 compounds. The frozen samples, however, showed a better retention of thymol, γ-terpinene and carvacrol than the air-dried samples at the end of the storage period.

The freeze-dried and frozen herbs suffered a loss of more than 50% for five compounds and three volatiles completely disappeared in the frozen sample, probably they were influenced by storage time and lose their stability, producing some other compounds which were detected in very small concentrations. The air-dried herbs showed a decrease of over 50% for only two compounds,

Table 2
Percentage retention[a] after stabilization and during storage of the characteristic volatile compounds of rosemary stabilized by different unit operations.

Compounds	Stabilized samples (%)			Stored samples (months)											
				3			6			9			12		
	AD**	FD	FR	AD	FD	FR	AD	FD	FR	AD	FD	FR	AD	FD	FR
Verbenone	109.4Ad	99.7Bh	98.6Bh	81.8k	121.4a	91.2i	111.4bc	109.7cd	102.2g	110.8bcd	104.7f	107.4e	112.6b	86.0j	98.4h
α-Pinene	88.3Bk	92.2Aj	92.4Aj	112.1a	73.3l	96.3i	100.1g	110.9bc	98.7h	108.5c	106.6e	98.2h	108.2cd	102.6f	107.5d
1,8-cineole	96.0Bg	96.8Bg	101.9Ade	100.4ef	104.6bc	108.1a	87.9h	105.2bc	99.2f	99.5f	103.1cd	95.2g	96.3g	106.6ab	82.2i
Bornyl acetate	108.3Cde	111.7Bd	119.4Ac	96.4f	119.6b	97.8f	128.3a	77.2i	93.7fg	88.8gh	89.1gh	89.0gh	84.2h	103.4e	87.6h
Camphene	94.2Ch	100.7Ad	97.6Bg	112.8a	70.1i	105.3b	71.0i	95.3h	103.4c	100.2de	99.8def	98.4efg	102.8c	98.0fg	114.5a
Limonene	96.6A1i	98.7Ahi	98.6Ahi	131.0c	84.9j	112.5e	82.4k	100.1h	120.1d	109.1fg	120.0d	108.4g	111.1ef	160.2a	154.2b
Borneol	97.9Bh	101.8Ag	94.3Bi	114.1d	115.5d	114.4d	142.2b	107.4f	110.9e	118.9c	119.0c	25.0j	201.6a	140.7b	21.2k
Camphor	95.8Cfg	110.5Aa	103.7Bbc	103.4bc	95.5fgh	105.9b	80.1j	88.1i	101.8cd	93.8gh	89.1i	97.5ef	97.8ef	99.5de	92.6h
β-Pinene	99.8Acd	96.9Ad	96.7Ad	77.6e	56.4f	102.4bc	33.0h	55.7f	103.7b	54.6f	46.9g	97.1d	47.7g	45.3g	109.7a
Linalool	93.5Bi	98.6Agh	102.7Aefg	111.1c	139.8a	103.1ef	80.4j	102.8efg	106.1de	103.2ef	110.2cd	101.0fgh	97.1hi	134.4b	100.fgh
γ-Terpinene	99.8Bb	112.7Aa	116.2Aa	86.7d	72.6f	92.4c	56.4h	62.4g	86.4d	48.3i	44.6i	77.5e	35.6j	36.4j	80.2e
Geraniol	111.6Ad	90.0Cf	99.3Be	84.2g	155.1b	114.1d	161.3a	116.6d	97.2e	66.0h	99.1e	111.6d	0.0i	122.1c	95.8e
α-Terpineol	73.8Ci	117.8Bd	141.4Ab	139.4b	112.1e	78.0h	156.2a	96.7f	78.9h	141.6b	110.4e	92.2g	139.4c	132.4c	79.8
Terpinen-4-ol	90.2Cef	97.4Bd	105.4Ac	100.7cd	123.5a	113.7b	82.0g	85.1fg	95.3de	97.5d	95.9de	100.0cd	86.9fg	113.5b	87.3fg
Terpinolene	103.4Bab	102.2Bb	107.6Aa	86.0d	78.8e	96.8c	63.4f	63.5g	89.8d	63.1g	72.2f	87.5d	61.2g	60.5g	87.9d
Myrtenol	98.2Bde	96.1Be	105.2Ac	90.3fg	104.1c	82.5h	150.5a	93.1ef	95.6e	68.6i	0.0j	83.2h	101.8cd	116.6b	87.2gh
Percent mean	**97.7**	**99.0**	**100.0**	**97.6**	**99.3**	**98.6**	**100.5**	**100.0**	**100.4**	**99.8**	**98.4**	**96.9**	**101.4**	**99.4**	**97.1**

**Different capital letters within each row indicate significantly different means among stabilized samples according to the Tukey test at $P < 0.01$. Different lower case letters within each row indicate significantly different means among stabilized and stored samples according to the Tukey test at $P < 0.01$. **Air-dried (AD), freeze-dried (FD) and frozen (FR) samples. Three replicates of each sample were analyzed.

[a] The percentage retention is calculated as considering as 100% the percentage of each compound in the fresh samples.

Table 3
Relative abundance[a] after stabilization and during storage of the characteristic volatile compounds of thyme stabilized by different unit operations.

Compounds	Stabilized samples (%)			Stored samples (months)											
				3			6			9			12		
	AD**	FD	FR	AD	FD	FR	AD	FD	FR	AD	FD	FR	AD	FD	FR
Thymol	97.3Ch	119.8Ab	113.6Bc	99.7f	98.2g	97.0h	102.5e	109.7d	90.1k	94.8i	91.2j	90.9j	80.3l	91.2j	142.7a
p-Cymene	105.1Ah	88.3Bj	80.6Ak	96.3i	106.1h	107.4g	114.1e	115.1de	126.2c	108.8f	144.2a	116.0d	128.1b	145.2a	69.7l
γ-Terpinene	83.6Cg	95.8Be	110.0Ab	41.7l	68.3h	99.5d	33.9n	53.6j	103.3c	37.6m	62.8i	117.2a	36.6m	46.0k	92.1f
Carvacrol	87.1Ch	96.7Bg	101.2Af	116.8d	111.0e	78.5i	152.3b	174.9a	101.4f	100.8f	141.5c	75.6i	99.0g	139.4c	140.7c
Linalool	89.8Bf	77.8Cg	97.2Ae	120.9c	104.5d	93.0f	118.2c	144.7a	98.1e	127.8b	126.9b	81.2g	96.9e	130.0b	39.3h
β-Caryophyl	122.2Aab	89.5Cc	95.6Bbc	86.5c	93.9c	94.7bc	74.9c	83.1c	91.4c	73.9c	70.9c	71.4c	72.3c	41.6d	134.3a
Borneol	83.8Bf	81.0Bf	137.9Aa	72.5g	109.6de	69.9g	80.3f	115.0bcd	85.4f	111.1cde	106.3e	44.9h	120.7b	116.8bc	45.4h
α-Terpinene	78.0Bd	89.1Ac	95.4Ac	96.3c	63.7e	0.0g	0.0g	46.6f	112.3b	129.2a	72.9d	134.2a	0.0g	47.8f	62.6e
Caryoph. oxide	149.7Ad	45.0Cj	66.8Bi	107.2g	129.8e	119.2f	112.3fg	225.4a	93.7h	152.7d	196.0b	75.7i	118.4f	198.7b	166.0c
α-Thujene	81.9Ad	77.6Bd	69.5Ce	47.3g	69.6e	135.0b	61.0f	41.9gh	135.1b	66.2ef	77.0d	144.5a	62.2f	40.1h	91.2c
Myrcene	88.6Abcd	87.2Cbcde	90.2Bbcd	52.9f	79.9cdef	115.0ab	57.9def	53.6ef	130.3a	47.0f	67.5def	90.3bcd	106.4abc	46.3f	72.3def
Oct-1-en-3-ol	137.41Ab	85.9Bj	96.5Cgh	90.6ij	104.1e	94.5hi	123.8c	156.0a	100.8efg	109.6d	102.6e	97.4fgh	101.4ef	122.6c	0.0k
Camphene	81.6Af	60.4Bg	84.5Aef	90.4de	97.8c	97.2c	95.5cd	80.3f	124.9b	126.7b	143.2a	97.2c	131.2b	101.7c	50.8h
α-Pinene	82.1Bf	93.1Ae	76.4Cf	46.5i	80.7f	109.2d	121.7c	56.0h	120.9c	147.3b	104.2d	126.7c	159.9a	66.2g	111.6d
1,8-Cineole	37.3Cj	88.8Bg	124.0Ad	97.1ef	94.7fg	57.6i	166.0c	88.4g	93.5fg	304.7a	104.2e	74.9h	177.7b	182.8b	0.0k
Terpinen-4-ol	109.1Ab	96.4Bc	94.1Ac	122.1a	94.2c	15.00e	108.3b	80.3d	96.8c	122.7a	95.6c	131.7a	93.4c	102.4bc	0.0f
Percent mean	**97.3**	**99.5**	**100.7**	**91.1**	**95.0**	**96.5**	**99.8**	**102.8**	**102.3**	**99.1**	**101.3**	**100.0**	**96.4**	**98.4**	**106.1**

**Different capital letters within each row indicate significantly different means among stabilized samples according to the Tukey test at $P < 0.01$. Different lower case letters within each row indicate significantly different means among stabilized and stored samples according to the Tukey test at $P < 0.01$. **Air-dried (AD), freeze-dried (FD) and frozen (FR) samples. Three replicates of each sample were analyzed.

[a] The percentage retention is calculated as considering as 100% the percentage of each compound in the fresh samples.

including γ-terpinene, one of the most important constituents. Five of the seven oxygenated compounds decreased dramatically in the frozen samples. The two most important compounds, thymol and carvacrol, increased at the end of the storage and only two remained unchanged, whereas the opposite behavior was recorded in the air-dried and freeze-dried samples, with some exceptions (Table 3). This behavior can be due to the differences in packaging materials, as reported above for rosemary. Our data could be partially compared with those reported by Venskutonis et al. (1996), who summed the volatiles extracted from air-dried and freeze-dried samples. No data have been reported so far for the storage of frozen samples.

4. Conclusions

Freezing is the best method to maintain the composition of rosemary essential oil, while air-drying is the worst. However, air-dried and freeze-dried herbs can be stored with appropriate packaging up to one year with negligible quality loss, while storage at freezing temperature gives good results up to 6 months.

Freezing seems to be the best method to maintain the composition of thyme essential oil, while the worst results were obtained with freeze-drying, even though it was better at retaining thymol and carvacrol, the most important components of thyme aroma. The air-dried and freeze-dried samples had the best retention of volatile compounds during storage. The frozen samples showed the best retention of thymol, γ-terpinene and carvacrol.

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