KONYA FOOD AND AGRICULTURE UNIVERSITY

INSTITUTE OF GRADUATE EDUCATION

DEPARTMENT OF FOOD ENGINEERING

INVESTIGATION OF THE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *EUCALYPTUS RADIATA* LEAF ESSENTIAL OIL AND NANOEMULSION*: IN VITRO* AND MEAT APPLICATION

MASTER'S THESIS

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This study titled "**Investigation of the antimicrobial and antioxidant activity of *Eucalyptus radiata* leaf essential oil and nanoemulsion: *in vitro* and meat application**" prepared by A*****r Ö*****N has been accepted with consensus/ majority by the following jury on 10/06/2024 as a **MASTER'S THESIS** in the DEPARTMENT OF FOOD ENGINEERING.

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ABSTRACT

INVESTIGATION OF THE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *EUCALYPTUS RADIATA* LEAF ESSENTIAL OIL AND NANOEMULSION: *IN VITRO* AND MEAT APPLICATION

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Master of Science Thesis, Department of Food Engineering

Supervisor: Asst. Prof. A**a G*******U

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In this study, the antimicrobial and antioxidant activities of *Eucalyptus radiata* essential oil and its nanoemulsion were examined in in vitro assays. Besides, the antimicrobial potential of the nanoemulsion was assessed against pathogenic bacteria and spoilage organisms in fresh meat. The results of the GC-MS showed that o-cymene, 2-bornene, and 1,8-cineole were the most abundant compounds. The nanoemulsion characteristics were droplet size: 52.04±31.44 nm, zeta potential: −9.16 mV, viscosity: 82.67 mPa·s., and whiteness index: 48.91. The IC_{50} values of eucalyptus oil and its nanoemulsion were 53.71 and 240.41 µg/mL, respectively. *Bacillus cereus* NRRL B3711 and *Staphylococcus epidermidis* ATCC 12228 bacteria showed the highest inhibition area at 24 mm and 20.5 mm in the disc diffusion assay. Similarly, the same bacteria also had lower minimum inhibitory concentration values. In the time-kill assay, the nanoemulsion inhibited *Escherichia coli* 7 $\log_{10}$ CFU/g after 60 min of interaction time, while only a 1 $\log_{10}$ CFU/g reduction was achieved for *S. aureus*. The dipping of fresh beef pieces into the nanoemulsion was very successful as an antimicrobial application. The nanoemulsion caused a significant ($P \leq 0.05$) reduction in the *S. aureus* count in contaminated fresh beef pieces. In addition, the nanoemulsion controlled ($P \leq 0.05$) the growth of total coliform, *Pseudomonas* spp., yeast, and mold counts and extended the shelf life of fresh beef pieces by 4 days. This study has shown that *E. radiata* oil nanoemulsion can be used in the food industry to extend the shelf life and enhance the safety of meat products.

Keywords: biomaterial, natural antimicrobials, dipping, microwave extraction, beef, shelf life.

ÖZET

INVESTIGATION OF THE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *EUCALYPTUS RADIATA* LEAF ESSENTIAL OIL AND NANOEMULSION: *IN VITRO* AND MEAT APPLICATION

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Bu tez çalışmasında *Eucalyptus radiata* esansiyel yağı ve nanoemülsiyonunun antimikrobiyal ve antioksidan aktiviteleri in vitro analizlerde incelenmiştir. Ayrıca nanoemülsiyonun patojen bakterilere ve bozulmaya neden olan organizmalara karşı antimikrobiyal potansiyeli taze ette değerlendirilmiştir. GC-MS sonuçları o-simen, 2-bornen ve 1,8-sineolün en bol bulunan bileşikler olduğunu gösterdi. Nanoemülsiyon özellikleri damlacık boyutu: 52,04±31,44 nm, zeta potansiyeli: −9,16 mV, viskozite: 82,67 mPa.s. ve beyazlık indeksi: 48,91'dir. Okaliptüs yağı ve nanoemülsiyonunun IC_{50} değerleri sırasıyla 53,71 ve 240,41 µg/mL idi. *Bacillus cereus* NRRL B3711 ve *Staphylococcus epidermidis* ATCC 12228 bakterileri, disk difüzyon testinde 24 mm ve 20,5 mm'de en yüksek inhibisyon alanını gösterdi. Benzer şekilde aynı bakterilerin minimum inhibitör konsantrasyon değerleri de daha düşüktü. Zamana bağlı öldürme testinde nanoemülsiyon, 60 dakikalık etkileşim süresinden sonra *Escherichia coli*'yi tamamen inhibe ederken, *S. aureus* için yalnızca 1 log CFU/g azalma elde edildi. Taze sığır eti parçalarının nanoemülsiyona batırılması antimikrobiyal uygulama olarak oldukça başarılı olmuştur. Nanoemülsiyon, kontamine taze sığır eti parçalarındaki *S. aureus* sayısında önemli ($P \leq 0.05$) bir azalmaya neden oldu. Ayrıca nanoemülsiyon toplam koliform, Pseudomonas spp., maya ve küf sayımlarının büyümesini kontrol altına almış ($P \leq 0.05$) ve taze sığır eti parçalarının raf ömrünü 4 gün uzatmıştır. Bu çalışma, *E. radiata* yağı nanoemülsiyonunun gıda endüstrisinde raf ömrünü uzatmak ve et ürünlerinin güvenliğini arttırmak için yaygın olarak kullanılabileceğini göstermiştir.

Anahtar kelimeler: biyomateryal, doğal antimikrobiyeller, daldırma, mikrodalga ektraksiyonu, dana eti, raf ömrü

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A*****r Ö*****N

Jun, 2024

TEXT OF OATH

The study I presented as my master of science thesis titled “Investigation of the antimicrobial and antioxidant activity of *Eucalyptus radiata* leaf essential oil and nanoemulsion: in vitro and meat application” has been written without applying to any assistance inconsistent with the scientific ethics and traditions, and all sources I have benefited from were listed in bibliography and I have been used them by means of making references and I declare and confirm this with my honor.

10/06/2024

A*****r Ö*****N

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LIST OF ABBREVIATIONS

ABBREVIATONS

Eos	Essential oils
MAE	Microwave extraction
DPPH	2,2-Diphenyl-1-picrylhydrazyl
O/W	Oil in water
W/O	Water in oil
FSIS	Food Safety and Inspection Service
MHA	Mueller-Hinton agar
BPA	Baird-Parker agar
VRB agar	Violet red bile agar
MRS	de Man, Rogosa, and Sharpe
WI	Whiteness index
MIC	Minimum inhibitory concentration
Amu	Atomic mass units
CFU	Colony forming units
GC-MS	Gas Chromatography–Mass Spectrometry
E.radiata	*Eucalyptus radiata*
S.aureus	*Staphylococcus aureus*
L. monocytogenes	*Listeria monocytogenes*
E. coli	*Escherichia coli*
E. gillii	*Eucalyptus gilii*

SYMBOLS

Mm	Micrometer
Nm	Nanometer
Min	Minute
W	Watt
°C	Centidegrees
M	Meter
Mm	Milimeter
Cm	Centimeter
S	Second
%	Percent
G	Gram
Mg	Miligram
eV	Electron Volt
G	Gram
Hz	Hertz
W	Weight
V	Volume
Ml	Mililiter
L	Liter
L*	Lightness Value

a*	Red-Green Value
b*	Yellow-Blue Value
mPa.s	Millipascal Second
mV	Millivolt

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1. INTRODUCTION

Essential oils (EOs) obtained from different plants by different methods are secondary plant metabolites. Secondary metabolites are compounds that are not necessary for the survival of an organism in processes such as growth and reproduction. They play an important role in the interaction of the organism with its environment, can affect the smell and colors of the plant, help plants fight against pests, and protect plants against environmental stress (Bennett, & Wallsgrove, 1994; Pagare et al., 2015). Secondary metabolites in plants can be divided into different groups such as alkaloids, phenolic compounds, terpenoids, glycosides, etc. Essential oils obtained from plants are in the class of terpenoids produced from isoprene units (Gershenzon, & Dudareva, 2007; Pandita, & Pandita, 2021). Plant secondary metabolites can be classified in different ways based on their solubility and chemical structure. The basic classification is given in Figure 1.1.

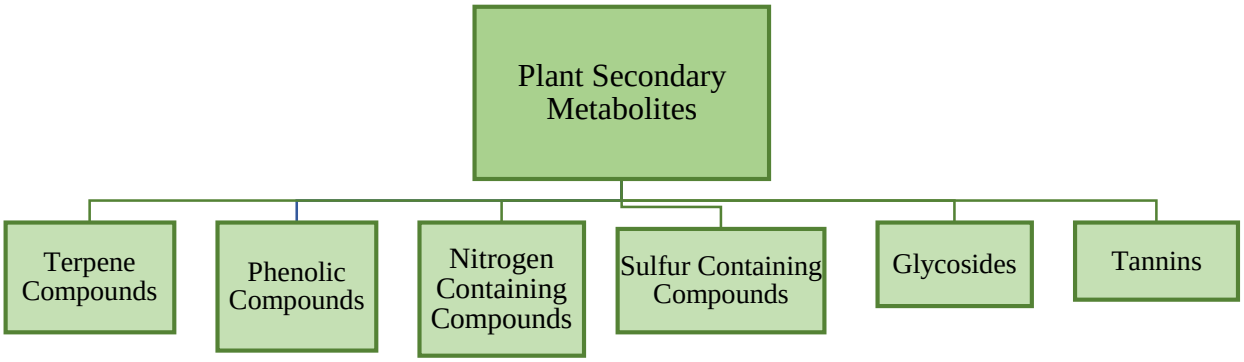

Figure 1.1. Classification of plant secondary metabolites

(Das, & Gezici, 2018; Nawrot-Chorabik et al., 2022)

The basic compounds of essential oils are terpenoids, which are organic compounds and aromatic molecules found naturally and widely in the plant kingdom. Terpenoids consist of multiple isoprene (C_5H_8) units and also contribute to the biological activities of essential oils. In addition to their therapeutic and antimicrobial activities, essential oils can be used for flavoring purposes, and those with a pleasant scent can be used in perfumery (Nazzaro et al., 2013; Sharmeen et al., 2021).

Essential oils have different chemical compositions depending on the nature of the plant where it grows and the season in which it is harvested (Iacobellis et al., 2005; McGimpsey et al., 1994). It has been seen in studies that essential oils obtained from plants such as eucalyptus, coriander, and cumin have antibacterial properties (Jahan et al., 2011; Sodzim, 2012).

Eucalyptus, belonging to the *Mytaceae* family, is an aromatic, fragrant tree. *Eucalyptus*, which generally grows in subtropical regions, is thought to secrete compounds that have antagonistic effects against various microorganisms (Takahashi et al., 2004). Due to its analgesic and anti-inflammatory effects, eucalyptus leaves are used in pharmacy, especially in the treatment of colds and throat and nose disorders.

The essential oil obtained from *Eucalyptus radiata*, which belongs to the *Eucalyptus* genus, is rich in 1,8-cineole (eucalyptol) (Ogunwande et al., 2003). The compound, which has anti-inflammatory and antioxidant effects, works by showing different pathways and mechanisms, and studies on this subject are continuing in the pharmacological field (Cai et al., 2021).

Emulsions, are thermodynamically unstable systems, in which two insoluble substances are held together with the help of an emulsifier. The term thermodynamically unstable describes the tendency of the dispersed phase to combine and separate from the continuous phase (Kabalnov, 1998).

Emulsifying agents ensure the stability of the system by reducing the interfacial tension between the dispersed and continuous phases (McClements & Jafari, 2018).

Emulsions are divided into three main groups according to particle sizes: macroemulsion, microemulsion, and nanoemulsion (Solans et al., 2003). Nanoemulsions have smaller particle sizes than macroemulsions and microemulsions; they are submicron-sized emulsions, and their effect is more effective because the penetration area is wider (Shah et al., 2010).

In this study, *in vitro* and *in situ* studies of *Eucalyptus radiata* essential oil and nanoemulsion on fresh red meat, which is microbiologically risky, are included. For this, *E.radiata* leaves were subjected to microwave extraction, and the chemical composition of the resulting essential oil was determined. Although essential oils are obtained with different methods, microwave extraction, which was used in this study, differs from other traditional methods in that it has a shortened extraction time, less or no solvent consumption, no need for toxic solvents, and less environmental pollution (Li et al., 2012; Michel et al., 2011; Veggi et al., 2012). Nanoemulsion was prepared with essential oil (EO) using pectin as a coating material, and the characteristic properties, antimicrobial properties, and antioxidant activity of essential oil nanoemulsion were determined. Broth dilution, disc diffusion, and time-kill assay procedures were applied in *in vitro* studies to assess antimicrobial activity. In the part of the study to be carried out with red meat, the efficacy of the nanoemulsion was tested against *Staphylococcus aureus*-contaminated meat as an antimicrobial dipping agent. Lastly, shelf-life studies were performed by evaluating the counts of total coliform, *Pseudomonas* species., lactic acid bacteria, yeast, and mold.

2. LITERATURE REVIEW

2.1. *Eucalyptus radiata* Plant

Eucalyptus is a large genus of the *Myrtaceae* family, containing approximately 900 species and subspecies. *Eucalyptus* species attract attention due to their potential therapeutic effects, such as antimicrobial activity, anti-inflammatory effects, and stress relief (Mahumane et al., 2016). Food and plants have been used for therapeutic purposes for centuries due to their health potential. Today, plants and plant extracts are frequently used in wide areas, such as health, cosmetics, and the food industry.

One of the plant genera whose essential oil is most researched in the literature is the *Eucalyptus* species. *Eucalyptus radiata,* or *Eucalyptus australiana,* also known as narrow-leaf mint and belonging to the *Myrtaceae* family, is a species of *Eucalyptus* native to southeastern Australia. *Eucalyptus radiata* is one of the well-known species from which eucalyptol-rich essential oil is obtained (Bendaoud et al., 2009; Luís et al., 2016). *Eucalyptus radiata* is a tall, perennial tree, typically growing to heights between 20 and 30 m. Its bark is smooth and can appear whitish or gray, shedding in strips to expose a light green or cream-colored trunk. The foliage consists of slender, lance-shaped leaves arranged alternately along the branches. This species bears clusters of petite, creamy white flowers, which are known to attract various pollinators, including bees (Boland et al., 2006).

2.1.1. Chemical Composition of *Eucalyptus radiata* Essential Oil

Essential oils are odorous, aromatic liquids extracted from different parts of the plant, such as flowers, roots, and leaves. They are volatile structures with a characteristic and persistent scent and are secondary metabolites with antimicrobial and antioxidant potential (Abdelhafed et al., 2015; Ahmad et al., 2021). The antimicrobial activity of essential oils is mostly associated with the presence of terpenes and phenolic compounds. Essential oils can be obtained from plants by different methods, such as

distillation, extraction, and pressing. They have been used in various cultures for centuries due to their therapeutic properties and pleasant scent (Bakkali et al. 2008). In recent years, essential oils have gained popularity as natural remedies for a wide variety of ailments and as ingredients in personal care products. They are also the subject of research due to their antimicrobial and antioxidant properties.

The essential oil obtained from *Eucalyptus* species is rich in eucalyptol, or 1,8-cineole. It generally constitutes a large part of the chemical composition of the oil. It is responsible for the dominant aroma and smell of eucalyptus oil (Bakkali et al., 2008; Dogan et al., 2017).

Eucalyptus radiata oil also contains significant amounts of α-pinene and limonene, which contribute to its scent and therapeutic benefits. α-pinene has been shown to have anti-inflammatory properties, while limonene exhibits antimicrobial effects, increasing the oil's effectiveness in fighting infections and supporting the immune system (Gupta et al., 2021; Khoshnazar et al., 2020).

Eucalyptus radiata essential oil contains a complex mixture of bioactive compounds, of which 1,8-cineole is generally the predominant component. The various chemical compositions of the oil are effective in showing different therapeutic effects.

2.1.2. The Bioactivity and Application Area of *Eucalyptus radiata* Essential Oil

For centuries, essential oils have been used in cosmetic applications, medical complementary therapies, the agricultural sector, pharmacy, and the food industry due to their antibacterial, antimicrobial, and fungicidal effects and have potential for use in different sectors (Nazari et al., 2019; Mihai & Popa, 2013). Essential oils that can be extracted by various methods from different parts of plants, such as roots and leaves, contain terpenes and aromatic and aliphatic compounds associated with low molecular weight. The properties of essential oils can be classified according to the compounds found in them. While chemical structures such as limonene and myrcene show antibacterial, antiviral, and stimulant effects in hydrocarbon-containing essential oils, structures classified as esters such as geranyl acetate and bornly acetate show

antifungal and anti-inflammatory effects (Jugreet et al., 2020). Due to the compounds EOs contain, they are expected to reduce or prevent the growth of microorganisms and show antioxidant properties (Abad et al., 2012; Bakkali et al., 2008).

Essential oils are also preferred in the cosmetic and pharmaceutical industries due to their different effects. It is known that essential oils obtained from plants and fruits containing vitamin C have an improving effect on skin health (Sharmeen et al., 2021). D-limonene, which is abundant in citrus peels, is used in cosmetics, aromatherapy, and household industries due to its pleasant lemon-like smell (Bakkali et al., 2008; Ibáñez et al., 2020). Because it has good solvent properties and antimicrobial activity, it can be used in cosmetic formulations and is therefore preferred in facial cleansers and make-up removers. In skin care formulations, D-limonene acts as a natural emollient, imparting moisturizing properties to lotions, creams, and serums. Its antioxidant properties also contribute to skin protection against oxidative stress and environmental aggressors and control skin rejuvenation (Haiyan et al., 2016; Thombare, & Shirsath et al., 2023).

The food industry frequently encounters microbial hazards such as bacteria, yeasts, and molds in different sectors. It is generally aimed at preventing such risks by applying heat treatment, pasteurization, or sterilization. Such practices affect the organoleptic properties of foods and, in some cases, can also affect their nutritional value (Salanță, & Cropotova, 2022). In addition to its disadvantages, some food products are not suitable for heat treatment or may require the use of hurdle technologies (Khan et al., 2017).

In order to prevent contamination in meat, different chemicals, such as chlorine and ozone, are used. The purpose of using chemicals is to provide a bactericidal effect by inhibiting glucose oxidation (Dokuzlu, 2006). Ozone application, generally recognized as safe, has a strong sanitizing effect (Khanashyam et al., 2022). This practice has also disadvantages; considering its toxic effects, it is not accepted by consumers. In addition, it may cause the polyphenol content of the product to decrease and negatively affect its sensory properties by affecting its color and hardness. Moreover, there are some concerns with the use of chemicals in food industry. There is a demand for natural source additives and eco-friendly solutions by the consumers. As targeted in

this study, decontamination effects can also be seen in methods such as dipping, spraying, and coating using emulsions (Brodowska et al., 2018; Frewer et al., 2003; Miller et al., 2013).

Due to their antimicrobial and antioxidant activity, essential oils are considered a natural solution alternative instead of synthetic additives and preservatives used in the food industry for purposes such as extending the shelf life of foods and improving their organoleptic properties (Saeed et al., 2022). In addition to concerns such as microbiological and chemical spoilage, food-grade oils can also be used as natural flavorings. The volatility of essential oils, their pungent odor, and their unstable structure may be a challenge for industrial use (Maurya et al., 2021).

The use of essential oils in edible films for food applications has also been explored. For example, when the edible coating containing a mixture of oregano and thyme was applied to fresh ground beef, it showed an inhibitory effect on *E. coli, E. coli* O157:H7, *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Lactobacillus plantarum* (Emiroğlu et al., 2010). In another study, it was observed that green tea extract reduced the water activity, number of *S. aureus*, *Lactococcus*, and total bacteria in quark cheese (Pluta-Kubica et al., 2021). With the increasing demand of consumers for eco-friendly and clean-label foods, areas of use can be investigated through studies in the field, natural preservatives can be obtained, and their use can be expanded.

2.1.3. Extraction Methods of Essential Oils

Essential oils are obtained from different parts of many plants through various extraction methods. Depending on the plant type and extraction method, the yield and quality of the oil obtained may vary, and even the properties and bioactivity of the oil may change. Essential oils can be obtained by solvent extraction, distillation, microwave extraction, or a combination of solvent and steam (Tongnuanchan, & Benjakul, 2014).

Solvent extraction is a method for the extraction of essential oils. In this method, plants are treated with a solvent that can dissolve them (Stratakos & Koidis, 2016). Following the extraction process, the liquid blend containing the essential oil, among other constituents, undergoes filtration and subsequent distillation. Commonly utilized solvents for extraction encompass alcohol, hexane, ethanol, petroleum ether, and methanol. In this process, heat is not used, so the chance of degradation of compounds and the production of artifacts is lowered. Besides these properties, solvent extraction comes forward with its simplicity, being less time consuming, and cost-effectiveness (Adepoju, 2014). However, the use of chemical solvents is not beneficial for the environment (Geow, 2021).

In the distillation method, for releasing essential oils from plants, plants are treated with boiling water or steam (Reyes-Jurado et al., 2015). To get back the essential oils, they are distilled with water, which they do not mix with each other because of their density differences. This process is accomplished when the boiling point, combined with the vapor pressure, equals the ambient pressure. For the separation of essential oils and water, essential oils are evaporated at the temperature of water. Distillation methods are a common method for the extraction of essential oils; however, they have some disadvantages, such as that during the process, plants are treated with boiling water (Stratakos, & Koidis, 2016). Also, by heat exposure, some compounds might go through degradation.

Microwave extraction (MAE) is a promising method that shows a high yield with a lower cost and time (Cardoso-Ugarte et al., 2013). In this method, microwave energy, which is electromagnetic energy, is used for the extraction of plants (Chan et al., 2011). This energy has a direct influence on molecules via ionic conduction and dipole rotation (Eskilsson & Björklund, 2000). Heating the sample facilitates the transfer of analytes from the sample matrix to the solvent. This method is considered environmentally friendly due to minimizing the use of solvents during the process (Teo et al., 2013).

2.2. Emulsions

Emulsions are colloidal structures consisting of two immiscible liquids in the presence of surfactants, stabilized by an emulsifier. Therefore, it shows the properties of colloidal structures (Bibette et al., 1999). Metastability is achieved in emulsions by Brownian motion, thus providing kinetic stability in emulsions created with essential oils (Helgeson, 2016; McClements & Rao, 2011).

Emulsions are used in different industries, such as food, medicine, cosmetics, and dye. It is important to ensure stabilization for all areas of use, so appropriate emulsifying materials should be used (Tadros, 2016). Emulsifiers used in emulsion preparation can be adsorbed at the oil-water interface, reducing interfacial tension and preventing the agglomeration of droplets in the emulsion. Amphilic surface active molecules, that is, having both hydrophilic and hydrophobic properties, are surfactants responsible for reducing attractive forces and helping develop the emulsion structure (Ghosh et al., 2020; Ozturk & McClements, 2016).

Although essential oils are used in many areas, it is necessary to use an appropriate structure to ensure their stability and preserve their effects. Therefore, it is preferred to use essential oils in emulsions (Buranasuksombat et al., 2011; Donsì et al., 2012). By using essential oils in emulsions, the effect of uncontrolled distribution can be eliminated. By creating appropriate colloidal distribution systems, the effects of problems such as high volatility and low solubility in water can be eliminated (Hou & Sheng, 2023).

2.2.1. Classification of Emulsions

Emulsions, which are systems in which an immiscible liquid phase is dispersed as spheres in the second phase of the immiscible liquid, are basically divided into three classes based on their type (Fatima et al., 2014). These are oil-in-water emulsions, water-in-oil emulsions, and multiple emulsions. Water in oil emulsion is formed when water particles are dispersed in the oil phase; oil in water emulsion is formed when oil particles are dispersed in the water phase; and multiple emulsions are formed when o/w or w/o emulsions are dispersed in another phase (Hou & Sheng, 2023; Khan et al., 2011; Taha et al., 2020; Wong et al., 2015).

Emulsions are divided into three categories according to droplet size: macroemulsions, microemulsions, and nanoemulsions as shown in Table 2.1. Droplet sizes affect the disruption process of emulsions. Thus, important properties such as stability, flow properties, and viscosity are determined (Karbstein & Schubert, 1995).

- **Macroemulsions:** These are systems with droplet sizes ranging from 0.1 to 100 μm. Macroemulsions have larger droplet sizes that are visible to the naked eye (McClements, 2004). Their color is white or milky because they allow light to be dispersed. Macroemulsions are thermodynamically unstable and kinetically stable systems (Kundu et al., 2013). Stabilizers or emulsifying agents are often added to macroemulsions to increase their stability by reducing droplet coalescence and phase separation (Fatima et al., 2014).

- **Microemulsions:** These are systems with droplet sizes ranging from 10 to 100 nm (Madhav & Gupta, 2011). Microemulsions are isotropic liquid mixtures of oil, water, and surfactant. They are transparent and more thermodynamically stable than macroemulsions (Kumar & Mittal, 1999).

Nanoemulsions: Nanoemulsions are systems with very small droplet sizes, have high kinetic stability, and are generally applied for controlled release and drug delivery. Kinetically stable structures can be thermodynamically unstable. Thermodynamic

instability defines the ability of nanoemulsions to have less energy than their current energy, while the ability to remain without visible agglomeration defines their physical stability. Although the diameters of droplet sizes vary in different sources, they are less than 100 nm, and nanoemulsions are thermodynamically unstable systems (Çınar, 2017; Fatima et al., 2014; Fryd & Mason, 2012; Souto et al., 2022). Since nanoemulsions have small particle sizes, attractive forces are weaker, providing kinetic stability (Tadros et al., 2004).

Table 2.1 Comparison of macroemulsions, microemulsions and nanoemulsions

	Macroemulsions	***Microemulsions***	***Nanoemulsions***	***References***
Particle Size	Large >400 nm	Small 100-400 nm	Very Small 10-100 nm	(Callender et al., 2017; Souto et al., 2022)
Shape	Spherical Droplets	Spherical Droplets	Spherical Droplets, Lamellar, Disruption of bicontinuous structures is responsible for nanoemulsion formation	(Deverajan & Ravichandran, 2011; Fernandez et al., 2004; Gupta et al., 2016).
Stability	Thermodynamically unstable, kinetically weakly stable	Thermodynamically unstable, kinetically stable	Thermodynamically stable, kinetically stable	(Gupta et al., 2016).
Appearance	Turbid	Transparent	Transparent	(Thakur et al., 2018)

2.3. Red Meat

High-moisture red meat provides some of the nutrients required for the daily diet of a healthy individual. It is rich in amino acids, B-complex vitamins, iron, and zinc. It contains approximately 20% protein, 5% fat, and 1.5% carbohydrates (Darwish et al., 2024; Mann, 2000).

Due to its high moisture content, fresh meat is susceptible to microbial contamination and can cause foodborne diseases (Biswas et al., 2008). Methods such as drying, smoking, fermentation, refrigeration, and canning have been used in the past to extend shelf life during storage, and in the food industry, chemical, biological, and nonthermal

techniques are additionally used (Nychas et al., 2008). The important thing is to prevent undesirable physical, chemical, and microbial changes while extending the shelf life of the food, preserving its nutritional value, and ensuring that added food additives do not adversely affect consumer health. For this reason, food additives and preservatives are generally used in industrial production. For example, ascorbic acid, or vitamin C, has antioxidant properties, thus preventing the oxidation of meat and preventing its color from deteriorating (Sánchez-Escalante et al., 2001). Phosphoric acid's sodium, potassium, or calcium salts, in the form of diphosphates (E451), triphosphates (E451), and polyphosphates (E452) are frequently employed as additives (Younes et al., 2021).They are used as stabilizers and texture improvers in processed meat products. Additives such as sodium nitrate (E251), potassium nitrate (E252), sodium nitrite (E250), and potassium nitrite (E249), which are the subject of controversy due to their transformation into potentially carcinogenic nitrosamines, are used in different products as both preservatives and coloring agents (Sindelar & Milkowski et al., 2011; Xie et al., 2016).

2.3.1. Microbial Properties of Fresh Red Meat

Red meat, which can be obtained from different animals such as sheep and cattle, shows microbial properties that will affect its shelf life, characteristics, and food safety. Due to high water activity of meat, the possibility of contamination during production and processing stages, and failure to ensure storage and distribution under appropriate temperature and humidity conditions may cause foodborne diseases in red meat (Sofos, 2014).

Meat safety problems are often associated with microbial pathogens. Especially bacterial pathogens have been linked with many of the foodborne-related outbreaks. According to the Food Safety and Inspection Service (FSIS) and recalls, *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* are pathogens of concern in this regard (Selim, 2011; Sofos, 2008).

E. coli are Gram-negative, facultative anaerobic microorganisms that belong to the *Enterobacteriaceae* family, are a diverse group of bacteria, and are microorganisms found in the intestinal flora of many animal species (Guabiraba & Schouler, 2015). Although many species are harmless, their harm and effects vary depending on their type. Enterohemorrhagic *E. coli* O157:H7 produces shiga toxin and causes diseases like hemorrhagic colitis and hemolytic uremic syndrome in humans. It is acquired into the body through consumption of contaminated food and water and in contact with infected creatures (Bosilevac, & Koohmaraie, 2011; Lim et al., 2010; Lynnes et al., 2014).

L. monocytogenes is a Gram-positive, facultative anaerobe, and sporeless bacterium that can even grow under refrigeration. It is known that it can cause contamination at stages such as production, processing, and storage because its serotypes can be found in every environment (Thakur et al., 2018*). L. monocytogenes* is a food-borne, pathogenic bacteria that can cause listeriosis in humans (Holch et al., 2013).

Salmonella spp. is a common bacterial pathogen that can contaminate various types of meat and pose significant risks to human health if consumed. The presence of *Salmonella* in meat products is a major concern for food safety authorities and meat producers worldwide (FDA, 2020). Cross-contamination may occur during meat processing through contaminated surfaces, equipment, or contact with other meat products (CDC, 2021; Kurpas et al., 2018). *Salmonella* species are Gram-negative, rod-shaped, and motile, belonging to the family Enterobacteriaceae. *Salmonella* causes symptoms such as diarrhea, fever, and vomiting in living organisms.

S. aureus, which is one of the leading contributors of foodborne illnesses (CDC, 2021), is a Gram-positive facultatively anaerobe. It causes staphylococcal food poisoning through the formation of enterotoxins in foods (Thwala et al., 2021). Contamination with *S. aureus* generally results from poor production techniques, the slaughterhouse process, food storage, and poor hygiene conditions in the production process (Abdalrahman et al., 2015). *S. aureus*, which can colonize different areas in humans, may cause cross-contamination due to a lack of hygiene on surfaces such as cutting

boards (Ebert, 2018). It has been isolated from different meat products, such as raw beef, sheep, poultry, and lamb meat (Şanlıbaba, 2022). The presence of antibiotic-resistant strains of *S. aureus* was observed in the examined foods, such as meat, lamb, and chicken. For this reason, it is one of the species that should be taken into consideration in the safety of food products (Gundogan et al., 2005).

3. MATERIALS AND METHODS

3.1. Materials

Eucalyptus radiata plant was collected from Tarsus, Mersin, Türkiye during September in 2022. Sodium sulfate anhydrous and 2,2-diphenyl-1-picrylhydrazyl (DPPH) used for antioxidant determination and microwave extraction were purchased from Sigma-Aldrich (Germany). Peptone water used in microbiological analysis; tryptic soy broth; Mueller-Hinton agar (MHA); Baird-Parker agar (BPA); egg yolk tellurite; potato dextrose agar; violet red bile (VRB) agar; *Pseudomonas* CFC/CN agar; de Man, Rogosa, and Sharpe (MRS) agar; Tween 80®; glycerol; and NaCl were obtained from Merck KgaA (Germany).

3.2. Methods

3.2.1. Microwave Extraction of *Eucalyptus radiata*

The method of Gedikoğlu et al. (2019) was used with minor changes for this analysis. *Eucalyptus radiata* leaves were cleaned, brought to a constant weight, and dried. The dried leaves were roughly ground. A sample was weighed and kept in distilled water (1:5, w/v) for 15 min. After resting, the extraction procedure was carried out for 45 min at 550 W using a NEOS microwave extractor (MA 125 Milestone, Italy). Then the collected eucalyptus oil was placed in a bottle, and anhydrous Na_2SO_4 was added to the bottle to bind to the remaining water. After the removal of water from the essential oil ,the eucalyptus oil was transferred to a new bottle. Finally, the vials were tightly capped and kept at 4 °C for further analysis.

3.2.2. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of *Eucalyptus radiata* Essential Oil

Qualitative analysis of *Eucalyptus radiata* essential oil was conducted utilizing a GC-MS QP2020 outfitted with an Rxi-5Sil MS column (5% diphenyl-95% dimethylpolysiloxane 30 m × 0.25 mm i.d., df = 0.25 μm), as supplied by RESTEK GC Columns, USA and analysis conditions are shown in Table 3.1. The injector and detector temperatures were established at 250 °C. The oven temperature was programmed to remain at 40 °C for 2 minutes before ramping up at a rate of 4 °C per minute to 240 °C and holding for 53 minutes. Helium, with a linear velocity of 43.4 cm/s, was employed as the carrier gas. Samples were diluted with n-hexane (in a 1:10, v/v ratio), and a 1.0-μL volume was introduced into the GC using the syringe in split mode (with a split ratio of 1:25). An ionization voltage of 70 eV (electron volts) was applied, and the mass-to-charge ratio (m/z) mass range spanned from 40 to 400 amu (atomic mass units). The National Institute of Standards and Technology (NIST), WILEY 7 mass spectral library data, and original standards were utilized to correlate the isolated constituents. Peak area integration facilitated the determination of combination percentages (Gedikoğlu et al., 2019). This analytical procedure was executed in duplicate.

Table 3.1. GC-MS qualitative analysis conditions

Properties	***Conditions***
Dilution ratio (Sample: n-hexane)	1:10 (v/v)
Split ratio	1:25
Volume of injection	1.0-μL
Carrier gas	Helium
Flow rate of carrier gas	43.4 cm/s
Ionization voltage	70 eV
Starting temperature	40 °C
End temperature	240 °C

3.3. Preparation of *Eucalyptus radiata* Oil Nanoemulsion

To formulate the nanoemulsion containing eucalyptus essential oil, a 1% solution of high-methoxyl pectin was dissolved in sterile Milli-Q® water at 90 °C under

continuous stirring. Subsequently, the pectin solution was cooled to 25 °C, and a mixture comprising 2% eucalyptus essential oil, 5% glycerol, and 5% Tween 80® was gradually incorporated. Following this, an o/w nanoemulsion was generated using the robust shearing action of the WiseTis® Homogenizer HG-15D (DAIHAN Scientific Co., Korea) at a speed of 13,415 × g for a duration of 8 minutes. The resultant emulsion was deaerated and was carefully transferred into sterile, opaque glass containers, where it was stored in light-shielded conditions at temperatures ranging between 20 and 25 °C for further analytical investigations.

3.4. Characteristics of *Eucalyptus radiata* Nanoemulsion

Particle size and zeta potential, viscosity, and whiteness index were investigated to determine the characteristic properties of the prepared emulsion.

3.4.1. Particle Size and Zeta Potential

The droplet size of the nanoemulsion containing eucalyptus essential oil and pectin was analyzed using a Zetasizer NanoZS laser diffractometer (Malvern Instrument Ltd., UK). Measurements were conducted at a wavelength of 633 nm on the laser diffractometer, which was equipped with a backscatter detector set at an angle of 173°, all at a constant temperature of 25 °C. Additionally, the surface electrical charge of the emulsion's continuous phase was assessed through phase analysis light scattering, utilizing the same Zetasizer NanoZS laser diffractometer, following the method described by Artiga-Artigas et al. (2018).

3.4.2. Viscosity and Whiteness Index (WI)

The viscosity of the nanoemulsion was assessed in triplicate utilizing a Vibrio SV-10 viscometer (manufactured in Japan) at a temperature of 25 °C. The gold sensor plates of the viscometer induced oscillatory vibration with an amplitude below 1 mm at a frequency of 30 Hz.

To determine the whiteness index (WI) of the nanoemulsion, first the *L* (black = 0 and white = 100), *a* (a+ = red and a– = green), and *b* (b+ = yellow and b– = blue) values were obtained using a Ser-Lab SL400 colorimeter (Turkey). Then, the following formula was used to calculate the WI (Chang et al., 2015):

$$WI = 100 - \sqrt{(100 - L*)^2 + (a*^2 + b*^2)}$$

By applying this equation, the value of the whiteness index was attained.

3.5. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Rree Radical Scavenging Activity Analysis of Essential Oil and Nanoemulsion

For this analysis, we employed the multi-plate method as previously outlined by Gedikoğlu (2022a). Initially, each well was filled with 100 µL of methanol. Subsequently, the first column of wells (excluding the final row) received 100 µL of eucalyptus essential oil. Following thorough mixing, half of the volume was transferred to the adjacent well, resulting in a 50% dilution for each subsequent well. The last row was designated for the blank, containing the methanol-DPPH radical. Subsequently, 100 µL of 0.004% (w/v) DPPH methanolic solution was added to all wells, and the multi-well plate was incubated at 25 °C in darkness for 30 min. Finally, the results were measured at 517 nm using a Synergy H1 microplate reader from

BioTek (manufactured by Agilent, USA). The following formula was applied to calculate the inhibitions (%) of the DPPH radical.

Equation (3.2)

$$I\ (\%) = \frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100$$

A_{blank}: absorbance of the methanol and DPPH radicals

A_{sample}: absorbance of a methanolic sample with the DPPH radical

Following the computation of inhibition percentages, the sample concentrations were graphed against them. Subsequently, the equation derived from the graph was employed to ascertain the sample concentration necessary for achieving 50% inhibition (IC_{50}). This process was carried out in triplicate.

3.6. Antimicrobial Activity of *Eucalyptus radiata* Essential Oil and Its Nanoemulsion

The antimicrobial activity of *E. radiata* essential oil was determined by an agar disc diffusion assay and a broth dilution assay, while the antimicrobial activity of the nanoemulsion was examined by a time-kill assay.

3.6.1. Preparation of Bacterial Cultures

Cultures of *Bacillus cereus* NRRL B3711, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 9144, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Salmonella* Enteritidis ATCC 13076, and *Salmonella* Typhimurium ATCC 14028 were used for the antimicrobial assays. For culture

activation, a loop was taken from the cell suspensions, transferred separately to tryptic strain broth, and kept at 37 °C for 24 h. The next day, the same process was repeated, and the transfer was carried out in two cycles. The tubes were centrifuged at 1714 × g for 10 min to obtain pellets, and the resulting pellets were washed.

3.6.2. Agar Disc Diffusion Assay

A disc diffusion assay was used to examine the activity of essential oils against pathogens. During the culture preparation phase, the pellets obtained by centrifugation were diluted in a 0.9% NaCl solution. A densitometer (Novolab, Belgium) was used to determine concentration. The concentration was adjusted to $1x10^8$ CFU/mL, or 0.5 McFarland number, with the densitometer. 10 µL of essential oil was dropped onto the discs and placed on the agar. Plates were incubated at 35 °C for 18 to 24 hours. The antimicrobial activity of eucalyptus oil was obtained by measuring the clean area around the disc in mm on incubated plates and subtracting the radius of the disc.

3.6.3. Broth Dilution Assay

Following the method of Gedikoğlu and Çıkrıkcı Erünsal (2023), sterile 96-well multiplates were used for this assay. A hundred µL of tryptic soybean water was added to the well and 100 µL of eucalyptus oil was added to the wells in the first column. Half of the volume in the well of the first column -(100 µL)- was transferred to the adjacent well by pipetting to ensure homogeneous mixing. This process was repeated, and the concentration was reduced by 50%. The oil concentration was in the range of 0.091- 46.5 mg/mL after the dilution process. In the plate with 12 columns, the $11t^h$ column was left for positive control (broth inoculated with microorganisms) and the 12^{th} column was left for negative control (sterile broth without any inoculation). Cultures were prepared and diluted to 1-2 × 10^6 CFU/mL as described in the preparation of cultures section. A hundred µL of microbial suspension was added to the negative control wells.

After the process was completed, the plates were placed in the incubator and incubated at 37 °C for 18-24 h. The minimum inhibitory concentration was determined by determining that there was no visible growth on the plates.

3.6.4. Time Kill Assay

The antibacterial activity of eucalyptus oil nanoemulsion was tested on *S. aureus* ATCC 9144 and *E. coli* ATCC 25922. Cultures of these species were mixed with nanoemulsion and sterile water. For this, cultures, nanoemulsion, and sterile water were added at a ratio of 1:1:9 (v/v/v). A hundred µL of bacterial cultures were added to the selective agars of the cultures, Baird Parker agar for *S. aureus* and purple red bile agar for *E. coli*, at 5, 30, and 60 minutes and incubated at 37 °C for 24 h, and the colonies were counted. From the dilutions prepared for planting in 5, 30 and 60 minutes, the results were obtained at the 5th minute by planting at the time of dilution preparation, the results at the 30th minute were obtained by waiting for 30 minutes, and the results at the 60th minute were obtained by waiting for 60 minutes.

3.7. Antimicrobial Activity of *Eucalyptus radiata* Essential Oil Nanoemulsion on Fresh Beef Chunks

3.7.1. *Staphylococcus aureus* Enumeration

3.7.1.1. Preparation of *Staphylococcus aureus*

As previously mentioned, a loop of *S. aureus* culture was taken from the cell suspension, transferred separately to tryptic soy broth, and kept at 37 °C for 24 h. The following day, the same process was repeated, and the transfer was carried out. Next, the cell suspension was centrifuged at 1714 × g for 10 min. The pellets were rinsed

and diluted in 0.1% sterile peptone water (w/v). A dilution series was prepared, and the final inoculum concentration was determined as 1×10^6 CFU/g.

3.7.1.2. Application of *Eucalyptus radiata* Essential Oil Nanoemulsion on Fresh Beef Chunks Contaminated with *Staphylococcus aureus*

Fresh beef pieces were provided for analysis from Konya province. Meats obtained from different delicatessens were weighed, and 10-g samples were prepared. The samples were divided into two: control and experimental groups. No application was done for the control group, and nanoemulsion was applied to the experimental group. The meat pieces were allocated to the nanoemulsion group were dipped into the nanoemulsion and kept for 10 min. The samples soaked in the nanoemulsion mixture were removed and dried in sterilized steel trays as shown in Figure 3.1. A hundred μL (1×10^6 CFU/g) of *S. aureus* ATCC 9144 was inoculated into the samples. After waiting for a while, the applications and their replications were placed in petri dishes individually and covered with parafilm to prevent air. In order to monitor the shelf life, it was kept at 4 °C for 8 days to be examined on days 0, 2, 4, 6, and 8. Thus, the antimicrobial activity of the nanoemulsion was evaluated in fresh beef pieces contaminated with *S. aureus*. Ten g of sample was taken and homogenized with 90 mL of sterile peptone water for 2 minutes using the LS-700A stomacher automatic blending system (BNF, Korea). Serial dilutions were prepared from the homogenized mixture, spread on Baird-Parker agar, and incubated at 4 °C for 48 hours. The number of colonies was expressed as $\log_{10}$ CFU/g of meat sample.

Figure 3.1. Dipping and filtering process of fresh beef

3.7.2. Enumeration of Spoilage Microorganisms

3.7.2.1. Preparation of Meat Samples

To determine the shelf life of meat pieces immersed in nanoemulsion, spoilage microorganisms were counted. In this analysis, the inhibitory effect of the prepared eucalyptus oil nanoemulsion on total coliform, *Pseudomonas* spp., lactic acid bacteria, yeast, and mold was examined. The counting of these microorganisms was repeated on days 0, 2, 4, 6, and 8 for control and nanoemulsion.

Ten grams of fresh red meat pieces were cut, some of them dipped into the nanoemulsion, and some of them used as controls and added to sterile petri dishes. Petri dishes are labelled as days 0, 2, 4, 6, and 8, and they were stored under refrigerator conditions (+4 ℃). When the day indicated on the petri dish arrived, fresh meat pieces were mixed with sterile peptone water at a ratio of 1:9 (w/v), and 10 serial dilutions were prepared. The prepared peptone water-meat mixtures (0.1 mL) were spread onto agars using the spread plating method onto selective agars of microorganisms.

3.7.2.2. Total Coliform Count

VRB agar was used for total coliform bacteria, and the plates were incubated at 35 ºC for 48 h and repeated on 0, 2, 4, 6, and 8. days. The colony count was represented as $\log_{10}$ CFU/g of the meat sample.

3.7.2.3. *Pseudomonas* spp. count

For *Pseudomonas* spp., *Pseudomonas* CFC/CN agar was used, and the plates were incubated at 30 °C for 48 h and repeated on 0, 2, 4, 6, and 8. days. The colony count was represented as $\log_{10}$ CFU/g of the meat sample.

3.7.2.4. Lactic Acid Bacteria Count

MRS agar was used for lactic acid bacteria, and the plates were incubated at 35 ºC for 48 h and repeated on 0, 2, 4, 6, and 8. days. The colony count was represented as $\log_{10}$ CFU/g of the meat sample.

3.7.2.5. Yeast and Mold Count

Potato dextrose agar was employed for the yeast and mold counts, and the plates were maintained at 25 ºC for 72 h and repeated on 0, 2, 4, 6, and 8. days. The colony count was represented as $\log_{10}$ CFU/g of the meat sample.

3.8. Statistical Analysis

Using Stata IC 14 (produced by Stata Corp., USA), a two-sample t-test was used to examine the in vitro data. A two-way ANOVA (analysis of variance) was used to examine data from on-site trials. A generalized linear mixed model was used for statistical analysis, in which replicates were treated as random effects and treatments and storage duration were designated as fixed variables. To identify any significant differences between the same treatments at various storage intervals, a Tukey multiple comparison test was employed. Furthermore, significant differences between the means of the two treatments on the same day were found using the least significant difference (LSD) approach ($P \leq 0.05$).

4. RESULTS AND DISCUSSION

4.1. Chemical Composition of *Eucalyptus radiata* Essential Oil

The chemical profile of *E. radiata* essential oil was determined using gas chromatography coupled with mass spectrometry. As a result of GC-MS, 53 compounds were identified in the essential oil. Table 4.1 lists the chemical compounds with a peak area greater than 0.5%. The most abundant o-cymene (45,4%), 2-bornene (26,29%), 1,8-cineole (11,31%), α-pinene (9,25%), Neoalloocimene (1,37%), Myrcene (0,98%), β-pinene (0,75%), were found in *E. radiata* essential oil, respectively.

Table 4.1 Chemical composition of *Eucalyptus radiata* essential oil obtained by microwave extraction

No	***Compounds***	***RI***	***Relative Peak Area (%)****
1	α-Pinene	12.911	9.25 ± 0.01
2	β-Pinene	14.644	0.75 ± 0.01
3	Myrcene	15.095	0.98 ± 0.02
4	o-Cymene	17.338	45.4 ± 0.05
5	2-Bornene	17.525	26.29 ± 0.02
6	1,8-Cineole	17.610	11.31 ± 0.01
7	Neoalloocimene	32.736	1.37 ± 0.01
	Total peak area (%)		95.35%

* Values are expressed as mean ± standard deviation (n = 2).

Bendaoud et al. (2009) investigated the chemical composition of Tunisian *E. radiata* essential oil and discovered that the largest concentrations were 1,8-cineole (69.53%), α-pinene (11.94%), and trans-pinocarveol (4.81%). Studies on *E. radiata* have shown that 1,8-cineole and o-cymene are the most abundant compounds in the essential oils obtained from this plant (Mulyaningsih et al., 2011).

Luís et al. (2016) determined the main components of E. radiata obtained from a pharmacy as limonene (68.51%), α-terpineol (8.60%), α-terpinyl acetate (6.07%), and α-pinene (3.01%).

Pinenes are utilized to create flavor and odor and have fungicidal activity in plants. α- and β-pinene are the two active constitutional isomers of pinenes and their chemical structures are shown in Figure 4.1. The composition of essential oils is rich in α-pinene, also known as monoterpene, with the formula $C_{10}H_{16}$. α- and β-pinene isomers are used as antibacterial agents due to their toxic effects on cell membranes (da Silva Rivas et al., 2012). The antiviral, antibacterial, antifungal, anti-inflammatory, antioxidative, and neuroprotective properties of the compound have been proven by different studies (Allenspach, & Steuer, 2021; Astani, & Schnitzler, 2014; da Silva Rivas et al., 2012).

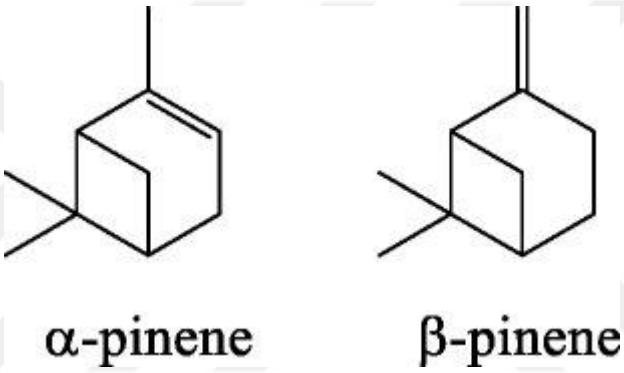

Figure 4.1. Chemical structure of α-Pinene and β-pinene

O-cymene, which belongs to the terpene group, is an abundant compound in *E. radiata* essential oil. O-cymene exhibits antibacterial, antifungal, and antiparasitic properties and is present in the essential oils of over 100 different plants (Marchese et al., 2017). The antimicrobial activity of *Thymus vulgaris* has been associated with o-cymene and its ability to dissolve in the membrane of the bacterial cell. Its antimicrobial activity may be associated with this feature (Al Maqtari et al., 2011).

The typical source of eucalyptol, or 1,8-cineole, is the leaves of eucalyptus species and its chemical structure is shown in Figure 4.2. It has been found that 1,8-cineole causes structural alterations in the bacterial cell, which is how it is assumed to be effective in the oil's antibacterial activity (Campos, & Berteina-Raboin, 2022; Li et al., 2014).

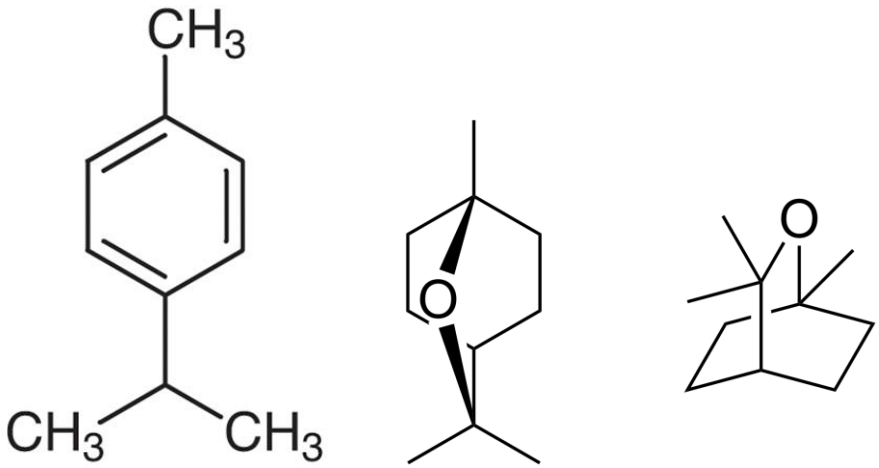

Figure 4.2. Chemical structure of o-cymene (left) and eucalyptol (right)

4.2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity of Essential Oil and Its Nanoemulsion

The free radical scavenging activity of *E. radiata* essential oil and nanoemulsion was measured using the DPPH method, and the IC_{50} value was determined. IC_{50} refers to the sample concentration required to capture fifty percent of free radicals, meaning a higher IC_{50} value is associated with lower antioxidant activity (Shahbazi, 2019). *E. radiata* essential oil showed high antioxidant activity with an IC_{50} value of 53.71 µg/mL, as shown in Table 4.2. It was observed that the oil obtained by microwave extraction showed higher antioxidant acitivity (Fernández-Marín et al., 2021).

In a study, the IC_{50} (%) value of *E. radiata* essential oil was found as 4.56 ± 0.70 (*v/v*) by DPPH method (Luís et al., 2016). When other species belonging to the Eucalyptus genus were examined, the antioxidant activity of *E. gillii* essential oil had an IC_{50} of 163.5 ± 10.7 mg/L with DPPH method (Hassine et al., 2012). The IC_{50} value of *E. globulus* oil was found as 33.72 (v/v) (Mishra et al., 2010). In another study conducted

by Elkolli et al. (2023), the IC_{50} value of *E. radiata* was obtained as 0.19 + 0.03 mg/mL.

The IC_{50} value for eucalyptus nanoemulsion was determined as 240.41 µg/mL, which was lower than that of pure essential oil. In studies, essential oils generally showed higher antioxidant activity than their nanoemulsions. The IC_{50} value for the DPPH scavenging activity of *Ocimum basilicum* L. essential oil was 13.21 µg/mL and nanoemulsion was 10.47 µg/mL, indicating that nanoemulsion showed higher antioxidant activity (Sundararajan et al., 2018). The IC_{50} value of essential oil for *Vitex negundo* L. and nanoemulsion were determined as 28.87 µg/mL and 23.26 µg/mL, respectively (Balasubramani et al., 2017). In this case, the preparation method of the nanoemulsion and the behavior of the oil in the nanoemulsion can be related to the characteristic features of the nanoemulsion.

4.3. Characteristics of *Eucalyptus radiata* Nanoemulsion

To evaluate the characteristic properties of the emulsion, size, zeta potential, viscosity, and whiteness index values were determined and displayed in Table 4.2.

Table 4.2 Characterization of the *Eucalyptus radiata* essential oil nanoemulsion

Properties	***Nanoemulsion****
Size (nm)	52.04 ± 31.44
Zeta potential (mV)	9.16 ± 0.63
Viscosity (mPa.s)	82.67 ± 3.68
Whiteness index (WI)	48.91 ± 1.24
DPPH (IC_{50}, µg/mL)	240.41 ± 0.05

* Values are expressed as mean ± standard deviation (n = 3).
DPPH: 2,2-diphenyl-1-picrylhydrazyl, IC_{50}: half of the maximal inhibitory concentration.

4.3.1. Size of Nanoemulsion

The particle size of the *E. radiata* nanoemulsion was 52.04±31.44 nm and result is shown in Figure 4.3. Since this is in agreement with previos studies for nanoemulsion, the emulsion we obtained is called nanoemulsion (Jaiswal et al., 2015). As discovered previously, nanoemulsions have small particle sizes; their surface areas are large, so they dissolve more easily and act faster. In addition, they show high stability against agglomeration due to their small particle sizes (Aswathanarayan &Vittal, 2019; Çınar, 2017).

Particle size can be evaluated as an indicator of the homogeneity, stability and therefore effectiveness of the nanoemulsion. In addition, the purpose of the surfactant used in nanoemulsion preparation is to stabilize oil droplets, and it does this by using its ability to adsorb at the oil-water interface (Kralova, & Sjöblom, 2009). It can be said that the materials used in the nanoemulsion formulation and the recipe of the formulation affect all analyses.

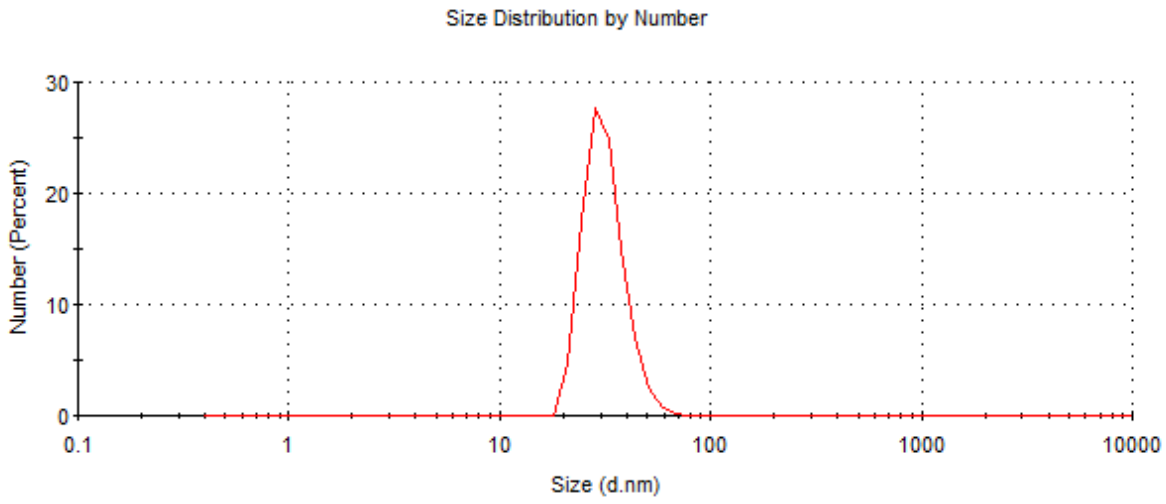

Figure 4.3. Droplet size of the *E. radiata* essential oil nanoemulsion

4.3.2. Zeta Potential of Nanoemulsion

The zeta potential of the eucalyptus nanoemulsion was measured at −9.16 mV, as shown in Figure 4.4. Nanoparticles show negative zeta potential due to phosphates on the surface; the results support nanoemulsions (Sharifi et al., 2021). Zeta potential is a parameter that affects emulsion stability (Wiącek & Chibowski, 1999). Zeta potential is important as the nanoemulsion formulation affects the surface charge and the consistency of the emulsion (Almasi et al., 2021). Aggregation occurs when the zeta potential is below 5 mV and above 30 mV, producing a dispersed excess of uniformly sized particles (Gumustas et al., 2017). Since the nanoemulsion we obtained is not in this range, there may be doubts about its stability. This problem can be prevented by examining the nanoemulsion and adding stabilizers.

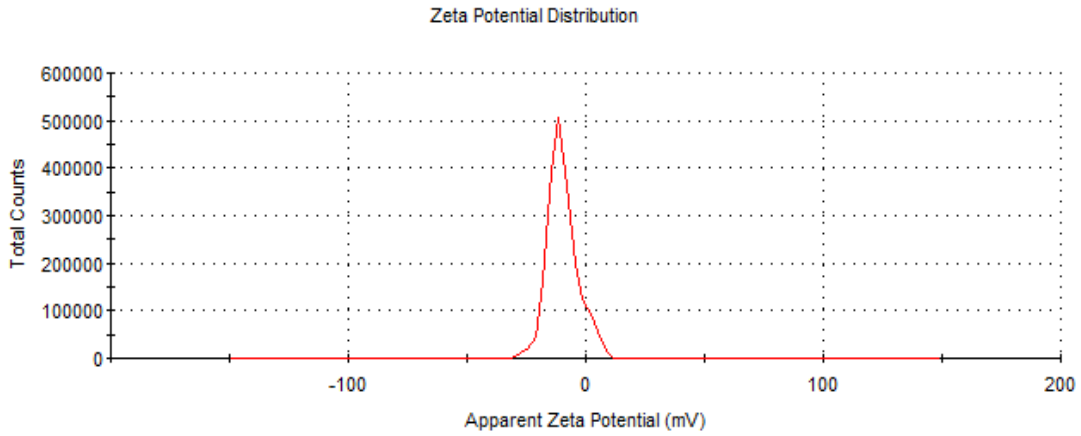

Figure 4.4. Zeta potential of the *E. radiata* essential oil nanoemulsion

4.3.3. Viscosity of Nanoemulsion

The viscosity of the nanoemulsion was measured at 82.67 mPa·s. Viscosity, a crucial thermophysical characteristic of nanoemulsions, is influenced by various factors such as the biopolymer type, surfactant presence, essential oil variety and concentrations, and the methods employed in emulsion preparation, which are crucial for their stability, flow behavior, and application performance (Salvia-Trujillo & McClements,

2016). The viscosity of the nanoemulsion is affected by many parameters: composition, droplet size, surfactant properties, temperature, and shear rate. High oil content increases viscosity due to higher interfacial tension between oil and water phases (Gupta et al., 2016; Tontul, 2019). Small droplet sizes generally result in low viscosity. Since surfactants reduce interfacial tension, they also reduce viscosity. To obtain sufficient viscosity, high phospholipid usage is required (Salvia-Trujillo & McClements, 2016; Tadros, 2015).

4.3.4. Whiteness Index of Nanoemulsion

The whiteness index of the prepared nanoemulsion was measured at 48.91 (WI). Nanoemulsions have a lower whiteness index than macroemulsions. There are many studies investigating the relationship between particle size and optical properties in emulsions. For example, in the food industry, the particle size distribution of emulsions can affect their opacity, light scattering, and overall visual appearance (Hu et al., 2017). Emulsifiers capable of producing smaller and more uniform droplets can lead to emulsions with higher whiteness indices (Dickinson, 2012). Lower WI values correlate with improved transparency, a crucial characteristic that can influence the acceptability of coated food items. (McClements, 2011).

4.4. Evaluation of the Antimicrobial Activity of Essential Oil and Nanoemulsion *In Vitro* Assays

The antimicrobial activity of *E.radiata* essential oil and nanoemulsion was determined by in vitro studies. The microorganisms on which the studies were carried out are shown in Figure 4.5.

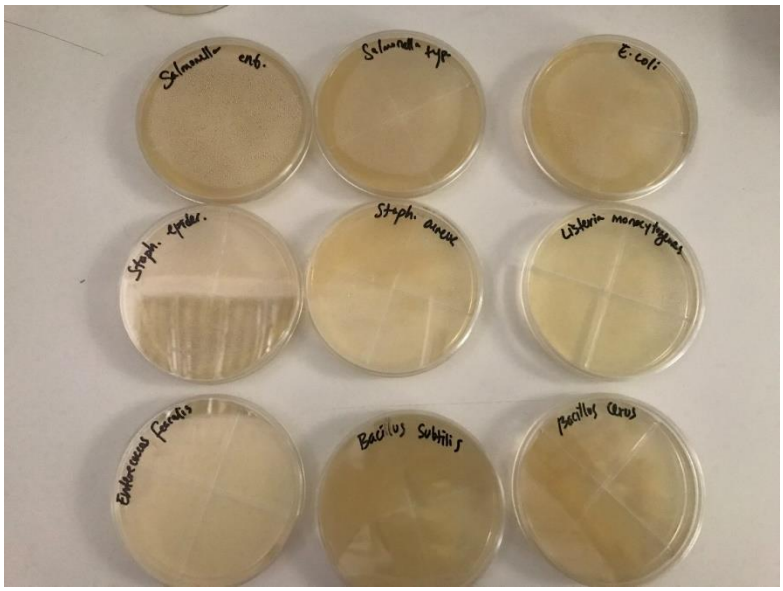

Figure 4.5 Plating for antimicrobial activity assays

4.4.1. Results of Disc Diffusion Assay

The *in vitro* antibacterial activity analysis findings for *E. radiata* essential oil are shown in Table 4.3. In the disk diffusion test results, it was seen that the largest inhibition area belonged to *B. cereus* NRRL B3711 and *S. epidermidis* ATCC 12228 bacteria, 24 mm and 20.5 mm, respectively; both bacteria are Gram-positive. The ones with the lowest inhibition area are Gram-positive *E. faecalis* ATCC 29212 and Gram-negative *S.* Typhimurium ATCC 14028. From these results, it can be deduced that the inhibition effect of *E. radiata* essential oil on Gram-positive is more effective than on-Gram negative.

Table 4.3 Antibacterial activity of the microwave-extracted *Eucalyptus radiata* essential oil

Bacterial species	***Disc diffusion assay Inhibition zone (mm)****	***Broth dilution assay MIC (mg/mL)***
Bacillus cereus NRRL B3711	24 ± 2.00	11.63 ± 0.00
Enterococcus faecalis ATCC 29212	5.5 ± 0.50	11.63 ± 0.00
Staphylococcus aureus ATCC 9144	15 ± 0.50	11.63 ± 0.00
Staphylococcus epidermidis ATCC 12228	20.5 ± 0.50	11.63 ± 0.00
Escherichia coli ATCC 25922	15 ± 0.50	11.63 ± 0.00
Salmonella Enteritidis ATCC 13076	9.5 ± 0.50	23.25 ± 0.00
Salmonella Typhimurium ATCC 14028	4 ± 0.00	23.25 ± 0.00

*Values are expressed as mean ± standard deviation (n = 2).

4.4.2. Results of Broth Dilution Assay

The results of the broth dilution test are shown in Table 4.3, and when the results were examined, the MIC values for all Gram-positive bacteria and *E. coli* tested were obtained as 11.63 mg/mL. When the results for Gram-negative bacteria and *Salmonella* were examined, a result of 23.25 mg/mL was obtained. In other words, *E. radiata* essential oil is more effective on Gram-positives and *E. coli* than on Gram-negatives. The antibacterial activities of essential oils can be attributed to the compounds they contain. 1,8-cineole and pinenes found in *E. radiata* essential oil are compounds that show antimicrobial activity (Allenspach & Steuer, 2021; Bachir & Benali, 2012; Leite et al., 2007).

4.4.3. Results of Time-Kill Assay

The results of the antibacterial activity of *E. radiata* essential oil nanoemulsion against Gram-positive and Gram-negative bacteria obtained by the time kill assay are shown in Figure 4.6. Colonies incubated at 37 °C for 24 h after inoculation of *S. aureus* and *E. coli* microorganisms at 0, 30 and 60 min were counted as explained in the methods session.

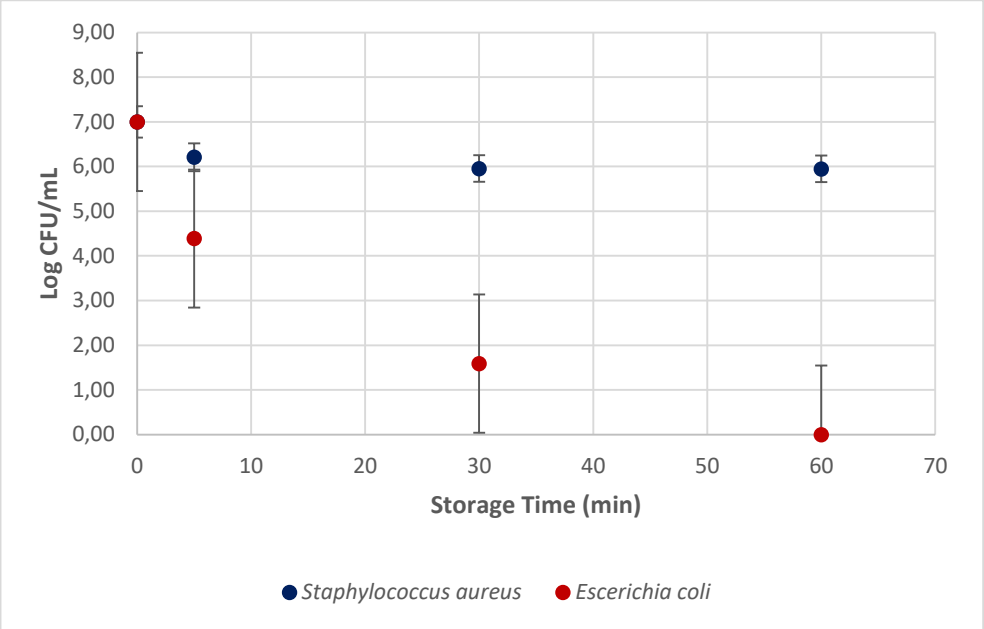

Figure 4.6. Antibacterial activity of the *E. radiata* essential oil nanoemulsion against Gram-positive and Gram-negative bacteria

With this assay, the inhibitory effect of the nanoemulsion against *Staphylococcus aureus* and *Escherichia coli* cultures was measured at 5, 30, and 60 min. While a decrease of 1 $\log_{10}$ CFU/mL was observed in Gram-positive *S. aureus*, a decrease of 7 log CFU/mL was observed in Gram-negative *E. coli* from 5th minutes to 60th minutes. From these results, it was seen that eucalyptus oil nanoemulsion was more effective against Gram-negative bacteria. Similar results were reported in a previous study by Gedikoğlu and Çıkrıkcı Erünsal (2023). It was found that *Thymbra spicata* essential oil was effective against Gram-positive bacteria, while in the nanoemulsion form, antimicrobial potential improved against Gram-negative bacteria. It is thought that chemical components such as terpenes found in essential oils and terpenoids, which represent a group of phytochemicals with known antimicrobial effects, react with the lipids in the bacterial membrane, disrupting the bacterial cell structure, causing leakage of cell material, and ultimately leading to cell death (Mahizan et al., 2019; Mazarei & Rafati, 2019; Zengin & Baysal, 2014). The thinner cell walls of Gram-negative microorganisms compared to Gram-positive microorganisms may enable essential oils and the resulting emulsions to have a high inhibitory effect.

The antimicrobial activity of essential oils is mainly evaluated based on cell membrane and cell wall destruction, but our studies have also shown that the effectiveness of Gram-positive and Gram-negative bacteria varies. When Gram-positive bacteria are compared with Gram-negative bacteria, the difference in the peptidoglycan layer is remarkable (Angane et al., 2022), this difference is shown in Figure 4.7. Differences in the cell walls of Gram-positive and Gram-negative bacteria particularly explain that. Porin proteins, which are water-filled channels in the outer membrane of Gram-negative bacteria, serve as hydrophilic pores and facilitate the uptake of substances into the cell (Galdiero et al., 2012). Intracellular substance uptake is affected depending on whether porins are specific or non-specific, while non-specific porins allow the passage of low molecular weight substances (Vergalli et al., 2020).

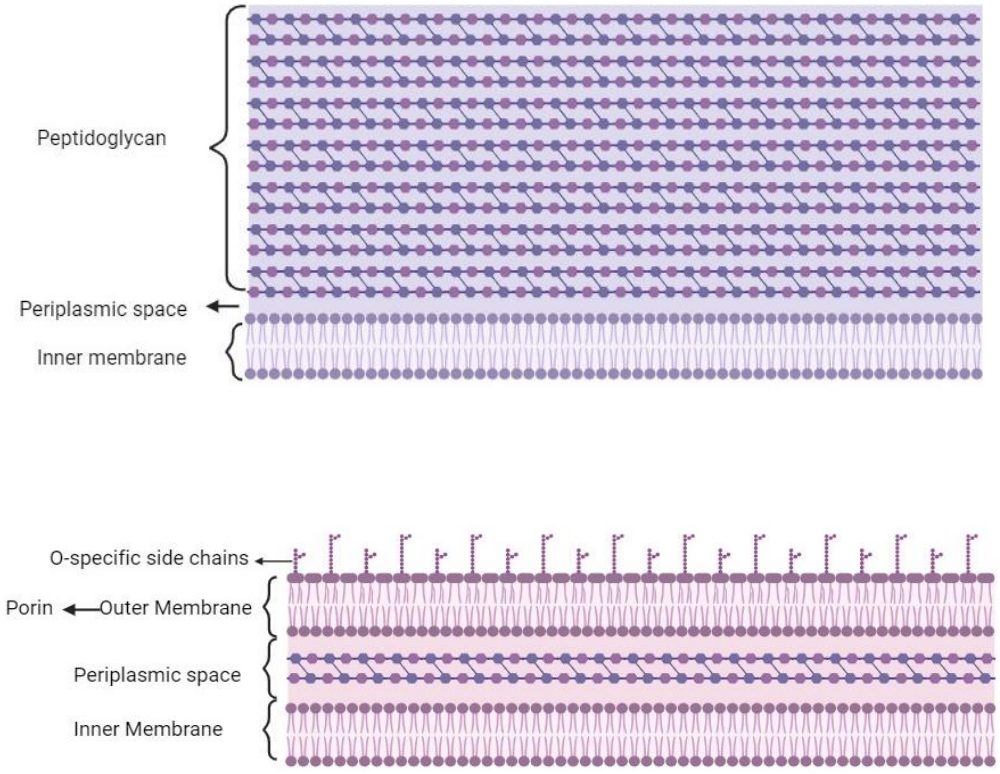

Figure 4.7. Schematic view of the cell wall structure of Gram-positive(up) and Gram-negative bacteria (down)

Since the surfactants used in the nanoemulsion prepared with essential oil affect the hydrophilicity, they may be infused into the cell more easily compared to oil. This structural difference seen in gram-negative cells may also explain the higher antimicrobial effect of *E. radiata* essential oil nanoemulsion on *E. coli* compared to *S. aureus.*

4.5. In Situ Antimicrobial Activity of *Eucalyptus radiata* Essential Oil Nanoemulsion

In addition to what we obtained as a result of *in vitro* studies, the antimicrobial activity of eucalyptus essential oil nanoemulsion was also evaluated on the food matrix. For this purpose, the inhibitory effect of the nanoemulsion was assessed against a foodborne pathogen and spoilage microorganisms, which affected the safety and shelf life of fresh meat. Testing was conducted on fresh beef pieces that are aerobically stored at 4 ºC, and data was collected on days 0, 2, 4, 6, and 8.

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4.5.1. Antimicrobial Activity of *Eucalyptus radiata* Essential Oil Nanoemulsion on Fresh Beef Chunks Against *Staphylococcus aureus*

The antimicrobial effectiveness of the nanoemulsion prepared with *E. radiata* essential oil on beef pieces contaminated with *S. aureus* was determined by the applying dipping process. Fresh beef pieces (10 g) were contaminated with 10^6 CFU/g of *S. aureus* for two treatment which are control and meat pieces dipped in *E. radiata* essential oil nanoemulsion. When colonies were counted at different days, significant differences ($P \leq 0.05$) were observed between control and nanoemulsion-treated meat pieces, as shown in Figure 4.8. While the number of *S. aureus* observed in the control on day 0 was 5.65 $\log_{10}$ CFU/g, it was observed to increase to 6.62 $\log_{10}$ CFU/g on day 8; while the nanoemulsion was 3.47 $\log_{10}$ CFU/g on day 0, it increased to 5.08 $\log_{10}$ CFU/ml on day 8. There was a significant ($P \leq 0.05$) reduction in the *S. aureus* counts in nanoemulsion treatments compared to the control throughout the storage period. This

study showed that eucalyptus oil nanoemulsion is an effective antimicrobial agent, and application of it as a dip is a feasible method.

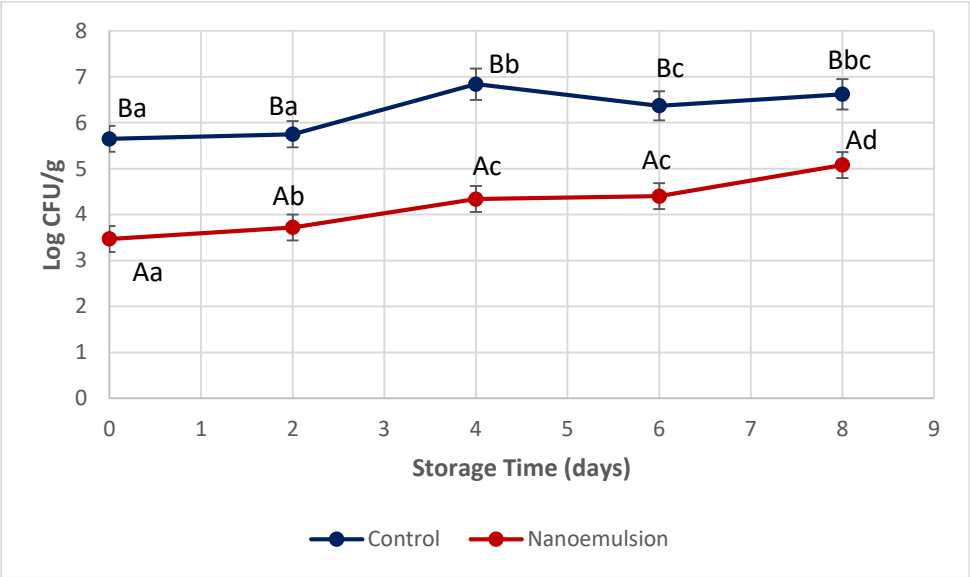

Figure 4.8. Effect of the E. radiata essential oil nanoemulsion as an antimicrobial dipping solution on the inhibition of *Staphylococcus aureus* ATCC 9144-contaminated fresh beef chunks.

Different uppercase letters indicate significant ($P \leq 0.05$) difference between two groups. Different lowercase letters indicate a significant ($P \leq 0.05$) difference within the treatment at different storage times.

Similarly, in a previous study by Hu et al. (2011), the antimicrobial activity of the extract obtained from the *Magnolia officinalis* plant was tested on *S. aureus*. It was observed that *S. aureus* was effectively and significantly inhibited by the extract. The result of the investigation showed that the extract damaged the cell wall and membrane, altering the structure of the cell. As a result, the cell membrane's permeability increased, which caused the cells to die, as demonstrated by a scanning electron microscope (Hu et al., 2011).

4.5.2. Antimicrobial Activity of *Eucalyptus radiata* Essential Oil Nanoemulsion on Fresh Beef Chunks Against Total Coliform

The findings of this study for total coliform are given in Table 4.4. There were two treatments. The first one was the control treatment, in which the meat pieces had no application of an antimicrobial. The second treatment was the nanoemulsion, in which the meat pieces were immersed in the *E. radiata* nanoemulsion for 10 min before packaging. Data were collected on days 0, 2, 4, 6, and 8 and stored under refrigerator conditions.

Table 4.4 Effect of the *Eucalyptus radiata* essential oil-nanoemulsion as an antimicrobial dipping application on the microbial count of fresh beef chunks during cold storage

Treatments	***Storage time (days)***				
	0	2	4	6	8
			Total coliform count (log CFU/g)		
Control	4.44 ± 0.06Ba	5.68 ± 0.07Bb	7.48 ± 0.08Bc	7.67 ± 0.15Bc	8.63 ± 0.14Bd
Nanoemulsion	3.14 ± 0.03Aa	4.45 ± 0.01Ab	5.52 ± 0.03Ac	6.69 ± 0.03Ad	7.07 ± 0.02Ae
			***Pseudomonas* spp. count (log CFU/g)**		
Control	4.76 ± 0.04Ba	5.86 ± 0.02Bb	7.60 ± 0.04Bc	7.90 ± 0.07Bc	8.49 ± 0.21Bd
Nanoemulsion	3.40 ± 0.03Aa	4.86 ± 0.02Ab	5.51 ± 0.03Ac	6.96 ± 0.03Ad	7.62 ± 0.14Ae
			Lactic acid bacteria count (log CFU/g)		
Control	ND	2.20 ± 0.03Ab	3.42 ± 0.09Ac	3.34 ± 0.02Ac	3.43 ± 0.06Ac
Nanoemulsion	ND	2.51 ± 0.24Bb	3.28 ± 0.09Ac	3.74 ± 0.03Bd	4.10 ± 0.07Bd
			Yeast and mold count (log CFU/g)		
Control	4.97 ± 0.01Ba	5.80 ± 0.06Bb	7.51 ± 0.13Bc	7.79 ± 0.11Bc	8.29 ± 0.06Bd
Nanoemulsion	3.69 ± 0.05Aa	3.82 ± 0.27Aa	5.33 ± 0.08Ab	6.54 ± 0.16Ac	7.84 ± 0.02Ad

Values are expressed as mean ± standard errors (S.E.). Different uppercase letters in the same column indicate a significant ($P \leq 0.05$) difference between two groups. Different lowercase letters in the same row indicate a significant ($P \leq 0.05$) difference within the treatment at different storage times. ND = not detected.

The effectiveness of *E. radiata* essential oil-nanoemulsion on total coliform count was examined in fresh beef pieces during cold storage. Significant changes ($P \leq 0.05$) in total coliform counts were observed between the control and eucalyptus oil nanoemulsion samples during the 8-day storage period. For total coliform count, samples retained in the emulsion ranged from 3.14 to 7.07 $\log_{10}$ CFU/g, while control

samples ranged from 4.44 to 8.63 $\log_{10}$ CFU/g, providing significant ($P \leq 0.05$) differences between the treatments on all the days of storage. Additionally, the number of coliform colonies increased significantly ($P \leq 0.05$) for both treatments during the course of the storage days. It was also observed that the control group's colony count reached the spoiling level on day 4, whereas the nanoemulsion treatment required an additional 4 days to reach that level. These changes can also be seen in Figure 4.9. The largest difference between the groups was on day 4. The dipping application of the eucalyptus nanoemulsion slowed down the increase in the total coliform count on fresh beef pieces. In another study, the effect of essential oils obtained from different parts of different plants, such as flowers or bark, on the total coliform count during the shelf life of pork back fat was examined. It was reported that the total coliform count was reduced by a minimum 16 days with *Cinnamomum zeylanicum* essential oil and by a minimum 6 days with a mixture of *Origanum compactum* and *Thymus zygis* essential oils (Van Haute et al., 2016). These findings demonstrate the potential of essential oils and their emulsions as an antimicrobial agent for meat products.

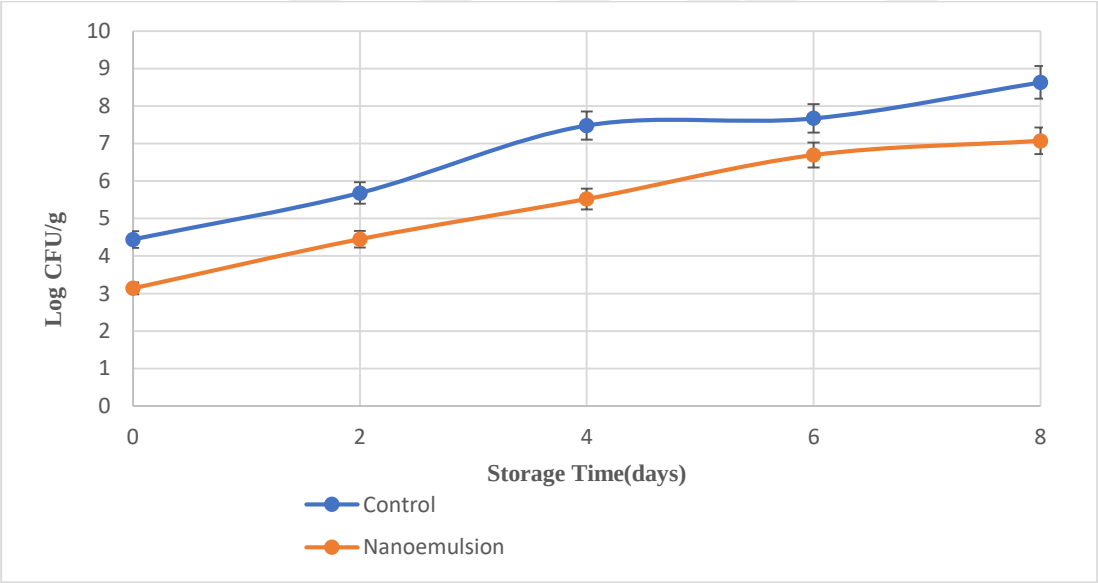

Figure 4.9. Effect of the *E. radiata* essential oil nanoemulsion as an antimicrobial dipping solution on the delay the growth of total coliform

4.5.3. Antimicrobial Activity of *Eucalyptus radiata* Essential Oil Nanoemulsion on Fresh Beef Chunks Against *Pseudomonas* spp.

The results of the *Pseudomonas* spp. counts are displayed in Table 4.4. Similarly, we found that *E. radiata* oil nanoemulsion had significant ($P \leq 0.05$) antimicrobial activity against *Pseudomonas* spp. throughout the storage period. The differences in the number of colonies between groups ranged from 0.87 to 2.09 $\log_{10}$ CFU/g. In addition, the biggest difference between the two groups was observed on day 4, as seen in Figure 4.10. Once more, the nanoemulsion group achieved the spoiling level on day 8, but the control group's *Pseudomonas* spp. levels reached that level on day 4.

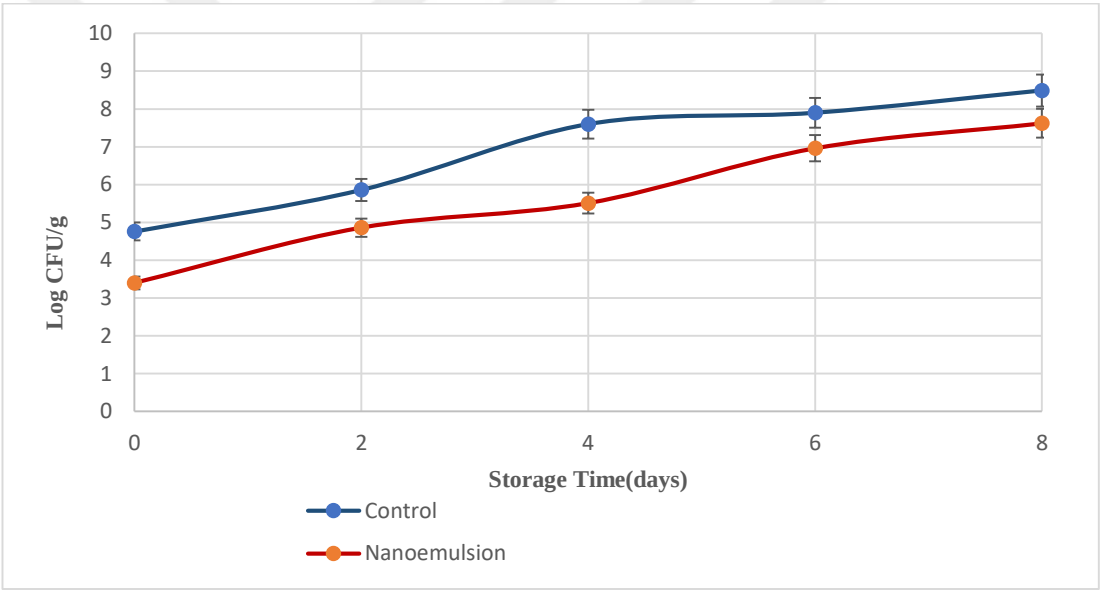

Figure 4.10. Effect of the *E. radiata* essential oil nanoemulsion as an antimicrobial dipping solution on the delay the growth of *Pseudomonas* spp.

In a study examining the antibacterial activity of essential oils obtained from different plants on fish against *Pseudomonas* species, it was reported that *Cinnamomum zeylanicum*, *Cymbopogon nardus*, *Origanum vulgare*, *Foeniculum vulgare*, and *Thymus serpyllum* essential oils showed strong antimicrobial activity. When the GC-MS results of *Cinnamomum zeylanicum*, the most effective of these essential oils, were examined, it was seen that it contained the most (E)-cinnamaldehyde, Linalool, Eugenol, and 1,4-cineole compounds, respectively (Kačániová et al., 2017). This

suggests that the potent antibacterial action of the *E. radiata* essential oil nanoemulsion against *Pseudomonas* spp. in fresh beef is probably caused by the abundance of monoterpenoids (o-cymene, 2-bornene, 1,8-cineole, and α-pinene) present in the eucalyptus essential oil.

4.5.4. Antimicrobial Activity of *Eucalyptus radiata* Essential Oil Nanoemulsion on Fresh Beef Chunks Against Lactic Acid Bacteria

Table 4.4 displays the impact of applying the nanoemulsion on the lactic acid bacteria count. The results demonstrate that the eucalyptus nanoemulsion had no antimicrobial effect on the lactic acid bacteria. Besides, due to the presence of other predominant spoilage microorganisms, the lactic acid bacteria count has been low in both groups; nonetheless, it has varied between 2.20 and 3.43 $\log_{10}$ CFU/g in the control group and 2.51 and 4.10 log CFU/g in the nanoemulsion group. When the results are examined at different days, on day 0, no lactic acid bacteria were found in either the control or the emulsion. While significant differences were found on day 2, similar results were obtained on day 4, but after that, samples kept in the emulsion had higher lactic acid bacteria counts, which is seen in Figure 4.11. Contrarily to our findings, a recent study by Gedikoğlu (2022b) reported that the essential oils of *Thymus vulgaris* and *Thymbra spicata* have exhibited strong inhibitory effects against lactic acid bacteria. Only 1% of the essential oil mixture has provided a 3.02 log CFU/g reduction in the lactic acid bacteria count for the essential oil-coated bologna samples in comparison to the control bolognas. Despite the fact that the eucalyptus nanoemulsion has shown no antimicrobial activity against lactic acid bacteria in this study, it has demonstrated strong antimicrobial activity against a wide range of microorganisms.

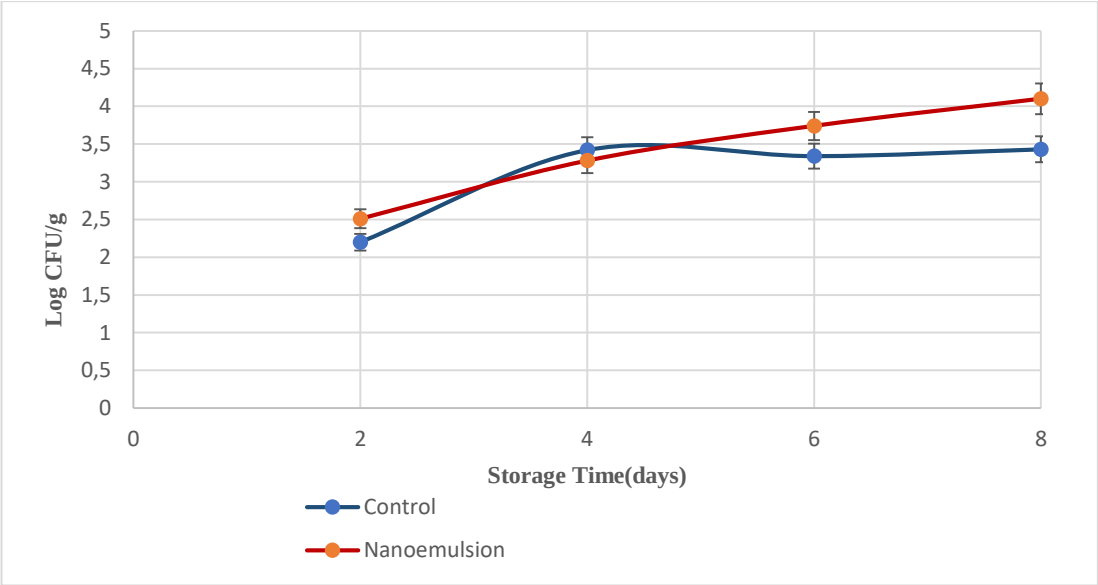

Figure 4.11. Effect of the *E. radiata* essential oil nanoemulsion as an antimicrobial dipping solution on the growth of lactic acid bacteria

4.5.5. Antimicrobial Activity of *Eucalyptus radiata* Essential Oil Nanoemulsion on Fresh Beef Chunks Against Yeast and Mold

The changes in the number of yeasts and mold counts of fresh beef samples treated with the eucalyptus nanoemulsion in comparison to the control groups are displayed in Table 4.4. Similar to the results of total coliform and *Pseudomonas* spp. counts, eucalyptus oil nanoemulsion treatment has shown a significant ($P \leq 0.05$) reduction in yeast and mold counts during 8 days of cold storage. Maximum antifungal activity was observed on the 4th day, proving a 2.18 log CFU/g difference between the control and the nanoemulsion treatments (Figure 4.12).

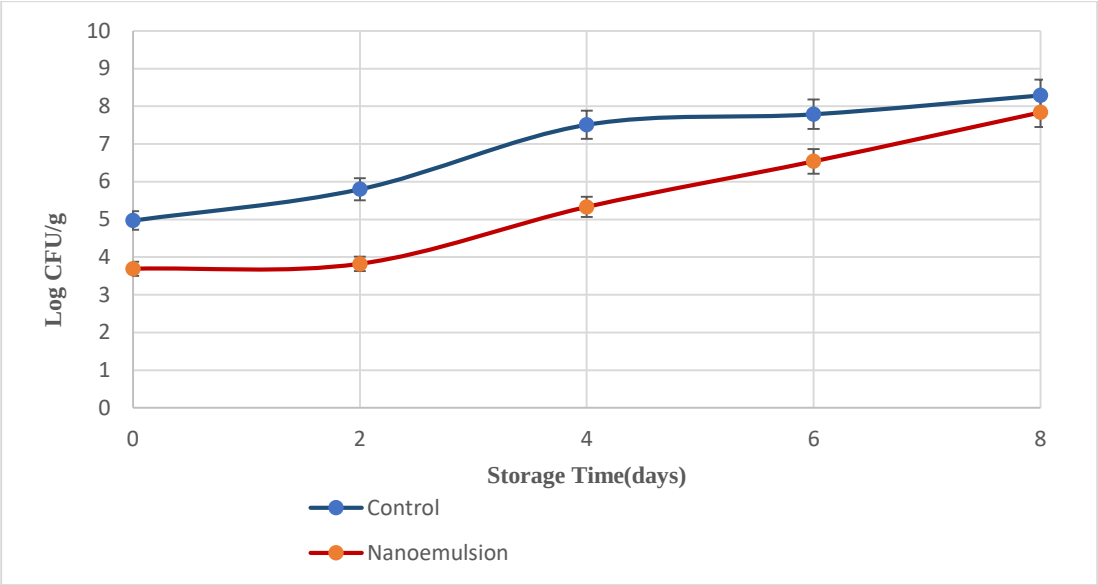

Figure 4.12. Effect of the *E. radiata* essential oil nanoemulsion as an antimicrobial dipping solution on the growth of yeast and mold

In the study conducted by Noori et al. (2018), a sodium caseinate-based nanoemulsion containing 6% *Zingiber officinale* essential oil exhibited antimicrobial activity against mold and yeast in chicken fillets. It was observed that the formation of the number of organisms was delayed until the 4th day. Furthermore, Gedikoğlu (2022b) found that a 1% thyme oil-pectin coating effectively inhibited the growth of yeast and mold on bologna samples and slowed down their growth by 3.56 $\log_{10}$ CFU/g at the end of the storage. Effective results occur when the antimicrobial activity of bioactive compounds found in essential oils is combined with a nanoemulsion formulation. The smaller size of nanoemulsions results in a larger surface area, the entry of nanoemulsions into the cells of microorganisms becomes easier; the binding tendency increases; and thus, the effectiveness of essential oils increases.

Despite the fact that research on the *in vitro* antimicrobial activities of essential oils of different plants and their by-products has been ongoing, to the best of our knowledge, no studies have been found comparing the antimicrobial activity of eucalyptus oils' or their nanoemulsions' in meat products. As a result, this study demonstrates the antimicrobial potential of eucalyptus nanoemulsion by controlling the growth of *S.*

aureus and delaying the spoilage of fresh red meat, while also providing new information to the body of literature in this field.

5. CONCLUSION

Red meat is susceptible to microbial contamination because of its high nutritional value and its highly available water content. Natural substances, such as essential oils, can be used in food applications to reduce the risk of pathogen contamination and delay the spoilage of meat products. In this study, both the *in vitro* and *in situ* antimicrobial activity of an essential oil obtained from natural sources, namely the *E. radiata* plant, were examined. Eucalyptus oil was extracted using microwave extraction technology with a microwave power of 550 W for 45 min. The eucalyptus oil nanoemulsion was prepared using a high-shear blending of 2% *Eucalyptus radiata* essential oil, 1% high-methoxyl pectin, 5% glycerol, and 5% Tween 80®. To evaluate the nanoemulsion's characteristic properties, particle size, zeta potential, viscosity, and whiteness index values were determined. Results showed that the eucalyptus oil nanoemulsion had a particle size of 52.04 nm and a low zeta potential of -9.16 mV. Additionally, the *in vitro* antibacterial activity of eucalyptus essential oil and nanoemulsions was examined against foodborne pathogens. The results of the *in vitro* studies showed that the eucalyptus oil exhibited an inhibitory effect, especially against Gram-positive bacteria, while the nanoemulsion had a stronger antimicrobial effect against Gram-negative bacteria. For the testing of the food matrix, the application of the nanoemulsion as a dipping solution was evaluated against *S. aureus*-contaminated meat samples. In addition, the effect of the nanoemulsion on the shelf life of fresh beef chunks was determined by assessing total coliform, *E. coli*, *Pseudomonas* spp., lactic acid bacteria, yeast, and mold counts. The results demonstrated that the nanoemulsion application had a significant ($P \leq 0.05$) effect on controlling the growth of *S. aureus* on fresh beef pieces, providing a 1.54 to 2.5 $\log_{10}$ CFU/g decline in the bacteria count in comparison to control samples. Moreover, the nanoemulsion application negatively affected the growth of spoilage microorganisms and delayed the spoilage of fresh beef pieces by 4 days. In conclusion, this study demonstrated that use of *E. radiata* essential oil nanoemulsions as a dipping application can be used as a hurdle technology to improve the food safety and shelf life of fresh meat products. New strategies can be developed by increasing studies on the synergistic mechanism between different components.

6. REFERENCES

Abad, M. J., Bedoya, L. M., Apaza, L., & Bermejo, P. (2012). The *Artemisia* L. genus: a review of bioactive essential oils. *Molecules*, 17(3), 2542-2566. https://doi.org/10.3390/molecules17032542.

Abdelhafed, E. A., Miladi, K., Badri, W., Sala, M., Ait Addi, E. H., Casabianca, H., El Mousadik, A., Hartmann, D., Jilale, A., Renaud, F. N. R., & Elaissari, A. (2015). Essential oils: From extraction to encapsulation. *International Journal of Pharmaceutics, 1-2(483)*, 220-243. https://doi.org/10.1016/j.ijpharm.2014.12.069.

Abdalrahman, L. S., Wells, H., & Fakhr, M. K. (2015). *Staphylococcus aureus* is more prevalent in retail beef livers than in pork and other beef cuts. *Pathogens*, 4(2), 182-198. https://doi.org/10.3390/pathogens4020182

Adepoju, T. F., Olawale, O., Okunola, A. A., & Olatunji, E. A. (2014). Solvent extraction of oil from soursop oilseeds & its quality characterization. *International Journal of Sustainable Energy and Environmental Research*, 3(2), 80-89.

Ahmad, A., Elisha, I. L., van Vuuren, S., & Viljoen, A. (2021). Volatile phenolics: A comprehensive review of the anti-infective properties of an important class of essential oil constituents. *Phytochemistry*, 190, 112864. https://doi.org/10.1016/j.phytochem.2021.112864.

Al Maqtari, M. A. A., Alghalibi, S. M., & Alhamzy, E. H. (2011). Chemical composition and antimicrobial activity of essential oil of *Thymus vulgaris* from Yemen. *Turkish Journal of Biochemistry*, 36(4), 342-349.

Allenspach, M., & Steuer, C. (2021). α-Pinene: A never-ending story. *Phytochemistry*, 190, 112857. https://doi.org/10.1016/j.phytochem.2021.112857

Almasi, L., Radi, M., Amiri, S., & McClements, D. J. (2021). Fabrication and characterization of antimicrobial biopolymer films containing essential oil-loaded microemulsions or nanoemulsions. *Food Hydrocolloids, 117*, 106733. https://doi.org/10.1016/j.foodhyd.2021.106733.

Angane, M., Swift, S., Huang, K., Butts, C. A., & Quek, S. Y. (2022). Essential oils and their major components: An updated review on antimicrobial activities, mechanism of action and their potential application in the food industry. *Foods*, 11(3), 464. https://doi.org/10.3390/foods11030464

Artiga-Artigas, M., Guerra-Rosas, M. I., Morales-Castro, J., Salvia-Trujillo, L., & Martín-Belloso, O. (2018). Influence of essential oils and pectin on nanoemulsion formulation: A ternary phase experimental approach. *Food Hydrocolloids, 81*, 209–219. https://doi.org/10.1016/j.foodhyd.2018.03.001.

Astani, A., & Schnitzler, P. (2014). Antiviral activity of monoterpenes beta-pinene and limonene against herpes simplex virus in vitro. *Iranian Journal of Microbiology, 6(3), 149*.

Aswathanarayan, J. B., & Vittal, R. R. (2019). Nanoemulsions and their potential applications in food industry. *Frontiers in Sustainable Food Systems*, 3, 95. https://doi.org/10.3389/fsufs.2019.00095

Bachir, R., & Benali, M. (2012). Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and *Staphylococcus aureus*. *Asian Pacific Journal of Tropical Biomedicine, 2*, 739–742. https://doi.org/10.1016/S2221-1691(12)60220-2

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils–a review. *Food and Chemical Toxicology, 46*(2), 446-475. https://doi.org/10.1016/j.fct.2007.09.106.

Balasubramani, S., Rajendhiran, T., Moola, A. K., & Diana, R. K. B. (2017). Development of nanoemulsion from *Vitex negundo* L. essential oil and their efficacy of antioxidant, antimicrobial and larvicidal activities (*Aedes aegypti* L.). *Environmental Science and Pollution Research*, 24, 15125-15133. https://doi.org/10.1007/s11356-017-9118-y

Başer, K. H. C., Gülbaba, A. G., Azcan, N., Kara, M., Kırımer, N., Kürkçüoğlu, M., Özek, T., & Özkurt, N. (1998). Study of the technology, yield and composition of essential oils from some *Eucalyptus* species grown in Turkey. *Eastern Mediterranean Forestry Research Institute, 7.*

Bendaoud, H., Bouajila, J., Rhouma, A., Savagnac, A., & Romdhane, M. (2009). GC/MS analysis and antimicrobial and antioxidant activities of essential oil of *Eucalyptus radiata*. *Journal of the Science of Food and Agriculture*, 89(8), 1292-1297. https://doi.org/10.1002/jsfa.3585

Bennett, R. N., & Wallsgrove, R. M. (1994). Secondary metabolites in plant defence mechanisms. *New Phytologist*, 127(4), 617-633. https://doi.org/10.1111/j.1469-8137.1994.tb02968.x

Bibette, J., Calderon, F. L., & Poulin, P. (1999). Emulsions: basic principles. *Reports on Progress in Physics, 62(6), 969*. https://doi.org/10.1088/0034-4885/62/6/203

Biswas, A. K., Kondaiah, N., Bheilegaonkar, K. N., Anjaneyulu, A. S. R., Mendiratta, S. K., Jana, C., Singh, H., & Kumar, R. R. (2008). Microbial profiles of frozen trimmings and silver sides prepared at Indian buffalo meat packing plants. *Meat Science*, 80(2), 418-422. https://doi.org/10.1016/j.meatsci.2008.01.004

Boland, D. J., Brooker, M. I. H., Chippendale, G. M., Hall, N., Hyland, B. P. M., Johnston, R. D., Kleinig, D. A., & Turner, J. D. (2006). *Forest Trees of Australia* (5th ed.). CSIRO Publishing.

Bosilevac, J. M., & Koohmaraie, M. (2011). Prevalence and characterization of non-O157 Shiga toxin-producing Escherichia coli isolates from commercial ground beef in the United States. Applied and environmental microbiology, 77(6), 2103-2112. https://doi.org/10.1128/AEM.02833-10

Brodowska, A. J., Nowak, A., & Śmigielski, K. (2018). Ozone in the food industry: Principles of ozone treatment, mechanisms of action, and applications: An overview.

Critical Reviews in Food Science and Nutrition, 58(13), 2176-2201. https://doi.org/10.1080/10408398.2017.1308313

Buranasuksombat, U., Kwon, Y., Turner, M., & Bhandari, B. (2011). Influence of emulsion droplet size on antimicrobial properties. *Food Science and Biotechnology, 20*, 793–800. https://doi.org10.1007/s10068-011-0110-x.

Cai, Z. M., Peng, J. Q., Chen, Y., Tao, L., Zhang, Y. Y., Fu, L. Y., Long, Q. D., & Shen, X. C. (2021). 1, 8-Cineole: A review of source, biological activities, and application. *Journal of Asian Natural Products Research*, 23(10), 938-954. https://doi.org/10.1080/10286020.2020.1839432

Callender, S. P., Mathews, J. A., Kobernyk, K., & Wettig, S. D. (2017). Microemulsion utility in pharmaceuticals: Implications for multi-drug delivery. *International Journal of Pharmaceutics*, 526(1-2), 425-442.

Campos, J. F., & Berteina-Raboin, S. (2022). Eucalyptol, an all-purpose product. *Catalysts*, 12(1), 48.

Cardoso-Ugarte, G. A., Juárez-Becerra, G. P., SosaMorales, M. E., & López-Malo, A. (2013). Microwave-assisted extraction of essential oils from herbs. *Journal of Microwave Power and Electromagnetic Energy*, 47(1), 63-72. https://doi.org/10.1080/08327823.2013.11689846

Centers for Disease Control and Prevention (CDC). (2021). *Salmonella*. Retrieved from https://www.cdc.gov/salmonella/index.html

Chan, C. H., Yusoff, R., Ngoh, G. C., & Kung, F. W. L. (2011). Microwave-assisted extractions of active ingredients from plants. *Journal of Chromatography A*, 1218(37), 6213-6225. https://doi.org/10.1016/j.chroma.2011.07.040

Chang, Y., McLandsborough, L., & McClements, D. J. (2015). Fabrication, stability and efficacy of dual-component antimicrobial nanoemulsions: Essential oil (thyme oil) and cationic surfactant (lauric arginate). *Food Chemistry, 172*, 298–304. https://doi.org/10.1016/j.foodchem.2014.09.081.

Çınar, K. (2017). A review on nanoemulsions: preparation methods and stability. *Trakya Üniversitesi Mühendislik Bilimleri Dergisi*, 18(1), 73-83.

da Silva Rivas, A. C., Lopes, P. M., de Azevedo Barros, M. M., Costa Machado, D. C., Alviano, C. S., & Alviano, D. S. (2012). Biological activities of α-pinene and β-pinene enantiomers. *Molecules*, 17(6), 6305-6316. https://doi.org/10.3390/molecules17066305

Darwish, W. S., El Bayoumi, R. M., Mohamed, N. H., & Hussein, M. A. (2024). Microbial contamination of meat at a low temperature storage: A review. *Journal of Advanced Veterinary Research*, 14(2), 322-325.

Das, K., & Gezici, S. (2018). Secondary plant metabolites, their separation and identification, and role in human disease prevention. *Annals of Phytomedicine*, 7(2), 13-24. https://doi.org/10.21276/ap.2018.7.2 .

Devarajan, V., & Ravichandran, V. J. I. J. C. P. (2011). Nanoemulsions: as modified drug delivery tool. *IDevarajan V / Pharmacie* Globale (IJCP), 4(1), 1-6.

Dickinson, E. (2012). "Emulsions and Emulsifiers." In Food Colloids: Self-Assembly and Material Science (pp. 167-198). *Royal Society of Chemistry*.

Dogan, G., Kara, N., Bagci, E., & Gur, S. (2017). Chemical composition and biological activities of leaf and fruit essential oils from *Eucalyptus camaldulensis*. *Zeitschrift für Naturforschung* C, 72(11-12), 483-489. https://doi.org/10.1515/znc-2016-0033

Dokuzlu, C. (2006). Kesimhanelerde broiler karkasların mikrobiyal yükü üzerine ozon ve klor uygulamalarının etkileri. *Uludağ Üniversitesi Veteriner Fakültesi Dergisi*, 25(1-2), 7-11.

Donsì, F., Annunziata, M., Vincensi, M., & Ferrari, G. (2012). Design of nanoemulsion-based delivery systems of natural antimicrobials: Effect of the emulsifier. *Journal of Biotechnology*, *159*(4), 342–350. https://doi.org/10.1016/j.jbiotec.2011.07.001.

Ebert, M. (2018). Hygiene principles to avoid contamination/cross-contamination in the kitchen and during food processing. In *Staphylococcus aureus* (pp. 217-234). Academic Press. https://doi.org/10.1016/B978-0-12-809671-0.00011-5

Emiroğlu, Z. K., Yemiş, G. P., Coşkun, B. K., & Candoğan, K. (2010). Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. *Meat Science*, 86(2), 283-288. https://doi.org/10.1016/j.meatsci.2010.04.016

Elkolli, H., Elkolli, M., Ataya, F. S., Salem-Bekhit, M. M., Zahrani, S. A., Abdelmageed, M. W., Ernst, B., & Benguerba, Y. (2023). In vitro and in silico activities of *E. radiata* and *E. cinerea* as an enhancer of antibacterial, antioxidant, and anti-inflammatory agents. *Molecules*, 28(20), 7153. https://doi.org/10.3390/molecules28207153

Eskilsson, C. S., & Björklund, E. (2000). Analytical-scale microwave-assisted extraction. *Journal of Chromatography A*, 902(1), 227-250. https://doi.org/10.1016/S0021-9673(00)00921-3

Fatima, M., Sheraz, M. A., Ahmed, S., Kazi, S. H., & Ahmad, I. (2014). Emulsion separation, classification and stability assessment. *RADS Journal of Pharmacy and Pharmaceutical Sciences*, 2(2), 56-62.

Fernandez, P., André, V., Rieger, J., & Kühnle, A. (2004). Nano-emulsion formation by emulsion phase inversion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 251(1-3), 53-58. https://doi.org/10.1016/j.colsurfa.2004.09.029

Fernández-Marín, R., Fernandes, S. C., Andrés, M. A., & Labidi, J. (2021). Microwave-assisted extraction of *Curcuma longa* L. Oil: Optimization, chemical structure and composition, antioxidant activity and comparison with conventional soxhlet extraction. *Molecules*, 26(6), 1516.

Food and Drug Administration (FDA). (2020). U.S. Department of Health and Human Services: Rockville, MD, USA.

Frewer, L., Scholderer, J., & Lambert, N. (2003). Consumer acceptance of functional foods: issues for the future. *British Food Journal*, 105(10), 714-731. https://doi.org/10.1108/00070700310506263

Fryd, M. M., & Mason, T. G. (2012). Advanced nanoemulsions. *Annual Review of Physical Chemistry*, 63, 493-518. https://doi.org/10.1146/annurev-physchem-032210-103436

Galdiero, S., Falanga, A., Cantisani, M., Tarallo, R., Elena Della Pepa, M., D'Oriano, V., & Galdiero, M. (2012). Microbe-host interactions: structure and role of Gram-negative bacterial porins. *Current Protein and Peptide Science*, 13(8), 843-854.

Gedikoğlu, A., Sökmen, M., Çivit, A. (2019). Evaluation of *Thymus vulgaris* and *Thymbra spicata* essential oils and plant extracts for chemical composition, antioxidant, and antimicrobial properties. *Food Science & Nutrition*, 7(5), 1704–1714. https://doi.org/10.1002/fsn3.1007

Gedikoğlu, A. (2022a). Antimicrobial and antioxidant activities of commercialized Turkish propolis extract, and application to beef meatballs. *Turkish Journal of Agriculture - Food Science and Technology*, 10(10), 2021–2029. https://doi.org/10.24925/turjaf.v10i10.2021-2029.5340

Gedikoğlu, A. (2022b). The effect of *Thymus vulgaris* and *Thymbra spicata* essential oils and/or extracts in pectin edible coating on the preservation of sliced bolognas. *Meat Science*, 184, 108697. https://doi.org/10.1016/j.meatsci.2021.108697

Gedikoğlu, A., Çıkrıkcı Erünsal, S. (2023). Characterization of a *Thymbra spicata* essential oil–pectin nanoemulsion, and antimicrobial activity against foodborne pathogenic bacteria. *Food Measure* 17, 3195–3206. https://doi.org/10.1007/s11694-023-01855-2

Geow, C. H., Tan, M. C., Yeap, S. P., & Chin, N. L. (2021). A review on extraction techniques and its future applications in industry. *European Journal of Lipid Science and Technology*, 123(4), 2000302. https://doi.org/10.1002/ejlt.202000302

Gershenzon, J., & Dudareva, N. (2007). The function of terpene natural products in the natural world. *Nature Chemical Biology*, 3(7), 408-414.

Ghosh, S., Ray, A., & Pramanik, N. (2020). Self-assembly of surfactants: An overview on general aspects of amphiphiles. *Biophysical Chemistry*, 265, 106429. https://doi.org/10.1016/j.bpc.2020.106429

Guabiraba, R., & Schouler, C. (2015). Avian colibacillosis: still many black holes. *FEMS Microbiology Letters*, 362(15), fnv118. https://doi.org/10.1093/femsle/fnv118

Gumustas, M., Sengel Turk, C. T., Gumustas, A., Ozkan, S., & Uslu, B. (2017). Effect of polymer-based nanoparticles on the assay of antimicrobial drug delivery systems. In A. M. Grumezescu (Ed.), *Multifunctional systems for combined delivery, biosensing and diagnostics* (pp. 67–108). Elsevier. https://doi.org/10.1016/B978-0-323-52725-5.00005-8

Gupta, A., Eral, H. B., Hatton, T. A., & Doyle, P. S. (2016). Nanoemulsions: formation, properties and applications. *Soft Matter*, 12(11), 2826-2841.

Gupta, A., Jeyakumar, E., & Lawrence, R. (2021). Journey of limonene as an antimicrobial agent. *Journal of Pure & Applied Microbiology*, 15(3). https://doi.org/10.22207/JPAM.15.3.01

Gupta, A., Narsimhan, V., Hatton, T. A., & Doyle, P. S. (2016). Kinetics of the change in droplet size during nanoemulsion formation. *Langmuir*, 32(44), 11551-11559. https://doi.org/10.1021/acs.langmuir.6b01862

Gundogan, N., Citak, S., Yucel, N., & Devren, A. (2005). A note on the incidence and antibiotic resistance of *Staphylococcus aureus* isolated from meat and chicken samples. Meat science, 69(4), 807-810. https://doi.org/10.1016/j.meatsci.2004.10.011

Haiyan, L. U., Chongxin, X. U., Zhang, X., Liang, Y., & Xianjin, L. I. U. (2016). Antibacterial effect of limonene on food-borne pathogens. *Journal of Zhejiang University (Agriculture and Life Sciences), 42(3),* 306-312.

Hassine, D. B., Abderrabba, M., Yvon, Y., Lebrihi, A., Mathieu, F., Couderc, F., & Bouajila, J. (2012). Chemical composition and in vitro evaluation of the antioxidant and antimicrobial activities of *Eucalyptus gillii* essential oil and extracts. *Molecules,* 17(8), 9540-9558. https://doi.org/10.3390/molecules17089540

Helgeson, M. (2016). Colloidal behavior of nanoemulsions: Interactions, structure and rheology. *Current Opinion in Colloid & Interface Science*, 25. https://doi.org/10.1016/j.cocis.2016.06.006.

Holch, A., Webb, K., Lukjancenko, O., Ussery, D., Rosenthal, B. M., & Gram, L. (2013). Genome sequencing identifies two nearly unchanged strains of persistent Listeria monocytogenes isolated at two different fish processing plants sampled 6 years apart. *Applied and Environmental Microbiology*, 79(9), 2944-2951. https://doi.org/10.1128/AEM.03715-12

Hou, X., & Sheng, J. J. (2023). Properties, preparation, stability of nanoemulsions, their improving oil recovery mechanisms, and challenges for oil field applications—A critical review. *Geoenergy Science and Engineering*, 221, 211360. https://doi.org/10.1016/j.geoen.2022.211360.

Hu, Y. T., Ting, Y., Hu, J. Y., & Hsieh, S. C. (2017). Techniques and methods to study functional characteristics of emulsion systems. *Journal of Food and Drug Analysis*, 25(1), 16-26. https://doi.org/10.1016/j.jfda.2016.10.021

Hu, Y., Qiao, J., Zhang, X., & Ge, C. (2011). Antimicrobial effect of Magnolia officinaliss extract against *Staphylococcus aureus. Journal of the Science of Food and Agriculture*, *91*(6), 1050-1056. https://doi.org/10.1002/jsfa.4280

Iacobellis, N. S., Lo Cantore, P., Capasso, F., & Senatore, F. (2005). Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. *Journal of Agricultural and Food Chemistry*, 53(1), 57-61. https://doi.org/10.1021/jf0487351

Ibáñez, M. D., Sanchez-Ballester, N. M., & Blázquez, M. A. (2020). Encapsulated limonene: A pleasant lemon-like aroma with promising application in the agri-food industry. A review. *Molecules*, 25(11), 2598. https://doi.org/10.3390/molecules25112598

Jahan, M., Warsi, M. K., & Khatoon, F. (2011). Studies on antibacterial property of Eucalyptus-The aromatic plant. *International Journal of Pharmaceutical Sciences Review and Research*, 7(2), 86-8.

Jaiswal, M., Dudhe, R., & Sharma, P. K. (2015). Nanoemulsion: an advanced mode of drug delivery system. *3 Biotech*, 5, 123-127. https://doi.org/10.1007/s13205-014-0214-0

Jugreet, B. S., Suroowan, S., Rengasamy, R. K., & Mahomoodally, M. F. (2020). Chemistry, bioactivities, mode of action and industrial applications of essential oils. *Trends in Food Science & Technology*, 101, 89-105. https://doi.org/10.1016/j.tifs.2020.04.025

Kabalnov, A. (1998). Thermodynamic and theoretical aspects of emulsions and their stability. *Current Opinion in Colloid & Interface Scienc*e, 3(3), 270-275. https://doi.org/10.1016/S1359-0294(98)80071-X

Kačániová, M., Terentjeva, M., Vukovic, N., Puchalski, C., Roychoudhury, S., Kunová, S., Klūga, A., Tokár, M., Kluz, M., & Ivanišová, E. (2017). The antioxidant and antimicrobial activity of essential oils against *Pseudomonas* spp. isolated from fish. *Saudi Pharmaceutical Journal*, 25(8), 1108-1116. https://doi.org/10.1016/j.jsps.2017.07.005

Karbstein, H., & Schubert, H. (1995). Developments in the continuous mechanical production of oil-in-water macro-emulsions. *Chemical Engineering and Processing: Process Intensification*, 34(3), 205-211. https://doi.org/10.1016/0255-2701(94)04005-2

Khan, B. A., Akhtar, N., Khan, H. M. S., Waseem, K., Mahmood, T., Rasul, A., Iqbal, M., & Khan, H. (2011). Basics of pharmaceutical emulsions: A review. *African Journal of Pharmacy and Pharmacology*, 5(25), 2715-2725. https://doi.org/10.5897/AJPP11.698

Khan, I., Tango, C. N., Miskeen, S., Lee, B. H., & Oh, D. H. (2017). Hurdle technology: A novel approach for enhanced food quality and safety–A review. *Food Control*, 73, 1426-1444. https://doi.org/10.1016/j.foodcont.2016.11.010

Khanashyam, A. C., Shanker, M. A., Kothakota, A., Mahanti, N. K., & Pandiselvam, R. (2022). Ozone applications in milk and meat industry. *Ozone: Science & Engineering*, 44(1), 50-65. https://doi.org/10.1080/01919512.2021.1947776

Khoshnazar, M., Parvardeh, S., & Bigdeli, M. R. (2020). Alpha-pinene exerts neuroprotective effects via anti-inflammatory and anti-apoptotic mechanisms in a rat model of focal cerebral ischemia-reperfusion. *Journal of Stroke and Cerebrovascular Diseases*, 29(8), 104977. https://doi.org/10.1016/j.jstrokecerebrovasdis.2020.104977

Kralova, I., & Sjöblom, J. (2009). Surfactants used in food industry: a review. *Journal of Dispersion Science and Technology*, 30(9), 1363-1383. https://doi.org/10.1080/01932690902735561

Kumar, P., & Mittal, K. L. (Eds.). (1999). Handbook of microemulsion science and technology (pp. 1-12). *New York: Marcel Dekker*.

Kundu, P., Agrawal, A., Mateen, H., & Mishra, I. M. (2013). Stability of oil-in-water macro-emulsion with anionic surfactant: Effect of electrolytes and temperature. *Chemical Engineering Science,* 102, 176-185. http://dx.doi.org/10.1016/j.ces.2013.07.050

Kurpas, M., Wieczorek, K., & Osek, J. (2018). Ready-to-eat meat products as a source of. Journal of Veterinary Research, 62(1), 49-55. https://doi.org/10.2478/jvetres-2018-0007

Leite, A., Lima, E., Souza, E., Diniz, M., Trajano, V., & Medeiros, I. (2007). Inhibitory effect of β-pinene, α-pinene and eugenol on the growth of potential infectious endocarditis causing Gram-positive bacteria. *Revista Brasileira de Ciências Farmacêuticas, 43*, 121–126. https://doi.org/10.1590/S1516-93322007000100015

Li, Y., Han, L., Ma, R., Xu, X., Zhao, C., Wang, Z., ... & Hu, X. (2012). Effect of energy density and citric acid concentration on anthocyanins yield and solution temperature of grape peel in microwave-assisted extraction process. *Journal of Food Engineering*, 109(2), 274-280. https://doi.org/10.1016/j.jfoodeng.2011.09.021

Li, L., Li, Z. W., Yin, Z. Q., Wei, Q., Jia, R. Y., Zhou, L. J., Xu, J., Song, X., Zhou, Y., Du, Y. H., Peng, L.C., Kang, S., & Yu, W. (2014). Antibacterial activity of leaf essential oil and its constituents from *Cinnamomum longepaniculatum. International Journal of Clinical and Experimental Medicine*, 7(7), 1721.

Lim, J. Y., Yoon, J., & Hovde, C. J. (2010). A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. *Journal of Microbiology and Biotechnology*, 20(1), 5–14.

Luís, Â., Duarte, A., Gominho, J., Domingues, F., & Duarte, A. P. (2016). Chemical composition, antioxidant, antibacterial and anti-quorum sensing activities of *Eucalyptus globulus* and *Eucalyptus radiata* essential oils. *Industrial Crops and Products*, 79, 274-282. https://doi.org/10.1016/j.indcrop.2015.10.055

Lynnes, T., Horne, S. M., & Prüß, B. M. (2014). ß-phenylethylamine as a novel nutrient treatment to reduce bacterial contamination due to Escherichia coli O157: H7 on beef meat. *Meat Science*, 96(1), 165-171. https://doi.org/10.1016/j.meatsci.2013.06.030

Madhav, S., & Gupta, D. (2011). A review on microemulsion based system. *International Journal of Pharmaceutical Sciences and Research*, 2(8), 1888.

Mann, N. (2000). Dietary lean red meat and human evolution. *European Journal of Nutrition*, 39, 71-79. https://doi.org/10.1007/s003940050005

Mahizan, N. A., Yang, S. K., Moo, C. L., Song, A. A. L., Chong, C. M., Chong, C. W., ... & Lai, K. S. (2019). Terpene derivatives as a potential agent against antimicrobial resistance (AMR) pathogens. *Molecules*, 24(14), 2631. https://doi.org/10.3390/molecules24142631

Mahumane, G., van Vuuren, S., Kamatou, G. P., Sandasi, M., & Viljoen, A. (2016). Chemical composition and antimicrobial activity of Eucalyptus radiata leaf essential

oil, sampled over a year. *Journal of Essential Oil Research*, 28, 1–14. https://doi.org/10.1080/10412905.2016.1175386.

Marchese, A., Arciola, C. R., Barbieri, R., Silva, A. S., Nabavi, S. F., Tsetegho Sokeng, A. J., Izadi, M., Jafari, N. J., Suntar, İ., Daglia, M., & Nabavi, S. M. (2017). Update on monoterpenes as antimicrobial agents: A particular focus on p-cymene. *Materials*, 10(8), 947. https://doi.org/10.3390/ma10080947

Maurya, A., Prasad, J., Das, S., & Dwivedy, A. K. (2021). Essential oils and their application in food safety. *Frontiers in Sustainable Food Systems*, 5, 653420.

Mazarei, Z., & Rafati, H. (2019). Nanoemulsification of *Satureja khuzestanica* essential oil and pure carvacrol; comparison of physicochemical properties and antimicrobial activity against food pathogens. *LWT - Food Science and Technology*, *100*. https://doi.org/10.1016/j.lwt.2018.10.094

McClements, D., & Rao, J. (2011). Food-grade nanoemulsions: Formulation, fabrication, properties, performance, biological fate, and potential toxicity. *Critical Reviews in Food Science and Nutrition*, *51*, 285–330. https://doi.org/10.1080/10408398.2011.559558.

McClements, D. J. (2004). Food emulsions: principles, practices, and techniques. CRC press. https://doi.org/10.1201/9781420039436

McClements, D. J., & Jafari, S. M. (2018). Improving emulsion formation, stability and performance using mixed emulsifiers: A review. *Advances in Colloid and Interface Science*, 251, 55-79. https://doi.org/10.1016/j.cis.2017.12.001

McGimpsey, J. A., Douglas, M. H., Van Klink, J. W., Beauregard, D. A., & Perry, N. B. (1994). Seasonal variation in essential oil yield and composition from naturalized *Thymus vulgaris* L. in New Zealand. *Flavour and Fragrance Journal*, 9(6), 347-352. https://doi.org/10.1002/jsfa.3585

Michel, T., Destandau, E., & Elfakir, C. (2011). Evaluation of a simple and promising method for extraction of antioxidants from sea buckthorn (*Hippophaë rhamnoides* L.) berries: Pressurised solvent-free microwave assisted extraction. *Food Chemistry*, 126(3), 1380-1386. https://doi.org/10.1016/j.foodchem.2010.09.112

Mihai, A. L., & Popa, M. E. (2013). Essential oils utilization in food industry-a literature review. *Biotechnology Journal*, 17, 187-192.

Miller, F. A., Silva, C. L., & Brandao, T. R. (2013). A review on ozone-based treatments for fruit and vegetables preservation. *Food Engineering Reviews*, 5(2), 77-106.

Mishra, A. K., Sahu, N., Mishra, A., Ghosh, A. K., Jha, S., & Chattopadhyay, P. (2010). Phytochemical screening and antioxidant activity of essential oil of *Eucalyptus* leaf. *Pharmacognosy Journal*, 2(16), 25-28. https://doi.org/10.1016/S0975-3575(10)80045-8

Mulyaningsih, S., Sporer, F., Reichling, J., & Wink, M. (2011). Antibacterial activity of essential oils from eucalyptus and of selected components against multidrug-resistant

bacterial pathogens. *Pharmaceutical Biology, 49*, 893–899. https://doi.org/10.3109/13880209.2011.553625.

Nawrot-Chorabik, K., Sułkowska, M., & Gumulak, N. (2022). Secondary metabolites produced by trees and fungi: Achievements so far and challenges remaining. *Forests*, 13(8), 1338. https://doi.org/10.3390/f13081338

Nazari, M., Ghanbarzadeh, B., Samadi Kafil, H., Zeinali, M., & Hamishehkar, H. (2019). Garlic essential oil nanophytosomes as a natural food preservative: Its application in yogurt as food model. *Colloid and Interface Science Communications*, 30, 1–7. https://doi.org/10.1016/j.colcom.2019.100176

Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*, 6(12), 1451–1474. https://doi.org/10.3390/ph6121451

Noori, S., Zeynali, F., & Almasi, H. (2018). Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (*Zingiber officinale*) essential oil and its effect on safety and quality attributes of chicken breast fillets. *Food Control*, 84, 312-320. https://doi.org/10.1080/87559129.2019.1573829

Nychas, G. J. E., Skandamis, P. N., Tassou, C. C., & Koutsoumanis, K. P. (2008). Meat spoilage during distribution. *Meat Science*, 78(1-2), 77-89. https://doi.org/10.1016/j.meatsci.2007.06.020

Ogunwande, I. A., Olawore, N. O., Adeleke, K. A., & Konig, W. A. (2003). Chemical composition of the essential oils from the leaves of three *Eucalyptus* species growing in Nigeria. *Journal of Essential Oil Research*, 15(5), 297-301. https://doi.org/10.1080/10412905.2003.9698595

Ozturk, B., & McClements, D. J. (2016). Progress in natural emulsifiers for utilization in food emulsions. *Current Opinion in Food Science*, 7, 1-6. https://doi.org/10.1016/j.cofs.2015.07.008

Pagare, S., Bhatia, M., Tripathi, N., Pagare, S., & Bansal, Y. K. (2015). Secondary metabolites of plants and their role: Overview. *Current Trends in Biotechnology and Pharmacy*, 9(3), 293-304.

Pandita, D., & Pandita, A. (2021). Secondary metabolites in medicinal and aromatic plants (MAPs): potent molecules in nature's arsenal to fight human diseases. Medicinal and Aromatic Plants: *Healthcare and Industrial Applications*, 41-84.

Pluta-Kubica, A., Jamróz, E., Juszczak, L., Krzyściak, P., & Zimowska, M. (2021). Characterization of furcellaran-whey protein isolate films with green tea or Pu-erh extracts and their application as packaging of an acid-curd cheese. *Food* and *Bioprocess Technology*, 14(1), 78–92. https://doi.org/10.1007/s11947-020-02570-2

Reyes-Jurado, F., Franco-Vega, A., Ramírez-Corona, N., Palou, E., & López-Malo, A. (2015). Essential oils: antimicrobial activities, extraction methods, and their modeling. *Food Engineering Reviews*, 7, 275-297. https://doi.org/10.1007/s12393-014-9099-2

Saeed, K., Pasha, I., Jahangir Chughtai, M. F., Ali, Z., Bukhari, H., & Zuhair, M. (2022). Application of essential oils in food industry: Challenges and innovation. *Journal of Essential Oil Research*, 34(2), 97-110. https://doi.org/10.1080/10412905.2022.2029776

Salanță, L. C., & Cropotova, J. (2022). An update on effectiveness and practicability of plant essential oils in the food industry. *Plants*, 11(19), 2488. https://doi.org/10.3390/plants11192488.

Salvia-Trujillo, L., & McClements, D. J. (2016). Improvement of β-carotene bioaccessibility from dietary supplements using excipient nanoemulsions. *Journal of Agricultural and Food Chemistry*, 64(22), 4639-4647. https://doi.org/10.1021/acs.jafc.6b00804

Sánchez-Escalante, A., Djenane, D., Torrescano, G., Beltrán, J. A., & Roncalés, P. (2001). The effects of ascorbic acid, taurine, carnosine and rosemary powder on color and lipid stability of beef patties packaged in modified atmosphere. *Meat Science*, 58(4), 421–9. https://doi.org/10.1016/s0309-1740(01)00045-6

Selim, S. (2011). Antimicrobial activity of essential oils against vancomycin-resistant *Enterococci* (vre) and *Escherichia coli* O157: H7 in feta soft cheese and minced beef meat. *Brazilian Journal of Microbiology*, 42, 187-196. https://doi.org/10.1590/S1517-83822010005000005

Shah, P., Bhalodia, D., & Shelat, P. (2010). Nanoemulsion: A pharmaceutical review. *Systematic Reviews in Pharmacy*, 1(1). https://doi.org/10.4103/0975-8453.59509

Shahbazi, Y. (2019). Antioxidant, antibacterial, and antifungal properties of nanoemulsion of clove essential oil. *Nanomedicine Research Journal*, 4(4), 204-208.

Sharifi, F., Jahangiri, M., Nazir, I., Asim, M. H., Ebrahimnejad, P., Hupfauf, A., ... & Bernkop-Schnürch, A. (2021). Zeta potential changing nanoemulsions based on a simple zwitterion. *Journal of Colloid and Interface Science*, 585, 126-137.

Sharmeen, J. B., Mahomoodally, F. M., Zengin, G., & Maggi, F. (2021). Essential oils as natural sources of fragrance compounds for cosmetics and cosmeceuticals. *Molecules*, 26(3), 666. https://doi.org/10.3390/molecules26030666

Sindelar, J. J., & Milkowski, A. L. (2011). Sodium nitrite in processed meat and poultry meats: a review of curing and examining the risk/benefit of its use. *American Meat Science Association White Paper Series*, 3, 1-14. https://doi.org/10.22034/NMRJ.2019.04.001

Sodzim, A. (2012). Extension of shelf life of pork using spices (Plant Extracts: Xylopia aethiopica, *Allium sativum* and *Piper nigrum*) (*Doctoral Dissertation, School of Graduate Studies, Kwame Nkrumah University of Science and Technology*).

Sofos, J. N. (2008). Challenges to meat safety in the 21st century. Meat Science, 78(1-2), 3-13. https://doi.org/10.1016/j.meatsci.2007.07.027

Sofos, J. N. (2014). Meat and meat products. In *Food Safety Management* (pp. 119-162). *Academic Press*.

Solans, C., Esquena, J. O. R. D. I., Forgiarini, A. M., Uson, N. U. R. I. A., Morales, D., Izquierdo, P., ... & Garcia-Celma, M. J. (2003). Nano-emulsions: formation, properties, and applications. *Surfactant Science Series*, 525-554.

Souto, E. B., Cano, A., Martins-Gomes, C., Coutinho, T. E., Zielińska, A., & Silva, A. M. (2022). Microemulsions and nanoemulsions in skin drug delivery. *Bioengineering*, 9(4), 158. https://doi.org/10.3390/bioengineering9040158

Stratakos, A. C., & Koidis, A. (2016). Methods for extracting essential oils. In *Essential Oils in Food Preservation, Flavor and Safety* (pp. 31-38). Academic Press.

Sundararajan, B., Moola, A. K., Vivek, K., & Kumari, B. R. (2018). Formulation of nanoemulsion from leaves essential oil of *Ocimum basilicum* L. and its antibacterial, antioxidant and larvicidal activities (Culex quinquefasciatus). *Microbial Pathogenesis*, 125, 475-485. https://doi.org/10.1016/j.micpath.2018.10.017

Şanlıbaba, P. (2022). Prevalence, antibiotic resistance, and enterotoxin production of *Staphylococcus aureus* isolated from retail raw beef, sheep, and lamb meat in Turkey. *International Journal of Food Microbiology*, 361, 109461. https://doi.org/10.1016/j.ijfoodmicro.2021.109461

Tadros, T. F. (2015). "Emulsion Formation, Stability, and Rheology." In Emulsion Formation and Stability (pp. 1-44). John Wiley & Sons, Ltd

Tadros, T. F. (2016). Emulsions: Formation, stability, industrial applications. Walter de Gruyter GmbH & Co KG.

Tadros, T., Izquierdo, P., Esquena, J., & Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science*, 108, 303-318. https://doi.org/10.1016/j.cis.2003.10.023

Taha, A., Ahmed, E., Ismaiel, A., Ashokkumar, M., Xu, X., Pan, S., & Hu, H. (2020). Ultrasonic emulsification: An overview on the preparation of different emulsifiers-stabilized emulsions. *Trends in Food Science & Technology*, 105, 363-377. https://doi.org/10.1016/j.tifs.2020.09.024

Takahashi, T., Kokubo, R., & Sakaino, M. (2004). Antimicrobial activities of eucalyptus leaf extracts and flavonoids from *Eucalyptus maculata*. *Letters in Applied Microbiology*, 39(1), 60-64. https://doi.org/10.1111/j.1472-765X.2004.01538.x

Teo, C.C., Chong, W.P.K. & Ho, Y.S. Development and application of microwave-assisted extraction technique in biological sample preparation for small molecule analysis. *Metabolomics*, 9, 1109–1128 (2013). https://doi.org/10.1007/s11306-013-0528-7

Thakur, M., Asrani, R. K., & Patial, V. (2018). *Listeria monocytegenes*: A food-borne pathogen. *Foodborne Diseases*, Academic Press, 157-192. https://doi.org/10.1016/B978-0-12-811444-5.00006-3

Thombare, S., & Shirsath, P. (2023). Herbal cosmetics and skin care formulations. *International Journal of Pharmaceutical Sciences*, 1(10), 1-1.

Thwala, T., Madoroba, E., Basson, A., & Butaye, P. (2021). Prevalence and characteristics of *Staphylococcus aureus* associated with meat and meat products in African countries: a review. *Antibiotics*, 10(9), 1108.

Tongnuanchan, P., & Benjakul, S. (2014). Essential oils: extraction, bioactivities, and their uses for food preservation. *Journal of Food Science*, 79(7), R1231-R1249. https://doi.org/10.1111/1750-3841.12492

Tontul, İ. (2019). Nanoencapsulation techniques in food industry. *Food Science and Technology*, 7(2): 220-233. https://doi.org/10.24925/turjaf.v7i2.220-233.2205

Van Haute, S., Raes, K., Van Der Meeren, P., & Sampers, I. (2016). The effect of cinnamon, oregano and thyme essential oils in marinade on the microbial shelf life of fish and meat products. *Food Control*, 68, 30-39.

Veggi, P. C., Martinez, J., & Meireles, M. A. A. (2012). Fundamentals of microwave extraction. In *Microwave-assisted extraction for bioactive compounds: theory and practice* (pp. 15-52). Boston, MA: Springer US.

Vergalli, J., Bodrenko, I. V., Masi, M., Moynié, L., Acosta-Gutiérrez, S., Naismith, J. H., Davin-Regli, A., Ceccaralli, M., Berg, B. V. D., Winterhalter, M., & Pagès, J. M. (2020). Porins and small-molecule translocation across the outer membrane of Gram-negative bacteria. *Nature Reviews Microbiology*, 18(3), 164-176.

Wiącek, A., & Chibowski, E. (1999). Zeta potential, effective diameter and multimodal size distribution in oil/water emulsion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 159(2-3), 253-261. https://doi.org/10.1016/S0927-7757(99)00281-2

Wong, S. F., Lim, J. S., & Dol, S. S. (2015). Crude oil emulsion: A review on formation, classification and stability of water-in-oil emulsions. *Journal of Petroleum Science and Engineering*, 135, 498-504. https://doi.org/10.1016/j.petrol.2015.10.006

Xie, L., Mo, M., Jia, H.-X., Liang, F., Yuan, J., & Zhu, J. (2016). Association between dietary nitrate and nitrite intake and sitespecific cancer risk: evidence from observational studies. *Oncotarget*, 7(35), 56915–56932. https://doi.org/10.18632/oncotarget.10917

Younes, M., Aquilina, G., Castle, L., Engel, K., Fowler, P., Frutos Fernandez, M. J., … Gundert-Remy, U. (2019). Re-evaluation of phosphoric acid–phosphates – di-, tri- and polyphosphates (E 338–341, E 343, E 450–452) as food additives and the safety of proposed extension of use. *EFSA Journal*, 17(6). https://doi.org/10.2903/j.efsa.2019.5674

Zengin, H., & Baysal, A. (2014). Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. *Molecules, 19,* 17773–17798. https://doi.org/10.3390/molecules191117773

7. APPENDIX

APPENDIX-1

Eucalyptus radiata											
	Compounds	Ret Time	Start Time	End Time	m/z	Area	Area %	Height	Height %	A/H	Ret. Index
1	Furan, tetrahydr	4.998	4.970	5.045	TIC	72862	0,01	37565	0,03	1,94	700
2	Furan, tetrahydr	5.234	5.205	5.295	TIC	109208	0,01	48757	0,04	2,24	709
3	Pinene <alpha->	12.911	12.685	13.130	TIC	108965724	9,25	15216041	12,73	7,16	936
4	Bicyclo[2.2.1]he	13.390	13.345	13.430	TIC	177603	0,02	48287	0,04	3,68	947
5	Camphene (CAS	13.477	13.430	13.575	TIC	690472	0,06	177249	0,15	3,9	949
6	Pinene <beta->	14.644	14.540	14.860	TIC	8829452	0,75	1554236	1,3	5,68	977
7	Myrcene	15.095	14.985	15.435	TIC	11591489	0,98	1029491	0,86	11,3	988
8	o-Cymene	17.338	16.520	17.375	TIC	534520222	45,4	26911334	22,52	19,9	1042
9	BICYCLO[2.2.1]	17.525	17.375	17.555	TIC	309661040	26,29	30092212	25,18	10,3	1047
10	EUCALYPTOL (	17.610	17.555	17.745	TIC	133168022	11,31	30510686	25,55	4,36	1049
11	.gamma.-Terpine	18.122	18.005	18.245	TIC	3399401	0,29	995588	0,83	3,41	1061
12	Epoxylinalol	18.697	18.545	18.795	TIC	565189	0,05	105025	0,09	5,38	1075
13	.ALPHA.-TERPI	19.203	19.105	19.285	TIC	402533	0,03	84800	0,07	4,75	1087
14	Benzene, 1-meth	19.404	19.285	19.585	TIC	1223154	0,1	230692	0,19	5,3	1092
15	BETA-PINENOX	19.805	19.595	20.035	TIC	1638850	0,14	315525	0,26	5,19	1102
16	(S)-cis-Verbenol	20.298	20.205	20.395	TIC	222970	0,02	54455	0,05	4,09	1114
17	D-Fenchyl alcoh	20.717	20.635	20.835	TIC	372230	0,03	70743	0,06	5,26	1124
18	Limonene oxide	21.137	21.055	21.245	TIC	408710	0,03	95022	0,08	4,3	1135
19	TRANS-LIMONI	21.305	21.245	21.505	TIC	397093	0,03	51400	0,04	7,73	1139
20	trans-Pinocarveo	21.630	21.505	21.955	TIC	9404052	0,8	1713642	1,43	5,49	1147
21	Pinocarvone	22.366	22.205	22.515	TIC	1871102	0,16	380684	0,32	4,92	1166
22	3-CYCLOHEXE	22.745	22.675	22.815	TIC	225938	0,02	42528	0,04	5,31	1175
23	Pinocamphone <	22.916	22.815	23.005	TIC	1051239	0,09	167536	0,14	6,27	1179
24	3-Cyclohexen-1	23.137	23.005	23.255	TIC	1687465	0,14	289980	0,24	5,82	1185
25	cis-p-Mentha-1(	23.458	23.255	23.615	TIC	1862710	0,16	276929	0,23	6,73	1193
26	.ALPHA. TERPI	23.747	23.615	23.975	TIC	4355607	0,37	698282	0,58	6,24	1200
27	Carveol <trans-2	24.665	24.565	24.865	TIC	655972	0,06	76775	0,06	8,54	1224
28	6-METHYL-.GA	24.909	24.865	24.985	TIC	278980	0,02	63116	0,05	4,42	1231
29	trans-p-Mentha-	25.075	24.985	25.275	TIC	848662	0,07	120078	0,1	7,07	1235
30	10-PINANOL, A	25.560	25.435	25.675	TIC	1373584	0,12	224030	0,19	6,13	1248
31	Camphenol, 6-	27.335	27.275	27.445	TIC	214634	0,02	32815	0,03	6,54	1295
32	Phenol, 5-methy	27.524	27.445	27.655	TIC	818335	0,07	148738	0,12	5,5	1300
33	1-P-MENTHEN-	28.043	27.945	28.135	TIC	240432	0,02	48181	0,04	4,99	1314
34	1-(.alpha.-Hydro	28.631	28.545	28.785	TIC	334499	0,03	63723	0,05	5,25	1330
35	1-(.alpha.-Hydro	28.966	28.875	29.065	TIC	270670	0,02	57053	0,05	4,74	1340
36	Terpinyl acetate	29.267	29.175	29.355	TIC	3755404	0,32	924397	0,77	4,06	1348
37	FARNESYL ACE	30.239	30.155	30.345	TIC	270719	0,02	57696	0,05	4,69	1375
38	1H-Cycloprop[e	31.578	31.485	31.675	TIC	572168	0,05	135112	0,11	4,23	1414
39	TRANS(.BETA.)-	32.048	31.965	32.185	TIC	461378	0,04	86068	0,07	5,36	1428
40	5-BETA-H,7-BE	32.283	32.185	32.355	TIC	317806	0,03	71913	0,06	4,42	1435
41	(+)-CALARENE	32.442	32.355	32.555	TIC	859584	0,07	176700	0,15	4,86	1439
42	NEOALLOOCIM	32.736	32.555	32.845	TIC	16161100	1,37	3352229	2,81	4,82	1448
43	.alpha.-Guaiene	32.911	32.845	32.985	TIC	312356	0,03	82079	0,07	3,81	1453
44	NEOALLOOCIM	33.441	33.345	33.565	TIC	2752485	0,23	640630	0,54	4,3	1469
45	Naphthalene, 1,2	33.819	33.715	33.965	TIC	319210	0,03	55045	0,05	5,8	1480
46	1H-Cycloprop[e	34.441	34.305	34.565	TIC	1660045	0,14	319433	0,27	5,2	1498
47	3-METHYL-5-(2	36.441	36.345	36.555	TIC	291675	0,02	60539	0,05	4,82	1561
48	Veridiflorol	36.872	36.765	36.975	TIC	1398267	0,12	277004	0,23	5,05	1574
49	Veridiflorol	37.128	36.975	37.245	TIC	459851	0,04	71364	0,06	6,44	1582
50	Veridiflorol	37.648	37.545	37.795	TIC	3841774	0,33	777479	0,65	4,94	1599
51	Veridiflorol	37.937	37.825	38.045	TIC	769536	0,07	154317	0,13	4,99	1608
52	Nootkatone (CA	38.365	38.305	38.485	TIC	175393	0,01	46471	0,04	3,77	1622
53	Rosifoliol	38.793	38.705	38.915	TIC	289378	0,02	55283	0,05	5,23	1636
54	HEXATRIACON	53.359	53.195	53.535	TIC	1069567	0,09	116381	0,1	9,19	2173

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