



Research Article

Packaging and storage effects on essential oil chemometric analysis of dried rosemary herbs.

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Received: 19 May 2024 / Revised: 17 June 2024 / Accepted: 23 June 2024

Abstract: *Rosmarinus officinalis* L. (rosemary) is a medicinal plant that is native to the Mediterranean region and is grown worldwide. In addition to being used therapeutically, it is frequently used as a condiment and food preservative. In addition to its significance in the food industry, rosemary essential oil (EO) is used worldwide to cure a wide range of maladies, including respiratory, gastrointestinal, and inflammation-related problems. Therefore, how this plant is stored is crucial. This study examined the multiple effects of packaging materials, storage period, and their interactions on the quantity and chemical components of rosemary EO. The net results revealed that these factors caused a decrease in the amount of extracted oil during the storage period (2–4 months). The results of GC-MS and the chemometric analysis, including principal component analysis (PCA) and agglomerative hierarchical clustering (AHC), revealed that the concentrations of the main compounds, including camphor, α -pinene, eucalyptol, camphene, and *D*-limonene, varied from 18.87%–30.31%, 14.09%–19.40%, 15.82%–17.56%, 3.36%–6.06%, and 4.82%–8.31%, respectively, in the EOs of all the different treatments. Additionally, the results showed that polypropylene and linen bags are the best bags for saving the rosemary plants without significant variations compared to those of rosemary plants before storage.

Keywords: rosemary; essential oil storage; polypropylene and linen bags; camphor; α -pinene

Citation: Amer H. M., El-Gendy A. N. G., Elshamy A. I., et al. Packaging and storage effects on essential oil chemometric analysis of dried rosemary herbs. *Food & Medicine Homology*, 2027, 4: 9420141. <https://doi.org/10.26599/FMH.2027.9420141>

1 Introduction

Interest in sources of natural products, such as essential oils (EOs) and diverse plant extracts has increased. They have undergone testing to determine whether they might be used as complementary treatments for a variety of infectious disorders^[1]. In particular, the medicinal properties of plant EOs and extracts have served as the foundation for a variety of applications, such as pharmaceuticals, alternative medicines, and natural therapies, as well as raw and processed food preservation^[2]. Additionally, plant EOs and extracts are rich sources of aromatic, volatile, and bioactive compounds^[3,4]. The powerful antioxidant and antimicrobial properties of these substances play a crucial role in agricultural, food, pharmaceutical, cosmetic, and health issues^[5].

In many nations, the agricultural sector of the national economy is a significant source of income for medicinal and aromatic plants. The well-known EO of these medications are derived from aromatic and medicinal plants. One of the most significant medicinal and aromatic perennial plants, rosemary (*Rosmarinus officinalis* L.) is used externally as a parasiticide and cicatrizes for muscle pain and rheumatism, dermatitis, dandruff,

and eczema^[6,7]. It has hepatoprotective, antimutagenic, and choretic properties and is utilized in traditional medicine, cosmetics, and food flavoring^[8].

Historically, renal colic and dysmenorrhea have been treated with rosemary as a medicine. Additionally, it has been used to stimulate hair growth and alleviate respiratory disorder symptoms. In aromatherapy, rosemary extracts are used to treat illnesses linked to anxiety and to improve alertness^[9,10]. As a result, rosemary extracts are highly sought after by both the pharmaceutical and food industries as sources of active compounds for medications. The storage of aromatic and therapeutic plants serves as the link between producers and customers. Storage of medicinal and aromatic plants occurs for marketing purposes as well as for use as a raw material in the production of pharmaceuticals, cosmetics, and food additives^[11].

The effectiveness of storage conditions, such as air temperature, light, humidity, and package material, affects the quality of stored parts^[12]. It is essential to assess the degree of physical and chemical changes in aromatic and medicinal plants during storage to minimize adverse effects on humans and to maximize the possible economic return of stored plants^[13,14]. The production of

extremely secure, efficient, and high-quality herbal goods as a result of standardizing the storage and packaging of unprocessed herbal medicines will hasten their popularity around the world^[15,16]. Thus, to avoid adverse effects on humans that could result from changes in chemical active constituents during storage and in the type of packaging materials and to achieve the maximum economic return of the storage process, it was crucial to investigate the extent of changes in the content and chemical constituents of rosemary EO during storage in different types of packaging materials for 4 months

2 Materials and methods

2.1 Plant authentication, collection and preparation

The mother rosemary plants (*R. officinalis* L.) were identified and authenticated by Prof. A.M. Abd-ElGawad (Prof. of Taxonomy, Mansoura University, Egypt) according to the books of the flora of Egypt following Tackholm^[17] and Boulos *et al.*^[18]. Additionally, the voucher sample was prepared and dropped into the herbarium of the National Research Center (Egypt) with specimen code 350 NRC-20xYD-AN-20-610. Rosemary plants grown in the Medicinal and Aromatic Plants Farm (Adlya Farm) Sekem Co., El-Sharkia Governorate (Egypt), 80 km east of Cairo, were harvested in the morning (6:00 am) at approximately 5 cm above the soil surface on the 15th of July, 2021. Harvested plants were subjected to open-shade air drying for 2 weeks. A total of 100 g of the air-dried herbs (4 replicates for every treatment) were packed in different packaging materials namely polyethylene, brown kraft paper, polypropylene, and linen bags. Where the packed samples were stored for three periods: 0, 2, and 4 months at room temperature.

2.2 Extraction of EO

The EO content of each sample was determined according to the AOAC^[19] and expressed as mL/100 g dried sample. The EO was extracted according to Hendawy *et al.*^[20] using the hydrodistillation method in a Clevenger-type apparatus for 3 h. The resulting oil was dried over Na₂SO₄ and stored at -20 °C in amber glass vials until analysis.

2.3 EO analysis

The gas chromatography-mass spectrometry (GC-MS) analysis of the rosemary EO samples was carried out using TRACE GC Ultra Gas Chromatographs (THERMO Scientific™ Corporation, USA), coupled with a single quadrupole mass spectrometer (Thermo Scientific ISQ™ EC) at the Department of Medicinal and Aromatic Plants Research, National Research Centre, Egypt. The GC-MS system was equipped with a TR-5 MS column (30 m × 0.32 mm, 0.25 µm) and helium as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was held at 60 °C for 1 min; then increased by 4.0 °C/min to 240 °C and held for 1 min, while the temperature of the injector and ion source were held at 210 °C.

The EO samples were dissolved in hexane (1:10, V/V) and 1 µL was injected with a split ratio of 1:10. Mass spectra were recorded by electron ionization (EI) at 70 eV with a scan range of *m/z* 40–450.

The separated compounds were identified using two different methods: (a) Kovats indices (KI) in reference to *n*-alkanes (C₉–C₂₂) by using AMDIS software (<https://www.amdis.net>), and (b) based on the mass spectra of the National Institute of Standards and Technology (NIST) and Wiley spectral library collection.

2.4 Statistical analysis

The experiment was arranged in a complete randomized block design with three replicates. A matrix of the rosemary EO samples, formed from the main components analyzed during the experimental period, was subjected to principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) using the XLSTAT statistical computer software package (version 2018, Addinsoft Inc., New York, NY, USA).

3 Results and discussion

3.1 EO percentage

The proportion of EO from stored dried herbs is affected by storage time and packing type, as shown by the data in Table 1. Regarding the impact of storage time, the percentage of rosemary EOs clearly decreased as the storage time increased, reaching its lowest percentage after 4 months. Regarding the impact of packing materials, the findings revealed variances across the various materials. The tested packaging materials were ranked according to their ability to maintain the oil content of the rosemary plant in the following order: polyethylene > kraft paper > polypropylene > linen bags, as illustrated in Fig. 1.

The combination of several treatments revealed that the lowest percentage of oil was recorded for the rosemary plants stored in polyethylene packages and kraft paper bags for only 2 months, followed by those stored in polypropylene bags and linen bags. These findings were consistent with other studies on preserved plants, such as those on *Ocimum basilicum*, where Baritau *et al.*^[14] reported that 19%, 62%, and 66% of the total EO was lost after 3, 6, and 7 months of storage, respectively. In contrast to paper packets, which recorded the highest values, Abd El-Wahab and Salem^[21] discovered that the EO percentage decreased in dried leaves held in polypropylene or cotton packages for 6 and 12 months. Additionally, it was noted that jute sacks experienced relatively minor quality degradation during storage due to the lengthening of the storage period, which should not exceed two months^[21]. Additionally, the weight and percentage of Siwa mint EO decreased noticeably as the storage time increased, and the chemical composition of the oil changed.^[22]

3.2 EO composition analysis

The chemical components of the rosemary EO used as a

Table 1 Rosemary EO amount during the storing period, packing materials and their interaction

Storage period (month)	Bag type				Mean
	Polyethylene	Kraft paper	Linen	Polypropylene	
0	1.34 ± 0.01	1.34 ± 0.02	1.34 ± 0.01	1.34 ± 0.02	1.34
2	1.33 ± 0.01	1.33 ± 0.01	1.29 ± 0.01	1.32 ± 0.02	1.32
4	1.29 ± 0.02	1.27 ± 0.01	1.21 ± 0.02	1.25 ± 0.01	1.26
Mean	1.32	1.31	1.28	1.30	

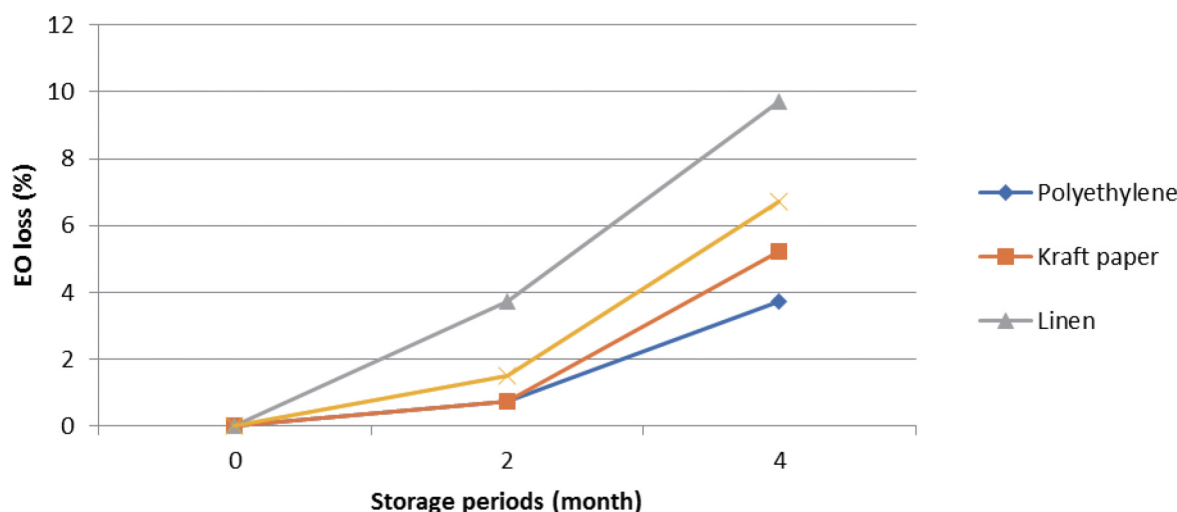


Figure 1 Rosemary EO loss during the storage period as affected by packing materials and their interaction.

standard sample, as well as its components following a period of storage, the materials used for packaging, and their interactions with one another, are shown in Table 2. In total, 23 compounds were identified from all analyzed samples. According to the GC-MS analysis, camphor, α -pinene and eucalyptol made up the majority of the components in all the treatments and varied from 18.87% to 30.31%, 14.09% to 19.40%, and 15.82% to 17.56%, respectively. In addition, camphene and *D*-limonene, which had concentrations ranging from 3.36% to 6.06% and 4.82% to 8.31%, respectively, were also identified as major constituents in the EOs of all the different treatments. All these findings were in complete agreement with those of all previous studies of rosemary worldwide, with differences in compound concentrations.

Several studies on the chemical and biological composition of various parts of various rosemary cultivars worldwide have been published^[3]. In this regard, Dellacassa *et al.*^[23] examined the composition of the EOs extracted from rosemary cultivars growing in various regions of Uruguay and southern Brazil. The EOs of cultivated Brazilian plants contained 32.2% α -pinene and 14.7% 1,8-cineole, while the EOs of wild Brazilian species contained myrcene, 1,8-cineole and α -pinene, (22.7%, 15.3%, 12.4%, respectively). In the same context, Porte *et al.*^[24] extracted and studied the EO from fresh rosemary leaves cultivated in Rio de Janeiro, Brazil. There were 36–45 different compounds found in the oil, which were dominated by camphor, 1,8-cineole, myrcene, and α -pinene representing 26.0%, 22.1%, 12.4% and 11.5%, respectively.

The EOs composition and quality can also be influenced by the environment in which the plant develops. In this context, the Spanish rosemary EO investigated by Guillen *et al.*^[25] had higher proportions of camphor, verbenone, and linalool but appeared to have richer flavor notes and a more complicated composition than those reported in the previous studies. It also contained intermediate amounts of 1,8-cineole and α -pinene compared to the EOs of rosemary plants from other geographical origins. Camphor, α -pinene and eucalyptol were found to be the main components in the EOs of rosemary plants growing in Egypt representing 32.36%, 18.82% and 15.79%, respectively^[20,26].

These results are in agreement with those reported by Tomei *et al.*^[27] for the EOs extracted from flowers and leaves of wild rosemary collected in Southern Spain. They found that camphor (32.33%) and α -pinene (11.56%) were the main components. Additionally, the EOs of Spanish rosemary were examined by Chalchat *et al.*^[28], who reported that α -pinene and

camphor were abundant, representing 24.7% and 18.9%, respectively; however, they also included high amounts of 1,8-cineole (21.8%) and some borneol (4.5%). These findings are consistent with the findings of the present study.

The EOs of freshly harvested rosemary from Siani and Giza were analyzed by Soliman *et al.*^[29] using GC-MS. They found that camphor, α -pinene and 1,8-cineole made up 14.9%, 9.3% and 9.0% of the EO of the Giza sample, respectively, while the majority of the Siani sample represented by verbenone, camphor, bornyl acetate, and limonene (12.3%, 11.3%, 7.6% and 7.1%, respectively)^[29]. Furthermore, Benhabiles *et al.*^[30] reported that the main components of the EOs from rosemary flowering aerial parts that were collected in several countries were camphor, borneol, α -terpineol, δ -cadinene, β -caryophyllene, α -humulene bornyl acetate, and muurolene^[30,31].

Herein, the camphene concentration increased after 4 months of storage after a decreased in the α -pinene contents and camphene content whereas this trend was observed during storage. Additionally, as the storage time increased, the eucalyptol content increased, while the camphor content decreased. For polyethylene, kraft paper, polypropylene, and linen bags, the percentages of camphor ingredients were 21.30%, 20.90%, 19.32%, and 30.31%, respectively, after 2 months of storage and 20.98%, 18.87%, 20.71%, and 30.09%, respectively, after 4 months of storage. Additionally, the eucalyptol contents of polyethylene, kraft paper, polypropylene, and linen bags were 16.29%, 16.21%, 16.03%, and 16.99%, respectively, after 2 months and 17.01%, 17.01%, 17.00%, and 17.56%, respectively, after 4 months.

The chemometric analysis, including the cluster analysis (Fig. 2) and PCA of the identified components of the rosemary EOs during all the storage experiments (Fig. 3), revealed strong correlations between all these samples, with a correlation ratio of 0.88%. In more detail, the results showed that most of the oil samples from all the storage packages were correlated within all the storage periods. The oils derived from the linen (within two periods, 2 and 4 months) and kraft (within 4 months) storage samples showed the greatest correlation via the abundance of several compounds, especially camphor. However, polyethylene (within all storage periods) and kraft (within 4 months) were strongly correlated with the presence of some of the main components, especially eucalyptol.

Numerous researchers have stated that all EOs have thermolabile components and are sensitive to oxidative agents such as air and light, which determine the deterioration of the

Table 2 EO constituents (%) of rosemary before and after storage and/or packing

KI		Constituents	Before storage	2 months				4 months					
Lit	Exp.			Polyethylene bags	Kraft paper bags	Linen bags	Polypropylene bags	Mean	Polyethylene bags	Kraft paper bags	Linen bags	Polypropylene bags	Mean
939	939	α -Pinene	19.40 $\pm$ 0.36	18.03 $\pm$ 0.29	16.90 $\pm$ 0.28	17.31 $\pm$ 0.28	16.05 $\pm$ 0.31	16.90	17.55 $\pm$ 0.32	15.99 $\pm$ 0.23	17.95 $\pm$ 0.34	14.09 $\pm$ 0.21	16.40
954	955	Camphene	6.06 $\pm$ 0.12	4.09 $\pm$ 0.19	4.10 $\pm$ 0.21	4.16 $\pm$ 0.16	3.36 $\pm$ 0.15	4.35	5.00 $\pm$ 0.19	5.01 $\pm$ 0.21	5.10 $\pm$ 0.15	5.01 $\pm$ 0.18	5.03
960	962	2,4(10)-Thujadien	0.54 $\pm$ 0.02	-	-	0.33 $\pm$ 0.01	0.23 $\pm$ 0.01	0.14	-	-	-	-	-
979	980	β -Pinene	2.33 $\pm$ 0.09	-	2.29 $\pm$ 0.07	2.30 $\pm$ 0.10	1.14 $\pm$ 0.04	1.91	0.35 $\pm$ 0.01	2.04 $\pm$ 0.08	2.30 $\pm$ 0.13	1.34 $\pm$ 0.06	1.51
991	993	β -Myrcene	1.34 $\pm$ 0.04	1.34 $\pm$ 0.04	1.39 $\pm$ 0.06	1.40 $\pm$ 0.05	1.38 $\pm$ 0.03	1.38	1.51 $\pm$ 0.05	1.55 $\pm$ 0.08	1.62 $\pm$ 0.06	1.62 $\pm$ 0.06	1.58
1,003	1,001	α -Phellandrene	0.31 $\pm$ 0.01	-	0.25 $\pm$ 0.01	0.29 $\pm$ 0.01	0.23 $\pm$ 0.01	0.19	0.15 $\pm$ 0.01	0.28 $\pm$ 0.01	0.31 $\pm$ 0.01	0.28 $\pm$ 0.01	0.26
1,029	1,026	D-Limonene	6.34 $\pm$ 0.16	5.99 $\pm$ 0.07	5.03 $\pm$ 0.12	6.02 $\pm$ 0.18	4.82 $\pm$ 0.11	5.47	6.04 $\pm$ 0.21	8.01 $\pm$ 0.26	8.31 $\pm$ 0.24	8.12 $\pm$ 0.23	7.62
1,031	1,035	Eucalyptol	15.82 $\pm$ 0.27	16.29 $\pm$ 0.24	16.21 $\pm$ 0.31	16.99 $\pm$ 0.37	16.03 $\pm$ 0.31	16.21	17.01 $\pm$ 0.31	17.01 $\pm$ 0.35	17.56 $\pm$ 0.36	17.00 $\pm$ 0.37	17.15
1,060	1,060	γ -Terpinene	2.03 $\pm$ 0.08	-	1.33 $\pm$ 0.04	1.55 $\pm$ 0.04	1.02 $\pm$ 0.07	0.98	0.01 $\pm$ 0.00	1.30 $\pm$ 0.03	1.59 $\pm$ 0.08	1.34 $\pm$ 0.07	1.06
1,088	1,085	Terpinolene	2.01 $\pm$ 0.07	1.54 $\pm$ 0.06	-	1.63 $\pm$ 0.03	-	0.79	-	-	-	-	-
1,096	1,095	Linalool	1.39 $\pm$ 0.06	0.83 $\pm$ 0.02	0.73 $\pm$ 0.01	0.95 $\pm$ 0.01	0.73 $\pm$ 0.03	0.81	0.83 $\pm$ 0.03	0.80 $\pm$ 0.03	0.85 $\pm$ 0.02	0.79 $\pm$ 0.03	0.82
1,126	1,124	α -Campholenal	0.42 $\pm$ 0.01	-	-	-	-	-	-	-	-	-	-
1,121	1,120	β -Fenchol	3.32 $\pm$ 0.14	1.82 $\pm$ 0.05	1.73 $\pm$ 0.06	2.02 $\pm$ 0.07	1.05 $\pm$ 0.03	1.66	1.39 $\pm$ 0.04	1.43 $\pm$ 0.03	1.39 $\pm$ 0.03	1.01 $\pm$ 0.02	1.31
1,141	1,143	cis-Verbenol	0.17 $\pm$ 0.01	-	-	-	-	-	-	-	-	-	-
1,146	1,148	Camphor	29.31 $\pm$ 0.47	21.30 $\pm$ 0.37	20.90 $\pm$ 0.35	30.31 $\pm$ 0.41	19.32 $\pm$ 0.30	22.96	20.98 $\pm$ 0.28	18.87 $\pm$ 0.31	30.09 $\pm$ 0.48	20.71 $\pm$ 0.37	22.66
1,164	1,166	Pinocarvone	0.40 $\pm$ 0.02	-	-	0.34 $\pm$ 0.01	-	0.09	-	-	-	-	-
1,188	1,187	α -Terpineol	0.20 $\pm$ 0.01	-	-	0.17 $\pm$ 0.01	0.15 $\pm$ 0.00	0.08	-	-	-	-	-
1,189	1,192	α -Terpinene	0.70 $\pm$ 0.03	0.73 $\pm$ 0.02	0.63 $\pm$ 0.01	0.69 $\pm$ 0.03	0.59 $\pm$ 0.01	0.66	0.76 $\pm$ 0.02	0.71 $\pm$ 0.03	0.74 $\pm$ 0.02	0.63 $\pm$ 0.01	0.71
1,205	1,203	1-Verbenone	4.35 $\pm$ 0.21	2.13 $\pm$ 0.06	2.20 $\pm$ 0.09	2.88 $\pm$ 0.06	1.99 $\pm$ 0.04	2.28	2.19 $\pm$ 0.11	2.31 $\pm$ 0.09	2.43 $\pm$ 0.12	2.43 $\pm$ 0.13	2.34
1,229	1,231	cis-Carveol	0.46 $\pm$ 0.02	0.32 $\pm$ 0.01	0.29 $\pm$ 0.01	0.35 $\pm$ 0.01	0.30 $\pm$ 0.01	0.32	0.29 $\pm$ 0.01	0.27 $\pm$ 0.01	0.31 $\pm$ 0.01	0.27 $\pm$ 0.01	0.29
1,252	1,250	Geraniol	0.31 $\pm$ 0.01	0.09 $\pm$ 0.00	0.12 $\pm$ 0.00	0.21 $\pm$ 0.00	0.08 $\pm$ 0.00	0.13	0.09 $\pm$ 0.00	0.15 $\pm$ 0.01	0.21 $\pm$ 0.01	0.12 $\pm$ 0.00	0.14
1,280	1,281	Nopol	2.36 $\pm$ 0.09	-	-	-	-	-	-	-	-	-	-
1,421	1,424	trans-Caryophyllene	0.24 $\pm$ 0.01	0.19 $\pm$ 0.01	0.23 $\pm$ 0.01	0.23 $\pm$ 0.01	0.23 $\pm$ 0.00	0.22	0.29 $\pm$ 0.01	0.26 $\pm$ 0.01	0.29 $\pm$ 0.01	0.25 $\pm$ 0.01	0.27
Total identified			99.99	74.69	74.33	89.35	68.63		74.44	75.99	91.05	75.01	

Note: -, Component absent.

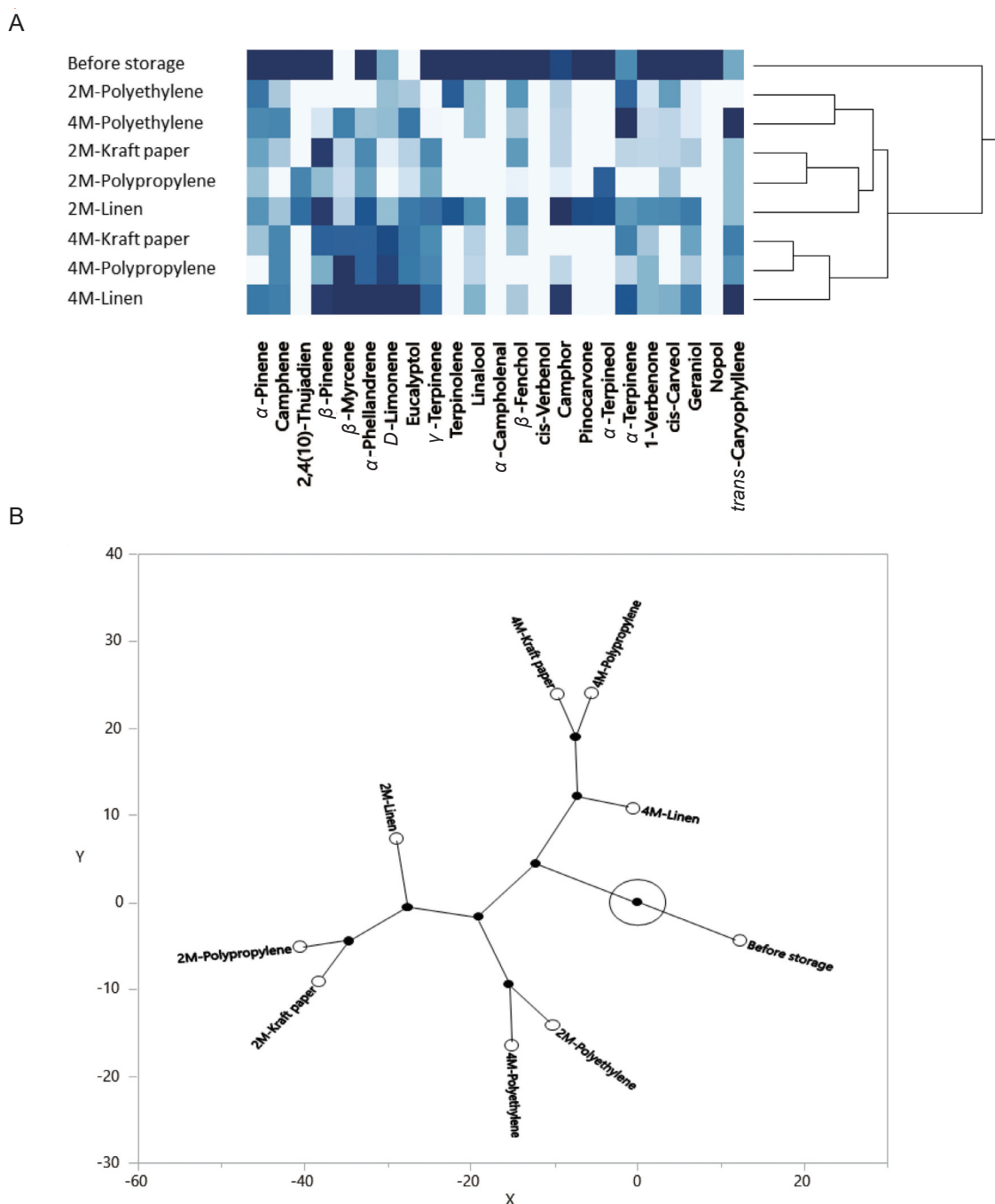


Figure 2 Cluster analysis of the rosemary EOs depending upon the EOs' components during all the storage experiment. (A) Constellation plot and (B) hierarchical clustering. 2M, 2 month storage; 4M, 4 month storage. The same below.

quality of EOs during storage^[30]. Since most flavoring ingredients are somewhat molabile or heat sensitive, these changes typically have a negative impact on flavor quality. During storage, EO components may change due to the rearrangement, hydrogenation, or dehydrogenation of other components^[32,33].

Despite the fact that polyethylene material consistently demonstrated greater preservation abilities and that polypropylene material had preservation abilities that were greater than those of linen, both are no longer advised for use as packaging materials because of concerns about the environment and human health^[34]. Plastic bags may be a problem because they are typically manufactured from petroleum derivatives, which are all finite resources that need to be preserved. They may also be problematic

because they produce many harmful pollutants during the manufacturing process^[35].

Additionally, the main chemicals used to make plastic bags are extremely hazardous and have been linked to a wide range of diseases, including cancer, birth deformities, immunological and nerve issues, as well as blood and kidney problems^[36]. However, linen and kraft paper bags represent healthy and eco-friendly sustainable alternatives. They are manufactured from renewable materials that are easily regrown, extremely biodegradable, recyclable, toxic-free, and cannot undergo any adverse chemical reactions within the herb or packet. As a result, both linen and kraft paper represent secure packing materials, especially for medicinal and fragrant plant storage.

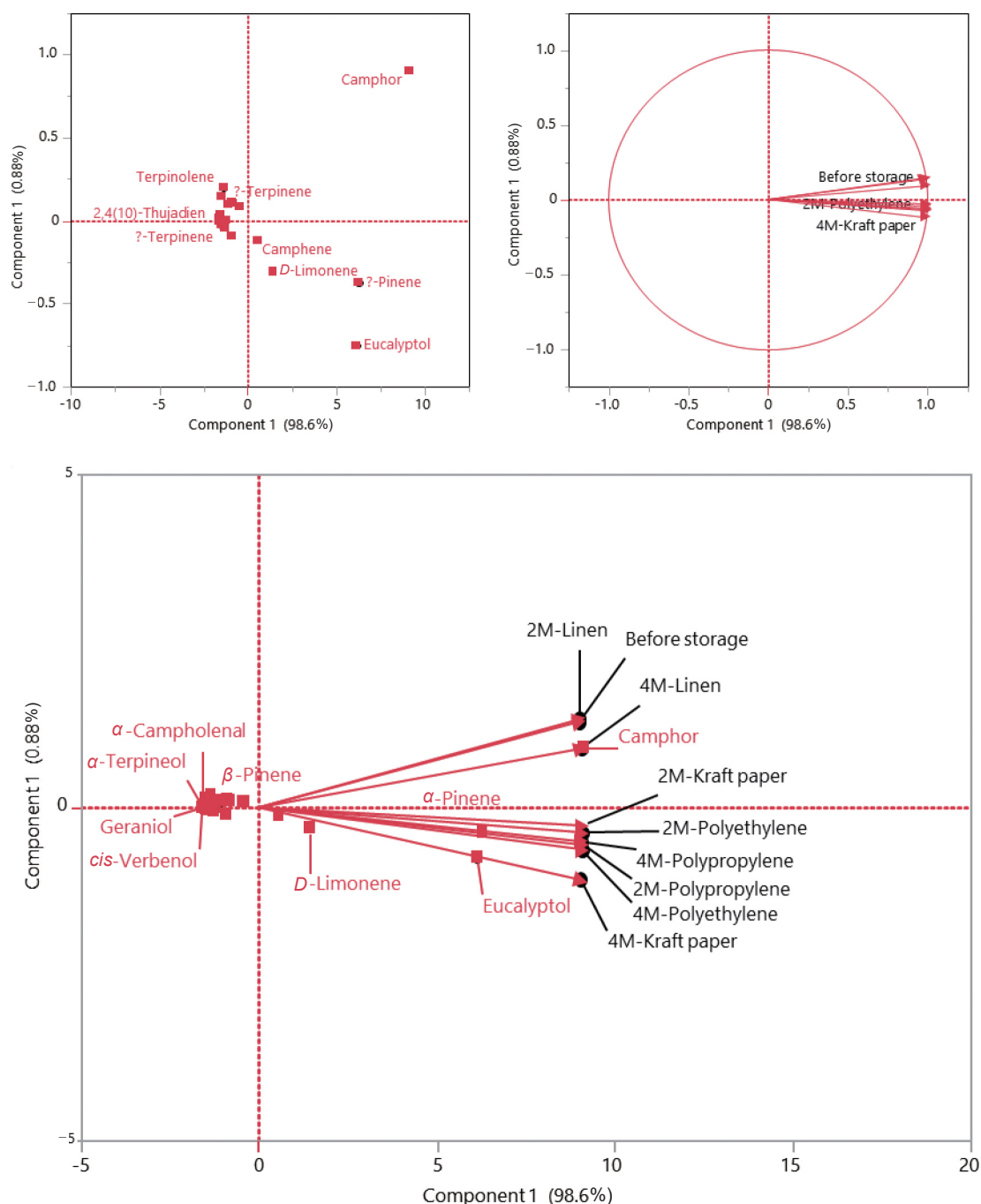


Figure 3 PCA of the identified components the rosemary' EOs during all the storage experiment, comprising of PCA space observation (A), correlation circle (B), and the biplotting diagram (C), respectively.

4 Conclusions

The results showed that kraft paper and polypropylene bags are the best bags for maintaining the oil content, while kraft paper linen bags are the best for saving rosemary plants without significant variations in the chemical constituents of rosemary EO compared to fresh plants. Additionally, the obtained results revealed that kraft paper and linen bags are potential healthy, eco-friendly, sustainable and renewable alternatives for polyethylene. Kraft paper bags were superior to linen bags in this context and still need more development and modifications.

Conflicts of interest

The authors declare that there are no conflicts of interest in this

work. Mohamed S. Hussein is an Editorial Board Member of Food & Medicine Homology, but was not involved in the processing, peer review or decision making of this article.

Acknowledgements

The authors gratefully acknowledge the National Research Center, Egypt fund (No. 12050103) for their financial support of this work.

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