



## RESPONSE OF ROSEMARY (*Rosmarinus officinalis* L.) PLANTS CULTIVATED IN SANDY SOILS TO POSTHARVEST TREATMENTS AND MICROBIAL LOAD

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**ABSTRACT:** This investigation was carried out during the period (2017/2020), at El-Quassassin Horticultural Research Station, Ismailia Governorate, Agricultural Research Center, Ministry of Agriculture, Egypt. The aim of this research was to arrive at the best methods of post-harvest treatments for rosemary (*Rosmarinus officinalis* L.) plants, such as the optimum drying method, the best storage package and the storage period that maintain the content of dry leaves of volatile oil, chlorophyll a, b, a+b and carotenoids, through three years. From the results of this research, drying in the shade for a period of 7 days was the best method, followed by drying in the oven at a temperature of 40° for a period of 6 hours, and that storage in glass jars was the best, followed by storage in carton bags, for a storage period of up to two or three years. The microbial load results indicated that the best drying method was sun drying with storage in glass containers, followed by oven drying and storage in glass containers, followed by carton bags, and that increased the storage period, reduced the percentage of microbial content, especially with oven drying, followed by sun drying, especially in packages glass jars followed by carton pages.

**Key words:** Rosemary (*Rosmarinus officinalis* L.), shade, oven, sun, drying, cotton page, carton page, glass jars, storing, storage, chlorophyll, carotenoids, microbial load.

### INTRODUCTION

One of the most important aspects to take into account in the commercialization of spices is their packaging and storage. The widespread idea is that the spices, once dry, possess a long shelf-life from the microbiological point of view, which can be attributed to their low water activity, which prevents the growth of the majority of the microorganisms. Nevertheless, the organoleptic properties of spices can be highly influenced by packaging material and duration of the storage.

Although the effects of drying on the quality of spices have been studied, little information regarding the effects of their storage exists, and this is a topic of great interest for both industry and the consumer. As it happens, during the drying of the spices, valuable constituents, such as volatile compounds, are fairly stable in the intact plant tissue; however,

they become sensitive during storage (Schweiggert *et al.*, 2007).

The family *Lamiaceae* includes many common species, some so similar that differentiation can be difficult. One of the most widely diffuse species of this family is *Rosmarinus officinalis* L. Staruch *et al.* (2011) indicated that, rosemary extract had a very effective and higher antioxidant activity. Also, the volatile oil of *R. officinalis* has hyperglycaemic and insulin release inhibitory effects on rabbits (Al-Hader *et al.*, 1994), and the leaves were used to decrease blood glucose levels (Erenmemisoglu *et al.*, 1997), an antispasmodic, to relieve respiratory disorders and stimulate growth of hair. Also, the leaves extract was choleric, hepatoprotective and anticancer activities (Al-Sereiti *et al.*, 1999).

The main components of *R. officinalis* essential oil were  $\alpha$ -pinene (4.1-20.14%), camphene (5.2-8.6%),  $\beta$ -pinene (5.3-13.7%), limonene (2.0-3.8%)

1,8-cineole [eucalyptol] (7.43-26.54%), camphor (13.0-31.0%),  $\alpha$ -terpineol (1.2-2.5%), borneol (3.2-13.03%), bornyl acetate (0.2-23.0%) and  $\beta$ -caryophyllene (1.8-5.1%) as determined by **Domokos et al. (1997)**; **Latif Gachkar et al. (2007)**; **Yang et al. (2011)**; **El-bastawesy et al. (2009)** and **Szumny et al. (2010)**.

**Oztekin and Martinov (2007)** demonstrated that, drying of aromatic and medicinal plants must meet the following requirements: (1) Moisture content has to be brought down to be at an equilibrium level that is defined for certain relative air humidity and temperature. This is defined as storage condition by standards; (2) minimum quality reduction in terms of active ingredients, color, flavor and aroma; and (3) microbial count must be below the prescribed limits. Drying is basically defined as the decreasing of plant moisture content, aimed at preventing enzymatic and microbial activity, and consequently preserving the product for extend shelf life (**Rocha et al., 2011**).

The sun has been used for drying as long as humans have inhabited the planet and laying a product out in the sun to remove its moisture is known as sun drying, which is a low-cost drying method. When sun drying, the temperature of the product is raised by the direct absorption of solar radiation. Although sun drying is still by far the most common method of drying, it does have several inherent disadvantages. The unprotected crop can be damaged by rain, contaminated by dirt and animals and/or eaten by birds and insects. This usually results in poorer final quality because of crop discoloration caused by enzymic and non-enzymic browning and often because of the formation of moulds. **Oztekin and Martinov (2007)** showed that intensive solar radiation adversely affects quality, causing losses in essential oils or color changes in dried plants.

As for drying in the shade (air drying), it has been discussed in many studies for its limited effect on the final product, especially for medicinal and aromatic plants, but the drying process in it continues for several days. **Refaat and Wahba (1998)** indicated that, lavender (*Lavandula officinalis*) plants which were shade-dried at 19-25°C had higher oil yields (1.3%) than sun- or oven-dried plants. **Dambrauskiene and Viskelis (2003)** found that, the quality of raw material changed at least when aromatic plants lavender (*Lavandula angustifolia*), balm (*Melissa officinalis*), oregano (*Origanum vulgare*) and sage (*Salvia officinalis*) were dried naturally. By this method, the highest amount of essential oils was preserved.

Most essential oils are volatile and sensitive in the air conditions (humidity, temperature and velocity). Drying temperatures is the most important parameter to preserve the active ingredients of volatile oil in gland cells, which are very sensitive to temperature increase. Drying encourages moisture loss from the whole tissue, including gland hairs (**Oztekin and Martinov, 2007**). Generally, high temperatures influence essential oil quantity and quality in aromatic and medicinal plants not only during drying; but also, reduction in active ingredients continues during storage period as well (**Martinazzo et al., 2009**).

Oven drying had a positive effect in the other times, as **Deans and Svoboda (1992)** which pointed out that, thyme (*Thymus vulgaris*) and rosemary (*Rosmarinus officinalis*) oils did not change significantly, when dried at temperatures between 40 and 100 degrees C for 24 h. and **Buggle et al. (1999)** stated that, for the best results of essential oil content of lemon grass (*Cymbopogon citratus*), were obtained when drying at 50°C (1.43%). Also, **Soares et al. (2007)** showed that, the higher essential oil contents of *Ocimum basilicum* L. were obtained in the drying process with an air temperature at 40°C. and **Saeidi et al. (2016)** showed that the highest essential oil contents (w/w) of *Mentha longifolia* (L.) Hudson were obtained by oven-drying at 40°C (0.94%), followed by the shade-drying (0.93%).

Oven drying has had a negative effect sometimes, as **Shalaby et al (1988)** who concluded that, oven drying reduced the essential oil content of the mint (*Mentha arvensis* L.) samples by 89.5-91.0%. furthermore, **Sankat and Maharaj (1994)** showed that, there was the strong positive influence of the air temperature (with natural convection drier at 35, 45, 55 and 65 °C) on the drying rate of the herb *Eryngium foetidum* (Fam. Apiaceae), however higher temperatures had a deleterious effect on the odour and flavour of the oil extracted from the herb. **David et al. (2006)** they found that, increasing temperature of air drying above 40°C reduced the levels of components in essential oil of *Ocimum selloi* Benth.

The major components of the rosemary (*Rosmarinus officinalis* Linn.) leaves oil were 1,8-cineole (31.7%), camphor (27.4%),  $\alpha$ -pinene (12.7%), verbenone (6.5%), camphene (5.2%)  $\beta$ -pinene (2.9%), bornyl acetate (2.9%)  $\beta$ -myrcene (2.5%) and  $\alpha$ -terpineol+borneol (4.2%). The quantitative composition of the oils was significantly affected by mode of drying. The monoterpene hydrocarbons which dominated in the fresh, shade dried and sun-dried leaves (24.7-25%) was reduced

to 23.4 and 16.4 in the hot air dried and oven dried leaves, respectively. whereas oxygenated monoterpenes were found to be higher in oven dried leaves (82.4%). The concentration of  $\alpha$ -pinene was slightly higher in shade and sun-dried leaves (13.4 and 13.9%), respectively. The percentage of 1,8-cineole was higher (31.8-33.9%) in other methods of drying than fresh leaves. However, the amount of camphor was observed to be higher in oven dried leaves (31.7%), while in shade and sun-dried leaves it was 26.6 and 26.9%, respectively. The concentration of verbenone was found to increase on oven dried leaves (9.4%). The changes in the regimes of volatile compounds during drying have been reported to depend on several factors such as drying method and change to species or family (Loughrin and Kasperbauer, 2003 and Rao *et al.*, 1998). The components of the essential oils that lost in the dried leaves were those stored on or near the leaf surface (Moyler, 1994). While, Diaz-Maroto *et al.* (2009) indicated that, the negative effect of the storage time in room temperature of dried rosemary leaves was observed in the majority of the volatile components identified, except in  $p$ -cymene, isopropenyl-4-methylbenzene, camphor, pinocampnone, thymol and carvacrol.

Concerning the effect of rosemary essential oil on microorganisms, Yang *et al.* (2011) indicated that essential oil of rosemary showed pronounced antimicrobial activity against Gram-positive bacteria (*Staphylococcus epidermidis*, *Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Proteus vulgaris*, *Pseudomonas aeruginosa* and *Escherichia coli*) and fungus (*Candida albicans* and *Aspergillus niger*). Also, Ozcan and Chalchat (2008) showed that, rosemary oil exhibited an inhibitory effect of (*Alternaria alternata*, *Botrytis cinerea* and *Fusarium oxysporum*) fungal growth.

Medicinal plants can be contaminated by a wide range of microorganisms, such as fungi, yeasts, protozoa, and viruses, most of which are transferred from soil (Kosalec *et al.*, 2009 and Kneifel *et al.*, 2002). Total microbial count is an important factor in determining the health status or probable detection of a contamination source (Khan *et al.*, 2013). Contaminants such as microorganisms, heavy metals, and pesticides affect the quality and the efficacy of herbal products. Since it is impossible to remove all contaminants, precautionary measures should be taken to prevent or limit contamination (Kosalec *et al.*, 2009 and Kneifel *et al.*, 2002). Therefore, our study aimed to show the effect of this contaminations on consumer's health. The microbial contaminants of herbal products are simply transferred through air, soil, animal-and human-

based fertilizers, and rally infected staff and workers producing units. Otherwise, a host of agricultural, environmental, industrial, and urban factors, together with less than good harvesting, storage, and processing procedures, are additional reasons for contamination in herbal products (Kneifel *et al.*, 2002). In these cases, medicinal plants and herbal products with confirmed therapeutic effects not only do not improve the patient's condition, but also lead to diverse kinds of foodborne diseases and disorders. Finally, as pointed out earlier, the attack of microorganisms, fungi and insects affect both qualitatively and qualitatively the medicinal plants. The microbial load depends on the temperature, humidity, handling and storage of the processed or unprocessed medicinal plants, and may increase morbidity and mortality, especially in patients with compromised immune systems and who are vulnerable to infections (Kneifel *et al.*, 2002; Araujo and Bauab, 2012 and Vuuren *et al.*, 2014).

Only few investigations are known about the effect of drying, packaging and period of storing upon the quality of aromatic herbs. The aim of this investigation is to finding the suitable drying methods, packaging types and the storing period to obtain a desirable essential oil quality and pigment content of rosemary leaves.

## MATERIALS AND METHODS

### Plant material

This investigation was carried out during (2017/2020) season, at El-Quassassin Horticultural Research Station, Ismailia Governorate, Agricultural Research Center, Ministry of Agriculture, Egypt.

The present study aimed to evaluate the effect of drying methods (shade, sun and oven) and packing materials (carton bags, glass jars and cotton bags) during the storage period at 3 years on volatile oil percentage, moisture content and pigments content (chlorophyll a, b, a+b and carotene), in the fresh and dried herb of *Rosmarinus officinalis* plants.

The cuttings of rosemary were collected from the Medicinal and Aromatic Plant Section, Horticultural Research Institute, and planted in the Experimental Farm of El-Quassassin Horticultural Research Station in October 2016. The plants of rosemary were cutting in April 2017, and the fresh leaves were separated and collected from stems and divided into four portions. The three portions were dried with different drying methods as follow:

1- In the sun to constant weight during 3 days.

- 2- In the oven which was kept at 40 °C to constant weight during 6 hours.
- 3- In the laboratory under normal air in the shade at room temperature conditions to constant weight during 7 days.
- 4- While the fourth portion left fresh.

Every portion of dried leaves (with the three methods of drying) was divided into 3 parts. The three parts of every method of drying stored in different three packaging types (carton bags, glass jars and cotton bags) and stored even 3 years.

Packaging and storage experiments were carried out at room temperature ( $\approx 22$  °C) under light and darkness conditions. After 36 months of storage, the trials were sampled to know and evaluate their volatile composition. Three replications of each experiment were performed.

#### **The treatments were:**

- 1- Fresh leaves (Control).
- 2- Dried leaves in the shad (zero time), (shade zero time).
- 3- Dried leaves in the sun (zero time), (sun zero time).
- 4- Dried leaves in the oven (zero time), (oven zero time).
- 5- Dried leaves in the shad, kept in carton bags (shade in carton bags).
- 6- Dried leaves in the shad, kept in cotton bags (shade in cotton bags).
- 7- Dried leaves in the shad, kept in glass jars (shade in glass jars).
- 8- Dried leaves in the sun, kept in carton bags (sun in carton bags).
- 9- Dried leaves in the sun, kept in cotton bags (sun in cotton bags).
- 10- Dried leaves in the sun, kept in glass jars (sun in glass jars).
- 11- Dried leaves in the oven, kept in carton bags (oven in carton bags).
- 12- Dried leaves in the oven, kept in cotton bags (oven in cotton bags).
- 13- Dried leaves in the oven, kept in glass jars (oven in glass jars).

#### **Recorded data**

##### **1- Volatile oil percentage**

Volatile oil percentage of fresh and dry leaves, were done before and after storing. About 100 g

each of fresh and dried leaves of rosemary plants were separately subjected to hydro-distillation for 3 h, using a Clevenger-type apparatus (**British Pharmacopoeia, 1980**).

The losing percentage of volatile oil percentage from fresh weight of leaves to dried leaves in zero time and after three years storing in different types of packaging.

##### **2- Gas Liquid Chromatographic (GLC)**

The G.L.C. analysis of rosemary essential oil samples were carried out at the Laboratory of Medicinal and Aromatic plants Department, Horticulture Research Institute, Agricultural Research Center, Dokki, Ministry of Agriculture, Egypt. The relative retention time (RT) of each peak was compared with the reference authentic sample to identify the unknown samples. The quantitative estimation for each compound was based on the peak area measurement by triangulation (**Guenther and Joseph, 1978**).

3- The weight of 100g leaves of rosemary after drying and during the three storage years under different treatments of drying and packaging

##### **4- Leaf Pigments**

Chlorophyll contents A, B, A+B and total carotenoides (mg/100 g as fresh weight) were determined according to the procedure described by **Mazumdar and Majumder (2003)**.

##### **5- Microbial load**

###### **Source of samples**

###### **Preparation and sterilization of media and Samples**

The media used were nutrient agar (NA) and (PDA) for enumeration of fungi, respectively. They were prepared according to the manufacturer's guide and sterilized in an autoclave at 121 °C for 15 min. Plant samples taken for the microbial pregnancy test was taken every year.

###### **Microbial analysis of plant samples**

The pour plate method used to cultivate serially diluted portions of the medicinal plant samples, was determined by transferring 10g leaves samples into 250 ml Erlenmeyer flasks containing 100 ml of distal water as diluents. Each flask was shaken at 140 RPM for 20 minutes on an orbital shaker. Serial dilutions up to 1:104 were made and 1 ml aliquots were plated on 20 ml of glucose yeast extract agar (GYE) plate count agar. All the plates were incubated at 30 °C for 5 days. Colonies of mycoflora that appeared after 5 days of incubation

were counted and calculated as log<sub>10</sub> CFU/g sample. The colonies were purified, isolated and stored for morphological and biochemical characterization. These were further identified with the Illustrated Genera of Imperfect.

### Statistical analysis

All data were statistically analyzed according to **Snedecor and Cochran (1980)** and mean values of the treatments were compared by LSD. Test according to **Steel and Torrie (1980)**.

## RESULTS AND DISCUSSION

### Effect of drying methods, packaging types and the interaction between them on essential oil

#### Oil percentage in leaves

Data presented in Table (1) showed that oil percentage in dried leaves of rosemary plant affected with different methods of drying in zero time. There were non-significant between them. The highest oil percentage (0.733%) obtained from the shade drying methods, while the least percentage (0.727%) observed with the sun drying method. Such results were in harmony with the findings of **Ahmed et al. (2018)** who showed that the highest yield was obtained from aerial parts of *Mentha pulegium* dried in the shade.

The effect of drying methods through 3 years storage and tabulated in Table (1) indicate that there was gradually decrease in essential oil from zero time to 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> storage years. The highest oil percentage found in leaves dried with shade method (0.707, 0.691 and 0.658%) respectively, over the other methods, with highly significant with sun drying method (0.675, 0.585 and 0.557%), and non-significant with oven drying method (0.675, 0.633 and 0.603%) respectively during the three storage years. These results are coincided with those reported by **Usai et al. (2010)** who demonstrated that appropriate packaging of air-dried herbs of rosemary (*Rosmarinus officinalis* L.) resulted in negligible quality loss up of the essential oil compositions to one year of storage.

The packaging types affected on oil percentage in dried rosemary leaves through 3 years storing with non-significant between them. The highest oil percentage obtained from glass jars type (0.698, 0.655 and 0.623%), while the lightest percentage observed with cotton bag type (0.658, 0.617 and 0.587%) during the three storage years, respectively.

Concerning the effect of interaction between different methods of drying and packaging types after 3 years storing on oil percentage in rosemary

dried leaves, the results in Table (1) show that, there were highly significant differences between the interaction treatments. The highest volatile oil percentage (0.777, 0.728 and 0.693%) resulted from the combined treatment of (shade drying method and packaging in glass jars type), while, the least oil percentage (0.560, 0.533 and 0.533%) found with the treatment of (sun drying method + cotton bags type packaging) with highly significant difference between them in all three storage years.

In the same trend, there were non-significant differences between different packaging types with shade drying method treatments, but there was significant difference between the combined treatments of (shade drying method + packaging in glass jars) and (sun drying method + packaging in glass jars). Furthermore, there was non-significant difference between (shade drying method + packaging in glass jars) and (oven drying method + packaging in glass jars) treatments, during 3 years storing.

On the other way, the average of oil percentage in dried Rosemary leaves after 2 and 3 years storing (Group 2 and 3) had highly significant decreasing from the average of oil percentage in zero time. While there was non-significant difference of essential oil percentage between the average zero time and average group (1). Obtained results in this study were in harmony with those reported by **Shalaby et al. (1988)** who found that the essential oil content of the stored samples of mint (*Mentha arvensis* L.) remained the same throughout the storage period (12 months) in all bag types (kraft paper bags, polyethylene bags or synthetic jute-like bags).

#### Essential oil constituents in dried rosemary leaves

We can observe from Table (2) that the combined treatment (oven drying method + carton pages) recorded highly percentage from  $\alpha$ -pinene (6.93%), camphene (4.51%), limonene (19.68%), 1,8 cineol (3.97%), camphor (19.95%) and eugenole (14.96%), after three years storage in dried leaves of rosemary. While the combined treatments (shade drying method + cotton pages), (shade drying method + glass jars), (sun drying method + cotton pages), (sun drying method + glass jars) and (oven drying method + glass jars) gave the highest percentage from camphene (4.58%), bornyle acetate (16.09%),  $\beta$ -caryophyllene (10.63%),  $\beta$ -pinene (7.51%) and  $\alpha$ -terpeniol (10.37%), respectively. Also, the interaction treatment (oven drying method + cotton pages) gave the highest percentage of  $\alpha$ -pinen (7.52%) and borneol (17.37%) over of the essential oil constituents of fresh leaves.

**Table 1.** Effect of drying methods, packaging types and the interaction between them on essential oil percentage in leaves of *Rosmarinus officinalis*, L. plant in the zero time and after 3 years storing from 2017 to 2020

Fresh weight of leaves (g)	<b>0.260</b>			
<b>Zero time</b>				
<b>Drying treatments in zero time</b>				
Shade Zero Time	0.733			
Sun Zero Time	0.727			
Oven Zero Time	0.731			
Average zero time	0.731			
Drying treatments	<b>Packaging treatments</b>			<b>(A) Means of Drying</b>
	<b>Carton bags</b>	<b>Cotton bags</b>	<b>Glass jars</b>	
<b>Group (1)</b>				
<b>Drying, packaging and its combined treatments after 1 year</b>				
Shade	0.731	0.704	0.777	0.737
Sun	0.637	0.597	0.638	0.624
Oven	0.675	0.672	0.679	0.675
<b>(B) Means of Packaging</b>	0.681	0.658	0.698	
<b>Group 1</b>	0.679			
<b>Group (2)</b>				
<b>Drying, packaging and its combined treatments after 2 years</b>				
Shade	0.685	0.660	0.728	0.691
Sun	0.597	0.560	0.599	0.585
Oven	0.633	0.630	0.637	0.633
<b>(B) Means of Packaging</b>	0.638	0.617	0.655	
<b>Group 2</b>	0.637			
<b>Group (3)</b>				
<b>Drying, packaging and its combined treatments after 3 years</b>				
Shade	0.653	0.629	0.693	0.658
Sun	0.569	0.533	0.570	0.557
Oven	0.603	0.600	0.607	0.603
<b>(B) Means of Packaging</b>	0.608	0.587	0.623	
<b>Group 3</b>	0.606			
<b>LSD between:</b>	<b>5%</b>			<b>1%</b>
Average zero time	NS			NS
Zero time and Groups (1)	NS			NS
Zero time and Groups (2)	0.0513			0.0701
Zero time and Groups (3)	0.050			0.068
<b>After 1 year</b>				
(A) Drying Treatments	0.0655			0.0895
(B) Packaging Treatments	NS			NS
(AB) Interaction	0.1135			0.1551
<b>After 2 years</b>				
(A) Drying Treatments	0.0628			0.0859
(B) Packaging Treatments	NS			NS
(AB) Interaction	0.1088			0.1487
<b>After 3 years</b>				
(A) Drying Treatments	0.061			0.083
(B) Packaging Treatments	NS			NS
(AB) Interaction	0.106			0.144

**Table 2. Effect of the interaction between drying methods and packaging types on essential oil constituents in fresh and dried leaves of *Rosmarinus officinalis* L. stored 3 years from 2017 to 2020**

Treatments	$\alpha$ -Pinen	Camphene	$\beta$ -Pinen	Limonen	1,8 Cineole	Camphor	$\alpha$ -Terpineol	Borneol	Bornyle acetate	Eugenole	$\beta$ -Caryophyllene
Fresh leaves	10.00	3.91	3.45	7.25	11.18	2.14	2.00	2.00	7.65	0.10	6.37
Shade drying + Cartoon	2.93	1.21	0.76	13.28	2.48	2.61	0.59	14.69	4.2	4.73	1.39
Shade drying + Cotton	6.91	4.58	1.87	15.8	3.09	0.15	2.12	2.52	14.3	4.52	4.10
Shade drying + Glass	3.6	4.21	3.16	0.71	2.39	2.69	1.53	0.52	16.09	1.76	2.15
Sun drying + Carton	4.54	3.18	2.53	10.06	2.31	4.18	2.57	9.16	6.95	6.58	4.65
Sun drying + Cotton	5.34	3.13	2.39	11.46	2.83	0.36	0.29	15.94	2.97	10.75	10.63
Sun drying + Glass jars	0.34	0.15	7.51	2.42	2.37	4.71	3.07	15.27	4.76	4.00	9.15
Oven drying + Carton	6.92	4.51	2.03	19.68	3.97	19.95	2.25	4.25	9.42	14.96	4.75
Oven drying + Cotton	7.52	3.87	1.36	4.53	0.08	2.03	0.35	17.37	1.02	3.12	3.55
Oven drying + Glass jars	2.75	3.77	1.18	12.56	1.24	0.94	10.37	2.75	2.81	8.77	1.51

On the other way, the combined treatments of (Shade drying method + Cartoon pages) decreased  $\beta$ -Pinen (0.76%) &  $\beta$ -Caryophyllene (1.39%); (Shade drying method + Cotton pages) decreased Camphor (0.15%); (Shade drying method + Glass jars) decreased Limonen (0.71%), Borneol (0.52%) & Eugenole (1.76%); (Sun drying method + Glass jars) decreased  $\alpha$ -Pinen (0.34%) & Camphene (0.15%) and (Oven drying method + Cotton pages) decreased 1,8 Cineole (0.08%),  $\alpha$ -Terpineol (0.35%) & Bornyle acetate (1.02%) less from the essential oil constituents of fresh leaves.

**Effect of drying methods, packaging types and the interactions on leaf pigments contents in rosemary leaves**

**Chlorophyll a content (mg/ 100 g f.w.)**

The results showed in Table (3) reveal that the treatments of drying methods in zero time significantly affected on chlorophyll a content in rosemary leaves. The highest content observed with the shade drying method followed by oven drying method with non-significant difference between them. While the least content showed when using sun drying method with highly significant with shade drying method.

Concerning the effect of drying methods during three years storing the data show that the highest content of chlorophyll a found in the leaves dried with oven drying method followed by shade drying method with non-significant differences between them. The sun drying method decreased the chlorophyll a content in leaves with highly significant difference with oven drying method.

The packaging types affected on chlorophyll a content for three years storing. The highest content showed in leaves stored in glass jars followed by

carton bags treatments. The least content observed in leaves stored in cotton bags and had significant difference with storage in glass jars during the third storage year. The differences between all treatments were non-significant during the first and second storage years.

Storing rosemary leaves for 2<sup>nd</sup> and 3<sup>rd</sup> years led to a highly significant decrease in their chlorophyll a content compared to zero time, and had significant decrease in the first year compared to zero time.

**Chlorophyll b content (mg/ 100 g f.w.)**

Data presented in Table (4) indicate that the treatment of shade drying method had highly significant content of chlorophyll b in rosemary leaves at zero time comparing with both oven and sun drying methods.

In the same Table there were no significant effects of the three drying methods on chlorophyll b content in drying leaves through 3 storing years. The highest content observed with shade drying method. The packaging methods during three years also had non-significant effects on chlorophyll b in leaves. Rosemary leaves stored in cartons held the largest chlorophyll b content.

Concerning the interactions, the data show that there were non-significant differences between the combined treatments. The highest chlorophyll b content in rosemary leaves found in the interaction treatment (shade drying method + carton bags packaging) in all storage years. Rosemary leaves content of chlorophyll b gradually decreased with non-significant, significant and highly significant differences for the average of 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> years (group 1, 2 and 3) when compared to zero time respectively.

**Table 3.** Effect of drying methods, packaging types and the interaction between them on chlorophyll a content (mg/ 100 g f.w.) in leaves of *Rosmarinus officinalis*, L. plant in the zero time and after 3 years storing from 2017 to 2020

<b>Fresh weight of leaves (g)</b>	<b>0.397</b>			
<b>Zero time</b>				
<b>Drying treatments in zero time</b>				
Shade Zero Time	0.099			
Sun Zero Time	0.080			
Oven Zero Time	0.093			
Average zero time	0.091			
<b>Drying treatments</b>	<b>Packaging treatments</b>			<b>(A) Means of Drying</b>
	<b>Carton bags</b>	<b>Cotton bags</b>	<b>Glass jars</b>	
<b>Group (1)</b>				
<b>Drying, packaging and its combined treatments after 1 year</b>				
Shade	0.075	0.073	0.080	0.076
Sun	0.069	0.046	0.073	0.063
Oven	0.083	0.081	0.093	0.086
<b>(B) Means of Packaging</b>	0.076	0.067	0.082	
<b>Group 1</b>	0.075			
<b>Group (2)</b>				
<b>Drying, packaging and its combined treatments after 2 years</b>				
Shade	0.063	0.061	0.066	0.063
Sun	0.057	0.038	0.061	0.052
Oven	0.069	0.067	0.078	0.071
<b>(B) Means of Packaging</b>	0.063	0.056	0.068	
<b>Group 2</b>	0.062			
<b>Group (3)</b>				
<b>Drying, packaging and its combined treatments after 3 years</b>				
Shade	0.050	0.049	0.053	0.051
Sun	0.046	0.031	0.049	0.042
Oven	0.055	0.054	0.062	0.057
<b>(B) Means of Packaging</b>	0.050	0.044	0.055	
<b>Group 3</b>	0.050			
<b>LSD between:</b>	<b>5%</b>		<b>1%</b>	
Average zero time	0.0107		0.0146	
Zero time and Groups (1)	0.0133		0.0181	
Zero time and Groups (2)	0.0110		0.0150	
Zero time and Groups (3)	0.0087		0.0119	
<b>After 1 year</b>				
(A) Drying Treatments	NS		NS	
(B) Packaging Treatments	NS		NS	
(AB) Interaction	NS		NS	
<b>After 2 years</b>				
(A) Drying Treatments	NS		NS	
(B) Packaging Treatments	NS		NS	
(AB) Interaction	0.0233		0.0319	
<b>After 3 years</b>				
(A) Drying Treatments	0.0107		0.0146	
(B) Packaging Treatments	0.0107		0.0146	
(AB) Interaction	0.0185		0.0253	



**Table 4.** Effect of drying methods, packaging types and the interaction between them on chlorophyll b content (mg/ 100 g f.w.) in leaves of *Rosmarinus officinalis*, L. plant in the zero time and after 3 years storing from 2017 to 2020

Fresh weight of leaves	<b>0.496</b>			
<b>Zero time</b>				
<b>Drying treatments in zero time</b>				
Shade Zero Time	0.082			
Sun Zero Time	0.028			
Oven Zero Time	0.049			
Average zero time	0.053			
Drying treatments	<b>Packaging treatments</b>			<b>(A) Means of Drying</b>
	<b>Carton bags</b>	<b>Cotton bags</b>	<b>Glass jars</b>	
<b>Group (1)</b>				
<b>Drying, packaging and its combined treatments after 1 year</b>				
Shade	0.055	0.047	0.053	0.052
Sun	0.051	0.034	0.046	0.044
Oven	0.049	0.038	0.047	0.045
(B) Means of Packaging	0.052	0.040	0.049	
Group 1	0.047			
<b>Group (2)</b>				
<b>Drying, packaging and its combined treatments after 2 years</b>				
Shade	0.046	0.039	0.044	0.043
Sun	0.043	0.028	0.039	0.037
Oven	0.041	0.032	0.039	0.037
(B) Means of Packaging	0.043	0.033	0.041	
Group 2	0.039			
<b>Group (3)</b>				
<b>Drying, packaging and its combined treatments after 3 years</b>				
Shade	0.037	0.031	0.035	0.034
Sun	0.034	0.023	0.031	0.029
Oven	0.033	0.025	0.031	0.030
(B) Means of Packaging	0.035	0.026	0.032	
Group 3	0.031			
<b>LSD between:</b>	<b>5%</b>			<b>1%</b>
Zero time	0.0114			0.0156
Zero time and Groups (1)	NS			NS
Zero time and Groups (2)	0.0105			0.0144
Zero time and Groups (3)	0.0093			0.0127
<b>After 1 year</b>				
(A) Drying Treatments	NS			NS
(B) Packaging Treatments	NS			NS
(AB) Interaction	NS			NS
<b>After 2 years</b>				
(A) Drying Treatments	NS			NS
(B) Packaging Treatments	NS			NS
(AB) Interaction	NS			NS
<b>After 3 years</b>				
(A) Drying Treatments	NS			NS
(B) Packaging Treatments	NS			NS
(AB) Interaction	NS			NS

**Chlorophyll a+b content (mg/ 100 g f.w.)**

The shade drying method achieved the highest content of chlorophyll a+b in rosemary leaves immediately after drying with a high significance compared to both oven and sun drying methods (Table 5). Sun drying method recorded the least chlorophyll a+b content. Similar findings were obtained by **Arslan and Ozcan (2008)** who demonstrated that oven drying (50 °C) resulted in a considerable decrease in the colour quality of the rosemary (*Rosmarinus officinalis* L.) leaves.

In the same Table, oven drying method was the best treatment followed by the shade drying method with significant differences between them during the 1<sup>st</sup> and 2<sup>nd</sup> year storage, and non-significant differences between all drying treatments in the third storage year. Likewise, the packaging treatment with glass jars had significant effect on the rosemary leaves content of chlorophyll a+b comparing with storage in cotton pages during the first and second storage years, and achieved the best content in the 3<sup>rd</sup> storage year. While in the third year, there was no significant between all treatments. The reaction treatment (oven drying method + storage in glass jars) recorded the highest content of chlorophyll a+b with high significance compared to the treatment (sun drying method + storage in cotton bags) during all storage years.

Chlorophyll a+b content in Rosemary leaves significantly and gradually decreased through 3 years storage when compared to average zero time.

**Carotenoids content (mg/ 100 g f.w.)**

The results obtained in Table (6) indicate that the drying treatment in the shade retained the largest content of carotenoids in the rosemary leaves at zero time, and with a high significant in comparison with the other treatments. Similar trend was resulted by **Benhura and Chitsiku (1997)** who indicated that 92 and 93% of beta-carotene was lost from leaves of Guku (*Bidens pilosa*) after drying in the sun and shade, respectively.

Concerning the effect of drying methods through three years storing the data state that the highest content of carotenoids showed in leaves dried with shade drying method with highly significant between the other treatments. The sun drying method significantly decreased carotenoids content in leaves. The highest carotenoids content showed in the leaves stored in glass jars followed by carton bags and cotton bags treatments, respectively, with significant differences between them during all storage years.

The highest carotenoids content in Rosemary leaves found in the leaves dried with shade drying method and stored for three years in glass jars with significant differences with the other combined treatments, but there were non-significant differences between this treatment and the combined treatments (shade drying method with carton bags storage). The least content observed with the interaction treatment of sun drying method + storing in cotton bags, during the three storage years.

**Effect of the interaction treatments between drying methods and packaging types on microbial load in dried rosemary leaves stored three years**

The data tabulated in Table (7), show the effect of combined treatments of drying methods and packaging types on microbial load in rosemary dried leaves during three years storage period, and the results indicated that the dried leaves loaded during storage with three fungus (*Alternaria sp.*, *Aspergillus flavus* and *Penicillium sp.*). There is no big difference between treatments. The least loading observed with the leaves stored in glass jars under oven drying method, followed by sun drying method. The highest load was in leaves dried with shade method and stored in cotton pages. We can also observe that the microbial load relatively decreased through the three years storage under all interaction treatments.

**Table 5. Effect of drying methods, packaging types and the interaction between them on chlorophyll a+b content (mg/ 100 g f.w.) in leaves of *Rosmarinus officinalis*, L. plant in the zero time and after 3 years storing from 2017 to 2020**

<b>Fresh weight of leaves</b>	<b>0.893</b>			
<b>Zero time</b>				
<b>Drying treatments in zero time</b>				
Shade Zero Time	0.182			
Sun Zero Time	0.108			
Oven Zero Time	0.142			
Average zero time	0.144			
<b>Drying treatments</b>	<b>Packaging treatments</b>			<b>(A) Means of Drying</b>
	<b>Carton bags</b>	<b>Cotton bags</b>	<b>Glass jars</b>	
<b>Group (1)</b>				
<b>Drying, packaging and its combined treatments after 1 year</b>				
Shade	0.130	0.120	0.133	0.128
Sun	0.120	0.080	0.119	0.106
Oven	0.132	0.119	0.140	0.130
<b>(B) Means of Packaging</b>	0.128	0.106	0.131	
<b>Group 1</b>	0.121			
<b>Group (2)</b>				
<b>Drying, packaging and its combined treatments after 2 years</b>				
Shade	0.109	0.100	0.110	0.106
Sun	0.100	0.067	0.099	0.089
Oven	0.110	0.099	0.117	0.109
<b>(B) Means of Packaging</b>	0.106	0.089	0.109	
<b>Group 2</b>	0.101			
<b>Group (3)</b>				
<b>Drying, packaging and its combined treatments after 3 years</b>				
Shade	0.087	0.080	0.088	0.085
Sun	0.080	0.053	0.079	0.071
Oven	0.088	0.079	0.093	0.087
<b>(B) Means of Packaging</b>	0.085	0.071	0.087	
<b>Group 3</b>	0.081			
<b>LSD between:</b>	<b>5%</b>			<b>1%</b>
Average zero time	0.0161			0.0220
Zero time and Groups (1)	0.0192			0.0262
Zero time and Groups (2)	0.0160			0.0219
Zero time and Groups (3)	0.0132			0.0180
<b>After 1 year</b>				
(A) Drying Treatments	0.0235			0.0321
(B) Packaging Treatments	0.0235			0.0321
(AB) Interaction	0.0407			0.0556
<b>After 2 years</b>				
(A) Drying Treatments	0.0196			0.0268
(B) Packaging Treatments	0.0196			0.0268
(AB) Interaction	0.0340			0.0465
<b>After 3 years</b>				
(A) Drying Treatments	NS			NS
(B) Packaging Treatments	NS			NS
(AB) Interaction	0.0279			0.0381

**Table 6. Effect of drying methods, packaging types and the interaction between them on carotenoids content in leaves of *Rosmarinus officinalis*, L. plant in the zero time and after 3 years storing from 2017 to 2020**

<b>Fresh weight of leaves (g)</b>	<b>0.2900</b>			
<b>Zero time</b>				
<b>Drying treatments in zero time</b>				
Shade Zero Time	0.0482			
Sun Zero Time	0.0295			
Oven Zero Time	0.0306			
Average zero time	0.0361			
<b>Drying treatments</b>	<b>Packaging treatments</b>			<b>(A) Means of Drying</b>
	<b>Carton bags</b>	<b>Cotton bags</b>	<b>Glass jars</b>	
<b>Group (1)</b>				
<b>Drying, packaging and its combined treatments after 1 year</b>				
Shade	0.049	0.046	0.057	0.051
Sun	0.021	0.014	0.030	0.021
Oven	0.033	0.030	0.047	0.037
<b>(B) Means of Packaging</b>	0.034	0.030	0.045	
<b>Group 1</b>	0.0360			
<b>Group (2)</b>				
<b>Drying, packaging and its combined treatments after 2 years</b>				
Shade	0.041	0.038	0.048	0.042
Sun	0.017	0.011	0.025	0.018
Oven	0.027	0.025	0.039	0.031
<b>(B) Means of Packaging</b>	0.029	0.025	0.037	
<b>Group 2</b>	0.0300			
<b>Group (3)</b>				
<b>Drying, packaging and its combined treatments after 3 years</b>				
Shade	0.0329	0.0307	0.0381	0.0339
Sun	0.0138	0.0090	0.0202	0.0143
Oven	0.0219	0.0202	0.0311	0.0244
<b>(B) Means of Packaging</b>	0.0229	0.0200	0.0298	
<b>Group 3</b>	0.0242			
<b>LSD between:</b>	<b>5%</b>		<b>1%</b>	
Average zero time	0.0048		0.0065	
Zero time and Groups (1)	NS		NS	
Zero time and Groups (2)	0.0041		0.0056	
Zero time and Groups (3)	0.0039		0.0053	
<b>After 1 year</b>				
(A) Drying Treatments	0.0054		0.0073	
(B) Packaging Treatments	0.0054		0.0073	
(AB) Interaction	0.0093		0.0127	
<b>After 2 years</b>				
(A) Drying Treatments	0.0050		0.0068	
(B) Packaging Treatments	0.0050		0.0068	
(AB) Interaction	0.0086		0.0118	
<b>After 3 years</b>				
(A) Drying Treatments	0.0048		0.0065	
(B) Packaging Treatments	0.0048		0.0065	
(AB) Interaction	0.0083		0.0113	

**Table 7. Effect of drying methods, packaging types and the interaction between them on quality and microbial load in leaves of *Rosmarinus officinalis*, L. plant during 3 years storing from 2017 to 2020**

Drying methods	Glass jars			Carton pages			Cotton pages		
	1 <sup>st</sup> year	2 <sup>nd</sup> year	3 <sup>rd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year	3 <sup>rd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year	3 <sup>rd</sup> year
<b>Sun drying</b>									
<i>Alternaria sp.</i>	1.0	0.9	0.3	1.1	1.0	0.9	1.1	1.0	1.0
<i>Aspergillus flavus</i>	0.9	0.6	0.4	0.9	0.8	0.7	1.1	0.9	0.7
<i>Penicillium sp.</i>	1.1	0.8	0.3	1.2	1.0	0.9	1.2	1.1	0.9
<b>Oven drying</b>									
<i>Alternaria sp.</i>	0.9	0.5	0.3	1.0	0.9	0.8	1.22	1.1	1.0
<i>Aspergillus flavus</i>	0.2	0.4	0.6	1.0	0.8	0.7	1.15	1.10	1.0
<i>Penicillium sp.</i>	0.7	0.5	0.5	1.1	1.0	1.0	2.0	1.83	1.65
<b>Shade drying</b>									
<i>Alternaria sp.</i>	1.0	0.9	0.8	1.1	1.0	1.0	1.12	1.0	1.0
<i>Aspergillus flavus</i>	1.0	0.8	0.8	1.0	0.9	1.0	1.16	1.12	1.10
<i>Penicillium sp.</i>	1.0	0.9	0.9	1.0	1.0	1.0	1.13	1.10	1.10

Calculated as log<sub>10</sub> CFU/g sample

### Essential oil loosing

The overall results indicate that the volatile composition of dried rosemary leaves decreases considerably during storage years, independently of the packaging material and storage conditions.

Table (8) shows the loosing rate in oil percentage of dried leaves in zero time and through 3 years storage from fresh weight of leaves. The results pointed out that shade drying method in zero time had the least loosing percentage (9.2%) from fresh leaves, while the sun drying method had the highest loosing percentage (16.2%). Furthermore, the shade drying method through 3 years storing had the least loosing rate of oil percentage in dried leaves (15.0, 20.0 and 23.8%) from fresh leaves, and the highest loosing rate (28.1, 32.3 and 35.8%) observed with sun drying method during 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> years storing, respectively. Whereas, **Baritiaux et al. (1992)** found losses of total essential oil in basil (*Ocimum basilicum* L.) after drying at 45 °C by 19%, 62% and 66% at 3, 6- and 7-months storage, respectively. And **Venskutonis et al. (1996)** pointed out that, the largest changes in flavour composition in thyme herb (*Thymus vulgaris* L.) were observed

during storage up to 10 months, especially a reduction in the concentration of monoterpene hydrocarbons.

Concerning the effect of packaging types on the loosing percentage of oil percentage in dried leaves during 3 years storing, the data in Table (8) reveal that the highest loosing rate observed with using cotton bags packaging (23.8, 28.8 and 32.3%), while the lightest loosing percentage found with glass jar packaging (19.2, 24.2 and 28.1%) during first, second and third storage years, respectively. From the other way, **Paakkonen et al. (1990)** with air-dried basil (*Ocimum basilicum*), type of packaging (polyethylene-aluminium-polyethylene bags under N<sub>2</sub> atmosphere, under vacuum, in glass jars and paper bags) had no significant effect. Furthermore, Air-dried marjoram showed significant quality changes during long-term storage. They concluded that the intensity of odour and taste of dried herbs could be maintained for 2 years at 23 degrees in air-tight Packaging. After the drying process, the packing method is an important factor in the quality conservation of the product during storage (**Martinazzo et al., 2009**).

**Table 8. Oil percentage and loosing percentage in leaves fresh weight, leaves dry weight in zero time and in leaves dry weight after storing period under different drying methods and packaging types during 2017 to 2019 season**

Treatments	Weight of leaves from fresh to dry	Oil % in 100 g (fresh or dry) leaves weight	Convert leaves dry weight to fresh weight	Oil % in leaves weights after converting	Oil Loosing percentage from fresh weight	Oil Loosing percentage After storing from zero time
<b>Fresh leaves zero time</b>	100 g	0.260 %	--	0.260 %		
<b>Shade zero time</b>	32 g	0.733 %	312.5 g	0.235 %	9.2 %	
<b>Sun zero time</b>	30 g	0.727 %	333.0 g	0.218 %	16.2 %	
<b>Oven zero time</b>	31 g	0.731 %	322.6 g	0.227 %	12.3 %	
<b>Average zero time</b>	31 g	0.730 %	322.5 g	0.226 %	13.1 %	
<b>After 1-year storage</b>						
<b>Shade</b>	30 g	0.737 %	333.0 g	0.221 %	15.0 %	5.96 %
<b>Sun</b>	30 g	0.624 %	333.0 g	0.187 %	28.1 %	14.2 %
<b>Oven</b>	30 g	0.675 %	333.0 g	0.203 %	21.9 %	10.6 %
<b>Average after 1-year storage</b>	30 g	0.679 %	333.0 g	0.204 %	21.5 %	9.73 %
<b>After 2 years storage</b>						
<b>Shade</b>	30 g	0.691 %	333.0 g	0.208 %	20.0 %	11.5 %
<b>Sun</b>	30 g	0.585 %	333.0 g	0.176 %	32.3 %	19.3 %
<b>Oven</b>	30 g	0.633 %	333.0 g	0.190 %	26.9 %	16.3 %
<b>Average after 1-year storage</b>	30 g	0.637 %	333.0 g	0.191 %	26.5 %	15.5 %
<b>After 3 years storage</b>						
<b>Shade</b>	30 g	0.658 %	333.0 g	0.198 %	23.8 %	15.7 %
<b>Sun</b>	30 g	0.557 %	333.0 g	0.167 %	35.8 %	23.4 %
<b>Oven</b>	30 g	0.603 %	333.0 g	0.181 %	30.4 %	20.3 %
<b>Average after 3 years storage</b>	30 g	0.606 %	333.0 g	0.182 %	30.0 %	19.5 %
<b>Packaging after 1 year</b>						
<b>Carton pages</b>	30 g	0.681 %	333.0 g	0.205 %	21.2 %	9.29 %
<b>Cotton pages</b>	30 g	0.658 %	333.0 g	0.198 %	23.8 %	12.4 %
<b>Glass jars</b>	30 g	0.698 %	333.0 g	0.210 %	19.2 %	7.08 %
<b>Packaging after 2 years</b>						
<b>Carton pages</b>	30 g	0.638 %	333.0 g	0.192 %	26.2 %	15.0 %
<b>Cotton pages</b>	30 g	0.617 %	333.0 g	0.185 %	28.8 %	18.1 %
<b>Glass jars</b>	30 g	0.655 %	333.0 g	0.197 %	24.2 %	12.8 %
<b>Packaging after 3 years</b>						
<b>Carton pages</b>	30 g	0.608 %	333.0 g	0.183 %	29.6 %	16.4 %
<b>Cotton pages</b>	30 g	0.587 %	333.0 g	0.176 %	32.3 %	19.6 %
<b>Glass jars</b>	30 g	0.623 %	333.0 g	0.187 %	28.1 %	14.6 %

## Conclusion

The shade drying did not cause major variation in the essential oil percentage, whereas sun and oven drying methods moderately changed the essential oil percentage. It was also found that storage in glass jars was better in reducing the loss of rosemary leaves to the essential oil percentage than in carton and cotton bags. On the other hand, the different drying methods greatly affected the content of rosemary leaves of chlorophyll a, b, a+b and carotenoids, immediately after drying, and it was also more after storage for three years. Therefore, it is recommended to dry the rosemary leaves in a clean, shaded place for seven days, then packing

them in glass containers or carton bags for a period of up to less than three years.

In the final trial of this paper, the possible control of the fungus residing on the rosemary plant was studied using different methods of drying with the use of conservation methods for it that this microbial load is acquired from the exposure of plants in the field and drying places as a contaminated agent and that the methods of preservation led to a reduction in the rate of microbial load and this is clear in the non-impact of the amount of oil extracted during storage periods and may also be due to the containment of microbial load to the action of volatile materials in the leaves stored and there are many signs of that.

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