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RESEARCH

Novel Insights Into the Recovery and Stabilization of *Rosmarinus officinalis* Volatile Aroma Compounds Using Green Solvents

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Abstract

In this study, the integrated application of supercritical CO_2 and natural deep eutectic solvents (NADES) was investigated in order to establish a green procedure that enables obtaining and stabilizing the aroma volatile constituents of *Rosmarinus officinalis* L. Supercritical CO_2 was used to obtain rosemary extracts that possessed an abundance of terpenes, particularly monoterpenes 68.97–88.08% and sesquiterpenes 5.38–21.22%. The obtained extracts were further dispersed in different NADES (betaine/glycerol (Bet/Gly), betaine/ethylene glycol (Bet/EG), and betaine/glycerol/sucrose/water (Bet/Gly/Suc/W) and their stability was assessed at room temperature. The headspace profile of the samples and their antioxidant activity were monitored for 6 months. Changes in the chemical profile of the extract were detected, which corresponded to terpene transformation reactions. In the control (CO_2 extract), the development of non-terpene components such as acetic acid was detected, which make the product unsuitable for use. Conversely, the accumulation of acetic acid was not observed in the NADES samples. The antioxidant activity of the control was the most significantly decreased during 6 months, while among the NADES samples, the reduction of activity occurred only in Bet/Gly sample. In Bet/EG and Bet/Gly/Suc/W samples, activity remained the same during the same period. The results suggest that the NADES could serve as stabilization media for CO_2-extracted rosemary volatile components. Furthermore, this represents a simple, green process of obtaining readily applicable products with extended stability at room temperature.

Keywords Volatile aroma compounds · Aroma stabilization · Green solvents · Supercritical CO_2 · Natural deep eutectic solvents

Introduction

Rosmarinus officinalis L. is an aromatic medicinal plant of the Lamiaceae family that is native to the Mediterranean region and is cultivated globally. It is a very important plant species because it is highly widespread and used and represents an important product in the development of food, pharmaceuticals, fragrance products, cleaning products, cosmetics, candles, and incenses. Due to their widespread and diverse application, lipophilic products of *R. officinalis* have great economic and commercial importance (Borges et al., 2019). According to research, the global rosemary oil market was projected at USD 13.21 billion in 2021. Moreover, it is expected to reach USD 15.92 billion by 2028, with a CAGR of 3.16% during the forecast period (Business Research Insights, 2022).

The growing need for *R. officinalis* components and products is conditioned by the growing tendencies of consumers and end users to use products of natural origin. However, conventional processes from isolation of *R. officinalis* components to application in the final product can be non-optimal and complex. Namely, conventional processes for the isolation of aroma volatile components such as hydro- and steam distillation can imply poor efficiency in terms of low yield, inadequate use of natural resources,

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lack of selectivity, and so on. As a modern alternative for efficient isolation of lipophilic components of *R. officinalis*, supercritical CO_2 was applied. It has been shown that using supercritical CO_2 can achieve a rational isolation of the lipophilic components of *R. officinalis*, with the possibility of varying the chemical composition according to the purpose (Carvalho et al., 2005; Conde-Hernández et al., 2017; García-Risco et al., 2011). Supercritical CO_2 enables the isolation of components at a mild temperature (critical temperature 31.06 °C and critical pressure 7.39 MPa); thus, products are obtained without degradation of sensitive components and remain fragrantly authentic to natural sources. Therefore, the rational use of rosemary resources by using a green solvent is possible by applying CO_2.

Also, *R. officinalis* aroma volatile compounds are characterized by volatility, sensitivity, and instability; hence, after their isolation, further processing steps are necessary to ensure the stability of the isolated components. Otherwise, their application is very limited and product properties are easily and quickly hindered. Among the numerous procedures for the stabilization of essential oil and volatile components that have been investigated in literature, spray drying is reported to be one of the most common and conventional procedures for the encapsulation of volatile compounds (de Souza et al., 2022; Plati & Paraskevopoulou, 2022). Spray drying has been investigated to preserve the properties of rosemary lipophilic products/essential oil (Fernandes et al., 2013, 2017; Teodoro et al., 2014). One of the main disadvantages of spray drying is the high temperature of the process. On the other hand, a process characterized by milder operation conditions is the supercritical solvent impregnation. Furthermore, the investigated techniques for the preservation of sensitive components encompass the inclusion complexation, ionic gelation, the formation of liposomes, and so on (Plati & Paraskevopoulou, 2022). Liu et al. (2021) suggested the possibility of application of yeast cell–based encapsulation for Mānuka essential oil for maintaining thermal stability (Liu et al., 2021), while Tavassoli-Kafrani et al. (2018) encapsulated orange essential oil in gelatin and gelatin-cross-linked tannic acid nanofibers by emulsion electrospinning. The preservation of properties of *Anethum graveolens* essential oil was attempted by encapsulation within the chitosan nanomatrix (Nm-AGEO) using the ionic gelation technique (Das et al., 2021). Additionally, a recent solution for the preservation of sensitive components is emulsification (micro- and nanoemulsification) which involves the dispersion of two immiscible liquid phases with the addition of a surfactant which aids in reducing the interfacial tension between the two phases. Further preparation of emulsions involves the breakdown of droplets in small size which can be achieved by different low- and/or high-energy methods (Ling et al., 2022; Sharma et al., 2022). The main drawback of both the micro- and nanoemulsions is the rapid release of the encapsulated material due to their liquid nature (Plati & Paraskevopoulou, 2022).

The majority of these procedures represent separate, additional processes which require additional equipment, affecting large-scale operations. New simple procedures are necessary that can respond to the high demand of the market and attainment of a high-quality product with extended shelf life, by applying efficient and safe processes. Among the possible, high-potential solutions for the stabilization of different types of components are the deep eutectic solvents. Deep eutectic solvents are alternative, new-generation solvents which represent mixtures of two or more components in the appropriate molar ratio, prepared by simple mixing without generating by-products, thus achieving 100% atom economy. If they consist of components of natural origin, they are considered natural deep eutectic solvents (NADES). Due to the formation of interactions between the components, which are mainly hydrogen bonding, the melting point of the NADES mixture decreases, compared to the initial constituents. NADES have a growing application because they are cheap, easy to prepare, biodegradable, safe, and it is possible to adjust their characteristics (Popović et al., 2022). Due to the favorable safety profile of NADES, additional purification is not necessary and the products can be used directly in the final products. They are also characterized by thermal and chemical stability; so, several studies have shown that they can contribute to the stabilization of certain types of components, such as polyphenols and anthocyanins (Gómez-Urios et al., 2022; Ling et al., 2022; Panić et al., 2019). There are no reported studies exploring the capacity of NADES to stabilize volatile lipophilic compounds.

For the first time, this study explored the integrated application of supercritical CO_2 for the isolation of *R. officinalis* components and NADES for their stabilization, thereby creating a simple, green procedure for obtaining and stabilizing aroma volatile components. This approach offers superior process and product characteristics compared to conventional extraction and stabilization procedures, as it implies an environmentally clean and efficient process for more rational use of raw materials, absence of solvent waste, and a safe profile and high product quality with a long shelf life.

The stability of the rosemary components, recovered by supercritical CO_2 and further dispersed in different NADES, was evaluated by monitoring the headspace profile of samples stored at room temperature for 6 months. Also, antioxidant activity assessment of the samples was performed during the same storage time as an additional stability indicator. Patter recognition chemometric methods, hierarchical cluster analysis (HCA), principal component analysis (PCA), and a ranking approach—sum of ranking differences (SRD)—were used for the analysis, interpretation, and representation of data. The Wilcoxon matched pair test, a non-parametric statistical test,

was applied to evaluate the differences between the composition of the samples after 0, 3, and 6 months of monitoring and to statistically estimate their stability.

Material and Methods

Materials and Chemicals

R. officinalis leaves were provided by Aromaticas Vivas Ltd., Carreço, Portugal. The plants were grown in Viana do Castelo (41°44′27.4″N 8°51′55.9″W), in the sub-region of Alto Minho, Portugal, under optimal growth conditions defined by the company to meet high standards of food safety, quality, and sustainability. Moisture content of the plant material (8.97 ± 0.13%) was analyzed using a moisture analyzer (KERN DAB 100–3, Germany). The mean particle size (0.39 ± 0.15 mm) of the material was determined using the vibration sieve sets (CISA, Cedaceria, Spain).

Betaine (≥ 99% purity), sorbitol, sucrose, and Nile red were purchased from Sigma-Aldrich (St. Louis, MO, USA). Glycerol (99.5% purity) was purchased from Scharlau (Barcelona, Spain). L-proline (99% purity) was purchased from Alfa Aesar (Haverhill, MA, USA). Ethylene glycol (≥ 99.5% purity) and ethanol (99% purity) were obtained from Carlo Erba (Val-de-Reuil, France).

Preparation and Characterization of NADES

NADES mixtures were prepared by mixing components in the adequate molar ratio and then heated (40 °C) and stirred until a clear liquid was formed. The NADESs molar ratios were betaine/glycerol (Bet/Gly) 1:2, betaine/ethylene glycol (Bet/EG) 1:3, and betaine/glycerol/sucrose/water (Bet/Gly/Suc/W) 2:3:1:5.

The viscosity of Bet/Gly/Suc/W was determined using a MCR102 Modular Compact Rheometer (Anton Parr) fitted with a parallel plate geometry with 50 mm of diameter (PP50, Anton Parr) and 1 mm of gap. Measurements of viscosity of the systems were performed in the temperature range of 60–20 °C (2 °C/min). The measurements were conducted in triplicates. Viscosity of Bet/Gly and Bet/EG was determined in the previous work (Vladić et al., 2023).

Supercritical Carbon Dioxide Extraction and Dispersion in NADES

CO_2 extraction was carried out using the high-pressure extraction system (HPEP, NOVA-Swiss, Effretikon, Switzerland) with main specifications as follows: gas cylinder with CO_2, diaphragm type compressor (with a pressure range up to 1000 bar), extractor vessel with heating jacket (internal volume 200 mL, maximum operating pressure 700 bar), separator with cooling jacket (internal volume 200 mL and maximum operating pressure 250 bar), pressure control valve, temperature regulation system, and regulation valves. The extractions were performed under the following conditions: pressure 300 bar, temperature 40 °C, CO_2 flow 0.194 kg/h, and extraction time 4 h. The extraction parameters were selected based on the study by Carvalho et al. (2005). Each extraction was performed in triplicate. The extraction yield was calculated using the following equation (Eq. 1):

$$\text{Extraction yield } (\%) = \frac{\text{mass of obtained extract (g)}}{\text{mass of feed material (g)}} \times 100$$

After 4 h of extraction, depressurization was applied and the extract was dispersed in the prepared NADES and the mixture was homogenized by mixing on a vortex for 1 min. The ratio of NADES extracts was 0.05 g ± 10% extract:1 mL NADES. This ratio was selected because it allows easy homogenization of the mixture. The control and CO_2 extracts dispersed into NADES (Sc-NADES) were analyzed at the beginning (0 month), after 3 months, and after 6 months of storage in transparent containers at room temperature in the dark.

Headspace Solid-Phase Microextraction (HS-SPME) and Gas Chromatography with Mass Spectrometry Analysis (GC–MS)

The headspace volatiles were extracted by a manual SPME (solid-phase microextraction) fiber with a layer of divinylbenzene/carbon wide range/polydimethylsiloxane (DVB/Carbon WR/PDMS) from Supelco Co. (Bellefonte, PA, USA). The fiber was conditioned according to the manufacturer's instructions. For HS-SPME, the sample (1.5 g) was placed in a 15-mL glass vial and hermetically sealed with PTFE/silicone septa. The closed vial was placed in a water bath at 60 °C during equilibration (15 min), and the extraction time of 45 min was applied for HS-SPME. After the sampling, the SPME fiber was withdrawn into the needle, removed from the vial, and inserted into the injector (250 °C) of the GC–MS (gas chromatography–mass spectrometry) for 6 min for thermal desorption directly to the GC column. An Agilent 8890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to a mass spectrometer (series 5977E, Agilent Technologies, Palo Alto, CA, USA) was used for the analysis. The analyzed components were separated on HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm, Agilent Technologies, Palo Alto, CA, USA). The injector temperature was set at 250 °C with a split mode of 1:50. Helium was used as a carrier gas in a constant flow regime of 1 mL/min. The GC temperature program was set at 70 °C for 2 min followed by the temperature ramp of 3 °C/min to 200 °C, and afterwards, the temperature was

maintained constant for 15 min. The separated components were analyzed with a mass spectrometry (70 eV) with a scan mode and *m*/*z* range of 30–300. The injector and detector temperatures were 250 and 300 °C, respectively. Qualitative identifications of the compounds were performed using Wiley 9 (Wiley, New York, NY, USA) and NIST 17 (National Institute of Standards and Technology, Gaithersburg, MD, USA) mass spectral libraries as well as the literature data of retention indices calculated with C_9–C_{25} alkanes. The HS-SPME/GC–MS analysis of each sample was performed in three replicates and the results are expressed as mean data.

Antioxidant Activity

The antioxidant activity of extracts was analyzed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Espín et al., 2000). Different volumes of extracts were mixed with 95% methanol solution and 90 µM DPPH solution. After the 60-min incubation period at room temperature, absorption was measured at a wavelength of 515 nm (6300 Spectrophotometer, Jenway, Dunmow, UK). The antioxidant activity was expressed as IC_{50} (µg/mL) value which represents the concentration of the extract which inhibits 50% of DPPH radicals. All measurements were performed in triplicates.

Chemometric and Statistical Analysis

The statistical comparison of the NADES and control samples was done applying chemometric approaches, including principal component analysis (PCA), sum of ranking differences (SRD), and the data analysis in a form of box, dot, and density plots put together in a combined chart. The analysis was performed on non-scaled data.

The PCA was carried out by using Statistica v. 12.0 program (StatSoft, 2014) and it was based on the correlations. The results are presented in the form of scores and loading plots. The distribution of the samples in the space of the analyzed variables (the percentage of the identified compounds) is presented in a score plot, while the load of each variable on each PC is shown in a loadings plot. The selection of the most important PCs was carried out based on the Eigenvalues higher than one.

The SRD analysis was performed using the program designed by Héberger and Kollár-Hunek (2011, 2019). The program was created in Microsoft Excel software. The ranking of the samples was done based on the row average as the reference (consensus) ranking. The ranking validation was carried out applying sevenfold cross-validation approach and by Comparison of Ranks by Random Numbers (CRRN) (Héberger & Kollár-Hunek, 2011). All analyzes were carried out in triplicates and the results were expressed as means ±standard deviation (SD). Mean values were considered significantly different at $p < 0.05$ confidence level, after the performance of the one-way ANOVA statistical analysis followed by Tukey's HSD post hoc test.

Results and Discussion

Supercritical CO_2 Extraction of *R. officinalis*

R. officinalis material was submitted to the supercritical CO_2 extraction (300 bar and 40 °C) and the exhaustion of the material was achieved after 4 h of extraction with total yield of 4.87 ± 0.20% (w/w) (Fig. 1). The obtained yield was in accordance with the yield obtained in Carvalho et al. (2005), where using the same pressure and temperatures (300 bar and 40 °C), the yield was up to 5%. Conde-Hernández et al. (2017) reported approximately 2% yield of the supercritical CO_2 extraction at 174 bar and 40 °C, and Kessler et al. (2022) reported an extraction yield of 3.03 ± 0.06% at 80 bar and 50 °C.

After it was established that exhaustion had been achieved after 4 h of extraction, further extractions at the same conditions were conducted where recovered extract was dispersed into a prepared NADES mixture (Sc-NADES samples). As control, the extract collected without dispersion into NADES was used. Obtained samples were further analyzed by using HS-SPME and GC–MS during 6 months of storage at room temperature.

The identified components in the samples (control and Sc-NADES samples) belong to different classes of compounds and can be classified into three main groups: monoterpenes 68.97–88.08%, sesquiterpenes 5.38–21.22%, and non-terpene compounds 0.27–3.78%. A detailed composition is presented in Table 1.

Additionally, the group of terpenes (mono- and sesquiterpenes) can be divided into groups of oxygenated terpenes 57.42–83.75% and terpene hydrocarbons 8.11–27.49%. The most abundant oxygenated monoterpenes components were verbenone 12.08–29.76%, camphor 10.43–17.64%, and 1,8-cineole 2.03–17.61%. A significant percentage of borneol 4.96–9.89%, linalool 3.38–9.18%, and α-terpineol 1.97–5.89% were found.

Monoterpene hydrocarbons were represented in the samples from 0.39 to 11.91%, with *α*-pinene as the most dominant (0.15–7.25%), followed by limonene, *p*-cymene, and camphene. Among the most abundant sesquiterpene, hydrocarbons were *trans*-caryophyllene (2.2–8.75%), Δ-cadinene and *α*-humulene. Oxygenated sesquiterpenes were not identified in the samples at the start (0 month), while after 3 and 6 months of storage, two compounds from this group were identified, caryophyllene oxide and viridiflorol.

The components identified in supercritical extracts of *R. officinalis* in this study were previously identified in the extracts and essential oils of *R. officinalis*. Significant

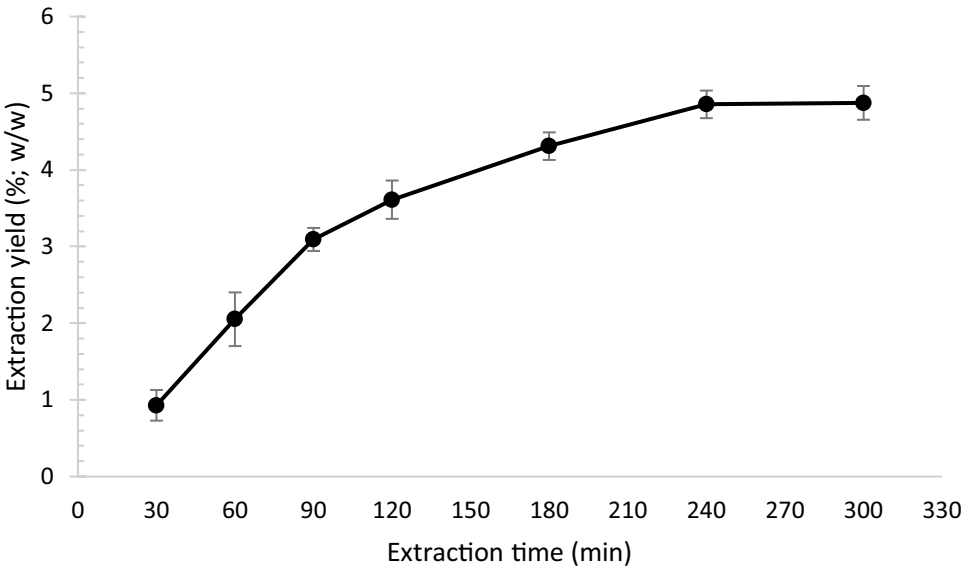

Fig. 1 Supercritical carbon dioxide extraction kinetics of *R. officinalis* (pressure 300 bar, temperature 40 °C, extraction time 5 h)

variability in the content of components may be caused by different conditions of growth or cultivation, and extraction. According to Conde-Hernández et al. (2017), the most dominant components of the supercritical extract were camphor, *β*-caryophyllene, and eucalyptol. Verbenone (34.16%) was the most dominant in the extract obtained by CO_2 extraction from Corsican *R. officinalis* (300 bar, 40 °C, 0.4 kg h^{-1}) (Mouahid et al., 2017). Kessler et al. (2022) found that in the CO_2 extract, the most dominant constituents were *α*-pinene, eucalyptol, verbenone, and camphor.

Chemometrics of the Sc-NADES and Control Samples

By monitoring the headspace profile of *R. officinalis* control and Sc-NADES samples during the 6-month period of this study (Table 1), quantitative changes were observed including changes in percentage of individual components, as well as changes such as the disappearance or occurrence of certain components. In the analysis of experimental data, chemometrics can provide insights into the stabilization efficacy and classification of the samples (da Silva Santos et al., 2020; Shojaei et al., 2021; Ghendov-Mosanu et al., 2022). Therefore, to analyze the data and observe groupings, deviations, and changes over time, different chemometrics analyses were applied—HCA, PCA, and SRD. Moreover, the Wilcoxon matched pair test, a non-parametric statistical test (Miller & Miller, 2010), was applied to statistically estimate stability, i.e., the differences between the headspace composition of the samples after 0, 3, and 6 months of monitoring.

In order to analyze the characteristics of each sample based on the presence of identified compounds, as well as to estimate the center around which the data were distributed and to examine extreme values, combination charts were formed. Figure 1 presents the combined charts of the control and Sc-NADES samples based on the percentage distribution (headspace profile of samples; Table 1) of the identified compounds in the samples. To gain an overview of the change in the headspace profile of the samples during 6 months, each particular graph contains the data of the same sample analyzed at the beginning of the experiment (month 0) and after 3 and 6 months. The Box-Whisker plot on each chart presents the Inter-Quartile Range (IQR). Whisker boundaries were based on ± 1.5 IQR, while severe outlier boundaries were set at ± 3.0 IQR. The vertical line in each green box represents the median. As it can be noticed, the median of each sample tends towards low values, since the majority of the extracted compounds were present in low percentage. All detected compounds in each sample in Fig. 2 are marked with pink dots below the green Box-Whisker plots. Among those dots, the outliers were the compounds that were present in very high concentrations (orange and red dots on the charts). In the case of control samples, the change was noticeable after 3 months when the presence of the most abundant compounds (such as verbenone) significantly increased. After 6 months, a significant shift in the median could be observed, as well as a decrease in the percentage of certain compounds. The presence of the most abundant compound did not change significantly.

Considering the Sc-NADES samples, the continuous increase of one of the most abundant compounds could be noticed only in the Bet/Gly samples. In other Sc-NADES samples, it can be noticed that after 3 months, there was an increase in the percentage of highly abundant compounds, but the change in their abundance was moderate even after 6 months. This may lead to the conclusion that those samples could be considered quite stable over time. The median change was insignificant for all the Sc-NADES samples.

Table 1 The volatile headspace compounds (%) of *Rosmarinus officinalis* extracted by headspace solid-phase microextraction and analyzed by gas chromatography–mass spectrometry using DVB/carbon WR/PDMS

Compound	RI	Control			Betaine/glycerol			Betaine/ethylene glycol			Betaine/glycerol/sucrose/water		
		0 month	3 months	6 months	0 month	3 months	6 months	0 month	3 months	6 months	0 month	3 months	6 months
Monoterpene hydrocarbons													
α-Pinene	940	4.14 ± 0.02	7.25 ± 0.80	5.33 ± 0.04	2.13 ± 0.10	3.22 ± 0.39	0.15 ± 0.19	1.79 ± 0.11	3.23 ± 0.08	3.21 ± 0.21	2.15 ± 0.13	3.53 ± 0.41	0.76 ± 0.60
Camphene	955	1.03 ± 0.22	1.41 ± 0.21	1.41 ± 0.18	0.49 ± 0.17	0.66 ± 0.06	0.06 ± 0.04	0.49 ± 0.19	0.83 ± 0.21	0.87 ± 0.13	0.50 ± 0.04	0.72 ± 0.10	0.15 ± 0.09
β-Pinene	984	0.10 ± 0.00	0.11 ± 0.04			0.06 ± 0.04					0.07 ± 0.04	0.07 ± 0.07	
β-Myrcene	993	0.83 ± 0.12	0.53 ± 0.20	0.52 ± 0.13	0.35 ± 0.05	0.15 ± 0.02		0.24 ± 0.10			0.36 ± 0.08	0.20 ± 0.12	
δ-3-Carene	1014	0.91 ± 0.18	0.88 ± 0.15	0.91 ± 0.15	0.48 ± 0.18	0.4 ± 0.10		0.45 ± 0.36	0.42 ± 0.20	0.47 ± 0.29	0.50 ± 0.12	0.55 ± 0.12	
α-Terpinene	1023	0.17 ± 0.11	0.08 ± 0.00	0.07 ± 0.02	0.09 ± 0.02	0.02 ± 0.01	0.03 ± 0.00	0.08 ± 0.02	0.03 ± 0.01	0.04 ± 0.01	0.06 ± 0.02	0.02 ± 0.01	0.01 ± 0.01
p-Cymene	1030	1.67 ± 0.28	0.88 ± 0.44	1.23 ± 0.26	1.01 ± 0.07	0.41 ± 0.13	0.09 ± 0.02	1.56 ± 0.33	0.72 ± 0.32	0.89 ± 0.12	1.05 ± 0.06	0.50 ± 0.12	1.56 ± 0.81
Limonene	1035	2.53 ± 1.45			1.33 ± 1.00		0.06 ± 0.02	1.30 ± 0.64		0.64 ± 0.09	1.35 ± 0.24		
(*Z*)-β-Ocimene	1043	0.22 ± 0.08		0.02 ± 0.02	0.11 ± 0.02						0.10 ± 0.01		
(*E*)-β-Ocimene	1054	0.20 ± 0.13			0.07 ± 0.01						0.07 ± 0.06		
γ-Terpinene	1065	0.11 ± 0.08	0.02 ± 0.00		0.05 ± 0.00			0.10 ± 0.05			0.06 ± 0.01		
Oxygenated monoterpenes													
Verbenene	968	0.46 ± 0.22	0.58 ± 0.12	0.78 ± 0.48	0.30 ± 0.02	0.23 ± 0.11	0.13 ± 0.10	0.32 ± 0.12	0.31 ± 0.12	0.50 ± 0.12	0.30 ± 0.06	0.22 ± 0.14	0.06 ± 0.03
1,8-Cineole	1037	7.86 ± 0.97	13.56 ± 2.14	11.24 ± 1.80	6.73 ± 3.41	10.52 ± 2.74	2.03 ± 1.04	10.13 ± 2.64	17.61 ± 5.09	15.07 ± 2.49	6.64 ± 1.64	10.81 ± 2.14	6.27 ± 3.01
trans-Linalool oxide	1078		0.08 ± 0.01				0.13 ± 0.04			0.06 ± 0.01	0.06 ± 0.00		0.12 ± 0.09
cis-Linalool oxide	1087		0.08 ± 0.02										
Linalool	1103	3.38 ± 0.93	6.53 ± 1.11	5.58 ± 1.02	4.94 ± 1.23	8.48 ± 1.71	6.94 ± 1.29	4.77 ± 1.32	6.32 ± 2.29	5.18 ± 2.15	5.42 ± 1.26	8.66 ± 2.87	9.18 ± 0.07
Filifilone	1108	1.43 ± 0.54	0.41 ± 0.13	0.77 ± 0.21	1.06 ± 0.09	0.38 ± 0.15	0.29 ± 0.09	1.00 ± 0.05	0.30 ± 0.16	0.37 ± 0.15	1.00 ± 0.58	0.37 ± 0.23	0.53 ± 0.11
α-Thujone	1104	0.72 ± 0.22	0.45 ± 0.42	1.14 ± 0.14	0.79 ± 0.14	0.41 ± 0.22	0.40 ± 0.12	1.12 ± 0.34	0.52 ± 0.23	1.39 ± 0.98	0.81 ± 0.14	0.36 ± 0.13	0.84 ± 0.05
β-Thujone	1114	0.53 ± 0.17		0.60 ± 0.22	0.50 ± 0.11		0.19 ± 0.12	0.71 ± 0.35		0.69 ± 0.25	0.51 ± 0.36		0.45 ± 0.03
α-Campholenal	1132	0.07 ± 0.00		0.01 ± 0.01									
Camphor	1150	12.31 ± 1.36	16.03 ± 2.17	11.91 ± 1.54	13.91 ± 2.14	17.43 ± 1.38	10.43 ± 4.41	14.13 ± 1.69	17.64 ± 2.59	15.08 ± 1.25	13.52 ± 0.26	16.46 ± 0.15	13.43 ± 3.98
Menthone	1160	0.08 ± 0.00											
(*Z*)-Pinocamphone	1167	1.34 ± 0.51			1.43 ± 1.01		0.87 ± 0.41	1.64 ± 0.25	1.72 ± 0.07	1.96 ± 0.36	1.43 ± 0.69		1.44 ± 0.12
Pinocarvone	1169	0.30 ± 0.18			0.29 ± 0.11			0.18 ± 0.09			0.27 ± 0.14		
Borneol	1170	6.30 ± 2.54	8.56 ± 1.36	6.61 ± 1.36	8.22 ± 1.02	9.89 ± 3.58	9.86 ± 0.36	4.96 ± 1.36	5.77 ± 1.36	5.18 ± 1.23	7.69 ± 0.69	8.26 ± 1.88	7.43 ± 1.58
Terpinene-4-ol	1182	0.92 ± 0.32	1.29 ± 0.28	1.45 ± 0.23	1.24 ± 0.08	1.78 ± 0.28	1.99 ± 0.28	1.00 ± 0.25	0.71 ± 0.09	1.18 ± 0.05	1.27 ± 0.19	1.46 ± 0.08	1.92 ± 0.02
p-Cymen-8-ol	1190	0.26 ± 0.21		0.54 ± 0.09	0.40 ± 0.16		1.99 ± 0.04	0.20 ± 0.10		0.28 ± 0.12	0.08 ± 0.01		0.76 ± 0.35
α-Terpineol	1194	1.99 ± 0.38	3.56 ± 1.78	3.43 ± 0.69	2.84 ± 0.16	4.45 ± 1.23	5.89 ± 2.36	1.97 ± 0.06	2.40 ± 0.99	2.54 ± 0.98	3.11 ± 1.14	4.00 ± 1.03	4.77 ± 2.08
Myrtenol	1212	0.33 ± 0.05		0.25 ± 0.02	0.44 ± 0.20		0.79 ± 0.12	0.22 ± 0.01		0.27 ± 0.15	0.41 ± 0.09		0.55 ± 0.35
Verbenone	1224	14.35 ± 3.47	20.56 ± 2.49	19.94 ± 2.98	18.06 ± 1.06	23.99 ± 4.68	29.76 ± 1.22	12.08 ± 0.29	15.7 ± 1.67	14.77 ± 1.00	17.55 ± 3.58	21.82 ± 2.69	21.45 ± 1.25
trans-Carveol	1230	0.11 ± 0.04		0.30 ± 0.15	0.13 ± 0.01	0.10 ± 0.00	0.49 ± 0.01	0.04 ± 0.02		0.09 ± 0.02	0.14 ± 0.01		0.30 ± 0.05
Bornyl acetate	1297	3.36 ± 0.39	2.87 ± 1.10	3.55 ± 0.45	4.95 ± 0.02	3.73 ± 1.0	2.15 ± 0.39	7.58 ± 1.39	6.68 ± 2.36	6.19 ± 1.36	5.29 ± 2.69	4.92 ± 1.28	3.83 ± 0.94
Piperitenone	1347	1.23 ± 0.87	1.05 ± 0.05	1.85 ± 0.69	1.67 ± 0.19	1.51 ± 0.26	3.22 ± 0.14	0.85 ± 0.18		1.42 ± 0.78	1.74 ± 1.01	1.52 ± 0.88	2.80 ± 1.36

Table 1 (continued)

Compound	RI	Control			Betaine/glycerol			Betaine/ethylene glycol			Betaine/glycerol/sucrose/water		
		0 month	3 months	6 months	0 month	3 months	6 months	0 month	3 months	6 months	0 month	3 months	6 months
Eugenol	1359				0.36 ± 0.12	0.17 ± 0.07	1.15 ± 0.09	0.06 ± 0.03		0.15 ± 0.08	0.19 ± 0.09		0.46 ± 0.12
Dihydroactinidiolide	1532	0.09 ± 0.08		0.22 ± 0.17		0.09 ± 0.02	0.28 ± 0.01			0.09 ± 0.00			0.17 ± 0.09
Sesquiterpene hydrocarbons													
α-Copaene	1378	1.10 ± 0.28	0.59 ± 0.09	0.12 ± 0.05	1.44 ± 0.12	0.46 ± 0.30	0.35 ± 0.04	1.28 ± 0.18	0.61 ± 0.05	0.80 ± 0.02	1.42 ± 0.36	0.74 ± 0.12	0.85 ± 0.07
trans-Caryophyllene	1419	5.10 ± 1.78	2.66 ± 0.23	3.12 ± 0.99	7.33 ± 01.32	3.15 ± 0.49	2.20 ± 0.84	8.75 ± 0.48	5.01 ± 0.58	4.58 ± 1.58	7.42 ± 1.25	4.64 ± 1.29	4.38 ± 0.36
α-Humulene	1456	1.66 ± 0.45	1.08 ± 0.26	1.36 ± 1.00	2.76 ± 0.12	1.39 ± 0.21	1.36 ± 0.62	3.51 ± 1.36	2.16 ± 1.23	1.99 ± 0.47	3.08 ± 0.88	1.96 ± 0.08	2.09 ± 0.58
γ-Muurolene	1477	0.91 ± 0.03			0.13 ± 0.08		0.53 ± 0.07	0.14 ± 0.06	0.87 ± 0.05	0.83 ± 0.03	1.63 ± 0.05	0.80 ± 0.11	0.95 ± 0.05
ar-Curcumene	1483	0.19 ± 0.09			0.32 ± 0.03	0.10 ± 0.10	0.16 ± 0.06	0.35 ± 0.08	0.08 ± 0.04		0.38 ± 0.05	0.06 ± 0.02	0.22 ± 0.18
β-Selinene	1486	0.14 ± 0.63			0.24 ± 0.01		0.13 ± 0.07			0.16 ± 0.08	0.28 ± 0.04		0.19 ± 0.04
α-Muurolene	1499	0.59 ± 0.49		0.48 ± 0.45	0.97 ± 0.36	0.28 ± 0.17	0.37 ± 0.18	0.97 ± 0.04	0.20 ± 0.02	0.46 ± 0.03	1.09 ± 0.06	0.57 ± 0.09	0.58 ± 0.04
β-Bisabolene	1500	0.38 ± 0.28		0.31 ± 0.06	0.61 ± 0.18	0.14 ± 0.11	0.20 ± 0.03	0.49 ± 0.01	0.14 ± 0.06	0.19 ± 0.03	0.71 ± 0.55	0.22 ± 0.10	0.34 ± 0.30
γ-Cadinene	1505	0.78 ± 0.06		0.66 ± 0.41	1.32 ± 0.84	0.44 ± 0.23	0.98 ± 0.09	1.50 ± 0.06	0.64 ± 0.04	0.90 ± 0.04	1.55 ± 0.22	0.61 ± 0.09	1.04 ± 0.81
Δ-Cadinene	1525	1.77 ± 0.34	1.05 ± 0.87	1.45 ± 1.00	3.32 ± 0.32	1.13 ± 0.84	1.44 ± 0.14	3.61 ± 1.36	0.84 ± 0.07	1.71 ± 1.20	3.66 ± 0.69	1.49 ± 0.88	1.91 ± 0.05
Oxygenated sesquiterpenes													
Caryophyllene oxide	1581			1.04 ± 0.26		0.59 ± 0.12	1.50 ± 0.36		1.01 ± 0.05	1.42 ± 0.98		0.67 ± 0.06	1.30 ± 0.16
Veridiflorol	1592						0.24 ± 0.20			0.18 ± 0.12			0.19 ± 0.10
Non-terpenes													
Acetic acid			0.61 ± 0.02	1.15 ± 0.87									
Dimethyl sulfoxide			0.04 ± 0.00	0.72 ± 0.12									
(*Z*)-Hex-3-en-1-ol	885	0.02 ± 0.00	0.02 ± 0.00				0.06 ± 0.00						
Tricyclene	949	0.04 ± 0.01	0.05 ± 0.01	0.08 ± 0.02							0.02 ± 0.00		
Oct-1-en-3-ol	986	0.26 ± 0.12	0.45 ± 0.10	0.89 ± 0.15	0.24 ± 0.02	0.44 ± 0.09	2.18 ± 0.08	0.22 ± 0.12	0.24 ± 0.06	0.34 ± 0.15	0.28 ± 0.07	0.39 ± 0.12	
Octan-3-one	989	0.09 ± 0.02		0.21 ± 0.00	0.07 ± 0.06			0.10 ± 0.02	0.07 ± 0.06	0.07 ± 0.06	0.07 ± 0.00		
Benzyl alcohol			0.19 ± 0.09	0.10 ± 0.03									
2-Phenylethanol	1112	0.26 ± 0.16		0.63 ± 0.15	0.14 ± 0.02		0.52 ± 0.15						0.27 ± 0.19

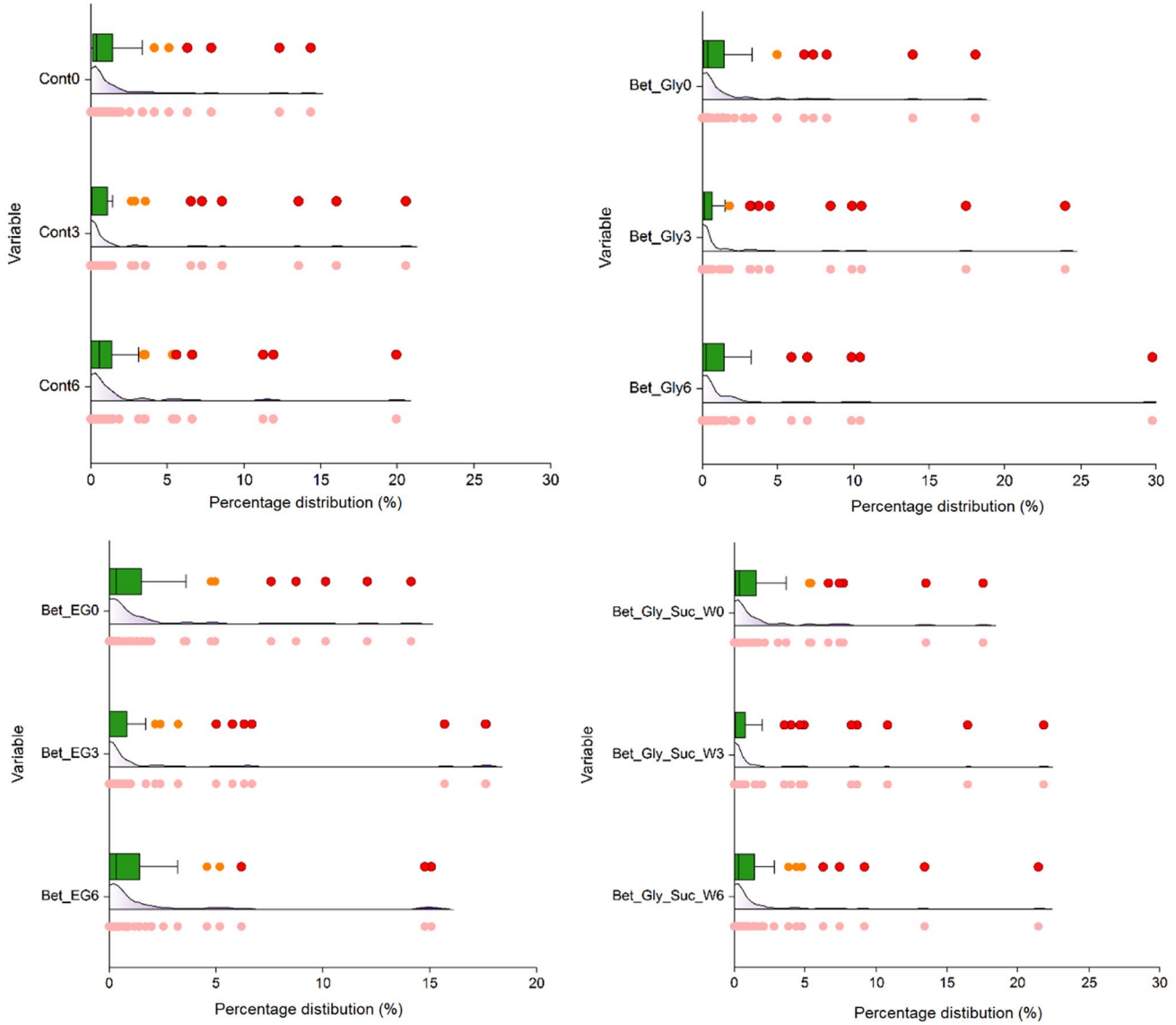

Fig. 2 Combined charts of the control samples and Sc-NADES based on the presence of the identified volatile headspace compounds. Pink dots – all identified compounds; orange and red dots – compounds present in higher percentage

Taking into account the group of the compounds (oxygenated monoterpenes, monoterpene hydrocarbons, oxygenated sesquiterpenes, sesquiterpene hydrocarbons, and non-terpenes) (Fig. 3) instead of individual compounds, it can be seen that the most prominent oscillations in composition and median were in the control samples. The NADES samples had relatively stable parameters over time with the exceptions of Bet/Gly and Bet/Gly/Suc/W samples between the 3rd and 6th month where the change of median was noticeable. However, the representation of the most abundant compounds (oxygenated monoterpenes) in those samples was stable even after 6 months.

The distribution of the samples in the space of the considered variables (oxygenated monoterpenes-OM, monoterpene hydrocarbons-MH, oxygenated sesquiterpenes-OS, sesquiterpene hydrocarbons-SH, and non-terpene compounds-NT) was obtained applying PCA (Fig. 4). The resulting PCA model is based on two principal components (PCs) which cover 77.42% of total variability. PC1 takes into account for 49.56%, while the PC2 covers 27.86% of total variance. Both PCs are described by Eigenvalues higher than 1 (PC1 = 2.5, PC2 = 1.4). The score plot (Fig. 4a) indicates the separation of the samples so along the PC1 axis, the samples analyzed at the start of the experiment are oriented on the negative end of the PC1, while the samples monitored after 3 and 6 months are inclined towards the positive end. The distribution of the samples along the PC1 axis was mostly influenced by oxygenated

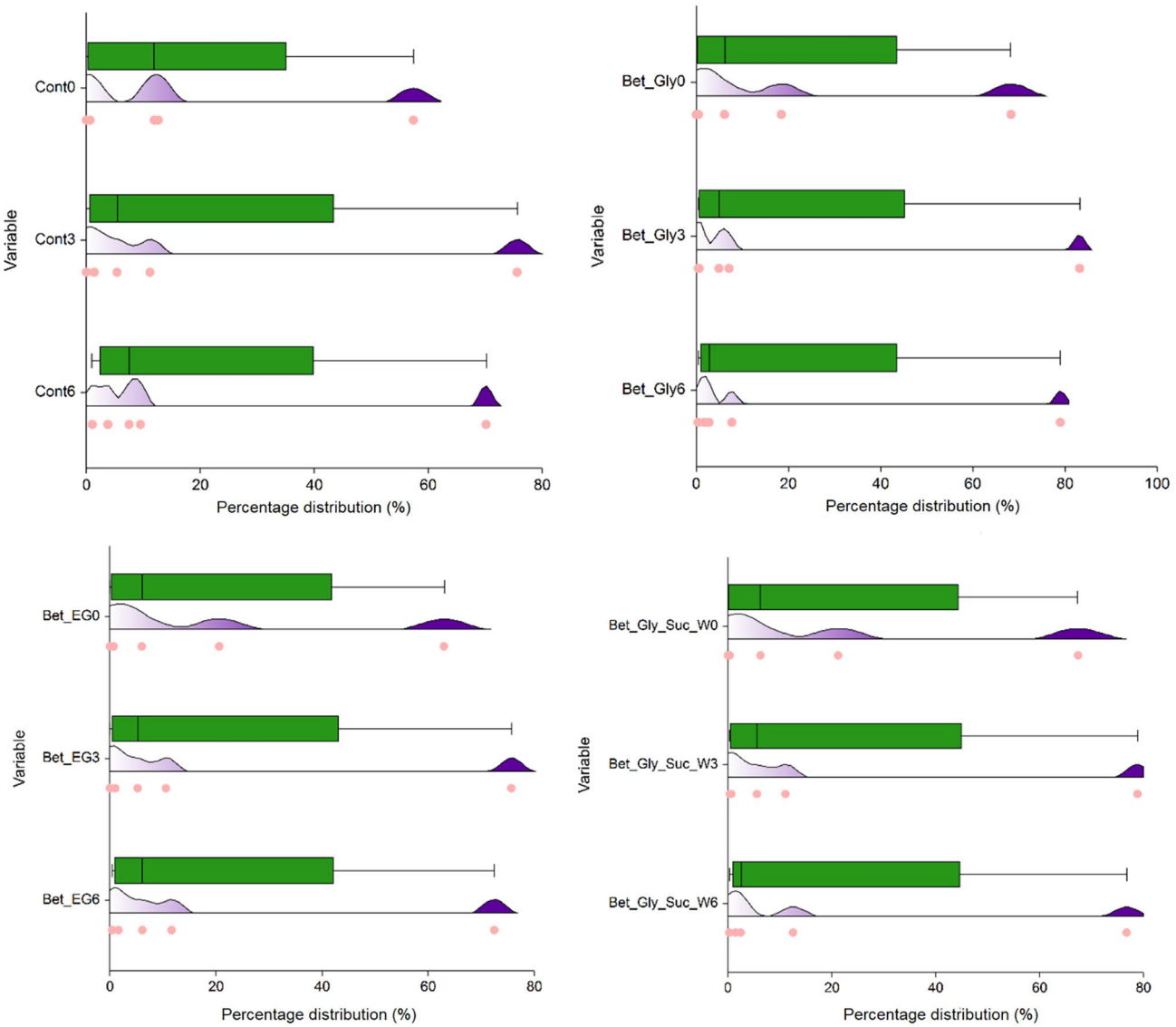

Fig. 3 Combined charts of the control samples and NADES based on the content of the group of the samples (oxygenated monoterpenes, monoterpene hydrocarbons, oxygenated sesquiterpenes, sesquiterpene hydrocarbons, non-terpenes). Pink dots – group of components

monoterpenes and oxygenated sesquiterpenes (they have the highest positive influence on the PC1 as it can be seen on the loadings plot in Fig. 3b). SH and MH had almost the same influence on both PC1 and PC2 axis. The control samples are separated from the Sc-NADES samples along the PC2 axis on which the sesquiterpene hydrocarbons, monoterpene hydrocarbons, and non-terpenes had the highest influence as the loading plot indicates (Fig. 4b). Generally, on the PC1 axis, there is a clear separation between the samples monitored at the start of the experiment and the samples monitored after 3 and 6 months. The grouping of the samples analyzed at the beginning was expected. Furthermore, the separation between the samples analyzed at the beginning and after 3 and 6 months indicates that after 3 months, there were changes compared to the initial analysis. However, after 3 and 6 months, there was no separation and stability remained. The changes are most likely caused by the transformation and/or evaporation of labile hydrocarbons, but since there was no separation between 3 and 6 months, no significant changes were noticeable. The sample Bet/Gly6 is separated from other Sc-NADES samples on the PC1 due to the highest OS content. On the PC2 axis, there is a separation of the control samples from the Sc-NADES, as a result of the influence of components that belong to the non-terpene group and monoterpene hydrocarbons. The PC2 does not separate the Sc-NADES samples; it projects them on a very close distance pointing on the similarity distribution components during time.

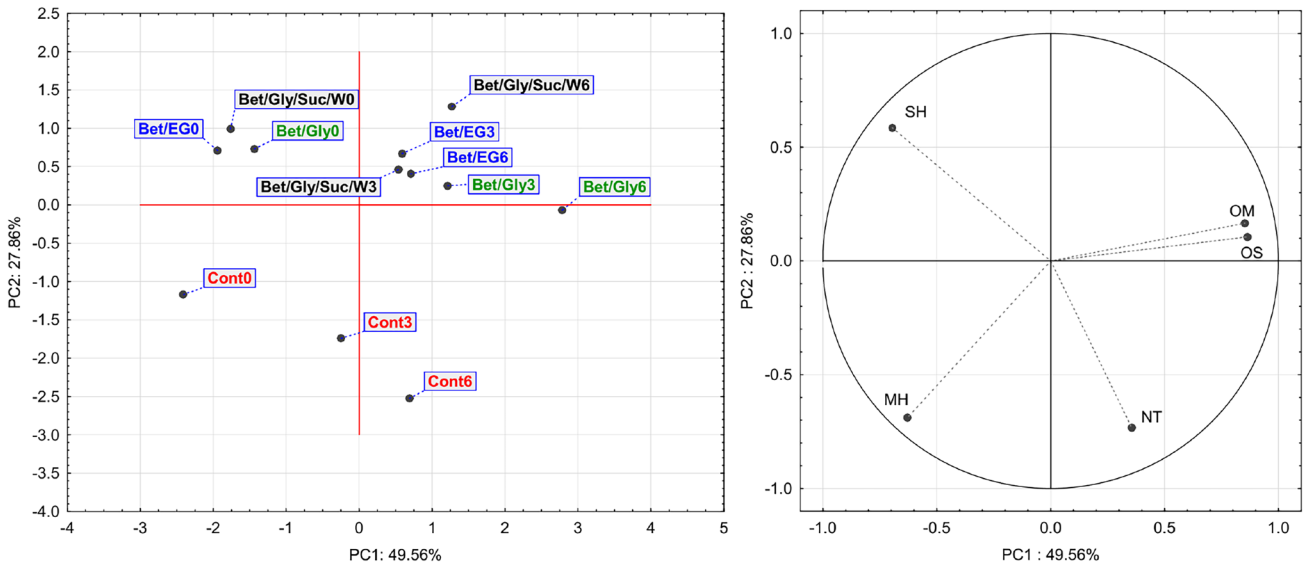

Fig. 4 The score (left) and the loadings plot (right) of the Sc-NADES and control samples based on the groups of compounds (OM oxygenated monoterpenes, MH monoterpene hydrocarbons, OS oxygenated sesquiterpenes, SH sesquiterpene hydrocarbons, NT non-terpenes)

The ranking analysis of the Sc-NADES and control samples was carried out based on the SRD approach with CRRN and sevenfold cross-validation (Fig. 5). The ranking was based on the presence of the individual compounds. The results indicate that the majority of the Sc-NADES samples were placed close to the reference ranking (row average or consensus ranking) and significantly far from the random number distribution. Bet/EG3 and Bet/EG6 samples were the closest to the reference ranking, while all control samples were quite far away from the reference ranking together with the Bet/Gly6 sample. All the samples, except for Bet/Gly6, were placed very close to each other meaning that they have quite similar ranking; therefore, there was no significant rank change over time (after 3 and 6 months of monitoring). On the contrary, the control samples had a significant rank change over time since all the control samples were distant from each other. The results of the SRD analysis point out that the Bet/Gly6 sample was significantly distant from other Sc-NADES samples, as it was previously shown in the results of PCA analysis. Namely, the reason behind it is that in this sample, the biggest oscillation in the presence of oxygenated terpenes and the greatest drop in hydrocarbons during time were detected.

The validation of the SRD procedure based on sevenfold cross-validation confirmed the validity of the SRD procedure. The rankings of the samples are presented in Fig. 6 in the form of a Box-Whisker plot. For each sample, there are 7 normalized SRD values for which the median and 25–75% percentile range were calculated. There were no outliers nor extreme values detected. The samples are placed in the same

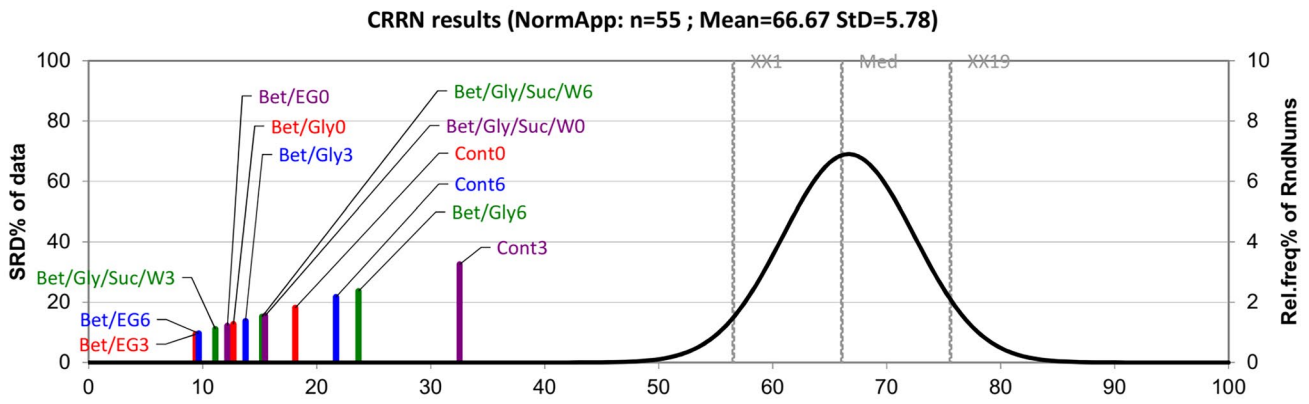

Fig. 5 The ranking of the NADES and control samples by SRD and CRRN with row average as a reference ranking

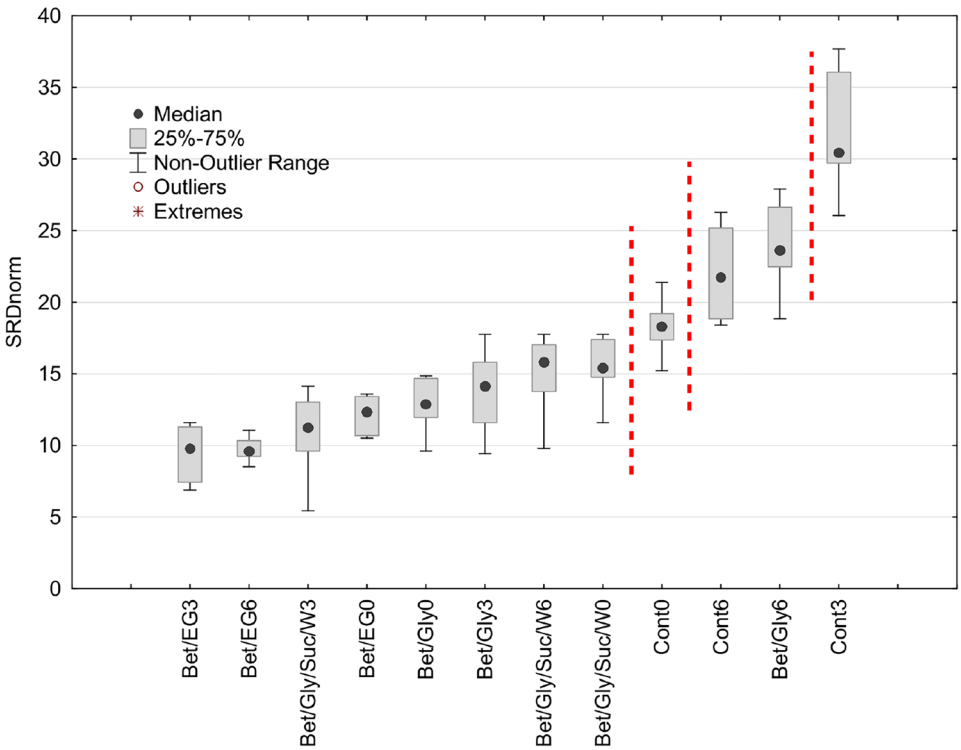

Fig. 6 The comparison of ranking of NADES and control samples in terms of sevenfold cross-validation of the SRD procedure (the red vertical dashed lines indicate the significant differences between neighboring samples in terms of the Wilcoxon matched pairs test)

order as in Fig. 5. The red vertical dashed lines in the graph indicate the significant differences between the samples Bet/Gly/Suc/W0 and Cont0, Cont0, and Cont6 as well as Bet/Gly6 and Cont3, based on the Wilcoxon matched pairs test at 0.05 confidence level. Therefore, the samples Cont0, Cont6, Bet/Gly6, and Cont3 significantly differ from other samples regarding their ranking based on SRD values.

By observing the headspace profiles of the Sc-NADES samples of *R. officinalis* and the control, the storage-induced changes observed by the hierarchical analysis can be analyzed in more detail. Susceptible to changes were monoterpene hydrocarbons; hence, the most significant decrease in monoterpene hydrocarbons was observed in Bet/Gly Sc-NADES samples, from 6.11 to 0.39%. Also, Bet/Gly/Suc/W samples had a decline from 6.27 to 2.48% over 6 months. Reduction of hydrocarbons has been reported previously during essential oil storage, *Majorana hortensis* (Misharina et al., 2003), *Melissa officinalis* (Najafian, 2014), *Thymus daenensis* (Rowshan et al., 2013).

The cause of fluctuations in the content as well as the disappearance of certain terpene hydrocarbons over time is the susceptibility to evaporation and changes. Hydrocarbons represented in *R. officinalis* samples, such as limonene, camphene, and pinene, are volatile and low molecular weight hydrocarbons (C_2–C_{10}), which are characterized by easy chemical transformations which can lead to the formation of their degradation products (McGraw et al., 1999). Also, their mutual change (transformation from one to the other) is possible, which might be the reason why the control and Bet/EG had oscillations in content within ± 2% during 6 months. Some of the hydrocarbons were significantly reduced or completely disappeared after 3 and/or 6 months, such as limonene, and some had an increase in percentage, such as pinene and camphene in control and Bet/EG.

Due to the lack of functional groups to interact more strongly between molecules, hydrocarbons are significantly more volatile and unstable in relation to oxygenated components (Silvestre et al., 2019). Additionally, due to hydrocarbon's unsaturated character, they are prone to oxidation and other chemical transformations, as well as resinification (Walid et al., 2019). On the other hand, components like alcohol, such as α-terpineol and borneol, or ketones such as verbenone, are less volatile and more stable due to stronger molecular interaction forces (hydrogen bonds) and dipole–dipole interactions, respectively (Silvestre et al., 2019). Therefore, changes in hydrocarbons affected the presence of other components and dictated their share in the samples. In all samples, an increase in oxygenated monoterpenes was observed after 3 months and then there was a decrease. The most prominent oscillations were in the control with growth after 3 months of 18.19% and after that a reduction of 5.44%. while Sc-NADES samples had less pronounced changes in the presence of oxygenated monoterpenes with the least prominent changes in Bet/Gly/Suc/W (increase after 3 months of 11.43% and decrease after 6 months of 2.1%).

Bicyclic monoterpene α-pinene is an unstable molecule that can be easily transformed into oxygenated monoterpenes (Silvestre et al., 2019). Furthermore, the increase of verbenone and terpineol was observed during storage. Also, the transformation of α-pinene can produce borneol and camphor (Bicas et al., 2009). Additionally, due to the rearrangement or oxidation of α-pinene, products such as β-pinene, pinocamphone, and myrtenol can be generated. By further oxidation of β-pinene, myrtenol can be produced (McGraw et al., 1999).

Additionally, verbenone, α-terpineol, and carveol can also be created as oxidation derivatives of limonene (Becerra et al., 2022; Bicas et al., 2009), so their growth can be partially caused by the decrease in the percentage of limonene due to its transformation.

Due to dehydrogenation, α-terpinene and limonene can form aromatic systems, such as *p*-cymene, which can potentially explain its oscillations during storage. The degradation of α-terpinene due to epoxide formation can lead to the formation (from α-terpinene) of 1,8-cineole, which had a trend in all samples—increase after 3 months and followed by its reduction. Also, it is possible that the growth of α-terpineol during 6 months might be a consequence of the rearrangement of 1,8-cineole (Walid et al., 2019). Moreover, the increase in α-terpineol might be caused by the conversion of linalool (Misharina et al., 2003). Additionally, terpenes can undergo allylic oxidation and form alcohols, ketones, and aldehydes, and be subject to carbon skeleton rearrangements; hence, it is possible that camphene yielded verbenone (McGraw et al., 1999).

Therefore, the headspace profile changes of *R. officinalis* samples which represent complex mixtures of terpene hydrocarbons, alcohols, ketones, aldehydes, and others chemical classes are very difficult to detect, because these components are prone to fast changes which are usually sequential, meaning one change initiates a string of further changes.

Oscillations in profiles of the samples over time point to the instability of the product, but depending on the product's purpose, the reduction in hydrocarbons can represent an advantage. For industrial applications, hydrocarbons are often removed from the product to ensure greater stability and avoid unwanted changes. Namely, it is considered that the presence of less stable hydrocarbons creates problems in terms of stability and can lead to the deterioration of aroma and flavor (Walid et al., 2019). On the other hand, hydrocarbons have important properties, which is why they are in high demand in certain industries. Depending on the application of the product, their presence can be unwanted or highly significant and relevant. For example, because of its fresh pine scent and woody flavor, the bicyclic monoterpene pinene is used as a fragrance substance that is used to improve the odor of industrial products (Bicas et al., 2009).

Also, changes were observed in the abundance of sesquiterpene hydrocarbons, so there was a decrease and disappearance of certain sesquiterpene hydrocarbons during storage. The most dominant of this group, β-caryophyllene, was reduced during storage compared to the start, which may be due to its oxidation. This coincides with the appearance and increase in the very important and biologically active component caryophyllene oxide.

The group in which the non-terpene components were classified was characterized by a small percentage, which increased most significantly in the control samples over time (Fig. 7). However, apart from the quantitative changes within this group, there were very significant qualitative changes. Namely, the emergence and increase in certain components were significant which can indicate instability and cause significant qualitative alterations in the characteristics of the samples. Namely, changes during storage that indicate chemical reactions of transformation can result in deterioration of organoleptic properties, as well as the development and growth of components that make the product unsuitable for use (Misharina & Polshkov, 2005).

Benzyl alcohol was identified only in control after 3 and 6 months. Although it is considered that alcohols in small concentration have a minor contribution to flavor, Voon et al. (2007) established the correlation between the increase in benzyl alcohol and green note and off-odor. Also, in the control after 3 months, the presence of acetic acid was detected and its content continued to grow until 6 months, while it was not detected in Sc-NADES samples. The appearance and growth of the acetic acid can potentially cause a pungent odor and taste of products; therefore, it limits their application in areas where aromatic/taste properties are significant. In addition, the presence and growth of acetic acid can be accompanied by the appearance of other organic acids and other components of low molecular weight which can be a consequence of processes such as oxidation of aldehyde, fermentation, and similar. Therefore, an increase in the presence of acetic acid can be an indicator of reduced stability, which further can potentially influence the reduction in quality and alternations in products' biological properties. The emergence and the growth of acetic acid represents a sign of lower stability of the control samples in relation to Sc-NADES samples. Longer monitoring of the headspace profile of stored samples is desirable to establish more precisely the stability of the product. A more significant increase in the non-terpene group in the Bet:Gly sample after 6 months was caused by an increase in the presence of oct-1-en-3-ol, which is characterized by a strong metallic mushroom-like odor. In the remaining two Sc-NADES samples, the presence of this component was below 0.04%.

Antioxidant Stability

In order to additionally evaluate the stability of the samples over time, antioxidant activity was monitored using

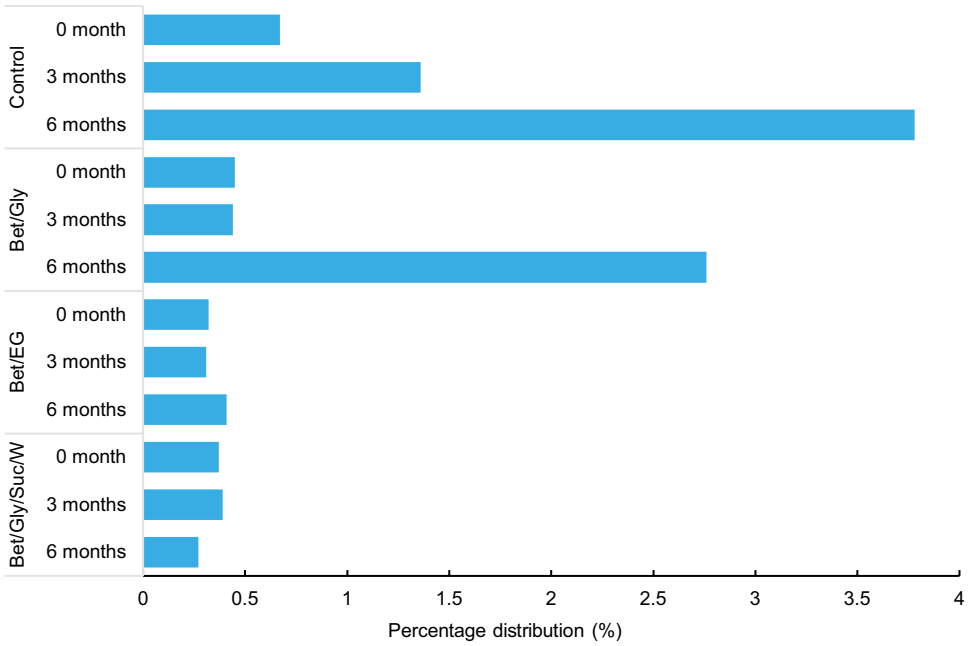

Fig. 7 Changes in the distribution (%) of components from groups of non-terpenes during 6 months of storage in control and Sc-NADES samples

the DPPH test. Initially, the control sample exhibited a stronger (statistically insignificant) antioxidant activity compared to the Sc-NADES samples with an IC_{50} value of 5.45 ± 0.50 µg/mL (Fig. 8), while the IC_{50} value of the Sc-NADES samples at the beginning (0 months) was in the range 8.34 ± 0.95–9.52 ± 0.84 µg/mL. Namely, the Sc-NADES samples were diluted due to the dispersion of extracts in NADES compared to the control, which may be the explanation of the lower initial activity.

Obtained antioxidative activities were in accordance with the activities reported in literature. The extracts obtained by supercritical fluid extraction at 100–300 bar and 30–50 °C had IC_{50} values 12.85–27.34 µg/mL (Genena et al., 2008). Cavero et al. (2005) determined IC_{50} values in the range 11.1–42.6 µg/mL for supercritical extracts obtained at 150 and 300 bar, and 40 and 60 °C. Moreover, essential oils from rosemary material collected at different locations. Palestine exhibited activity in the range 10.23 ± 0.11–158.48 ± 0.87 µg/mL (Al-Maharik et al., 2022).

During storage, there were significant changes in the activities of the control; after 3 months, the antioxidant activity decreased twofold, while after 6 months, the progressive and multifold decrease in antioxidant activity continued to IC_{50} value 48.68 ± 9.78 µg/mL.

In Bet/Gly samples, the reduction in antioxidant activities was observed over time, which indicates on the reduced stability of this sample, especially after 6 months of storage, while Bet/Eg and Bet/Gly/Suc/W had statistically insignificant change in activities. The maintained antioxidant activity potentially can be caused by the stabilization of extracts and

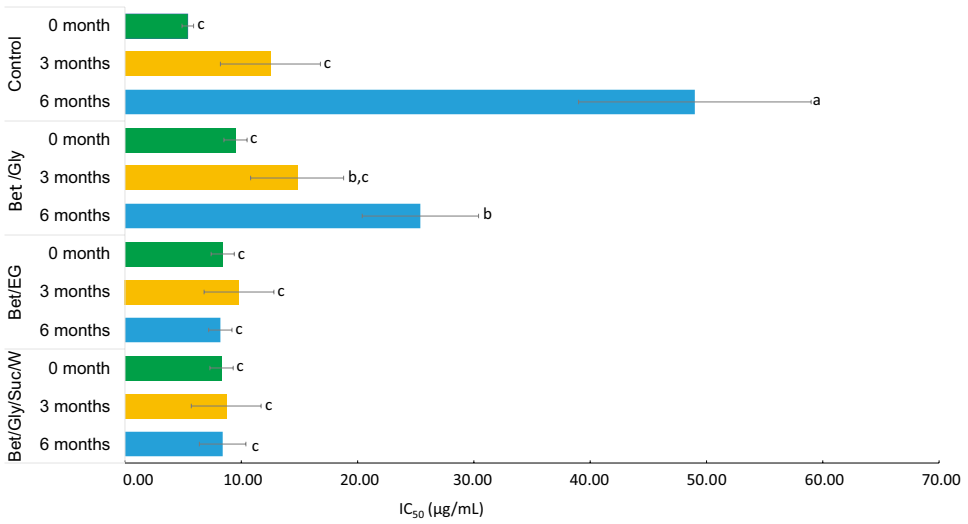

Fig. 8 Monitoring of antioxidant activity (expressed as IC_{50} value; µg/mL) of control and Sc-NADES samples over 6 months. Bars with different letters indicate significant differences based on Tukey's HSD test ($p < 0.05$)

activities because of the presence of NADES. Namely, due to presence of NADES, the components are potentially more protected from environmental conditions which can cause changes. Therefore, it might be assumed that the antioxidant activity of the samples was preserved due to the deceleration or stoppage of oxidation processes.

Also, it was previously shown that NADES can enhance the antioxidant activity of extracts (Durand et al., 2017; Nam et al., 2015; Radošević et al., 2018). Betaine has been shown in several studies to exhibit antioxidant activity in different systems (Alirezaei et al., 2012; Arumugam et al., 2021; Pourmehdi et al., 2020; Radošević et al., 2018). Therefore, it is possible that the presence of NADES contributed to the preservation of the antioxidant activity of the samples through an additive antioxidant effect together with the components of *R. officinalis* extracts and/or prevention of oxidation reactions.

Bearing in mind the results of monitoring the stability of the control and Sc-NADES samples, it can be concluded that betaine-based NADES were effective in preserving the stability of rosemary lipophilic extracts over time. Previous studies related the thermal stability of the components in NADES with the formation of network bonding between the extracts' and NADES' components (Dai et al., 2014; Zannou & Koca, 2020). Considering that the rosemary extract is a mixture of a large number of chemically diverse constituents, it can be assumed that the improved stability of Sc-NADES represents a consequence of established interactions between NADES components and components of *R. officinalis* extracts.

Additionally, according to earlier studies, viscosity has been linked to the stabilizing properties of NADES. It has been suggested that due to the lower viscosity of NADES solvents, the movement of molecules can potentially be slowed down and thus contribute to the preservation of the formed interactions, contributing to the stabilization of components (Dai et al., 2014). The viscosity of NADES used in this work was Bet/Gly (1278.1 ± 48.81 mPa.s), Bet/EG (48.630 mPa.s), and Bet/Gly/Suc/W (2246.767 mPa.s) at a temperature of 30 °C. Therefore, the correlation between viscosity and effectiveness of preserving the activity and properties of rosemary extracts was not determined.

The established procedure for obtaining and stabilizing *R. officinalis* aroma volatile compounds in NADES has multiple economic benefits for the downstream process. Namely, the effective isolation of *R. officinalis* components and their stabilization in NADES represents a more rational use of raw materials and obtaining products with an extended shelf life that can be stored and transported at room temperature. Additionally, *R. officinalis* extracts stabilized in NADES can be directly used in final formulations, due to the inherent properties of NADES. Therefore, the purification and separation steps are eliminated, which significantly reduces downstream costs.

Conclusions

Due to the diverse activity and wide field of application of *R. officinalis* products, green approaches to obtain and stabilize rosemary extracts are of great importance. In this work, the effectiveness of three different NADES for the stabilization of aroma volatile constituents isolated using supercritical CO_2 extraction was examined. Bet/EG and Bet/Gly/Suc/W NADES mixtures were found to be effective in maintaining stability. The additional confirmation of the stabilizing properties of NADES is the unchanged antioxidant activity during the storage period in these two samples.

The established procedure for the recovery of *R. officinalis* components and their stabilization, using CO_2 and NADES, is a process that can significantly contribute to environmental protection and resource sustainability due to the more rational use of natural resources and the non-generation and accumulation of solvent waste. Additionally, it contributes to the economic growth because stabilized, high-quality, and ready-to-use products, which can be stored and transported at room temperature, are obtained using simple and green procedure.

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Data Availability The data obtained during the study are available from the corresponding author upon reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

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