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Development of essential oil diffusion matrices using non-ionic surfactants-supported NADES and hydrophobic NADES†

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Natural deep eutectic solvents (NADES) represent a significant advance in green chemistry, offering an eco-friendly alternative to conventional organic solvents for applications in extraction, reaction media, and formulations. This study explores the application of NADES in essential oil formulations, using lavender essential oil (LEO) to investigate the solubilization and release of volatile organic compounds (VOCs). Two distinct NADES formulations were evaluated: hydrophilic NADES combined with surfactants, and hydrophobic NADES. The results show that hydrophilic NADES, when combined with non-ionic surfactants, form stable emulsions that effectively solubilize and release LEO's VOCs, overcoming the high hydrophilicity limitation of NADES. Headspace analysis and gas chromatography-mass spectrometry (GC-MS) identified 25 VOCs, with differential components analyzed through orthogonal partial least-squares discriminant analysis (OPLS-DA). VOCs were well-clustered in OPLS-DA and principal component analysis (PCA), with five clearly differentiated groups (C, N1, N3, N5, and N9). However, challenges remain, particularly in the solubilization and gradual release of VOCs. In contrast, hydrophobic NADES demonstrated a controlled release mechanism, effectively encapsulating and steadily releasing VOCs, making them advantageous for sustained fragrance delivery. The study further emphasizes the importance of the Hydrophilic–Lipophilic Balance (HLB) in NADES formulations, showing that adjusting HLB through surfactant concentrations significantly influences VOC release dynamics. In conclusion, this research highlights the potential of NADES as efficient, eco-friendly solvent systems for essential oil applications. By providing insights into optimizing NADES compositions for specific solubility and release profiles, the study identifies both challenges and opportunities, paving the way for further advancements in green chemistry.

Introduction

Advances in sustainable chemistry are driving research towards the development of green solvents. Traditional organic solvents, extensively used in various life science fields, are often toxic, volatile, and environmentally hazardous.[1] Initial studies indicated that ionic liquids (ILs) could be greener alternatives to these solvents. However, some ILs have exhibited issues such as non-biodegradability, high toxicity, resulting in them being scarcely sustainable.[2]

In this context, deep eutectic solvents (DESs) have emerged as a promising class of non-aqueous mixtures,[3] that are widely regarded as a viable replacement for ILs. These solvents are mixtures of hydrogen bond donors (HBD) such as urea, and a hydrogen bond acceptor (HBA) like choline chloride. Introduced in 2003, DESs share similar physicochemical properties with ILs as they can be easily prepared by simply mixing the HBA and HBD components. This feature facilitates atom-efficient production, potentially making DESs more cost-effective for future applications in the chemical industry.[4,5]

In 2011, natural products were acknowledged to be a source of components of deep eutectic solvents (DES) and ionic liquids (ILs) when hypothesizing the occurrence of DES in nature, leading to the introduction of natural deep eutectic solvents (NADES).[6] These solvents reproduce essential intracellular mixtures that have certain physiological roles,[7] in which the term "deep" stresses the significant decrease of the melting point of pure components resulting from the eutectic mixture.[8] Natural deep eutectic mixtures are formed by combining two or more natural primary metabolites such as sugars, carboxylic acids, amino acids, and bases, that allow

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hydrogen bonding interactions between the constituent molecules.[9]

It has been suggested that the NADES present in cells or tissues could act as media for enzymatic reactions and enhance the solubilization of water-insoluble compounds.[10] This feature suggests a potential application as new extraction solvents. They have proved to increase the yield in the extraction of metabolites like anthocyanins,[11,12] polyphenols,[13] flavonoids,[14] phenolic compounds,[15] and astaxanthin.[16] They have also been used successfully as biotin-conjugated solid–liquid nanocarriers (SLN) for the encapsulation of anticancer drugs,[17] and to solubilize a wide range of valuable compounds in foods,[9,13] cosmetics, and medicines; their role as adjuvants in therapeutic deep eutectic systems used to treat tuberculosis (TB) and colorectal cancer (CRC) has proved to be promising.[18] These applications are evidence of their versatility and potential impact in diverse areas as a means to enhance the efficacy of pharmaceuticals and nutraceuticals.[19]

Most previous studies have focused on the binary nature of NADES, which typically consist of two components that can be customized to create solvents tailored for specific applications.[4,20] However, many reported NADES include at least one ionic component with short alkyl chains (*e.g.*, acids, amines, alcohols, or amino acids), primarily governed by hydrogen bonding interactions. This composition conveys a hydrophilic character to the mixture. One of the advantages of using NADES to extract bioactive compounds from natural sources is their high polarity or hydrophilicity, which facilitates strong hydrogen bond interactions with the target compounds due to the abundance of hydroxyl groups in NADES. This characteristic makes NADES suitable for dissolving relatively polar or hydrophilic solutes but presumably less effective for non-polar or hydrophobic compounds, such as essential oils.

Essential oils are volatile organic substances primarily composed of terpenoids and nonpolar phenylpropanoids, which exhibit low water solubility and high lipophilicity.[21] Consequently, conventional hydrophilic NADES are unlikely to be effective in dissolving essential oils from plants or food by-products, as they are unable to form strong interactions with the molecules of the oil components. Additionally, the presence of water in NADES can negatively impact the quality and stability of essential oils, potentially promoting hydrolysis, oxidation, or microbial growth. Therefore, hydrophilic NADES are not the optimal solvent choice for essential oil applications unless they were modified with the addition of other components or techniques to enhance their lipophilicity or compatibility with the oil phase.[22] Furthermore, despite the extensive applications of NADES across various fields, the primary focus of most researchers is the optimization of the process design, enhancing product yield, or investigating their potential to replace conventional solvents, rather than exploring new applications of NADES themselves.[23]

In this study, natural deep eutectic solvents (NADES) were applied to obtain, stabilize, and diffuse the aroma volatile constituents of *Lavandula angustifolia* Mill. (Lamiaceae) essential oil (LEO) with the aim of developing a green procedure. Headspace gas chromatography/mass spectrometry (HS-GC/MS) has been used to characterize different varieties of LEO previously.[24,25] *Lavandula angustifolia* essential oil was selected due to its complex and diverse chemical composition, which includes key aromatic compounds such as linalool, linalyl acetate, and eucalyptol.[26] Other lipophilic compounds such as terpenoids, phenylpropanoids, and short-chain aliphatic hydrocarbon derivatives also contribute to its quality. This complexity makes LEO an ideal candidate to evaluate the effectiveness of NADES in solubilizing and controlling the release of various hydrophobic VOCs. Additionally, LEO is one of the most commercially important essential oils, widely used in aromatherapy, perfumery, cosmetics, and pharmacy, exhibiting various pharmacological effects such as improving insomnia, anxiety, and pain, reducing inflammation and allergy, inhibiting microbial growth, repelling insects, and killing mites.[27] According to recent market reports, the global lavender oil market was valued at approximately USD 138.2 million in 2024 and is projected to reach USD 267.2 million by 2034, growing at a CAGR of around 6.8%.[28] The high demand and economic significance of LEO underscore the need for eco-friendly and efficient delivery systems. While LEO serves as an excellent model, the methodologies developed herein are transferable to other essential oils, providing a proof of concept for broader applications.

This study aimed to explore the potential of NADES as a diffusion matrix for LEO and to compare the performance of two different types of NADES: hydrophilic and hydrophobic. Hydrophilic NADES were modified with an emulsifier to enhance their compatibility with the LEO phase, resulting in the formation of an oil/NADES system. Research on emulsions has grown significantly due to their versatile applications in food, cosmetics, polymers, agriculture, and pharmaceuticals. However, emulsions are inherently unstable systems prone to coalescence, sedimentation, and Ostwald ripening.[29] Emulsifiers such as surfactants, polymers, and solid particles are commonly incorporated to increase their stability. Surfactants play a crucial role by reducing interfacial tension at the oil/water interface. Emulsions stabilized by surfactants are well-established, with the Hydrophilic–Lipophilic Balance (HLB) being a key determinant of their type and stability. In many cases, mixtures of surfactants are used since they outperform individual ones rather than individual surfactants, highlighting the importance of investigating their interactions.

We provide evidence of well-defined mixtures formed by non-ionic surfactants (ethoxylated and non-ethoxylated esters of sorbitan), known as Span and Tween, with a NADES system. This discovery suggests new possibilities for the use of hydrophilic NADES as a dilution matrix for essential oils, particularly when combined with these non-ionic surfactants.

As another approach, the performance of hydrophobic NADES was evaluated. These NADES are composed of natural and non-toxic constituents, capable of dissolving oils without the need for surfactants, thanks to mechanisms involving entropy, hydrogen bonding, and dispersion forces. Terpenes, a diverse group of plant-derived compounds, are generally

known to be biodegradable and non-toxic, offering a sustainable alternative for various applications.[30] These include medical applications as antimicrobials, or have potential roles in cancer treatment and cholesterol management.[31] Moreover, owing to their aromatic attributes, terpenes are used in a wide range of products spanning from specialty chemicals to solvents, providing an eco-friendly substitute for petrochemical derivatives. Recent research has demonstrated that terpenes can form efficient hydrophobic eutectic solvents,[32] proficient in extracting a wide range of substances including metals, biomolecules, and phytochemicals.[20] This highlights their potential in green chemistry. However, their use in the area of hydrophobic NADES remains largely unexplored, requiring deeper characterization of terpene-based systems if they are to provide a valid alternative.

In this study, we extensively evaluated the release profiles of LEO constituents within various NADES formulations by analyzing the headspace composition over a period of four days at room temperature. Our analysis extended to the assessment of the integrity and stability of LEO when integrated in different NADES matrices, with a specific emphasis on elucidating how these solvents influence aroma release, without extensive exploration of their physical or chemical properties. By exploring how variations in the composition of surfactants and NADES affect the diffusion dynamics of essential oil molecules, we aim to enhance the release of essential oil aromas, contributing to green chemistry and sustainable practices.

Results and discussion

Selection of hydrophilic and hydrophobic natural deep eutectic solvents for Lavender oil

Two types of natural deep eutectic solvents (NADES) hydrophilic and hydrophobic were prepared to dissolve LEO. Numerous NADES have been reported, categorized as base–sugar, base–acid, acid–polyalcohol, and sugar–sugar groups, representing the primary families known for their efficiency in dissolving chemicals.[19] Among these, the following hydrophilic NADES: BE–SU (2 : 1); (N1), BE–MA (1 : 1); (N2), CA–XO (1 : 1) (N3), LA–PO (1 : 1) (N4), and SU–FR (1 : 1) (N5), were selected and evaluated using varying molar ratios of the components.

When hydrophilic NADES was mixed with LEO, a separation of phases was observed. All types of this type of NADES (N1–N5) exhibited this separation, indicating a lack of compatibility between the hydrophilic NADES and the hydrophobic LEO. This phase separation suggests that while hydrophilic NADES are able to dissolve a range of polar to less polar compounds, their application in dissolving hydrophobic essential oils such as LEO is limited. Consequently, it was necessary to explore alternative NADES formulations or modify the existing ones by adding other components that could enhance their hydrophobicity or compatibility with the oil phase. Among the possible options, one was the incorporation of emulsifiers to improve the interaction between the NADES and the oil phase.

In view of the failure of hydrophilic NADES to solubilize the LEO, hydrophobic NADES were then tested. Hydrophobic natural products have been explored for their potential in forming NADES, including plant volatiles such as menthol and thymol, which can act as hydrogen bond acceptors (HBA), and fatty acids like capric and lauric acids, which are hydrogen bond donors (HBD).[33]

This design is based on the principle of "like dissolves like", with the expectation that being lipophilic themselves, they should thus allow the efficient extraction or solubilization of non-polar/lipophilic compounds, such as essential oils, without the need for surfactants. The trials with all hydrophobic NADES (N6–N9) showed complete miscibility with LEO, demonstrating their effectiveness. Additionally, compared to hydrophilic NADES, the hydrophobic variants exhibit significantly lower viscosity due to the absence of coulombic charge interactions.

However, among these hydrophobic NADES, no further work was done with the terpene-based ones, since the results of headspace analysis revealed a significant presence of terpenes, indicating their substantial volatilization from the solutions (Fig. S1†). Such terpene diffusion could alter the intended properties of the final product, especially when LEO is added to the NADES. The presence of menthol or thymol, in particular, could significantly modify the final aroma of the product interfering with the desired LEO aromatic features. Therefore, the research with hydrophobic NADES was focused on fatty acid containing NADES like DO–DE (1 : 3, molar ratio) (N9) that preserved the intended chemical and sensory properties of the final product.

Revisiting hydrophilic natural deep eutectic solvents: cost-effectiveness and emulsifier potential

Despite the failure of hydrophilic NADES to solubilize LEO, their low cost and reduced toxicity were attractive enough to justify further efforts. Thus, hydrophilic NADES were tested but with the incorporation of emulsifiers to enhance their solubilizing capacity. This approach aimed to take advantage of the benefits of hydrophilic NADES while circumventing their limitations through the addition of suitable emulsifiers. The key parameter that affects the stability of hydrophilic emulsions is the hydrophilic–lipophilic balance (HLB). This parameter reflects the affinity of emulsifiers with oil or water; the higher the HLB value, the stronger the affinity with water. Typically, emulsifiers with higher HLB values are more suitable for oil-in-water (O/W) emulsions. Tween 20 is a hydrophilic non-ionic surfactant with an HLB value of 16.7 and a critical micelle concentration (CMC) of 0.05 mM.[34] According to Bancroft's rule, which states that the preferred external phase of an emulsion is the one in which the surfactant is more soluble,[35,36] O/W emulsions are favored by hydrophilic surfactants such as Tween 20. Conversely, Span 80, which is relatively lipophilic, is more suitable for stabilizing water-in-oil (W/O) emulsions. For the study, emulsions stabilized by a combination of hydrophilic Tween 20 and lipophilic Span 80 were prepared at room temperature and evaluated using the bottle

test. This test differentiates between O/W and W/O emulsions based on their tendency to cream or sediment, respectively.

A previous study described the preparation of an emulsion using a hydrophilic eutectic solvent (ES), with cyclohexane droplets stabilized by sodium dodecyl sulfate (SDS).[37] In that study, the hydrophilic ES, composed of urea and choline chloride, served as the continuous phase. However, the same study found that all three non-ionic surfactants tested (Triton X-100, Brij-35, and Tween-80) were virtually insoluble in the ES, thereby limiting their further investigation.

Our initial decision on the optimum emulsifier was determined by the results of screening with Span 80 and Tween 20 emulsifiers at a concentration of 20% in LEO (v/v), thus increasing the HLB from 4.3 to 16.7. Formulations (N3 with CA-XO) (Table S1†), and (N5 with SU-FR) (Table S2†), resulted in clear and stable emulsions with a 20% volume of Span 80-Tween 20 obtaining an HLB of 16.7, while (N1 with BE-SU) (Table S3†), achieved stability with a 40% Span-Tween ratio reaching an HLB of 14. Increasing the surfactant concentration has been demonstrated to reduce ripening rates and improve emulsion stability.[38]

Among the initially screened five NADES families in our study, only N1, N3, and N5 showed a potential for stable emulsification with LEO. In contrast, N2 and N4 did not exhibit stability or clear emulsion formation and were thus excluded from further evaluation (Fig. S2†). It is important to note that superficial and interfacial tension measurements could help corroborate these results, although they were not taken into consideration in this study.

The clear emulsions (N1, N3, and N5) were stored at room temperature in sealed glass tubes and showed no sedimentation or creaming for up to seven days during the release study.

Multivariate data analysis to overview chemical profile of released VOCs from NADES

After individual analysis of VOCs from NADES, the overall chemical profile of released chemicals (25 volatiles) was analyzed by principal component analysis (PCA), orthogonal partial least square (OPLS), and soft independent modeling of class analogy (SIMCA).

Firstly, to overview grouping of control and NADES samples (N1, N3, N5, and N9), an unsupervised multivariate data analysis, PCA, was applied to the headspace GC-MS data composed of 25 volatiles. As shown in the score plot of PC1 (71.5% variation) and PC2 (9.4% variation) (Fig. 1A), NADES samples were clearly distinguished from control ones. However, PCA is a data reduction method, and consequently, it is difficult to draw any conclusion from the separation. Also, the score plot can be built only based on 2–3 PCs. To overcome the limitation of PCA, SIMCA modeling was applied to the same set of data. Each class of samples (control, N1, N3, N5, and N9) was modeled separately by disjoin PC models. Based on the residual variation of each class, the distance to the model (DModXPs) was calculated based on five PCs (Fig. 1B) and N1, N3, and N5 showed significantly different released volatiles compared to that of control. The observation that for N1, N3,

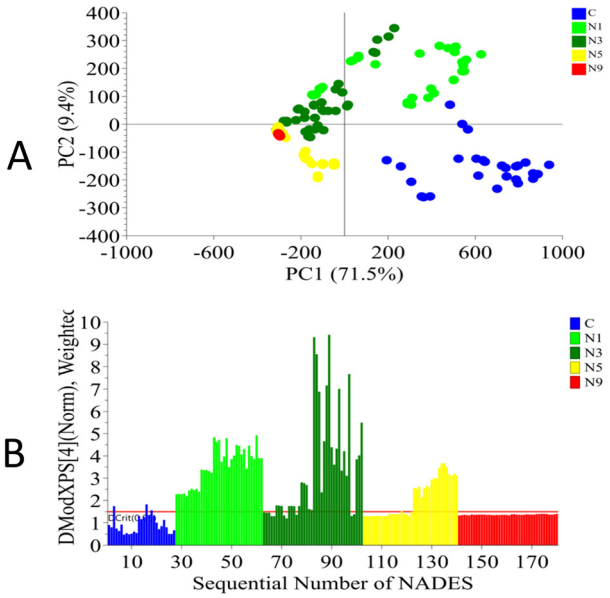

Fig. 1 Score plot (A) of principal component analysis (PCA) using PC1 and PC2, and the distance to the model (DModXPS) (B) obtained from soft independent model of class analogy (SIMCA) analysis based on headspace GC-MS data. Control (C), BE–SU (2 : 1, molar ratio) (N1), CA–XO (1 : 1, molar ratio) (N3), SU–FR (1 : 1, molar ratio) (N5), and DO–DE (1 : 3, molar ratio) (N9).

and N5, roughly half of the samples fall below the critical line while the other half exceed it, can be attributed to the effect of incubation time. As the incubation time increases (from T0 to T96), the samples tend to show more pronounced deviations from the model, reflected in the higher DModXPS values. This suggests that the VOCs' release dynamics and interaction with the NADES matrix evolve over time, leading to greater differentiation against the critical line. Interestingly, PCA results for N9 differed from those obtained with the SIMCA model. In PCA, N9 samples are close to N3 samples but in the SIMCA model, the difference is marginal. This may be explained by the number of PCs employed in each method: 2 PCs (PC1 and PC2) of PCA and 5 PCs of the SIMCA model.

This graphical analysis effectively underscores the variations and similarities within and between different sample classes. The control and N9 samples show consistent profiles with lower deviations, whereas N1, N3, and N5 samples exhibit higher variability, with many samples exceeding the critical limit. This variability is likely influenced by the incubation time, where prolonged incubation results in increased differentiation from the initial class model.

Importantly, this analysis provides a proof of concept that NADES can be used as effective diffusion matrices for the slow release of VOCs. The ability of NADES formulations to modulate VOC release profiles over time highlights their potential as versatile, eco-friendly solvent systems for sustained fragrance delivery and other applications requiring controlled release of volatile compounds. After overall comparison of released volatiles of each NADES sample, the volatiles responsible for the

group separation were investigated. For this, OPLS was carried out using two classes (class 1: control and class 2: N1, N3, N5, and N9 samples). As shown in Fig. 2A, two classes are undoubtedly separated and validated by the permutation test and high $Q2$ value (0.943). The S-plot was used to identify the discriminating metabolites, and β-phellandrene (5), hexylacetate (12), *o*-cymene (13), eucalyptol (15), (+)-camphor (21), and trifluoroacetyl-lavandulol (24) were found to be positive markers of NADES samples. Individual inspection revealed that N1 NADES exhibited higher levels of the compounds (5), (12), (13), (21), and (24). Other NADES formulations displayed different specific markers; for example, certain NADES showed elevated levels of (13), (15), and (21), with N3 NADES specifically characterized by increased levels of (5) and (13). Conversely, some compounds acted as negative markers which is clearly illustrated in the S-plot (Fig. 2B).

Effect of different hydrophilic–lipophilic balance on the VOCs Release monitored by headspace GC-MS

The release and retention of volatile organic compounds (VOCs) from natural deep eutectic solvents (NADES) are crucial for their application in various fields. To develop NADES as a matrix for VOC diffusion, the next step was to profile the released volatiles using headspace GC-MS. The experiment involved sampling 50 μL of NADES-LEO into 20 mL headspace vials, which were maintained at 25 °C, securely sealed, and incubated for durations of 0.0, 0.5, 1.0, 4.0, 24.0, 48.0, 72.0, and 96.0 hours.

The retention capacity of each NADES (N1, N3, and N5) varied according to the emulsifier concentration, which significantly influenced the total number of VOCs released over the four-day incubation period. Chromatographic peaks in the extracted-ion chromatogram (EIC) represented the VOCs, and VOCs components were quantified using the peak area normalization method, where 0.8% of the maximum peak area was selected as the minimum integral peak. Qualitative analysis was conducted using class percentage, and VOCs detected in each NADES are detailed in Tables S4–S8.† In total, 25 VOCs were identified based on their mass spectra and retention indices (RI), consistently with previously cited studies.[24,25]

The composition of headspace samples can vary significantly from the liquid phase due to factors such as temperature, vapor pressure, sample volume, and headspace volume (Fig. S3†). Notably, NADES samples N1, N3, and N5 shared 16 VOCs with the control sample, whereas N9 had only 4 compounds in common, highlighting the effect of the NADES composition on VOC release.

The control sample (C) exhibited a relatively stable VOCs release pattern, with cumulative percentages starting at 9.05%, peaking at 15.75% at four hours, and returning to 9.09% by the fourth day. In contrast, the other samples showed more pronounced fluctuations: N1 increased from 3.8% to 19.1% by the second day before stabilizing at 13.9%; N3 increased from 1.74% to a maximum of 24.21% by the first day, then decreased to 9.92% and N5 rose from 0.1% to a peak of 30.9% on the third day, subsequently falling to 25.0% (Fig. 3).

Within the control sample, specific compounds such as α-pinene and linalool increased consistently over time, with α-pinene rising from an initial 10.51% to 14.14%, and linalool

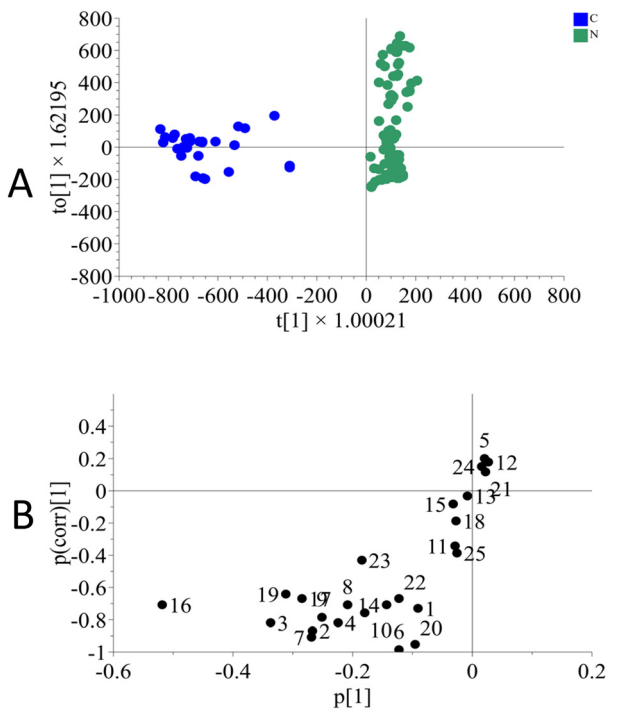

Fig. 2 Score plot (A) and S-plot (B) of orthogonal partial least square-discriminant analysis (OPLS-DA) using t1 and t01 using two classes (C: control and N: NADES samples). 1: α-thujene, 2: α-phellandrene, 3: α-pinene, 4: camphene, 5: β-phellandrene, 6: β-thujene, 7: β-pinene, 8: 3-octanone, 9: β-myrcene, 10: 3-carene, 11: β-cymene, 12: hexylacetate, 13: *o*-cymene, 14: D-limonene, 15: eucalyptol, 16: *trans*-β-ocimene, 17: β-ocimene, 18: γ-terpinene, 19: linalool, 20: *trans*-2-pinanol, 21: (+)-camphor, 22: (−)-4-terpineol, 23: linalyl acetate, 24: trifluoroacetyl-lavandulol, 25: (*Z*,*Z*)-α-farnesene.

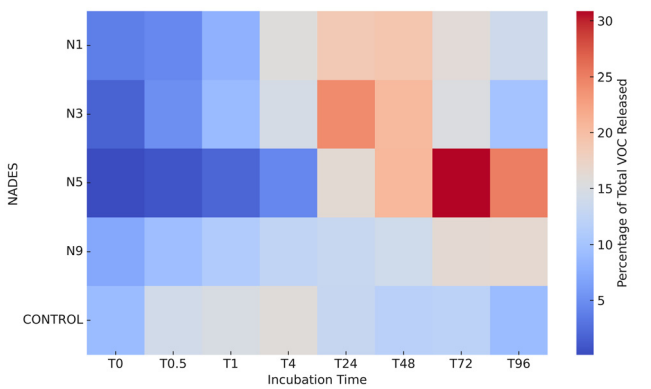

Fig. 3 Qualitative analysis representing the cumulative percentage of total VOCs released from the following: control (C), BE–SU (2 : 1 molar ratio) (N1), CA–XO (1 : 1 molar ratio) (N3), SU–FR (1 : 1 molar ratio) (N5), and DO–DE (1 : 3 molar ratio) (N9) throughout the incubation period.

from 9.83% to 15.23% by the fourth day. Conversely, *trans*-β-ocimene and linalyl acetate decreased substantially after their initial increase, with *trans*-β-ocimene falling from 29.74% to 14.97%, and linalyl acetate from 8.82% to 4.60% (Fig. 4) (Table S4†).

For N1, α-pinene initially started at 0.6% and exhibited a significant and steady increase to 8.9% by the fourth day, indicating a delayed but steady volatilization profile. Linalool, starting as the most abundant compound at 34.0%, showed a notable decrease to 10.9% by the fourth day. Conversely, *trans*-β-ocimene displayed a dynamic trajectory, beginning at 13.0% and peaking impressively at 28.6% by the four-hour mark, before gradually decreasing to 21.3% by the end of the study (Fig. 5) (Table S5†).

In N3, α-pinene had no initial detectable presence but rose to 8.1% by the fourth day. Eucalyptol started at 7.5%, peaked at 27.7%, and declined to 12% by the end of the period. Linalool, initially high at 46.9%, fell to 7.0%, and linalyl acetate, starting at 45.5%, significantly decreased to 2.7%. *trans*-β-ocimene in N3 exhibited a rapid increase to 30.1% within four hours, maintaining high levels of around 29.2% on the first day before a modest decline to 23.9% (Fig. 6) (Table S6†).

In N5, the total detected VOCs escalated dramatically from 0.1% at T0 to 30.9% by the third day, slightly falling to 25.0% by the fourth day. Among the compounds, α-pinene showed a significant rise from an initial non-detectable level to 24.31% by the fourth day. Camphene also displayed substantial dynamics, starting from zero and peaking at 14.99% at four hours before settling at 11.96%. Similarly, β-myrcene increased from 0% to 20.15% on the first day, then gradually decreased to 12.76%. Eucalyptol began at an unusually high 100% (very likely an anomaly), then dramatically reduced to 16.63% by the first day and further to 6.06% by the fourth day. Linalyl acetate emerged later in the observation period, increasing to 6.40% by the fourth day (Fig. 7) (Table S7†).

On the other hand, the analysis of the hydrophobic NADES-N9 reveals a significantly more limited range of VOCs compared to other samples. Specifically, only four compounds —α-pinene, camphene, eucalyptol, and α-phellandrene—were released, indicating a much narrower chemical profile (Fig. 8) (Table S8†).

The adsorbed layer of surfactants at the oil–NADES interface plays a pivotal role in this process, impacting not only the emulsion's stability but also the release patterns of encapsulated fragrance compounds. Surfactant concentration influ-

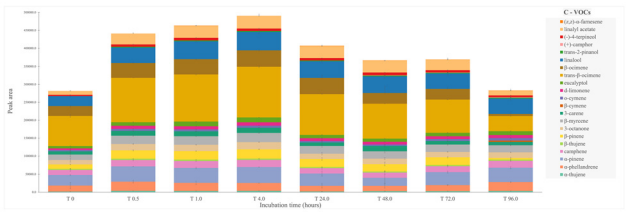

Fig. 4 Peak area distribution of 21 volatile organic compounds (VOCs) measured at incubation times of 0.0, 0.5, 1.0, 4.0, 24.0, 48.0, 72.0, and 96.0 hours for: control (C). The peak area segment for each compound represents the average of 5 repetitions measured at each time slot.

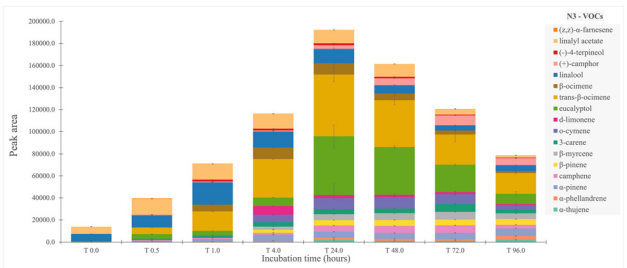

Fig. 6 Peak area distribution of 17 volatile organic compounds (VOCs) measured at incubation times of 0.0, 0.5, 1.0, 4.0, 24.0, 48.0, 72.0, and 96.0 hours for: CA–XO (1 : 1, molar ratio) (N3). The peak area segment for each compound represents the average of 5 repetitions measured at each time slot.

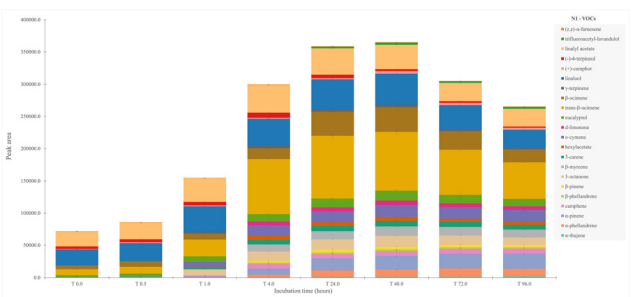

Fig. 5 Peak area distribution of 22 volatile organic compounds (VOCs) measured at incubation times of 0.0, 0.5, 1.0, 4.0, 24.0, 48.0, 72.0, and 96.0 hours for: BE–SU (2 : 1, molar ratio) (N1). The peak area segment for each compound represents the average of 5 repetitions measured at each time slot.

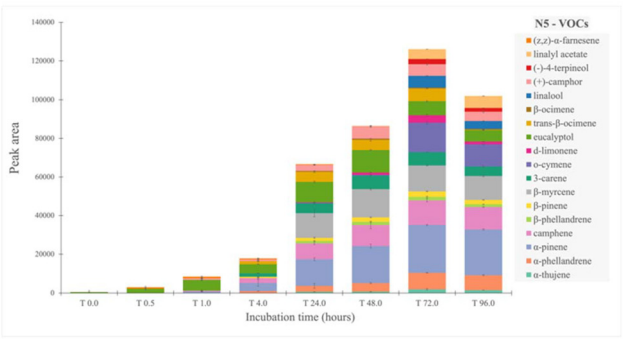

Fig. 7 Peak area distribution of 18 volatile organic compounds (VOCs) measured at incubation times of 0.0, 0.5, 1.0, 4.0, 24.0, 48.0, 72.0, and 96.0 hours for: SU–FR (1 : 1, molar ratio) (N5). The peak area segment for each compound represents the average of 5 repetitions measured at each time slot.

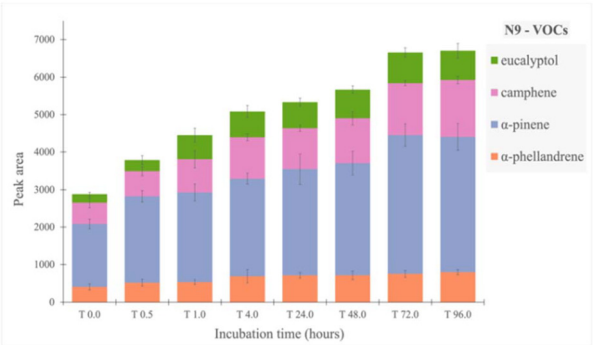

Fig. 8 Peak area distribution of 4 volatile organic compounds (VOCs) measured at incubation times of 0.0, 0.5, 1.0, 4.0, 24.0, 48.0, 72.0, and 96.0 hours for: DO–DE (1 : 3, molar ratio) (N9). The peak area segment for each compound represents the average of 5 repetitions measured at each time slot.

ences the nature of the interface, its interfacial area, and the amount of surfactant adsorbed, which in turn affects fragrance release. Bortnowska and coworkers highlight that emulsions formulated with a higher weight percentage of emulsifiers exhibit greater retention of aroma compounds, irrespective of compound hydrophobicity.[39] This suggests that the optimal coverage of the oil droplet surfaces is crucial in reducing the resistance to mass transfer of aroma compounds from the oil phase to the water phase, facilitating their release.

Our results show that LEO mixed with N1, N3, and N5 follow distinct behaviors as generally described. Variations in surfactant concentrations and the HLB adjustments among these NADES directly correlate with the different release dynamics and intensities of VOCs, as shown in Fig. 9. Notably, the control sample (LEO without NADES) released 21 VOCs. For example, N1, which required a higher emulsifier with Span 80–Tween 20 concentration to achieve emulsion stability (40%), facilitated a broader spectrum of VOCs release (22 volatiles). Conversely, N3 and N5, with lower Span 80–Tween 20 concentrations (20%), exhibit a selective release mechanism, releasing fewer compounds than N1, with 17 and 18 compounds, respectively (Fig. 9).

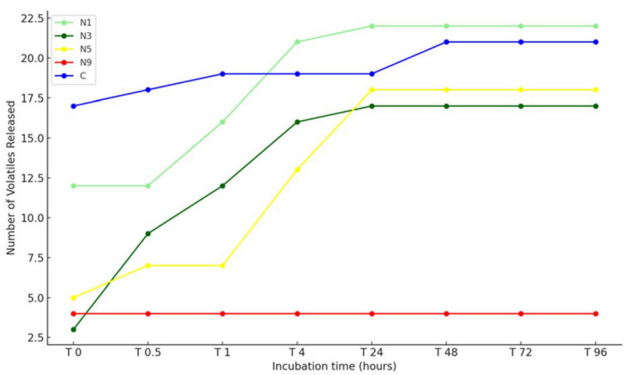

Fig. 9 Analysis of the total number of VOCs released over the complete incubation period for the following conditions: control (C), BE–SU (2 : 1, molar ratio) (N1), CA–XO (1 : 1, molar ratio) (N3), SU–FR (1 : 1, molar ratio) (N5), and DO–DE (1 : 3, molar ratio) (N9).

These results differ from those reported by Dadalı and coworkers, who observed a decrease in aromatic compound release with an increase in surfactant concentration within a model margarine matrix. This phenomenon indicates that surfactants adsorbed at the interface not only enhance the mechanical properties of the interface but sometimes pose additional barriers to the release of aromatic compounds, making their escape into the headspace more challenging.[40] It is assumed that these interactions between surfactant and NADES matrix ingredients which are defined by the physicochemical properties of the HBA and HBD (*e.g.*, polarity and hydrophilicity) of the NADES being used (acid–polyalcohol (N3) > sugar–sugar (N5) > base–sugar (N1)), might modify the release and intensity of LEO's volatiles.

Materials and methods

Chemicals and materials

Lavender essential oil (LEO) and all NADES components, (purity >99%) betaine (BE), sucrose (SU), malic acid (MA), citric acid (CA), xylitol (XO), lactic acid (LA), 1,2-propanediol (PO), fructose (FR), menthol (ME), thymol (TH), octanoic acid (OC), decanoic acid (DE), dodecanoic acid (DO), and non-ionic surfactants, specifically non-ethoxylated sorbitan esters (Span 80) and ethoxylated sorbitan esters (Tween 20) were purchased from Merck (Amsterdam, The Netherlands).

Preparation of natural deep eutectic solvents

All NADES were prepared following a previously published protocol.[41] Five hydrophilic NADES formulations were prepared in the following molar ratio of components: betaine (BE)–sucrose (SU) (2 : 1) (N1); BE–malic acid (MA) (1 : 1) (N2); citric acid (CA)–xylitol (XO) (1 : 1) (N3); lactic acid (LA)–1,2-propanediol (PO) (1 : 1) (N4); SU–fructose (FR) (1 : 1) (N5). Water was added to N1, N2, N3, and N5 at weight ratios of 26.0%, 33.4%, 20.7%, and 30.0%, respectively, to achieve a homogeneous liquid mixture while heating and stirring at 50 °C, followed by cooling to room temperature. For the hydrophobic NADES, different combinations of molar ratios of the terpenes (menthol (ME) or thymol (TH)) with fatty acids (octanoic acid (OC), decanoic acid (DE), or dodecanoic acid (DO)) were applied resulting in the following NADES: ME–TH (3 : 2) (N6); ME–OC (1 : 1) (N7); TH–OC (1 : 3) (N8); DO–DE (1 : 3) (N9). These mixtures were processed in glass vessels and stirred while heating at 50 °C until homogeneity.

Preparation of emulsifiers and natural deep eutectic solvents-infused Lavender essential oil emulsions

When LEO was mixed with NADES (N1–N5), two phases were formed: the oil phase of LEO and the aqueous phases of NADES. To enhance miscibility, emulsifiers were added to the

oil phase to achieve the desired Hydrophilic–Lipophilic Balance (HLB) values.

Preparation of emulsifiers

A selection of emulsifiers was tested to select the optimum one. Among those tested, *i.e.*, 2 types of Span (20 and 80), Tween 20, polyethylene glycol 200 (PEG-200), and Triton X 100, only Span 80 and Tween 20 showed a significant effect on the miscibility (Table S9†). The Hydrophilic–Lipophilic Balance (HLB) values were determined by blending specified amounts of a lipophilic emulsifier (A) (Span 80) with a hydrophilic emulsifier (B) (Tween 20) according to the mentioned equation below (eqn (1)).

$$\%\ (\mathrm{A}) = \frac{100\ \left(\mathrm{X} - \mathrm{HLB}_{(\mathrm{B})}\right)}{\mathrm{HLB}_{(\mathrm{A})} - \mathrm{HLB}_{(\mathrm{B})}} \qquad (1)$$

$$\%\ (\mathrm{B}) = 100 - \%\ (\mathrm{A})$$

where: X is the desired HLB of choice.

Accurate proportions of Span 80 and Tween 20 were combined in 5 mL tinted vials, gently heated in a microwave (Samsung, type M1712N) at 450 W for 15 seconds with 5-second breaks to avoid overheating. Following heating, the mixtures were thoroughly blended using an analog vortex mixer (Scientific Industries, INC. Bohemia, NY, 11716, USA) for 45 seconds. The mixtures were vacuum – degassed for 30 minutes (KnF, LABOPORT, N816.3KN.18, Germany) to remove air, ensuring uniform emulsifier preparation. Details of all HLB emulsifiers used are summarized in Table 1.

Optimization of HLB ratios for hydrophilic and hydrophobic NADES formulations: emulsifier selection and integration process

A screening test was conducted to identify the optimal HLB ratio for hydrophilic NADES formulations (N1–N5). Emulsifiers with HLB values ranging from 4.3 to 16.7 were mixed in 100 μL increments with 500 μL of LEO and 5 mL of NADES (N1–N5) (Tables S1, S2, and S3†). In the case of hydrophobic NADES formulations (N6–N9) 500 μL of LEO were added directly to 5 mL of NADES, as they did not require emulsifiers.

For N1 NADES, 200 μL of the mixture of Span 80–Tween 20 (HLB value of 14) was transferred to 15.5 mL Purex glass culture tubes and 500 μL of LEO were added (resulting in 28.6% (v/v) emulsifier to LEO). For N3 and N5 NADES, the same procedure was performed using 100 μL of a mixture of the emulsifiers with a HLB value of 16.7 and 500 μL of LEO (16.7% of emulsifiers in the total oil phase).

Table 1 Chemical name and HLB value of the emulsifiers investigated

	Emulsifier family	Type	Chemical name	HLB
(A)	Span	80	Sorbitan monooleate	4.3
(B)	Tween	20	Polyoxyethylene (20) sorbitan monolaurate	16.7

Each mixture was vortexed for 30 seconds to achieve a homogeneous oil phase. The different pre-prepared NADES were added incrementally in 5 mL portions, vortexing continually. This step was critical for the integration of the oil phase into the evaluated NADES. The final ratio between the oil phase and the aqueous (NADES) phase was 0.12 : 1 (v/v) (or 12% oil phase to NADES, for N3 and N5), and 0.14 : 1 (v/v) or 14% oil phase to NADES for N1.

To ensure the solubility of the emulsifier/LEO mixture and enhance the fluidity of the matrix, the tubes were subjected to a brief (15-second) microwave heating at 600 W. Intermittent breaks were introduced during the process to prevent overheating and potential degradation of the LEO and to avoid caramelization in sugar-based NADES, thus preserving the integrity of the emulsion formulations.

Headspace gas chromatography coupled managed to mass spectrometry (HS-GC-MS)

To test volatile recovery, 50 μL of NADES-infused LEO were sampled into 20 mL headspace vials, sealed securely and maintained at 25 °C, and for periods of 0, 0.5, 1, 4, 24, 48, 72, and 96 hours. Following the designated intervals, the vials were placed into a 7693 automatic headspace autosampler (Agilent, Wilmington, DE, USA), kept at 25 °C for 30 s and swirled at 500 rpm before their injection into the GC-MS. The GC-MS analysis was performed using a 7890A gas chromatograph, equipped with a 7693 automatic sampler and a 5975C single-quadrupole mass detector (Agilent, Folsom, CA, USA). Compounds were separated on a DB-5 column (30 m × 0.25 mm, 0.25 μm film, J&W Science, Folsom, CA, USA). Helium (99.9% purity) was used as a carrier gas at a flow rate of 1.6 mL min^{-1}. The oven temperature was held at 50 °C for 1 minute, ramped at 3 °C min^{-1} to 150 °C, then at 7 °C min^{-1} to 250 °C and held for 3 min. Five hundred microliter samples were injected in split mode (20 : 1 split) with a split flow of 8 mL min^{-1}. The ionization energy in EI mode was 70 eV, and the mass scan range was 50–550 amu. This procedure was replicated five times for each sample to ensure reliability and accuracy of the results.

Data processing and multivariate data analysis of GC-MS data

The data obtained were analyzed qualitatively using the NIST Chemical Structures library (2014), and the VOC components were quantified using the peak area normalization method, where 0.8% of the maximum peak area was selected as the minimum integral peak. MassHunter Quantitative Analysis (B.07.01, Agilent, Santa Clara, CA, USA) and SIMCA P (16.0.02, Sartorius, Göttingen, Germany) were used for data processing, plotting, and multivariate data analysis (principal component analysis (PCA), orthogonal partial least square-discriminant analysis (OPLS-DA)). To assess the detailed resemblance of released volatiles, a soft independent model of class analogy (SIMCA) analysis was performed as PCA-classes separately in each NADES family. In this model, the distances from each sample to each model are calculated and known as the distance to the model (DModXPS). For all the multivariate data analysis, data were normalized using Pareto scaling.

Conclusions

This study provides a comprehensive evaluation of natural deep eutectic solvents (NADES) as diffusion matrices for lavender essential oil (LEO), used here as a model essential oil to demonstrate the potential of NADES in green chemistry and essential oil applications. Hydrophilic NADES, modified with non-ionic surfactants such as Span and Tween, formed stable emulsions that effectively solubilized and released LEO's volatile organic compounds (VOCs), addressing their inherent hydrophilicity limitations. However, achieving optimal solubilization and controlled release over extended periods remains a challenge. In contrast, hydrophobic NADES demonstrated superior performance in controlled and steady VOC release, making them suitable for applications requiring sustained fragrance delivery. Up to 25 VOCs were identified using headspace gas chromatography-mass spectrometry (HS-GC/MS) and multivariate data analysis (PCA, OPLS-DA), and the significant influence of NADES composition on VOC release dynamics was demonstrated, with specific markers for different formulations. Adjusting the Hydrophilic–Lipophilic Balance (HLB) through the surfactant concentrations significantly impacted the stability and release profiles, enhancing the miscibility of LEO with hydrophilic NADES. This research underscores the versatility of NADES as eco-friendly solvent systems, offering valuable insights into optimizing their composition for specific solubility and release profiles. The methodologies and findings presented here provide a proof of concept that can be extended to other essential oils and hydrophobic compounds, highlighting advantages such as enhanced solubilization, controlled release, and sustainability over conventional formulations. They present a green chemistry alternative with potential applications in the pharmaceutical, cosmetic, and food industries. Future studies should address remaining solubilization challenges and explore additional applications of NADES in the agricultural, environmental, and energy sectors.

Author contributions

Y.H.C. and S.S, conceived and designed the experiment; S.S. performed the experiments, analyzed the data, and drafted the manuscript; Y.H.C., M.C.M., C.Z., and N.R.M. corrected and provided advice to the manuscript; Y.H.C. contributed data mining for the obtained results. All authors have read and agreed to the published version of the manuscript.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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