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Research article

Influence of choline chloride-based NADES on the composition of *Myristica fragrans* Houtt. essential oil

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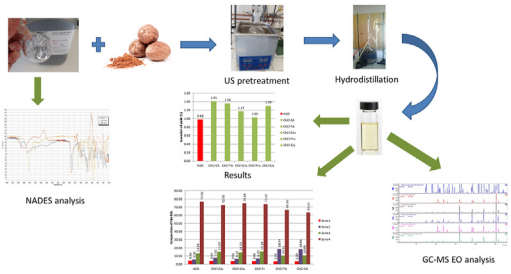
GRAPHICAL ABSTRACT



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ABSTRACT

Natural deep eutectic solvents (NADES) have emerged as green extracting solvents in recent years. In this study, a variety of choline chloride (ChCl)-based natural deep eutectic solvents (NADES) were used as co-solvents for the hydrodistillation of nutmeg with the aim to obtain *M. fragrans* essential oil (EO) in higher yield and with a lower content of toxic phenylpropenoids (e.g. myristicin and safrole). The influence of ChCl-based NADES as additives in the hydrodistillation process was studied. The results showed that NADES additives improved the yield of the extracted essential oil and influenced its composition leading to a decrease in toxic phenylpropenoids. Best results were achieved by using ChCl-CA NADES ultrasound-assisted pretreatment coupled with traditional 2 h Clevenger hydrodistillation that increased the yield of the EO from 0.98% (traditional) to 1.41% and a decrease of the phenylpropenoids amount in the essential oil.

1. Introduction

Myristica fragrans Houtt., Myristicaceae, is a small dioecious tree native to Indonesia that produces an oval fruit containing a kernel covered by a bright red aril called "mace". The dry kernel is known as "nutmeg" and is a common flavouring agent for food worldwide. The two main products of *M. fragrans*, nutmeg and mace, show several biological activities such as antioxidant and antibacterial (Gupta and Rajpurohit, 2011; Nurjanah et al., 2017; Olajide et al., 1999; Takikawa et al., 2002). In traditional medicine, they are used to improve appetite and treat rheumatism, nausea, flatulence, and other gastrointestinal problems (Abourashed and El-Alfy, 2016). Nutmeg essential oil finds a wide variety of applications thanks to its odorous features and cosmetic properties. Nutmeg essential oil is a common ingredient in perfumes, massage products, soaps, skincare, and hair care products; in particular, its spicy scent is popular for male products such as shaving creams and beards oils.

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Most of the biological activity studies have been performed on nutmeg essential oil (EO), in which 4-terpineol and myristicin are particularly abundant (Parthasarathy et al., 2008). Other important components of the EO are safrole and methyl eugenol, which are regarded to be carcinogenic (Al-Malahmeh Amer et al., 2017; Pflaum et al., 2016; SCF, 2002). Furthermore, since EO is used in aromatherapy and other direct applications on the skin, dermal adsorption of toxic components, especially when large parts of the body are interested, should be considered (Lis-Balchin, 2005). For this reason, the preparation of EO with a low percentage of these compounds can be considered as valuable.

Due to EO's economic importance, new extraction technologies have been developed, such as microwave-assisted (Nitthiyah et al., 2017) and ultrasound-assisted extractions (Li et al., 2018; Mejria et al., 2018; Richa et al., 2020) for the preparation of EO. In these last years, Ionic Liquids (IL) and Deep Eutectic Solvents (DES) have emerged as valuable additives for the hydrodistillation of essential oils (Bica et al., 2011; Lanari et al., 2018; Li et al., 2019; Mejria et al., 2018; Syahmina and Usuki, 2020).

Natural deep eutectic solvents (NADES) can be considered a new class of non-toxic, eco-friendly, biodegradable, and cheap solvents (Choi et al., 2011; Dai et al., 2013; Funari et al., 2019; González et al., 2018). They can be easily prepared from common natural cells components (sugars, organic acids, amino acids) by a hydrogen bond formation between a donor (HBD) and an acceptor (HBA) mixed in an appropriate ratio (Liu et al., 2018). Choline chloride (ChCl) is one of the most used HBA with different HBD, such as organic acids (Gontrani et al., 2019), polyols (Sakti et al., 2019), and sugars (Islamčević Razboršek et al., 2020). NADES can be easily prepared by freeze-drying, heating and stirring, and vacuum evaporation methodologies (Choi et al., 2011; Dai et al., 2013). This novel category of solvents finds different applications spanning from biomedical purpose to extraction and chromatographic use (Ivanović et al., 2020; Mustafa et al., 2021; Stanojević et al., 2021; Sutton et al., 2018). In the last few years, the peculiar properties of NADES, i.e. being able to dissolve significantly organic matrices (Nguyen et al., 2020), have made these compounds elective media for extraction processes (Choi and Verpoorte, 2019).

We already reported that Ionic Liquids (ILs) could influence the extraction yield and the composition of *M. fragrans* EO. Under optimized conditions, we observed an increase in the total extraction yield and a reduced amount of the toxic phenylpropanoids (Lanari et al., 2018). Recently, some concerns have arisen about their toxicity and poor degradability, so "greener" alternatives are desirable, and NADES, in our opinion, might be the most appropriate choice.

Despite a large number of researches on the use of NADES as extraction solvents of natural compounds (Cunha and Fernandes, 2018; Fernandez et al., 2018; Liu et al., 2018), as far as we know, little has been reported about the use of NADES in the preparation of essential oils (Stanojević et al., 2021; Xu et al., 2021; Yu et al., 2018; Zhao et al., 2019a).

This work presents the influence of different ChCl-based NADES in an ultrasound-assisted (UA) pretreatment, followed by hydrodistillation, on the yield and composition of nutmeg essential oil. The HBD components chosen as representative examples of the aforementioned classes are fructose, glucose, glycerol, citric acid, and tartaric acid.

2. Material and methods

2.1. Reagents and chemicals

Suppliers for chemical products and solvents were: Alfa Aesar (ThermoFisher Scientific-Alfa Aesar, Kandel, Germany) for choline chloride, fructose, glycerol, citric acid, tartaric acid, and glucose, VWR (VWR International Srl; Milan, Italy) for analytical grade hexane. Milli-Q water purification system from Merck-Millipore (Darmstadt, Germany) was employed to obtain deionized water.

2.2. Plant material

Commercial pulverized dried seeds of *M. fragrans* Houtt. were purchased from Italia Spezie, Formia (LT), Italy. After the opening of the package, the powder was kept in a desiccator ($CaCl_2$).

2.3. Instrumental

Attenuated total reflectance FT-IR spectra were recorded using an FT-IR Shimadzu IR-8000 spectrophotometer. The spectral range collected was 400–4000 cm^{-1} with a spectral resolution of 4 cm^{-1} using 100 scans.

GC-MS analyses were performed using a Varian system GC/MS Saturn 2100 equipped with a VF-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm). The spectrometer operated in electron-impact mode with ionization energy of 70 eV, the scan range was 40–400 amu, the scan interval was 0.5 sec, and the scan speed was 1000 amu sec^{-1}. The injector and MS transfer line temperatures were set at 250 and 280 °C, respectively. Pure helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. The GC programmer used was 60 °C (5 min) to 180 °C (5 min) at a rate of 8 °*C* min^{-1} and to 280 °C (10 min) at 6 °C min^{-1}. Two μL of the diluted samples (*n*-hexane) were injected in split mode 1:10. Identification of components was achieved by using the stored mass spectra libraries NIST and Wiley and literature data and by comparing their retention indices with published data. The relative quantity of the chemical compounds present in each sample was expressed as the percentage based on their peak area in the chromatogram. The percentage values are the mean ± SD (Standard Deviation) of three injections of the sample.

2.4. Preparation of NADES

The solid components of the NADES were dried on $CaCl_2$ under vacuum for 3 h before use, choline chloride was dried at 65 °C under vacuum for 24 h. All the NADES were prepared using the evaporating method according to Dai and coll (Dai et al., 2013). Hydrogen bond acceptor (ChCl) (1 mol) and hydrogen bond donor (glucose, fructose, glycerol, tartaric acid, and citric acid) (1 mol) were separately weighted. A small amount of water was added to each solid, and the two mixtures were stirred at room temperature until the compounds were wholly dissolved and subsequently sonicated at 25 °C for 10 min (Ultrasonic Cleaner, Model TH-10A, Vevor, China). The two solutions were mixed and stirred to ensure homogenization and evaporated at 50 °C with a rotatory evaporator.

The clear, viscous liquids were stored in a desiccator equipped with dry $CaCl_2$, under vacuum, until constant weight.

2.5. NADES characterization

All the prepared NADES are known and characterized (AlOmar et al., 2016; Altamash et al., 2017; Aroso et al., 2017; Koutsoukos et al., 2019; Shafie et al., 2019). The formation of NADES was evaluated by FT-IR technique (See Figures 1S–4S, Supplementary Material).

2.6. Conventional hydrodistillation

A conventional hydrodistillation experiment was performed as a reference for comparing the data obtained by ChCl-NADES extraction. Powdered nutmeg seeds (15 g) were suspended in 225 mL of deionized water (solid-liquid ratio: 1:15). Hydrodistillation was carried out using a Clevenger-type apparatus for 2, 3 or 4 h. Residual water was removed from the distilled oil by the addition of anhydrous Na_2SO_4. Each experiment was triplicated, and the yield is expressed as the mean ± SD. The oil was stored under a nitrogen atmosphere at 4 °C before the GC-MS analysis.

Table 1. Composition of the essential oils of *Myristica fragrans* obtained by conventional hydrodistillation (H_2O) and by using NADES.

Component[a]	CAS#	H_2O (%)[b,c]	ChCl-Gly (%)[b,c]	ChCl-Glu (%)[b,c]	ChCl-Fr (%)[b,c]	ChCl-CA (%)[b,c]	ChCl-TA (%)[b,c]	RI[d]	RI Lit.[e]
2-Octene	111-67-1		tr[f]	tr	tr	tr	tr	815	812
α-Thujene	2867-05-2	0.33 ± 0.09	0.38 ± 0.02	0.34 ± 0.01	0.35 ± 0.05	tr	tr	925	930
α-Pinene	80-56-8	0.68 ± 0.01	0.79 ± 0.15	0.72 ± 0.03	0.78 ± 0.06	0.53 ± 0.15	0.363 ± 0.05	933	937
Camphene	79-92-5	tr	tr			tr	tr	950	953
Sabinene	3387-41-5	0.51 ± 0.02	0.75 ± 0.10	0.60 ± 0.02	0.71 ± 0.10	tr	tr	973	977
β-Pinene	127-91-3	1.06 ± 0.17	1.46 ± 0.16	1.23 ± 0.10	1.34 ± 0.04	0.32 ± 0.16	0.25 ± 0.16	978	981
Myrcene	123-35-3	tr	0.10 ± 0.11	tr		0.11 ± 0.07	0.10 ± 0.08	988	994
α-Phellandrene	99-83-2	0.08 ± 0.06	0.11 ± 0.04	0.15 ± 0.11		1.01 ± 0.04	1.01 ± 0.08	1006	1007
3-Carene	13466-78-9	tr	tr					1009	1013
α-Terpinene	99-86-5	0.60 ± 0.09	0.89 ± 0.06	0.72 ± 0.04	0.83 ± 0.13	6.18 ± 0.06	6.68 ± 0.04	1017	1020
p-Cymene	99-87-6	0.30 ± 0.04	0.41 ± 0.09	0.37 ± 0.08	0.39 ± 0.02	0.65 ± 0.09	0.65 ± 0.07	1025	1025
Limonene	138-86-3	0.22 ± 0.06	0.35 ± 0.14	0.29 ± 0.07	0.31 ± 0.07	0.79 ± 0.14	0.70 ± 0.07	1030	1032
β-Phellandrene	555-10-2	0.30 ± 0.02	0.40 ± 0.05	0.34 ± 0.09	0.40 ± 0.02	0.87 ± 0.10	0.88 ± 0.11	1032	1031[h]
1,8-Cineol	470-82-6	0.12 ± 0.07	0.14 ± 0.02	0.12 ± 0.12	0.12 ± 0.02	0.30 ± 0.02	0.29 ± 0.05	1035	1032
γ-Terpinene	99-85-4	1.02 ± 0.18	1.50 ± 0.09	1.25 ± 0.05	1.41 ± 0.03	4.82 ± 0.09	5.11 ± 0.03	1059	1062
cis-Sabinene hydrate	15537-55-0	0.14 ± 0.03	tr	tr				1074	1076[h]
Terpinolene	586-62-9	0.31 ± 0.04	0.42 ± 0.02	0.35 ± 0.03	0.41 ± 0.14	2.22 ± 0.08	2.17 ± 0.06	1086	1092
p-Cymenene	1195-32-0	tr	tr			0.56 ± 0.07	0.60 ± 0.07	1091	1088
NI[g]						0.17 ± 0.05	0.19 ± 0.03	1096	
Linalool	78-70-6	0.62 ± 0.06	0.66 ± 0.13	0.64 ± 0.04	0.67 ± 0.01	0.10 ± 0.04	0.11 ± 0.08	1100	1101
6-Camphenol	3570-04-5	0.15 ± 0.06	tr					1105	1110[h]
Fenchyl alcohol	1632-73-1	tr				0.12 ± 0.08	0.10 ± 0.10	1124	1119[h]
NI			0.43 ± 0.07	0.42 ± 0.11	0.48 ± 0.04	tr	tr	1127	
1-Terpineol	586-82-3					0.69 ± 0.09	0.71 ± 0.02	1139	1140[h]
trans-*p*-Menth-2-en-1-ol	29803-81-4	0.35 ± 0.01	0.28 ± 0.04	0.29 ± 0.10	0.34 ± 0.03			1146	1144[h]
Camphene hydrate	465-31-6					0.20 ± 0.12	0.18 ± 0.12	1153	1155[h]
NI						0.18 ± 0.05	0.12 ± 0.09	1174	
Borneol	507-70-0	tr	0.10 ± 0.04	tr		0.18 ± 0.01	0.16 ± 0.08	1176	1171[h]
Terpinen-4-ol	562-74-3	9.53 ± 0.31	10.99 ± 0.01	10.57 ± 0.04	11.60 ± 0.01	5.60 ± 0.12	5.58 ± 0.02	1184	1184
p-Cymen-8-ol	1197-01-9	0.11 ± 0.07	0.13 ± 0.12	0.13 ± 0.07	0.15 ± 0.11	tr	tr	1190	1187[h]
α-Terpineol	98-55-5	1.53 ± 0.02	1.70 ± 0.01	1.69 ± 0.05	1.79 ± 0.01	2.41 ± 0.01	1.99 ± 0.07	1198	1193
γ-Terpineol	16721-38-3					0.40 ± 0.09	0.36 ± 0.10	1205	1198
trans-Piperitol	491-04-3		0.17 ± 0.08	0.18 ± 0.06	0.22 ± 0.01			1212	
Citronellol	106-22-9	tr						1230	1228[h]
Geraniol	106-24-1	tr	tr					1252	1253[h]
Bornyl acetate	76-49-3	0.36 ± 0.01	0.40 ± 0.13	0.37 ± 0.10	0.39 ± 0.08	0.41 ± 0.08	0.40 ± 0.01	1287	1285[h]
Anethol	4180-23-8	0.54 ± 0.10	0.62 ± 0.15	0.63 ± 0.06	0.60 ± 0.03	0.54 ± 0.03	0.55 ± 0.06	1289	1289[h]
Safrole	94-59-7	4.52 ± 0.21	4.86 ± 0.11	4.93 ± 0.07	4.86 ± 0.01	4.60 ± 0.13	4.70 ± 0.04	1293	1290
Sabinyl acetate	3536-54-7	tr	0.17 ± 0.13	0.21 ± 0.06				1297	1295[h]
Carvacrol	499-75-2	tr	0.11 ± 0.04	0.13 ± 0.02	0.12 ± 0.01	0.11 ± 0.10	0.071 ± 0.11	1300	1298[h]
4-Pentyl anisole	20056-58-0	tr	0.14 ± 0.06	0.12 ± 0.01	0.13 ± 0.11	3.31 ± 0.06	0.16 ± 0.09	1304	
NI						0.14 ± 0.09	0.14 ± 0.11	1329	
δ-Elemene	20307-84-0	0.85 ± 0.03	1.01 ± 0.03	1.09 ± 0.13	1.12 ± 0.10	0.56 ± 0.13	0.57 ± 0.07	1348	1344
Eugenol	97-53-0	0.81 ± 0.05	0.61 ± 0.16	0.65 ± 0.02	0.63 ± 0.02	0.65 ± 0.15	0.76 ± 0.10	1354	1363
α-Copaene	3856-25-5	1.14 ± 0.04	1.41 ± 0.14	1.37 ± 0.01	1.22 ± 0.05	1.27 ± 0.11	1.26 ± 0.08	1378	1400
Methyl eugenol	93-15-2	4.98 ± 0.03	5.49 ± 0.02	5.73 ± 0.13	5.73 ± 0.06	4.77 ± 0.13	4.83 ± 0.07	1401	1407
Carvone hydrate	60593-11-5	0.21 ± 0.01	0.22 ± 0.10	0.20 ± 0.09		0.17 ± 0.04	0.21 ± 0.03	1421	1424[h]
β-Caryophyllene	87-44-5	0.25 ± 0.03	0.41 ± 0.14	0.43 ± 0.02	0.44 ± 0.08	0.41 ± 0.06	0.38 ± 0.07	1423	1431
α-Bergamotene	17699-05-7	0.17 ± 0.06	0.25 ± 0.06	0.27 ± 0.10	0.25 ± 0.04	0.25 ± 0.01	0.26 ± 0.05	1432	1440
trans-Isoeugenol	5932-68-3	0.91 ± 0.16	0.79 ± 0.13	0.93 ± 0.06	0.76 ± 0.01	1.32 ± 0.16	1.29 ± 0.06	1448	1454
Germacrene-D	23986-74-5	0.10 ± 0.01	0.13 ± 0.07	0.13 ± 0.11	0.12 ± 0.02			1480	1481
Methyl isoeugenol	93-16-3		1.38 ± 0.04	1.62 ± 0.06	1.36 ± 0.16	0.99 ± 0.14	1.29 ± 0.03	1491	1491
α-Farnesene			tr				tr	1496	1537[h]
γ-Bisabolene	13062-00-5	1.60 ± 0.04	0.26 ± 0.01	0.03 ± 0.10	0.23 ± 0.02	0.24 ± 0.08	0.24 ± 0.02	1507	1512[h]
δ-Cadinene	483-76-1	0.19 ± 0.05	0.45 ± 0.06	0.23 ± 0.07		0.57 ± 0.02	0.54 ± 0.07	1518	1519[h]
Myristicin	607-91-0	61.36 ± 0.15	55.65 ± 0.13	56.88 ± 0.09	56.43 ± 0.02	48.01 ± 0.13	50.10 ± 0.09	1523	1521
Elemicin	487-11-6	3.26 ± 0.12	3.11 ± 0.05	3.27 ± 0.12	3.25 ± 0.02	2.60 ± 0.07	2.84 ± 0.07	1541	1560
Methoxy eugenol	6627-88-9	0.19 ± 0.06				0.12 ± 0.08	0.17 ± 0.08	1598	1610

(*continued on next page*)

Table 1 (*continued*)

Component[a]	CAS#	H_2O (%)[b,c]	ChCl-Gly (%)[b,c]	ChCl-Glu (%)[b,c]	ChCl-Fr (%)[b,c]	ChCl-CA (%)[b,c]	ChCl-TA (%)[b,c]	RI[d]	RI Lit.[e]
Total (%)		**99.35**	**99.66**	**99.64**	**99.96**	**99.12**	**99.47**		
Monoterpenes		**5.36**	**7.55**	**6.37**	**6.93**	**18.54**	**18.06**		
Oxygenated monoterpenoids		**13.14**	**15.07**	**14.53**	**15.39**	**10.11**	**10.69**		
Sesquiterpene s		**4.30**	**3.97**	**3.56**	**3.41**	**3.25**	**3.30**		
Phenylpropanoid		**76.56**	**72.50**	**74.64**	**73.62**	**66.55**	**63.61**		
Others			**0.57**	**0.54**	**0.60**	**0.67**	**3.80**		

[a] Identification: by comparing the mass spectrum with those of the computer NIST and WILEY libraries (99% matching).
[b] Percentage obtained by FID peak-area normalization. Values are presented as the mean of three extractions ±SD (Standard Deviation).
[c] Components are listed in order of their elution from a VF-5MS column.
[d] RI: Linear retention indices were determined relative to the retention times on VF-5MS column of homologous series of C8–C20 alkanes using the Retention Index Calculator reported by Lucero and coll. (Lucero et al., 2009).
[e] RI were taken from Zhao and coll. (Zhao et al., 2019a).
[f] tr, percentage below 0.1%.
[g] NIST Chemistry Webbook (NIST, 2018).

2.7. Ultrasound-assisted (UA) NADES pretreatment

Nutmeg powder (15 g) was treated with the NADES (75 g) composed of ChCl-HBD with 40% water. The liquid-solid ratio was 1:5 (w/w). The resulting suspension was irradiated in a US apparatus at 50 °C for 30 min.

2.8. Distillation of volatile compounds with NADEs as additives

After ultrasound-assisted (UA) pretreatment, 150 mL of deionized water were added, and the mixture was distilled with the same Clevenger-type apparatus used for the conventional hydrodistillation (2 h). The obtained essential oil was dried over Na_2SO_4 and stored under a nitrogen atmosphere at 4 °C before GC-MS analysis. The yield of the essential oils is expressed as the mean of three experiments ±SD.

2.9. Determination of the EO's composition

All the essential oil components were separated by using a GC-mass spectrometer, and they were identified by comparison of mass spectra from the NIST and Wiley Mass Spectral Databases installed in the instrument and by the comparison of their retention indexes (RI), calculated using the Retention Index Calculator reported by Lucero and coll (Lucero et al., 2009), relative to retention times on the VF-5MS column of a homologous series of C8–C20 alkanes (Sigma Chemical Co. (St. Louis, MO, USA)) with those reported in the literature (Zhao et al., 2019a) and in NIST WebBook (NIST, 2018) or on the basis of commercial standards, when available.

3. Results and discussion

NADES's ability to dissolve plant cells membranes and walls and increase the yield of extraction is well documented (Durand et al., 2020; Fu et al., 2021; Zainal-Abidin et al., 2017). In this work, we studied different HBDs to evaluate the influence of the choline chloride-based NADES on the extraction yield and the composition of *Myristica fragrans* EO.

3.1. Influence of the distillation time

Before studying the influence of NADES on the yield of the hydrodistilled EO, a traditional hydrodistillation of *M. fragrans* dried fruits was

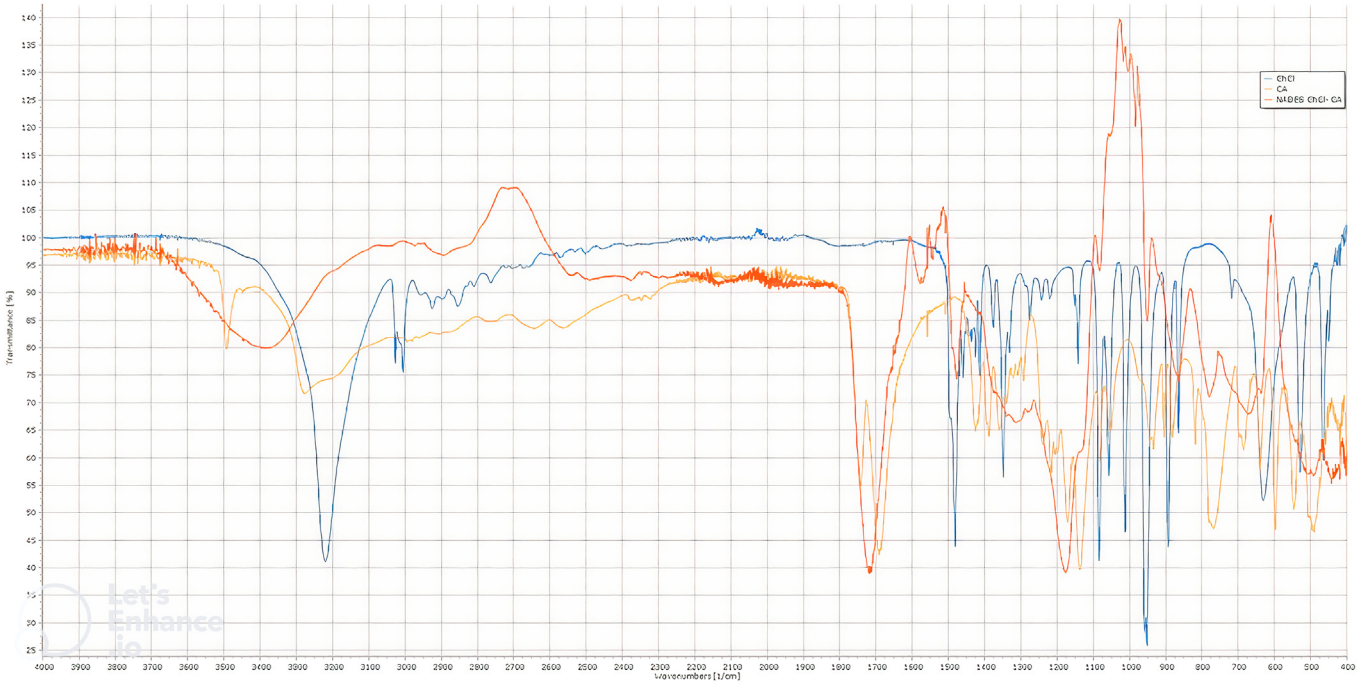

Figure 1. IR spectra of Citric acid (blue), Choline chloride (yellow) and NADES ChCl-CA (red).

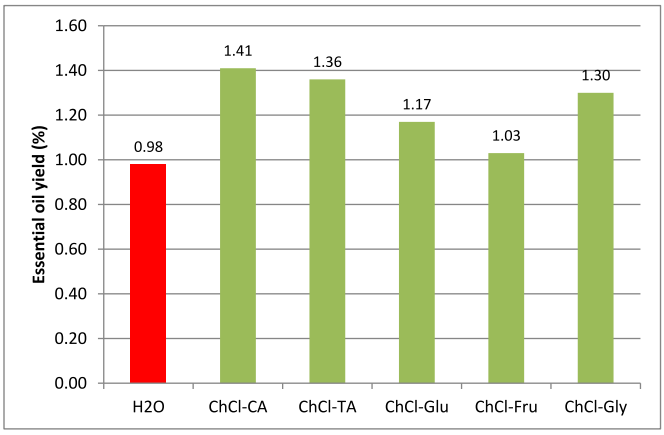

Figure 2. Influence of different NADES on the EO's extraction yield.

performed using a 1:15 ratio of plant material and water and 2, 3 and 4 h of distillation time. The ratio was chosen taking in account the final volume of the NADES solution in future experiments.

We obtained the EO in 0.98, 0.90 and 0.87% using this procedure, respectively. As the extraction yield did not improve extending the extraction time, we decided to choose 2 h as the extraction time. The EO obtained with these parameters contained 5.36% of monoterpenes hydrocarbons (MHs), 13.14% of oxygenated monoterpenes (OMs), 4.30% of sesquiterpenes hydrocarbons (SHs), 76.56% of phenylpropenes (PPs) and traces of 4-pentyl anisole (0.08%) (Table 1). These results were taken into account as reference data.

3.2. Different types of NADES and their dilution

For the preparation of choline chloride (ChCl)-based NADES, we considered tartaric acid (TA), citric acid (CA), glucose (Glu), fructose (Fru), and glycerol (Gly) as representative hydrogen bond donors (HBD). Using the evaporation method (Choi et al., 2011; Dai et al., 2013), we prepared binary NADES using a 1:1 ratio between ChCl and HBD.

All the NADES used in this study were already known and characterized, and molecular interaction between NADES components was determined by FT-IT spectroscopy. The obtained spectra are reported in Supplementary Material (Figures 1S–4S). Figure 1 shows the FT-IR spectrum for ChCl-CA as a representative.

It is well known that NADES are highly viscous solvents, and their viscosity could represent a detrimental parameter for the extraction of secondary metabolites. On the other hand, it has been disclosed that a 40% of water (w/w) in the NADES composition represents the maximum amount to still preserve the NADES supramolecular structure while working with an easy-handling solvent (Dai et al., 2015). For this reason, we decided to prepare NADES with 40% of water.

3.3. Pretreatment conditions

We decided to use an ultrasound-assisted (UA) method to ensure a rapid and an efficient pretreatment with NADES. In these last times, microwave-assisted pretreatment has mainly been chosen for the extraction of essential oils with NADES as co-solvents (Stanojević et al., 2021; Xu et al., 2021; Zhao et al., 2019b), while, as far as we know, UA pretreatment was used only for the extraction of essential oil of *Perilla frutescens* leaves (Chen et al., 2022). In our work, we decided to pretreat the suspension of nutmeg fruits and 40% NADES at 50 °C for 30 min.

3.4. Distillation results

The order of experiments was arranged randomly to avoid systematic errors. All the experiments were run in triplicate. The results of NADES-assisted hydrodistillation in terms of yield are reported in Figure 2.

ChCl-CA NADES, as an additive in the hydrodistillation process, allowed the highest yield of extraction (1.41%), followed by ChCl-TA (1.36%), ChCl-Gly (1.30%), while NADES with glucose and fructose as HBD gave the lower yield. These results can be ascribed to the higher

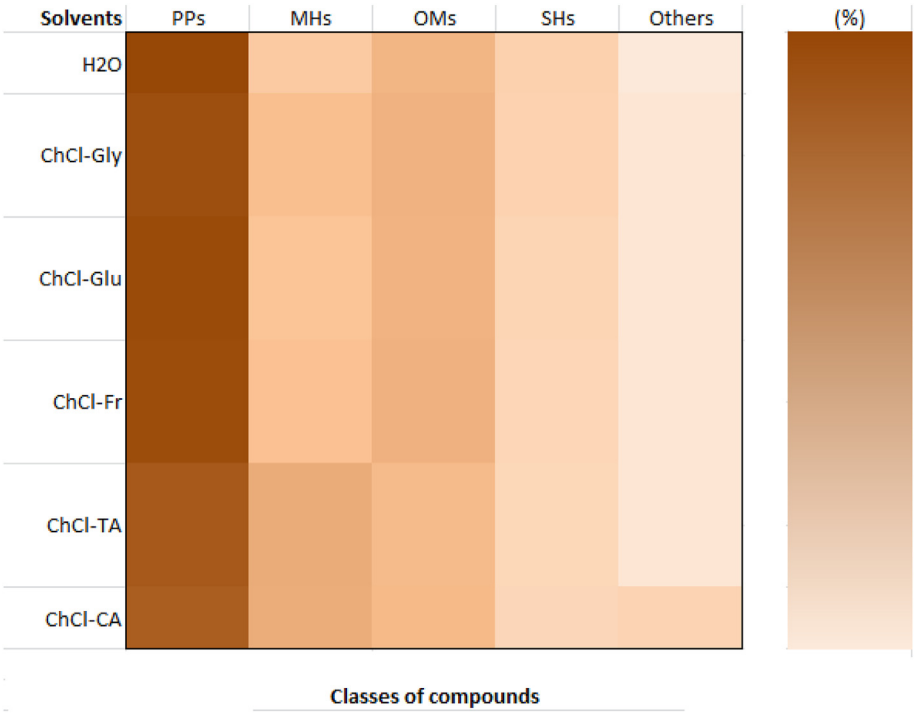

Figure 3. Relative area percentage of major classes of compounds in each used solvent.

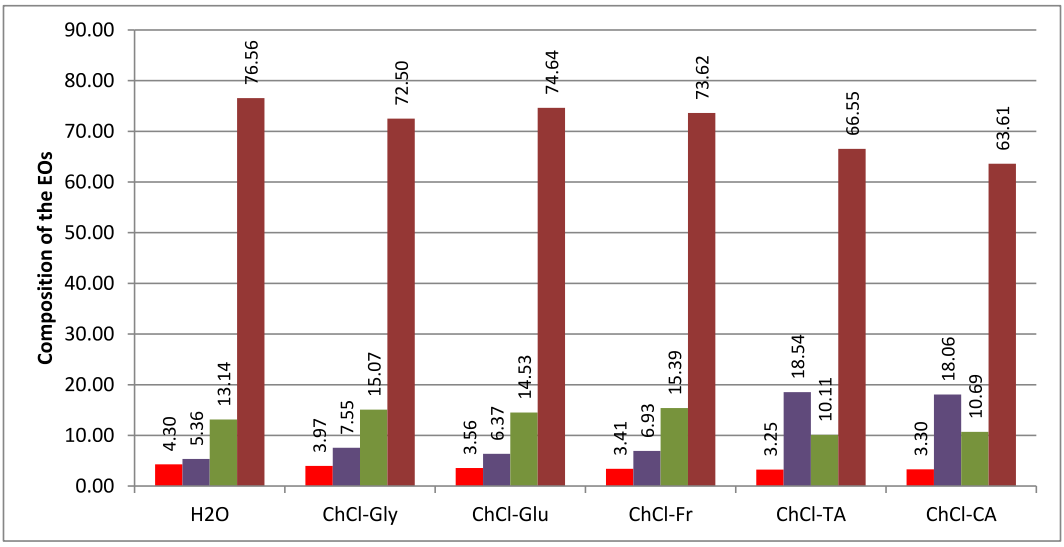

Figure 4. Composition of the EOs; Sesquiterpene hydrocarbons (red), Monoterpenes hydrocarbons (purple), Oxygenated monoterpenes (green), Phenylpropenoids (brown).

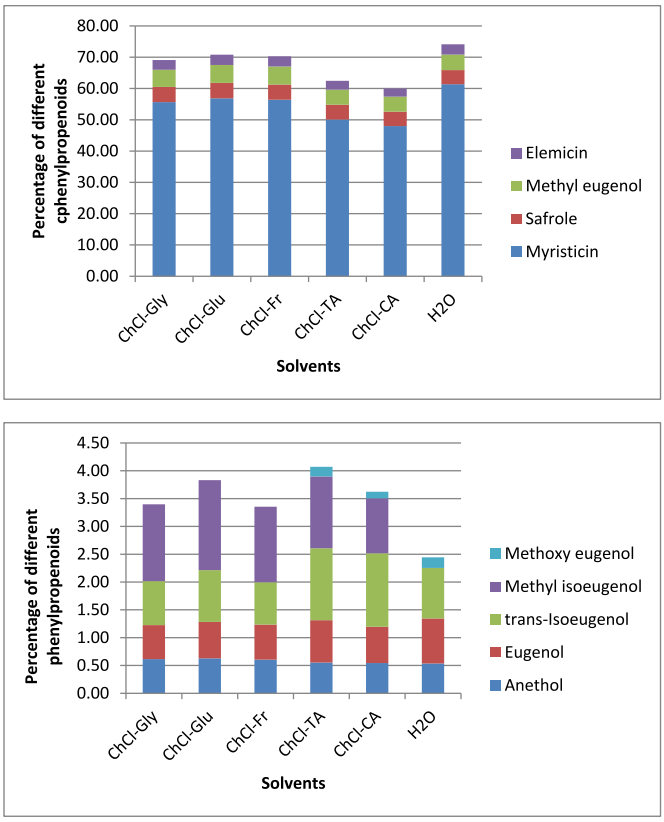

Figure 5. Relative area percentage of significant components in each solvent; Top: more abundant phenylpropenoids; Bottom: less abundant phenylpropenoids.

dissolution of cell wall cellulose by acidic NADES compared to neutral ones (Zhang et al., 2020).

3.5. Composition results

The chromatograms of all essential oils showed that their composition (Table 1 and Figure 5S) is influenced by the NADES used as the additive.

A total of 47 components (Table 1) were present in the EO obtained by the traditional method, some in traces (<0.10%). All the compounds were identified using mass spectra libraries research, retention indexes and reference compounds. Myristicin (61.36%) was the most abundant compound, followed by 4-terpineol (9.53%). The main compounds were similar to those already reported in the literature (Kapoor et al., 2013; Lanari et al., 2018; Muchtaridi et al., 2010).

The components were classified into five main groups: monoterpene hydrocarbons (MHs), oxygenated monoterpenes (OMs), sesquiterpene hydrocarbons (SHs), phenylpropenoids (PPs) and others and results are reported in Table 1 and in Figure 3.

Data demonstrate that ChCl-CA NADES is the most efficient NADES in reducing the amount of phenylpropenoids and increasing the terpenoidic fraction in the prepared EOs (Figure 4).

The EOs prepared using neutral NADES (ChCl-Gly, ChCl-Glu and ChCl-Fr) had a more similar composition to water EO (Figure 5). Interestingly, in all the EOs, safrole and anethole remained constant, methyl eugenol, isoeugenol and methyl isoeugenol increased, while myristicin, elemicin and methoxy eugenol decreased with respect to water EO. Furthermore, methyl eugenol was present only in the EOs obtained using NADES and methoxy eugenol only in the EOs obtained with water or acidic NADES.

4. Conclusions

In this work, *M. fragrans* EOs, obtained using different ChCl-based NADES, were studied. Compared with water, the use of NADES solution led to an improvement of the EO yield and a reduction of the extraction of phenylpropenoids. Particularly effective was ChCl-CA, which increased the yield of extraction up to 1.41% and decreased the percentage of phenylpropanoids in the essential oil (63.61% *vs* 76.56% in water). The developed process is an environmentally friendly approach that can be used to prepare nutmeg essential oil of the desired composition. In particular, this result could open the way to the sustainable extraction of a wide variety of EO that retains or maximizes the percentage of desired components and minimizes the unwanted or toxic elements.

Declarations

Author contribution statement

Maria Carla Marcotullio; Daniela Lanari: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Claudia Zadra: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Rima Njem; Francesca Negro: Performed the experiments.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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