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Essential Oil as Green Preservative Obtained by Ecofriendly Extraction Techniques

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Abstract

Essential oils are formed by a complex matrix of substances that are biosynthesized in the secondary metabolism of plants. Nowadays, different ecofriendly extraction techniques (e.g., ultrasound-, microwave-, enzyme-assisted extraction, and supercritical fluid by CO₂, etc.) have been adopted to obtain essential oils. These techniques provide unique quality of essentials oils or extracts from aromatic plants in a short time with high energy savings. Essential oils not only impart aroma, but also possess antimicrobial and antioxidant activities. Health limitations in the use of synthetic additives have drawn researchers' attention towards essential oils as safe natural preservatives. Therefore, this chapter summarizes novel technologies to recover essential oils or extracts. In addition, it focuses on application of essential oils and their constituents as green preservatives to retard microbial growth and oxidative spoilage.

Keywords: essential oil, green preservative, ecofriendly techniques, enzyme assisted extraction, ultrasound assisted extraction

1. Introduction

Conventional techniques (hydrodistillation, steam distillation) used for EO extraction from aromatic plants have several disadvantages such as long extraction time, high energy consumption and degradation of thermally labile aromatic compounds [1]. Characteristic natural flavor of an essential oil depends mainly on its components and their concentrations. Therefore, extraction procedure has to be sensitive enough to keep the proportions of its constituents in their natural state. The oxygenated compounds of EO is considered the main indicator for its quality [2]. The level of these compounds in the EO is affected by the extraction technique used. Recently, novel (green) techniques have been applied solely or in combination with other technique to recover essential oils with high quality in a short time [3]. These green techniques include: Enzyme (EAE), ultrasound (UAE), and microwave (MAE) and supercritical fluid extraction (SFE) [4].

Essential oils (EOs) are used in a wide range of food types as biopreservatives according to their antioxidant and antimicrobial properties [5]. Essential oils are

directly added to food matrix, incorporated into food packaging materials, applied in edible coatings or in modified atmosphere packaging [6]. Some EOs and their components such as carvacrol, carvone, cinnamaldehyde, citral, eugenol, linalool, limonene, thymol and vanillin, were accepted for use as flavorings and food additives by the European Commission. Application of essential oils at high concentration required in food preservation is limited by its sensorial characteristics, which would affect negatively the original organoleptic properties of foods [7–9].

2. Ecofriendly extraction techniques

2.1 Ultrasonic assisted extraction (UAE)

Ultrasonication is considered as a green extraction technique for EO [10]. Ultrasound (U) waves are successfully utilized in the extraction of essential oils, oleoresins, and other bioactive compounds from spice matrices [11]. The advantages of this technique include: low-temperature, short extraction time, low energy consumption, and superior quality of the extracted EO [12]. Microsecond pulses of ultrasound wave generated vapor bubbles within the liquid medium. The bubbles expanded to a large size during expansion cycles before implosion on the surface of plant material, that lead to micro cracking in the cell wall which helps to penetrate the solvent in the cell wall of the powder and release the intracellular components into the medium [13]. This observation could be noticed in **Figure 1**.

There are two types of UAE of EO: ultrasound (US) as a pretreatment prior to HD and simultaneous ultrasound-assisted HD extraction [14]. The UAE efficiency of EO is affected by power, sonication time, frequency, temperature, solvent type and liquid to solid ratio.

Lilia et al. [15] evaluated the effect of UAE time (10, 20, 30, 45 and 60 min) prior to HD on the EO yield from dried flowering tops of *Lavandula stoechas* L. Plants were collected from two regions: Keddara and Adekar in Algeria. The highest yield was obtained in the Adekar sample (1.59%) and Keddara sample (0.87%) after 10 min and 45 min of ultrasound (US), respectively. UAE pretreatment was followed by HD for 90 min. However, the yield of EO obtained by conventional HD (180 min) represented ~70% of that produced by US-HD technique.

Wu et al. [16] extracted EO from dried aerial parts of *Artemisia annua* by ultrasonic-assisted steam distillation extraction. The investigated independent factors were steam distillation (SD) time (1 h, 3.5, and 6 h), US time (0 h, 0.5, 1 h), and solid to liquid ratio (g/mL) (1:6, 1:10 and 1:14). The EO yield reached 0.71% with the optimal conditions (SD extraction time of 3.5 h, US time of 0.5 h, and solid to liquid ratio of 1:10 (g/mL) instead of 0.49% obtained with the conventional SD for 6 h.

Jadhav et al. [17] investigated the UAE of EO from *Piper betle* leaf powder in presence of water at different sonication time (20, 30 and 40 min), dissipated energy (5.64, 12.24, 19.8, 34.56, and 47.32 W) and temperature (30, 40, 50 and 60°C), with different leaf powder to solvent ratios (1:3, 1:4, 1:5 and 1:6). The maximum yield of EO (0.5%) was recorded at 30 min of US irradiation while it did not exceed 0.35% after 3 h of HD. Increasing temperature more than 30°C, dissipated energy higher than 34.56 W and solvent to solid ratio higher than 5 did not significantly increase the yield of EO. They attributed the obtained results at high US power to the formation of large bubble cloud in the solvent that shielded and scattered the bubble energy discharged during the collapse process.

Figure 1. Scanning electron micrographs of wild mint (*Mentha longifolia*) leaves (a) control without any treatment (b) after extraction with HD for 3 h (c) after extraction with U (60 W) for 10 min (d) after extraction with U (60 W) for 10 min + HD for 33 min ($\times 200$ – $\times 1000$ magnification, 25 kV).

Chen et al. [14] studied the effect of the US power on EO yield from cinnamon bark. The samples were subjected to US irradiation ranged from 100 to 500 W for 30 min prior HD. The EO yield (2%) increased with the increase of US power to 300 W. The yield decreased at higher US irradiation (>300 W). They attributed this decrement to high temperature generated, high acoustic pressure created, formation of big bubbles and increase of cavitation bubbles bursting that led to decomposition of EO constituents. Extending US time to 30°min caused a significant increase in EO yield and decrease HD time from 2 h to 1 h. Further increase in US time caused lower yield due to loss of volatile constituents. US at water to solid ratios ranged from 4 to 12 (mL/g) was evaluated. The highest EO yield was recorded for water to solid ratio of 6 (mL/g) and decreased with the continual increase of liquid to solid ratio due to the reduction of the ultrasound intensity needed for the breakage of cell walls. Statistical analysis of the data showed that the order of influence of the dependent factors on EO yield was US time > HD time > US power > liquid to solid ratio.

Ghule et al. [18] extracted eugenol and eugenol acetate from ground clove buds by ultrasound assisted hydrotropic extraction (UAHE). Sodium cumene sulfonate was used to prepare the hydrotropic solution, since solubility of eugenol in water was found to be 1.35 g/L instead of 500 g/L in the aqueous solution (1.8 M) of sodium cumene sulfonate. The extraction time (15–75 min), temperature (30–70°C), hydrotrope concentration (0.2–1.8 M), solid loading (6–22 g/150 mL of hydrotrope solution) and US power (120–200 W) were selected as independent variables. The combined yield of eugenol and eugenol acetate was used as a response. After sonication the mixture was filtered. Eugenol and its derivative were recovered from the filtrate by extraction with hexane. The highest extraction yield (20.04%) was obtained with the following optimal conditions; US power of 158 W, 38°C, hydrotrope concentration of 1.04 M, solid loading of 8.2 g, and extraction time of 30 min. The EO yield obtained by conventional hydrotropic extraction for 1 h was not significantly different than that resulted from UAHE technique.

Guo et al. [19] used ultrasound to enhance subcritical water extraction (USWE) of EO from ground cinnamon bark. The following independent factors were used, through Box-Behnken design, to optimize the extraction conditions: extraction time (20, 25, and 30 min), extraction temperature (120, 130, and 140°C), and US power (100, 125, and 150 W) with a pressure of 5 MPa. The yield of cinnamaldehyde was set as the dependent variable, while its content in the EO was used as a quality index. They compared USWE with the following extraction techniques: steam distillation for 4 h, ultrasound assisted extraction (UAE) by dichloromethane with US power of 150 W for 40 min, and subcritical water extraction (SWE) under pressure of 5 MPa, with a liquid to solid ratio of 12 mL/g, at 132°C for 38 min. Although UAE resulted in the highest EO yield (2.1%) compared to other extraction techniques (1.58–1.83%), the cinnamaldehyde content that obtained with UAE was the lowest (8.965 mg/g). The optimal conditions of USWE were found to be extraction time of 25 min, extraction temperature of 140°C, and US power of 145 W, a pressure of 5 MPa and liquid to solid ratio of 8 mL/g. Under these conditions the maximum yield of EO and cinnamaldehyde content were 1.78% and 12.662 mg/g, respectively. Results indicated that coupling ultrasound with SWE shortened the extraction time and improved the quality of the obtained EO.

Zhang et al. [20] extracted EO from dried citronella leaves with ultrasonic ohmic heating distiller in presence of distilled water. The EO yield of 18 mL/kg dry weight as obtained with liquid to solid ratio of 6. Increasing liquid to solid ratio to 12 caused significant decrease in the yield. They attributed this to difficult in distilling out the EO from large volume of solvent. Meanwhile, increasing US power from 36 to 144 W was accompanied by a significant increase in the EO yield. No significant increase in the EO yield was noticed by further increase in US power. Increasing the current intensity from 1 to 5 A significantly increased EO yield to 20 mL/kg dry weight. This yield was obtained when extraction process was conducted for 40 min, after which no further increase was observed. They suggested that excessive extraction time under the US and ohmic conditions used affected negatively the release of EO due to gelatinization of intracellular components. They used response surface method to optimize the extraction conditions. The EO yield reached 22.91 mL/kg dry weight under the optimal conditions (liquid to solid ratio of 7 mL/g, US power of 180 W, current of 5°A, and time of 30 min).

2.2 Enzyme-assisted extraction (EAE)

The EAE technique is considered as a green extraction technology [21]. In EAE, hydrolytic enzymes act on the polysaccharides of the cell wall, disrupt it and release

Figure 2.
 Scanning electron micrographs of lemon verbena (*Aloysia citrodora*) leaves (a) control without any treatment (b) after HD (c) pretreated with Cellulase (d) pretreated with pectinase (e) pretreated with Viscozyme.

the intracellular essential oil and other bioactive compounds [22]. This observation could be noticed in **Figure 2**.

However, low solubility of essential oil in the aqueous buffer system restrains complete extraction [23, 24]. Therefore, this technique is used as a pretreatment to enhance extraction of volatile oil. The extraction of essential oils (EOs) is carried out by a single enzyme or a combination of enzymes; i.e. cellulase, hemicellulase, pectinase, protease, amylase or viscozyme (mixture of cellulase, arabinase, β -glucanase, hemicellulase and xylanase) [25]. Mahmoudi et al. [26] found that treating sweet basil (*Ocimum basilicum* L.) leaves with viscozyme before HD increased the EO yield to 9.32% instead of 6.1% in the control samples. Enzymatic pretreatment was carried out with 5 mg of enzyme/50 g leaves in distilled water (250 mL) during stirring at 40°C for 1 h prior to HD for 2 h. Li et al. [27] reported that EAE of EO from *Mentha haplocalyx* leaves with a mixture of enzymes (cellulase and pectinase) was more efficient than each of them. They attributed this effect to the synergistic influence of enzyme mixture on the cell wall. Moreover, the efficiency of EAE is affected by type and activity of enzymes used, enzyme concentration, buffer to solid ratio, incubation temperature, and incubation time [28, 29]. In addition, difference in plant structures affects the enzyme efficiency with respect to the oil yield [30].

Baby and Ranganathan [31, 32] pretreated cardamom and fennel seeds with either of celluclast, pectinex, viscozyme and protease prior to steam distillation led to an increase in EO yield by 7–16% and 11–22.5%, respectively. The maximum yield was obtained by viscozyme at 1% (v/w) under optimized conditions of 50°C, pH 5 and incubation time of 90 min. GC-MS analysis showed that enzyme pretreatment increased significantly the main characteristic oxygenated compounds (1,8-cineole and α -terpinyl acetate) in cardamom EO and *trans*-anethole and fenchone in fennel EO.

Increasing enzyme concentration enhances the degradation of cell wall and the release of EO up to a level after which no significant increase of EO yield could be

obtained [32]. They ascribed this to the saturation of enzyme sites by the substrate. Shimotori et al. [33] treated peppermint (*Mentha arvensis*) leaves powder with enzyme aqueous solution at a ratio of 1:10 (*w/v*). Each of cellulase and hemicellulose was used at different concentrations (0.1, 1.0, 2.0, 5.0 and 10.0%, *w/w*), individually. The mixture was incubated for 3 h at 40°C, before subjecting to hydrodistillation for 1 h to obtain EO. Levels of L-menthol and L-menthone, the main components of EO, were used to examine the efficiency of the treatment. Maximum yields of L-menthol and L-menthone were obtained at each enzyme concentration of 2%. They found that the yield of EO increased by the combined use of 2% cellulase and 2% hemicellulase compared with the use of one enzyme.

Pretreatment of *Ocimum canum* aerial parts powder with viscozyme at 1% concentration before HD increased the EO yield to 1.2% compared to 0.83% with HD only [34]. This pretreatment decreased HD time from 180 to 30 min. They found that EAE increased the level of oxygenated monoterpenes in the obtained oil.

Vladić et al. [35] incubated *Origanum vulgare* aerial parts with viscozyme at 8%, pH 4.9, 45°C for 60 min before extraction for 4 h increased EO yield to 6.59% compared to 3.39% that obtained by the control. The EAE led to an increase of oxygenated compounds in the extracted EO to 94.67% compared to the control (88.06%).

2.3 Microwave-assisted extraction

Microwave-assisted extraction (MAE) accelerates the extraction of EO and saves energy and time without negative changes in the EO composition [36]. Microwaves (electromagnetic waves) rotate molecules with dipoles inside the plant cell that creates heat, generates high inwards pressure (as a result of the abrupt rise in temperature) on the cell wall disrupts it and releases cells' content into the extraction medium [37].

Hassanein et al. [38] extracted EO from the dried aerial parts of 7 plants from Lamiaceae family (*Origanum majorana* L., *Mentha piperita* L., *Mentha longifolia* L., *Origanum syriacum* L., *Lavandula angustifolia* L., *Rosmarinus officinalis* L., and *Thymus vulgaris* L.) using MAHD at 100°C and 800 W for 60 min. The yield and oxygenated constituents of EO extracted by MAHD were higher than those of EO obtained by the HD technique in 180 min, indicating the higher quality of MAHD EOs. They reported that long extraction time by HD enhanced decomposition of the oxygenated compounds.

Ghazanfari et al. [39] extracted EO from coriander seeds powder by microwave-assisted hydrodistillation (MAHD). The microwave oven was operated as follows; 10 min at 800 W up to 100°C, and then kept at 100°C for 60 min at 500 W, followed by 10 min of ventilation. The EO yield (*v/w*) obtained by MAHD (0.325%) was not significantly different ($p > 0.05$) from that extracted by HD (0.31%). The MAHD technique reduced extraction time from 240 min during HD to 60 min.

Memarzadeh et al. [40] studied the effect of microwave-assisted steam hydro-diffusion (MSHD) technique and extraction time on the EO yield of the Bakhtiari savory (*S. bachtiarica* Bunge.) aerial parts. The MSHD was carried out at 800 W for 75 min. The highest EO yield (1.80, *v/w* dry weight basis) was obtained after 60 min of MSHD vs 150 min by the HD. The maximum level of oxygenated monoterpenes (69%) was obtained by MSHD after 20 min instead of 65.5% that extracted by the HD after 150 min. The MSHD technique reduced energy required for EO recovery from 4.5 kWh by the HD to 0.26 kWh.

Yingngam et al. [41] used solvent-free microwave extraction (SFME) attached to a Clevenger apparatus to retrieve EO from the fresh *Shorea roxburghii* flowers. The

moisture content of the fresh flowers ranged between 69% and 74%. They subjected water inside plant cells to microwave energy. The effects of microwave power (480, 640, and 800 W) and irradiation time (10, 30, and 50 min) as independent variables, on the EO yield (% *w/w*) were evaluated. The highest yield of EO (0.0114%) was recorded at 780 W and 38 min. The oil obtained by HD for 8 h had the similar yield. The EO recovered by SFME technique was characterized by the same scent of fresh flowers. The energy consumption decreased from 3.60 kWh by HD to 0.58 kWh with the SFME. In other research, Yingngam et al. [42] used the same SFME technique to extract EO from fresh aerial parts of *Limnophila aromatic* (70.11–75.14% moisture content). The highest yield of EO (0.21% *v/w*) was recovered at 700 W and 25 min instead of 4 h by HD. Oxygenated monoterpenes of the EO produced by SFME technique (50.29%) was higher than that obtained by HD (39.09%). However, they reported that the quality of the EO obtained by both methods was similar. Peng et al. [43] used a combined technique of solvent-free MAE (SFME) and the screw extrusion of *Pinus pumila* fresh needles to obtain the EO. Response surface method was applied to optimize the extraction conditions. The investigated independent factors used were; moisture content (30, 40, and 50%), MAE time (20, 30, and 40 min), and MAE power (385, 540, and 700 W) while yield of EO was the response factor. Fresh needles were crushed in the extrusion treatment and the cell wall was ruptured with the increase of intracellular pressure in the cells due to the evaporation of water (100°C) by the microwave irradiation. Increasing the moisture content of needles from 20% to 40%, increased the yield of EO. The highest yield (12.00 mL/kg) was obtained at moisture content of 40%, power of 540 W and irradiation time of 30 min. On the other hand, the EO yield from *P. pumila* did not exceed 7.00 mL/kg after 4 h of conventional HD.

2.4 Supercritical fluid extraction by CO₂

Supercritical fluid extraction (SFE) is a green, non-selective, and solvent free technique [44]. Carbon dioxide is applied in the SFE because it is cheap, non-flammable, has low critical temperature (31.1°C) and pressure (73.8 bar) and has a polarity appropriate for extraction of non-polar materials such as EO [45]. **Figure 3** illustrates a flow diagram of SFE.

This extraction technique, avoid degradation of thermolabile compounds compared to other techniques. However, the SFE has the disadvantage of high investment and operating costs [46].

Quintana et al. [47] reported that SFE produces high yield extracts with higher quality but lower concentration of volatile compounds compared with HD technique.

The efficiency of SFE process is affected by the following independent variables: particle size of the material, pressure, temperature, co-solvent and time [48].

The most appropriate particle size of the ground material is within the range of 0.4–0.8 mm [49]. The decrease of particle size (diameter) increases surface area of the material that is subjected to fluid CO₂ and enhances the extractability of the target components. Too small particle size reduces extract yield due to agglomeration of particles and reduction of surface area [50]. Under SFE conditions, increasing pressure at a specific temperature increases the CO₂ density, and consequently the solubility of the target compounds. However, increasing pressure above a certain level could result in a higher solubility of waxes and other hydrocarbons besides essential oil components. Meanwhile, increased temperature at constant pressure decrease the CO₂ density, and reduce the extraction yield though it increases the vapor pressure of

Figure 3.
A flow diagram of supercritical fluid extraction by CO₂.

the EO [51, 52]. The SFE temperature is used in the range 35–50°C to avoid degradation of thermolabile compounds. Since CO₂ is nonpolar, addition of small amounts of co-solvents (polar modifiers) such as methanol and ethanol increase the solubility of more polar compounds (phenolic compounds). The moisture content of the plant material can be used as a modifier. However, the modifier has to be separated from the resulted extract [45].

Markom et al. [53] studied the effect of co-solvents on the efficiency of SFE of EO from *Polygonum minus* dried leaves. The co-solvents used were: water, methanol, ethanol, and aqueous solutions of methanol and ethanol. The SFE was performed at 40°C and pressure of 150 bars. The CO₂ flow rate was 3 mL/min while the co-solvents flow rate was adjusted at 0.3 mL/min. The static and dynamic periods were set for 20 min and 240 min, respectively. The highest extraction yields (>25%) were obtained by the aqueous solutions of methanol and ethanol while the lowest yields (<9%) were obtained by the pure alcohols. The yield reached ~20% when water was used as a co-solvent.

Ara et al. [54] used central composite design to optimize the extraction yield of EO from *Descurainia sophia* L. ground dried seeds by SFE. The independent factors were: pressure (100, 228 and 355 bar), temperature (35, 50 and 65°C), modifier volume (methanol) (50, 100 and 150 µL), dynamic time (10, 25 and 40 min) and static extraction time (10, 25 and 40 min). The EO yield was used as a response. They found that increasing static extraction time from 10 to 40 min at the same extraction conditions (100 bar, 35°C, dynamic time of 10 min, without modifier) caused a slight increase in the yield from 0.5 to 1.1%. Increasing extraction temperature from 35 to 65°C at the same extraction conditions (100 bar, dynamic time of 40 min, static time of 10 min and modifier, 100 µL) resulted the same extraction yield (1.2%). Therefore,

temperature and static time were fixed at 65°C and 10 min, respectively. Increasing pressure from 100 bar to 228 bar, at the same extraction conditions (methanol, 100 µL and dynamic time, 25 min), increased the extraction yield from 2.07 to 10.4%, due to increase of CO₂ density, which increases the solubility of the target components. Increasing modifier volume from 50 to 100 and 150 µL, at the same extraction conditions (228 bar and dynamic time, 25 min), increased the extraction yield from 9.24 to 10.4 and 12.72%, respectively. They attributed this increase to the ability of polar modifier to increase the solubility of polar compounds in the CO₂ and consequently, increases the extraction yield. The highest yield (18.48%) was obtained at the optimum conditions (355 bar, 65°C, static time of 10 min, dynamic time of 35 min and modifier volume of 150 µL).

Oliveira et al. [55] extracted EO fractions from *Piper divaricatum* dried leaves using SFE at temperature of 35°C and 55°C, and pressure of 100, 300 and 500 bar. Increasing pressure from 100 bar to 300 bar at 35°C caused an increase in the CO₂ density from 712.8 to 929.1 kg/m³, which consequently increased significantly the EO yield from 4.68 to 6.03% dry weight basis. Further increase in pressure to 500 bar, at the same temperature, did not significantly increase the yield, though the CO₂ density increased to 1005 kg/m³. The same trend was also noted when SFE was performed at 55°C using the same investigated levels of pressure. The yields at 55°C were found to be higher than those at 35°C. The highest yield (7.15%) was obtained at 55°C/300 bar instead of 3.03% that obtained after 3 h of HD. The highest concentrations of eugenol (21.7%) and methyl eugenol (61.85%) and the lowest concentration of eugenyl acetate (4.35%) were recorded for the EO obtained by HD. The concentration of eugenol did not exceed 13.2% in the extracts obtained by SFE. The SFE extracts were characterized by higher level of eugenyl acetate (>14.75%).

Marzlan et al. [56] extracted the EO from dried ground Torch ginger inflorescence with SFE at combinations of temperature of 34.7, 38, 46, 54, and 57.3°C and pressure of 83.6, 125, 225, 325 and 366.4 bar. The static and dynamic times were 2 h and 1 h, respectively. At a constant temperature (46°C), increasing pressure from 83.6 bar to 225 bar caused an increase in the EO yield from 0.65 to 5.2%, while continual increase in pressure to 366.4 bar resulted in a decrease in EO yield to 4.16%. On the other hand, at a constant pressure (225 bar), increasing temperature from 34.7 to 46°C caused an increase in the EO yield from 3.68 to 5.21%, while continual increase in temperature to 57.3°C resulted in a low increase in EO yield to 5.72%.

Silva et al. [57] obtained the EO from dried ground leaves of *Lippia thymoides* by SFE at 40 and 50°C, and pressures of 100, 200, and 300 bar. The static and the dynamic periods were 30 min and 120 min, respectively. The EO yield was 1.29% (w/w) at 200 bar and increased to ~1.6% (w/w) at 300 bar, regardless of the temperature used. The thymol (the major constituent, >74%) and oxygenated monoterpene contents of the EO obtained at 50°C were higher than those extracted at 40°C, regardless the level of pressure used.

3. Essential oil as a green preservative and flavoring agent

3.1 Essential oil as antioxidant agent

Oxidation of food products during processing and/or storage causes undesirable changes. It affects negatively nutritional quality and consumer acceptability (color changes and off-flavors). Antioxidants at low concentrations can delay

oxidative reactions and extend the shelf life of the food products [58]. Many essential oils have antioxidant properties through scavenging of free-radicals and singlet oxygen quencher [59–62]. Essential oil constituents like thymol, eugenol, carvacrol, linalool, 1,8-cineole, geranial/neral, citronellal, isomenthone, and menthone are potent antioxidants. They can convert free radicals into more stable compounds by the addition of hydrogen atoms [63]. Strong antioxidant activity of essential oils is attributed to their phenolic structure [64].

The peroxide value (PV) is used for assessing the early stages of fat oxidation. Meanwhile, the thiobarbituric acid (TBA) value represented the secondary product (Malondialdehyde (MDA) of oxidation of polyunsaturated fatty acids. Direct addition of peppermint essential oil to refined soybean oil (without synthetic antioxidant) at 200 ppm or packaging it in a high density polyethylene package incorporated with 3700 ppm peppermint essential oil kept its oxidative stability not significantly different from that containing 200 ppm butylated hydroxyl toluene (BHT) during storage for 45 days at 40°C [65]. They attributed this activity to the main constituents: L-menthol, menthone and isomenthone of essential oil. Mezza et al. [66] found that addition of rosemary essential oil, obtained by hydrodistillation, to refined sunflower oil at 0.1 g/100 g extended its shelf life from 26 days to 36 days during storage in a dark place at 23°C, in presence of air. Meanwhile, the residue fractions of the essential oil, obtained by molecular distillation, displayed a longer shelf life that reached 44 days at the same storage conditions. The levels of less volatile constituents (camphor, α -terpineol and *cis*-sabinene hydrate) increased progressively with successive stages of molecular distillation. Okhli et al. [67] investigated the antioxidant properties of Citron peel essential oil, obtained by steam distillation, on sunflower oil at 800 ppm during storage at 65°C for 5 days. Oxidative stability of oil samples was measured with a Rancimat apparatus. Oxidative stability of oil samples enriched with essential oil (3.39 h) was higher than that of oil supplemented with 200 ppm BHT (3.0 h) at the end of storage period.

Immersion of Atlantic mackerel (*Scomber scombrus*) fillets in 1% (*w/v*) basil (*Ocimum basilicum*) and rosemary (*Rosmarinus officinalis*) essential oils for 30 min at 2°C and stored at the same temperature after packing into air-proofed polyamide/polyethylene packs delayed the development of lipid oxidation. TBA of the treated samples was followed during storage. TBA value exceeded the acceptable level (~5 mg MDA/kg of fish flesh according to Bensid et al. [68] after 8, 10, and 11 days for the control, rosemary, and basil groups, respectively. The basil and rosemary essential oils extended the shelf life of the fish fillets by 2 and 3 days, respectively, compared to the control group [69].

Boskovic et al. [70] evaluated the efficacy of thyme and oregano essential oils in retarding lipid oxidation of minced pork stored in modified conditions (vacuum and 30% O₂ conditions) at 3 ± 1°C during 15 days of storage. Minced pork samples were homogenized with different concentrations (0%, 0.3%, 0.6%, and 0.9%) of thyme or oregano essential oils. The control mince was prepared without essential oils. Essential oils reduced significantly (*p* < 0.05) the TBARS (mg malondialdehyde/kg) values in the mince even at the low level (0.3%). Minced samples with this concentration of essential oil were sensory acceptable. The antioxidant activity was attributed to the phenolic compounds in the investigated volatile oils.

Dipping raspberry fruits into lemon verbena essential oil emulsion at 250 µl/L for 3 min reduced the damage caused by reactive oxygen species during cold storage at 4°C for 9 days and extended shelf life of the fruits. The use of this essential oil as an edible coating increased antioxidant activity measured by inhibition of 2,2-diphenylpicrylhydrazyl (DPPH) radicals from 50.99 to 85.63% [71].

The DPPH radical-scavenging activity of edible films based on konjac glucomannan (KGM) polysaccharide loaded with thyme essential oil (TEO) at 0.4, 0.8, 1.2 and 1.6% (*v/v*) increased significantly ($p < 0.05$) with the increase of TEO concentration. Loading KGM-based films with TEO at 1.6% increased its antioxidant capacity by about 50% [72]. They suggested using these films loaded with TEO in food packaging.

Food grade nano-emulsions have been widely used to enhance the water solubility and stability of essential oils [73]. The cellulose nanofibrils films prepared by O/W Pickering emulsion with oregano essential oil exhibit excellent antioxidant activity [74]. They attributed this activity to phenolic compounds of oregano essential oil and recommended these films for packaging of easily oxidized foods.

Cinnamon and clove essential oils improved the antioxidant capacity of mandarin (*Citrus reticulata*) essential oil nanocapsules by 4.43 and 3.52 times, respectively. This increment of antioxidant activity was attributed to the high antioxidant activity of cinnamon and clove essential oils components [75].

Nanoemulsion-based basil seed gum (NBSG) films containing clove essential oil (CEO) had higher antioxidant activity than that of BSG films prepared by conventional method with the same concentration of CEO. The DPPH and ABTS radical scavenging activities of NBSG-CEO films containing 10 mg CEO/mL were not significantly different from those of NBSG-BHT films containing 1 mg BHT/mL. Eugenol is the main constituent of CEO. Wrapping minced camel meat sample with NBSG films containing resveratrol (4 $\mu\text{g/mL}$) + CEO (10 mg/mL) kept its oxidative stability after 20 days of storage at 4°C better than that of the control group. Peroxide and thiobarbituric acid (TBA) values of the NBSG wrapped meat samples did not exceed 4.03 meq/kg lipid and 1.03 mg malondialdehyde/kg after 20 days of cold storage [76].

Kiralan et al. [77] flavored olive oil with peppermint, oregano, thyme and laurel essential oils at 0.05% (*v/w*). GC-MS analysis of the headspace of flavored samples was carried out after 15, 30, and 45 days of storage at 60°C. The major components of essential oil transferred into olive oil samples. *E*-2-hexenal and hexanal were the main volatile constituents of olive oils during oxidation. After 30 days of thermal oxidation the *E*-2-hexenal level of the control and the peppermint flavored oil exceeded its initial level by 30 and 90 times, respectively. Flavoring olive oil with oregano, thyme and laurel essential oils kept *E*-2-hexenal level in the headspace of flavored samples during thermal oxidation lower than 2 times its original level.

3.2 Essential oil as antimicrobial agent

EOs with antimicrobial activity inhibit the microbial cells reproductive ability, or damage bacterial cells [78, 79]. Hydrophobicity/lipophilicity property of EOs allows them to cross the cell cytoplasmic membrane and raising permeability of fatty acids, polysaccharides, and phospholipid layers [80]. This causes leakage of cell contents, and loss of macromolecules.

EOs have the ability to coagulate the cytoplasm and inhibit enzymes responsible for the synthesis of biologically active components [81]. Gram positive-bacteria are more sensitive to EOs effect than gram-negative ones, since the outer membrane surrounding the cell wall of gram negative-bacteria, restricts the penetration of EOs through the lipopolysaccharide layer [62].

EOs rich in phenolic compounds like thymol, carvacrol or eugenol display high antimicrobial activities against foodborne pathogens [82, 83]. Aromatic plants that are rich in these phenolic compounds are illustrated in **Figure 4**.

Figure 4. Examples of some aromatic plants rich in thymol, carvacrol and eugenol as phenolic components found in essential oils of these plants.

These phenolic compounds attack the amine groups in the cell membrane, alter its permeability leading to cell lysis [7, 84]. Moreover, a synergistic effect between EOs components enhances its antimicrobial efficiency. The synergistic effect between ρ -cymene and carvacrol, geraniol and menthol is a good example against wide bacteria range (Figure 5).

This antimicrobial efficiency was significantly weaker when each compound acted separately in the same medium. Some EO constituents do not possess antibacterial properties when used alone, but they enhance the bacterial inhibition of other compounds [85].

The incorporation of lemongrass (*Cymbopogon citratus*) essential oil (LEO) into chitosan-based films at 9% level controlled the growth of Gram-positive bacteria (*B. cereus* and *L. monocytogenes*) and Gram-negative bacteria (*E. coli* and *S. typhi*). The antibacterial activity of the films was evaluated using the disk-diffusion method. The chitosan/LEO composite film with 9% LEO completely inhibited the growth of *S. typhi* [86].

Chitosan, gum arabic, and polyethylene glycol composite film incorporated with black pepper essential oil or ginger essential oil possessed high antimicrobial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium* [87].

Figure 5.
Schematic representation the synergistic effect of *p*-cymene and carvacrol, geraniol and menthol mechanism of action as antimicrobial.

Fattahian et al. [88] investigated the antimicrobial activity of cumin essential oil (EO) against *Staphylococcus aureus* and *Escherichia coli* O157:H7. They found that *Staphylococcus aureus* was more sensitive to EO than *E. coli* O157:H7. Meanwhile, they studied the effect of coating fresh veal fillets with a biodegradable film of chitosan (CH) incorporated with cumin nanoliposomal EO at 1% level on the microbial properties of veal samples stored in modified atmosphere packages (20% CO₂ and 80% O₂) at 4°C for 21 days. The total microbial count, lactic acid bacteria, enterobacteriaceae, and pseudomonas were used as microbiological indicators. Encapsulation of cumin EO controlled the release of the antimicrobial compounds on coated samples that extended antimicrobial activity during cold storage for 21 days compared to free EO. At the end of storage, the investigated bacterial strains count of the CH + Nano EO coated samples were lower than those of CH + EO groups. Both coating films kept the bacterial load of meat fillets less than the CH group till the end of storage time.

The antibacterial activity was attributed to cuminaldehyde and the phenolic compounds in cumin essential oil and the synergistic effect of chitosan with EO.

Langroodi et al. [89] coated turkey breast fillet with chitosan incorporated with 1% (v/v) of *Origanum vulgare* essential oil and dried ethanolic extract of grape seeds (GSE, 2% v/v) before storage at 4°C for 20 days. Alterations in total viable count (TVC), lactic acid bacteria (LAB), Enterobacteriaceae, *Pseudomonas* spp., and yeast-mold counts of cold stored turkey meat samples and sensorial properties of roasted (10 min at 100°C) turkey samples were studied. Incorporation of oregano EO and GSE into chitosan increased the antibacterial activity of the coating film. The TVC counts of control and chitosan coated samples turned unacceptable (>6 CFU/g) after 12 and 16 days of storage, respectively. Coating with chitosan containing oregano essential oil and GSE kept TVC count of the samples less than 5 Log CFU/g after 20 days of storage. At the end of cold storage, the LAB and Enterobacteriaceae counts of samples coated with chitosan-oregano essential oil and GSE were ~ 4 and 4.39 Log CFU/g instead of 7.22 and 7.14 Log CFU/g for the chitosan coated samples, respectively. Furthermore, inclusion of oregano essential oil and GSE into chitosan coating reduced the Pseudomonads counts in the samples by ~3 Log CFU/g, at the end of storage time. Application of oregano EO at 1% level and GSE at 2% enhanced the antifungal activity of chitosan coating. Yeast-mold count of turkey meat samples coated with chitosan-oregano EO-GSE did not exceed 4.27 Log CFU/g at the end of storage time. They attributed this strong antibacterial activity of the coating film to the synergistic effect of oregano EOs and GSE. CH-GSE 2%-O coated samples depicted the highest consumer scores compared with other samples till the end of storage.

Sayadi et al. [90] packaged fresh chicken pieces (2 cm thickness and 20 cm length) in plain and nano composite edible films of gelatin (GE) containing 1% TiO₂ nanoparticles (GE + TiO₂), 2% cumin essential oil (GE + CEO), and 1% TiO₂ + 2% CEO (GE + TiO₂ + CEO) before storing in polyethylene plastic bags at 4 ± 1°C for 24 days. The population of total mesophilic bacteria, Enterobacteriaceae, lactic acid bacteria, and *Pseudomonas* spp., of packaged samples were evaluated. The bacterial growth (for different bacteria) in the control increased by ≥4 log CFU/g, after 24 days of storage. At the end of storage time the lowest population (<6 log CFU/g) of the tested bacteria was recorded for GE + CEO and GE + TiO₂ + CEO chicken samples. They attributed the antimicrobial activity to cuminaldehyde component and phenolic compounds of CEO in addition to reactive oxygen species generated by TiO₂-N that disrupt the bacteria membrane. Although the GE + CEO and GE + TiO₂ + CEO groups obtained the highest sensory scores among chicken samples, both groups showed unacceptable scores of sensory attributes (odor and overall acceptability) after 16 days of storage.

Sharma et al. [91] tested essential oils of clove bud, tagetes, thyme, eucalyptus, neem, cinnamon leaf, himalayan pine needle, and tea tree against the total bread molds by agar well-diffusion method. Thyme oil completely inhibited the growth of bread molds than other essential oils. They found that sealing fresh white slices of bread in a biodegradable film (poly, 3-hydroxybutyrate-co-4-hydroxybutyrate) incorporated with thyme essential oil at 30% (v/w) extended its shelf-life against molds to >5 days at ambient conditions (25–28°C and 35–45% RH), compared to 1–4 days in neat biopolymer film. The molds count of the bread packaged in this film after 5 days of storage was <1.00 log (CFU/mL), the same level at the zero-time storage.

3.3 Essential oil as flavoring agent

Flavoring is one of the main application of essential oils in the food and beverage industries [81]. Flavorings are used to improve the odor of foods in order to satisfy the consumer. EOs are used in the preparation of carbonated beverage to give the product its distinctive aroma.

Recently, flavored edible oils are produced in order to improve its sensory properties [92, 93]. Flavoring of oils is carried out by infusion or maceration of the aromatic plant into the oil [94, 95]. These flavoring techniques enhance the extraction of waxes and undesirable components into oil [96]. To overcome these defects, EOs have been used as flavoring materials [97]. However, strong flavors with some EOs may negatively affect the consumer acceptability of the food. The flavoring of edible oils improves their sensory properties [92], increase their use for the preparation of daily food condiments [98] and extend their usage by non-traditional consumers [99].

Porto and Decorti [100] flavored ricotta cheese with thyme essential oil by mixing at 0.26, 0.33 and 0.40% (*w/w*). The main constituents of the essential oil were carvacrol, carvacrol methyl ether, *p*-cymene, γ -terpinene and thymol. Sensory studies indicated that the minimum perception level of thyme essential oil in ricotta cheese was 0.20% (*w/w*). Aroma compounds of the flavored ricotta cheese were extracted by Headspace solid-phase micro-extraction at 30°C and were identified with GC-MS. Results showed that hydrocarbons monoterpene and hydrocarbons sesquiterpene were lower in the headspace of ricotta by 25% and 40%, respectively, compared to their original level in essential oil. They attributed these decrements to the binding capacity of fat and proteins to flavor compounds.

Benkhoud et al. [101] flavored extra virgin olive oil by homogenization with Eos (500 ppm) of thyme, rosemary, black pepper, fennel, and citrus peels. Flavored oil samples were stored for 12 months, at room temperature. The headspace of flavored oil enriched mainly with the major components of the flavoring essential oils. They attributed the bitterness of rosemary, thyme, and fennel flavored samples to the presence of 1,8-cineole and carvacrol while pungency of black pepper flavored samples was ascribed to β -caryophyllene and α -phellandrene. Citrus-flavored oil samples were distinguished by their fruity taste due to limonene. The highest acceptability scores were recorded for fennel and citrus flavored oil samples.

Moustakime et al. [99] flavored virgin olive oil (VOO) with the seeds of green anise. The main component of the anise seeds EO was found to be *trans*-anethole (76.16%). This compound was used as an indicator for the level of flavoring. Flavoring of VOO was carried out with anise seeds at a ratio of 15% (*w/w*) with maceration, sonication (intensity $\sim 1 \text{ W/cm}^2$) and direct addition of the EO (0.33 mL, equivalent to amount of oil from 15 g seeds) using stirring for 24 h. GC/MS analysis indicated that the diffusible *trans*-anethole reached 26.59% of the total volatile fraction of the flavored oil after 15 min of ultrasound treatment instead of 23.85% after 9 days of maceration. Meanwhile, *trans*-anethole level of the total volatile fraction reached 36.3% by direct addition of EO.

4. Conclusions

The ecofriendly techniques meet the terms of green extraction, reduces extraction time, with high yield, low energy consumption and solvent amount, allows the use

of renewable natural products, and ensures a safe and high-quality essential oil. The addition of essential oils to food as green preservative causes many positive effects such as antioxidant, antimicrobial activities and improve the flavor in food products. This effect could be due to the synergistic combination of the essential oil constituents rather than one component.

Conflict of interest

“The authors declare no conflict of interest.”

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