Chemical composition changes of main active components accompanying the conversion of some essential oils (EO) into essential oils nanoemulsion (EONE).

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Abstract

Four essential oils [Sweet Basil (*Ocimum basilicum L*, Fam: Lamiaceae), Geranium, (*Pelargonium graveolens,* Fam: Geraniaceae), Lemongrass (*Cymbopogon citratus,* Fam: Poaceae) and Peppermint (*Mentha piperita L*. Fam: Lamiaceae)] were selected to be converted into essential oil nanoemulsions (EONE). The four oils were analyzed in their natural (EO) and nano form (EONE) using GC-MS, and the results showed a significant increase in the percentage of the main components of the four oils in their nano form (EONE). The results also indicated an increase in the total content of TPC phenols (12.91, 12.04, 13.78, 12.88 (QE/g)) respectively, as well as an increase in the percentage DPPH% (48.22, 36.21, 44.68 and 30.83%) respectively in the four oils. The results showed that the conversion EO to EONE for the four oils under study had preserved their physical and chemical properties.

Key words: EO, EONE, GC-MS, TPC, DPPH%, PDI, Sweet basil, Geranium, Peppermint, Lemongrass.

Introduction

The presence of essential oils is one of the main factors in a plant's medicinal properties. The term "essential oil" was first used by Paracelsus von Hohenheim, the founder of toxicology, in his 16th century. Essential oils, uses have gradually changed, and it is used not only for culinary purposes to improve its sensory appeal, but also for therapeutic purposes, in addition to its use in the manufacture of fragrances and beauty products. Essential oils were used by the ancient Egyptians in medicine, perfumery, and the art of planning for preservation burials [1].

Essential oils are natural products extracted by hydro distillation (HD), steam distillation (SD), supercritical fluid (SFC) from plant materials, composed by small, volatile, and fairly hydrophobic molecules [2–4]. The functions of essential oils (EO) in plant organisms appear to be related to environmental interactions and protection of plants from pathogens and predators. [2,5,6]. In industry, essential oils (EO) are materials of great interest with a wide range of applications in agriculture, cosmetics, pharmacy and nutrition [7,8], due to their wide range of biological activities, including , including antibacterial, repellent, anti-inflammatory properties, analgesic and many others [2,3]. Essential oils can be extracted from plant matrices using different techniques classified as classical/conventional (hydro distillation (HD) that use water distillation by heat as a means to extract the whole volatile compounds) and innovative/advanced (which focus on the improvement of selectivity in extraction efficiency by reducing extraction time, use of energy, solvent, and CO_2 emissions (SFC) supercritical fluid). Finally, the steam distillation method (SD) is the simplest and the cost is low. Currently, 85% of the essential oils. [2].

Essential oils are natural products obtained from most aromatic plants. Due to their medicinal properties, essential oils have become an interesting product in many areas, including pharmaceutical. Growing interest in recent years in encapsulating essential oils into nanometric systems for different therapeutic approaches. Many techniques used to produce essential oil-loaded nanosystems involve heating or solvent evaporation procedures that can health compromise [9].

Emulsion definitions have been around since the 1930s, drawn from the International Union of Pure and Applied Chemistry (IUPAC). Emulsions contain liquid droplets and/or liquid crystals, dispersed in a liquid. The term emulsion was derived from the word emulgeo (meaning “to milk”). Milk is the best example of naturalness emulsion. Emulsions are either water-in-oil (W/O) or oil-in-water (O/W) or Oil-in-oil (O/O) type. Emulsifiers easily act as a third ingredient and it plays an important role in dispersing these two immiscible liquids (dispersion phases and sequential phases). The size of the formed globule is 0.25- 25 microns in diameter. Emulsion is a heterogeneous system. Classification by type of emulsifier used and emulsifier structure a system was formed macroemulsion, nanoemulsion, microemulsion, etc. [10].

Nanoparticles were first developed around 1970 for the delivery of vaccines and anticancer drugs [11]. In addition to controlling the release of drug molecules, nanocarriers protect EO from potential thermal or photochemical degradation, ensuring improved stability, palatability and functionality, and extended shelf life of the final product. [12]. In fact, given these properties, these systems could be an interesting approach to overcoming the limitations of essential oils. Nanocarriers may protect essential oils from oxidation and evaporation, and may facilitate their antibacterial activity by providing different diffusion properties across biological membranes depending on the size of the nanoscale particles. i.e. new routes of administration are possible [12].

Encapsulation of essential oils (EO) into nanoemulsions has emerged as method to improve their efficacy, stability, and utilization [13]. Nanoemulsions are classified as delivery systems for nano-sized lipophilic compounds [14] such as flavors [15], vitamins [16] and antibacterial agents [17]. Due to the small particle size (100 nm radius), nanoemulsions are usually cloudy [15]. Due to the small particle size, nanoemulsions are much more stable against aggregation, coalescence and creaming [18, 19], making those excellent candidates for the food industry and used as active ingredients in pharmaceuticals and drug delivery. Also, encapsulation of EO in nanoemulsions is an innovative technology that helps overcome the factors limiting the use of EO, lowering its volatility and increasing its stability and solubility to maintain therapeutic efficacy. Nanoemulsions stabilize and improve the antibacterial efficacy of oils in aqueous solutions due to their increased surface area [19].

The difference between essential oil emulsion and essential oil nanoemulsion is in the size of the oil particles. The stability of the emulsion is significantly improved when the size of the oil particles becomes small. Surfactants are added to oil-water mixture to enhance the kinetic stability of such a system. [20]

Due to their high nutritional value, fruit consumption is increasing rapidly. Fresh fruit provides all the necessary supplements such as fiber, vitamins, etc. that are lacking in today's daily diet. The short shelf life threatens the market with large quantitative and economic losses. Research is being conducted to find permanent and effective solutions to this social disorder. In this context, edible coatings have proven to be effective primary packaging materials that retard the ripening process while preserving nutritional properties. Furthermore, the incorporation of active ingredients such as essential oils has been observed to significantly improve the effectiveness of edible coatings. Essential oils (EO) are used in the form of nanoemulsions to provide positive properties as they exhibit antioxidant and antibacterial properties. This integration aims to preserve the freshness of the fruit by maintaining and preserving its shelf stability for as long as possible [21].

Four aromatic plants (Sweet Basil, Geranium, Lemongrass and Peppermint) were selected and essential oils were extracted from them in order to study the changes that occur in the chemical components of those four essential oils when they are converted to a nanoemulsion.

MATERIALS AND METHODS

1. Source and preparation of essential oils

The following four essential oils, Sweet Basil (*Ocimum basilicum L*, Fam: Lamiaceae), Geranium, (*Pelargonium graveolens*, Fam: Geraniaceae), Lemongrass (*Cymbopogon citratus,* Fam: Poaceae) and Peppermint (*Mentha piperita L*. Fam: Lamiaceae) were obtained from the steam distillation laboratory [22] at the farm of the Medicinal and Aromatic Plants Research Department in Alqanater Al-Khairia. It is affiliated with the Horticultural Research Institute, ARC in Giza, Egypt.

2. Preparation of nanoemulsions of the essential oils

The essential oils was prepared in the form of a nanoemulsions in the central laboratory the National Institute of Oceanography and Fisheries (NIOF) in Alexandria. Briefly, nanoemulsions of oil/water were prepared by combining the essential oils with Tween 80 as a surfactant (one oil to three T80); then adding water to obtain a concentration of 2.50%, mixing by using a magnetic stirrer (with a speed of 500 rpm, for 10 min). The prepared nanoemulsions was then sonicated for 5 min using an ultrasonicator (750 W, Branson Probe sonicator-Advanced model, 20 kHz). The resulting nanoemulsions were characterized by a UV-visible spectrophotometer (UV-2600, Shimadz, Japan) at 345 nm [23].

3. Measurement of Particle Size and Polydispersity index in EONE

Nanoemulsion droplets Diameters and Polydispersity index were measured using DLS (Dynamic Light Scattering instrument, MAL1008079, UK). The particle size were determined by DLS from intensity-time fluctuations (ITF) of a laser beam (633 nm) scattered from the sample [24]. The mean particles size or Z-Average size reported by DLS is the intensity-weighted mean diameter derived from the cumulants analysis [25]. Before measurements, the samples were diluted with distilled water to an appropriate concentration (1:10) to avoid multiple scattering effects.

4. GC-MS Analysis of essential oils

All essential oils (EO) and essential oil nanoemulsion (EONE) (Essential oils were extracted from the nanoemulsions by redistillation) analyzes were performed in a central laboratory network, National Research Centre, and Cairo, Egypt. The essential oils extract was carried out by GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A). Samples were diluted with hexane (1:19, v/v). The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness). The analysis was performed using helium as carrier gas, a flow rate of

1.0 ml/min, a split ratio of 1:10, an injection volume of 1 µl, and the following temperature program. 1 minute at 40°C. Heat up to 150°C at 4°C/min and hold for 6 minutes. Ramp at 4°C/min to 210°C and hold for 1 minute. The injector and detector were maintained at 280°C and 220°C, respectively. Mass spectra were acquired by electron ionization (EI) at 70 eV. A spectral range of m/z 50-550 and a solvent delay of 5 min. The identities of the various components were determined by comparing the spectral fragmentation patterns with those stored in Wiley and NIST mass spectral library data.

5. Measurement of the Refractive Index and Density

Essential oils were subjected to tests of index of refraction and density according to British Pharmacopoeia [26] using an Abbe refractometer (Atago, Tokyo, Japan), while density was determined by using density bottle at 25°C (Blaubrand, Germany).

6. Total phenolic content (TPC)

TPC of the extracted EO and EONE were determined spectrophotometrically with a slight modification of the Folin-Ciocalteu (FC) method described in [27]. TPC was measured and expressed as mg gallic acid equivalent (GAE/L) using the following standard curve as a reference. Mix 20 µl of extract (essential oil diluted 1:60 with methanol) with 1 ml of Folin-Ciocalteus phenol reagent, add 1 ml of saturated sodium Na_2CO_3 (20%) after 3 minutes, and adjusted to 980 µl with distilled water. After incubation for 100 minutes in the dark at room temperature, the absorbance at λ=765 nm was measured using UV-VIS. A standard curve was generated using gallic acid. A standard reference curve for gallic acid was constructed for the following concentrations: 0, 20, 40, 100, 160, and 200 mg L^{-1}, respectively. All measurements were performed in triplicates for precision and values were expressed in mean ± standard deviation in terms of Phenol Content (PC).

7. Total Flavonoid Content (TFC)

TFC in EO and EONE were determined according to method [27] using quercetin as a standard. 1ml of test sample (EO or EONE) and 10 ml volume (dd H_2O) distilled deionized water was added to a volumetric flask. Afterwards 0.3 ml of 5% $NaNO_2$ had been added, then 0.3ml of 10% $AlCl_3$ was added after 5 minutes. After 10 minutes incubation at 25^0C, 1 ml of NaOH was added to the reaction mixture. Immediately the final volume was making up to 10 ml with (dd H_2O). Absorbance of sample was measured against the blank at 515nm using a spectrophotometer. The standard reference curve for quercetin was made for the following concentrations: 0, 20, 40, 100, 160, and 200 mgL^{-1}, respectively. The total phenolic content was expressed as mg pyrocatechol equivalents (QE) per g of OE and OENE extract.

8. Measurement of antioxidant DPPH activity

The radical-scavenging activity of essential oils against stable DPPH radicals was measured spectrophotometrically according to [28], the radical scavenging capture percentage (% DPPH) radical of each essential oil was calculated by the following formula: %DPPH = (Ac-As)/As X 100. [Ac = Absorbance of control sample and As = Absorbance of a tested sample].

9. Statistical Design and Analysis

All measurements were conducted in three replicate. Data were reported as mean standard deviation (± SD). Analysis of significant differences between means were tested by the following tested by one way ANOVA followed by L.S.D to compare treatment means at a probability level of 0.05 [29].

Results

The results showed that the optical refractive index and density have no significant differences between them (EO and EONE) for four essential oils, Sweet basil, Geranium, Lemongrass and Peppermint, mentioning in Table (1). The results in Table (2) indicate that the particle size of the four EONE ranges from 39 to 62 nm. On the other hand, the results showed that the total phenols content (TPC) was distinguished by its concentration EONE compared to EO respectively, (Sweet basil, 12.91 and 12.74), (Geranium, 12.04 and 11.64), (Peppermint, 13.78 and 13.11), (Lemongrass, 12.88 and 11.83). While the total flavonoids content (TFC) in Table (3) has no significant differences between them (EO and EONE) for four essential oils. The results are shown in Table (4) for the antioxidant DPPH activity (DPPH %) significantly increasing of the percentage DPPH% in the four oils (EONE) compared to EO under this study, Sweet basil; 48.22%, Geranium; 36.21%, Peppermint; 44.68%, Lemongrass 30.83%, respectively.

Chemical composition of essential oils (EO) and essential oil nanoemulsions (EONE) Chemical composition of investigated essential oils and essential oil nanoemulsions analyzed by gas chromatography are presented in Table (5) and Fig. (1), changes in the essential oil components were observed in the nanoemulsions (EONE) compared with the corresponding original essential oils (EO). Data presented in Table (5) show that the main components percentage of the nanoemulsions of the following oils (EONE) were increased Sweet basil (Eucalyptol (5.88), Linalool (58.55), Z- Citral (7.83), E- Citral (10.35) and Caryophyllene (1.65)), Geranium (Linalool (11.05), α- Terpineol (1.10), Citronellol (34.55), Geraniol (19.37) and Geranyl acetate (4.20)), Peppermint (Menthone (21.89), Menthol (42.31), Menthyl acetate (6.49)), lemongrass (β-Myrcene (17.03), Linalool (1.62), Z- Citral (39.48) and E- Citral (42.44)), respectively, compared with EO (Essential oil) for all essential oils, while, Z-Citral and Caryophyllene (Sweet basil oil), Caryophyllene (Geranium oil), α-Pinene, Menthol and Menthyl acetate (Peppermint oil), and α- Pinene (Lemongrass oil) were decreased in essential oil nanoemulsions (EONE) compared with essential oil (EO).

Table (1): Physical Properties (Refractive index and Density) of EO and EONE in Sweet basil, Geranium, Peppermint and Lemongrass oils.

Physical Properties	Sweet Basil oil			Physical Properties	Geranium oil		
	EO	EONE	L.S.D $_{0.05}$		EO	EONE	L.S.D $_{0.05}$
Refractive index	1.47 ±0.03 a	1.47 ±0.01 a	0.018	Refractive index	1.46 ±0.01 a	1.46 ±0.02 a	0.015
Density	0.98 ±0.02 a	0.98 ±0.02 a	0.016	Density	0.89 ±0.04 a	0.88 ±0.02 a	0.013
Physical Properties	Peppermint oil			Physical Properties	Lemongrass oil		
	EO	EONE	L.S.D $_{0.05}$		EO	EONE	L.S.D $_{0.05}$
Refractive index	1.46 ±0.03 a	1.46 ±0.02 a	0.013	Refractive index	1.47 ±0.03 a	1.47 ±0.01 a	0.013
Density	0.91 ±0.01 a	0.91 ±0.03 a	0.015	Density	0.88 ±0.02 a	0.88 ±0.03 a	0.014

All values are expressed as mean ±SD (standard deviation) for three replicates. - Essential oil (EO) - Essential oil nanoemulsion (EONE)

Table (2): Particle Diameters and Polydispersity Index of Essential Oils Nanoemulsions (EONE) of Sweet Basil, Geranium, Peppermint and Lemongrass oils.

EONE	Sweet Basil oil	Geranium oil
Droplet size (nm)	45.7 ±6.1	62.3 ±7.4
Polydispersity index	0.234 ±0.014	0.314 ±0.025
EONE	Peppermint oil	Lemongrass oil
Droplet size (nm)	51.3 ±9.1	39.6 ±8.3
Polydispersity index	0.335 ±0.021	0.285 ±0.019

Table (3): TPC and TFC (QE/g) of EO and EONE in Sweet basil, Geranium, Peppermint and Lemongrass oils.

Bioactive (TPC and TFC)	Sweet Basil oil			Bioactive (TPC and TFC)	Geranium oil		
	EO	EONE	L.S.D $_{0.05}$		EO	EONE	L.S.D $_{0.05}$
TPC (QE/g)	12.74 ±0.02 b	12.91±0.04 a	0.15	TPC (QE/g)	11.64±0.04 b	12.04±0.03 a	0.21
TFC (QE/g)	26.81 ±0.05 a	26.90±0.03 a	0.24	TFC (QE/g)	24.49±0.02 a	24.41±0.05 a	0.32

Bioactive (TPC and TFC)	Peppermint oil			Bioactive (TPC and TFC)	Lemongrass oil		
	EO	EONE	L.S.D 0.05		EO	EONE	L.S.D 0.05
TPC (QE/g)	13.11 ±0.02 b	13.78±0.02 a	0.18	TPC (QE/g)	11.83±0.05 b	12.88±0.03 a	0.24
TFC (QE/g)	25.47±0.04 a	24.98±0.02 a	0.56	TFC (QE/g)	23.79±0.04 a	23.62±0.06 a	0.41

All values are expressed as mean ±SD (standard deviation) for three replicates. - Essential oil (EO) - Essential oil nanoemulsion (EONE)

Table (4): Antioxidant DPPH activity (DPPH %) of EO and EONE in Sweet basil, Geranium, Peppermint and Lemongrass oils.

Antioxidant DPPH activity	Sweet Basil oil			Antioxidant DPPH activity	Geranium oil		
	EO	EONE	L.S.D 0.05		EO	EONE	L.S.D 0.05
DPPH %	45.41 ±0.29 b	48.22 ±0.24 a	2.08	**DPPH %**	33.63 ±0.47 b	36.21 ±0.32 a	2.35
Antioxidant DPPH activity	**Peppermint oil**			**Antioxidant DPPH activity**	**Lemongrass oil**		
	EO	**EONE**	**L.S.D 0.05**		**EO**	**EONE**	**L.S.D 0.05**
DPPH %	40.34 ±0.31 b	44.68 ±0.34 a	1.89	**DPPH %**	28.21 ±0.26 b	30.83 ±0.34 a	2.41

All values are expressed as mean ±SD (standard deviation) for three replicates. - Essential oil (EO) - Essential oil nanoemulsion (EONE)

Table (5): Percentage chemical composition of the main compounds of EO and EONE in Sweet basil, Geranium, Peppermint and Lemongrass oils

Compounds	Sweet Basil oil			Compounds	Geranium oil		
	EO	EONE	L.S.D 0.05		EO	EONE	L.S.D 0.05
Cymene	0.56 ±0.02 b	0.67 ±0.02 a	0.08	**Linalool**	8.21 ±0.06 b	11.05 ±0.07 a	0.09
Eucalyptol	4.31 ±0.06 b	5.88 ±0.05 a	0.14	**α- Terpineol**	0.97 ±0.01 b	1.10 ±0.02 a	0.11
Linalool	49.62 ±2.11 b	58.55 ±2.14 a	1.18	**Citronellol**	32.10 ±1.94 b	34.55 ±2.06 a	1.12
Z- Citral	7.83 ±0.07 a	7.36 ±0.06 b	0.16	**Geraniol**	15.63 ±0.09 b	19.37 ±0.10 a	0.31
E- Citral	9.54 ±0.08 b	10.35 ±0.09 a	0.09	**Geranyl acetate**	4.20 ±0.05 a	4.19 ±0.04 a	0.03
Caryophyllene	1.65 ±0.03 a	0.28 ±0.01 b	0.11	**Caryophyllene**	1.31 ±0.02 a	0.16 ±0.01 b	0.01
Compounds	**Peppermint oil**			**Compounds**	**Lemongrass oil**		
	EO	**EONE**	**L.S.D 0.05**		**EO**	**EONE**	**L.S.D 0.05**
α- Pinene	1.52 ±0.02 a	0.83 ±0.03 b	0.07	**α- Pinene**	0.17 ±0.02 a	0.14 ±0.01 b	0.02
Menthone	21.30 ±1.64 b	21.89 ±1.62 a	0.24	**β- Myrcene**	12.46 ±0.04b	17.03 ±0.06a	1.29
Menthol	41.99 ±2.07 a	43.31 ±2.08 b	1.02	**Linalool**	1.18 ±0.02 b	1.62 ±0.01 a	0.18
α- Terpineol	0.41 ±0.01 b	0.55 ±0.01 a	0.06	**Citronellol**	0.34 ±0.01 b	0.43 ±0.03 a	0.05
Pulegone	0.51 ±0.01 b	1.50 ±0.02 a	0.08	**Z- Citral**	37.02 ±2.02 b	39.48 ±2.06 a	1.32

Menthyl acetate	6.49 ±0.05 a	4.83 ±0.04 b	0.91	**E- Citral**	39.07 ±2.06 b	42.44 ±2.08 a	1.42

All values are expressed as mean ±SD (standard deviation) for three replicates. - Essential oil (EO) - Essential oil nanoemulsion (EONE)

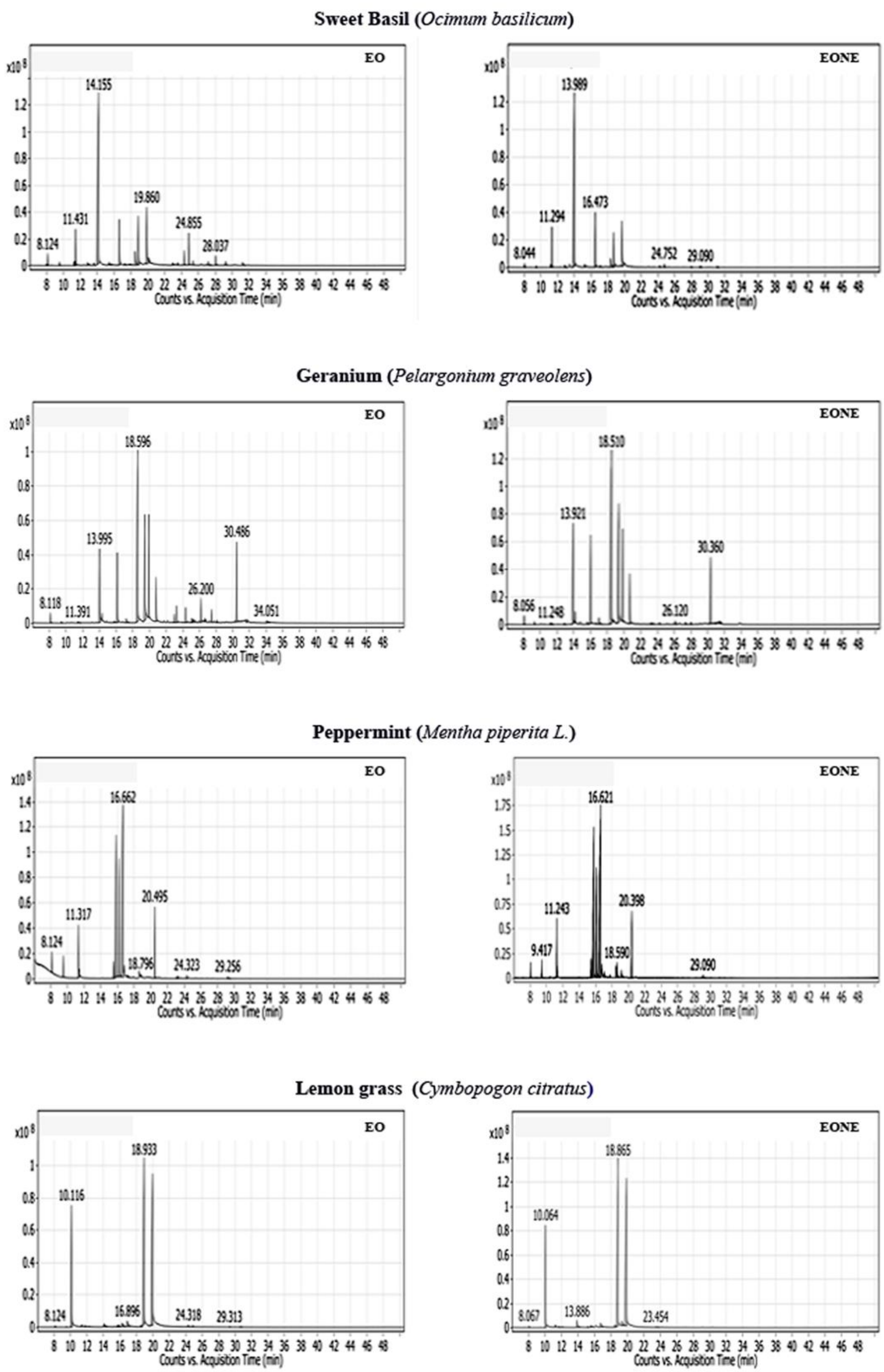

Fig (1): Typical chromatogrames of Essential oil (EO) and Essential oil nanoemulsion (EONE) for four aromatic plants by GC-MS

Discussion

The change in the concentration of the main components in essential oils nanoemulsion (EONE) may be there was a breakdown of ultrasonic chemical bonds during the manufacture of the nanoemulsion. Once again, the atoms are redistributed through the donor atom, which carries a positive charge such as the hydrogen atom and the recipient atom with a negative charge such as the oxygen atom. The compounds are then formed within aromatic oils again but at different concentrations [30]. indicated the results that the concentration of TPC in EONE increased significantly and this confirm that the conversion of EO essential oils to emulsified essential oils EONE preserves the properties of essential oils, whether they contain active substances and antioxidants, and this is consistent with what was mentioned [19,31].Also, the previous studies have confirmed nanoemulsion formulation is considered as a functional behavior for essential oils stuffs because of their increased surface area and small droplet size [32, 33].

Conclusions

Over time, increasing of applications and multiple uses of nanoemulsion, especially essential oils nanoemulsion (EONE), in various fields, the most important of which are food, medicine, pharmaceuticals, the prevention and treatments of some plant diseases... etc. The study showed that the conversion of essential oils from their natural form to essential oils nanoemulsion was accompanied by the preservation of their basic components and distinctive properties (main essential oils components %, TPC and DPPH%), which means that the use of essential oils nanoemulsion can be expanded in many different uses by a safe and effective manner.

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