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Research Article

Encapsulation of Essential Oils Using Complex Coacervation: A Study on Microcapsule Formation and Efficiency

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Abstract:

Encapsulation by Coacervation is a process used to create microcapsules. Coacervation is a process that has been used in the food and pharmaceutical industry to produce microspheres with an active ingredient, such as drugs, flavors or fragrances encapsulated by them. It is common in pharmacies, food preparation, cosmetics, and agriculture. This phase separation process is called Coacervation, where a colloidal (in this case polymer) solution will separate into two distinct liquid phases: a Polymer-rich phase, which we refer to as the coacervate, and the other is known as Polymer-poor or solvent-based. This can be accomplished by varying the temperature or pH of a non-solvent being introduced. In this study, essential oils of lemon and eucalyptus were encapsulated by the complex coacervation process using gum Arabic, gelatin, and chitosan as wall materials. Glutaraldehyde was used as a cross-linking agent in the methodology. FT-IR and GC characterized the essential oils used. The microcapsules were analyzed using a digital microscope, scanning electron microscopy (SEM), and thermogravimetric analysis. Conclusively, microcapsules were formed in spherical form. Encapsulation efficiencies were obtained between 75-78%.
In conclusion, microencapsulated essential oils offer a technology that makes essential oils more effective, long-lasting, and customized. These advantages reveal their widespread impact, offering various uses for a variety of industries and applications.

1. Introduction

Microencapsulation is an emerging technology that prevents unwanted reactions, loss of volatile components, and nutrients in valuable extracts with the protective structure it creates, as well as masking unwanted taste and aroma [1].
Microencapsulation techniques are used in various industries to encapsulate active ingredients within a protective coating, offering benefits such as controlled release, enhanced stability, and protection from environmental factors. These industries include pharmaceuticals, food and beverages, cosmetics and personal care, agriculture, textiles, paints and coatings, household products, biomedical applications, chemical and materials industry, printing and paper, energy, and environmental applications [2]. There are many methods for encapsulation, such as spray drying [3], interfacial polymerization [4], extrusion coating [5], fluidized

bed coating, lyophilization, coacervation [6] and centrifugal suspension separation [5] [7, 8, 9].
Essential oils are secondary metabolites obtained from the leaves of plants and contain volatile components [10, 11, 12]. There are 17,500 plant species with essential oils belonging to the Myrtaceae, Lauracea, Lamiaceae, and Asteraceae families. Essential oils are pure compounds that are volatile under normal conditions, defined as odor-containing or odorless substances or mixtures of substances ([13]. Essential oils are concentrated extracts from plants that capture the plant's scent and beneficial properties. They have a wide range of uses across various industries owing to their therapeutic, aromatic, and antimicrobial properties. Essential oils offer versatile and natural solutions across various domains, making them valuable components in everyday life and specialized applications [14, 12].

Jun-Xiaa et al. 2011, used a complex coacervation method to encapsulate sweet orange oil. In the study, soy protein isolate (SPI) and gum Arabic were used as wall materials. The results showed that microcapsules with spherical structures without voids were produced [15]. Jangam and Thorat (2010) encapsulated ginger extracts by drying them in a spray dryer. They optimized the process parameters [16]. Altiok et al. (2010), in their study on chitosan-based films to which oregano oil was added, determined that the minimum antimicrobial effect dose was 1.2% in films to which oregano oil was added [17]. Djihar et al. (2024) showed that a complex coacervation method could be used to develop microcapsules that can protect Citrus lemon essential oil (CEO) from oxidation reactions and modulate the release of volatile compounds during digestion. CEO was tested with different amounts of Gelatin and Carrageenan. Optimum microencapsulation conditions were determined. Microcapsules were examined and characterized under an optical microscope [18]. Napiorkowska et al. used oat-soluble protein and gum Arabic as wall materials to encapsulate by the complex coacervation of juniper. They used EO Response Surface Methodology (RSM) to optimize the microcapsule production process and characterized physical and chemical properties of the produced powders [19].
In this study, it was aimed to encapsulate lemon and eucalyptus oil by using different coating materials such as gelatin, Arabic gum, and chitosan with complex coacervation and to reach the most efficient formulation. GC-MS and FTIR were used to characterize essential oils. The morphological structure and encapsulation efficiency of the powder products obtained to reach the most stable formulation were examined.

2. Material and Methods

Citrus Lemon (Rutaceae) and *Eucalyptus globulus* (Myrtaceae) essential oil was provided by Art de Huile Company, Türkiye. Gelatin, Arabic gum, and glutaraldehyde were food-grade and obtained from Alfasol (a local supplier). Acetic acid was obtained from Sigma-Aldrich Company. All chemicals are used without any pre-treatment.

2.1 Preparation of microencapsulated lemon and eucalyptus oils

Gelatin in warm water was dissolved (around 40-50°C) under continuous stirring until a homogeneous solution was formed. Tween 80 was added to the gelation solution, and the mixture was stirred for 15 min at 400 rpm. Gum Arabic was dissolved gum Arabic in warm water (around 40-50°C) under continuous stirring until fully dissolved. The gum Arabic solution was slowly added to the gelatin solution under continuous stirring in the chemical reactor (Mixture A)(Fig 1).

Figure 1. *Chemical reactor*

The temperature was maintained around 40-50°C. Chitosan was dissolved in 1 M acetic acid solution, and this solution was added to mixture A. The lemon oil was slowly added to the final mixture while stirring vigorously to form an emulsion. The pH of the emulsion was gradually adjusted to around 4.0-4.5 using acetic acid. The pH adjustment would induce coacervation, causing the gelatin and gum Arabic to form a coacervate around the oil droplets (Fig 2). The mixture was gradually cooled to around 5-10°C while stirring to solidify the coacervate droplets. This can be done using a cooling bath or ice. Glutaraldehyde was added as a cross-linking agent to the mixture to harden the coacervate and stabilize the microcapsules. The mixture was stirred gently to ensure uniform cross-linking.

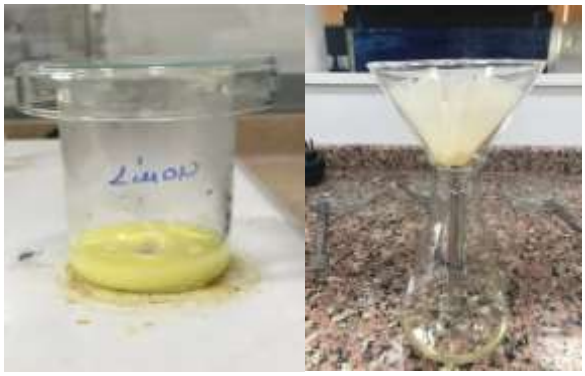

Figure 2. *Mixing and filtration of mixture*

The microcapsules were separated from the mixture by filtration (Fig 2). The microcapsules were washed with cold water to remove any residual materials and unreacted cross-linking agents. The washed microcapsules were dried conducting air drying, to obtain free-flowing microencapsulated powders. The same procedures were applied for eucalyptus oil.

Figure 3. *Microcapsules of essential oils*

2.3. Characterization of microencapsulated lemon and eucalyptus oils

2.3.1. Identification of essential oil compounds

The compositions of lemon and eucalyptus essential oils were analyzed using gas chromatography–mass spectrometry (GC–MS). Agilent (Agilent 5975 C Agilent 7890a Gc system) at Burdur Mehmet Akif Ersoy University Scientific and Technology Application and Research Center (MAKU BİLTEKMER) was used for GC analysis.

2.3.2. Chemical Structure (FTIR)

FT-IR analysis of the essential oils used was performed via Perkin Elmer Spectrum Two. FT-IR is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid, or gas one. It determines the chemical composition of a sample by matching its spectrum to reference spectra. FTIR spectra were registered in the range of 400–4000 cm^{-1}.

2.3.3. Thermogravimetric Analysis

DSC/TGA of the essential oils were analyzed using NETZSCH STA 449 F3 JUPITER MODEL. The experiments were carried out in a nitrogen gas atmosphere.

2.3.4. Morphology

The microcapsules were observed in a digital microscope (7 inc IPS high-definition screen). At the same time, SEM photographs of the produced microcapsules were taken using LEO 1430 VP model electron microscope.

2.3.5. Encapsulation Efficiency (EE) and Yield of Microcapsules

Encapsulation efficiency and yield are essential parameters to evaluate the success of the microencapsulation process. Encapsulation efficiency is the percentage of the total oil successfully encapsulated within the microcapsules. Five grams of microcapsules were weighed. The microcapsules were dissolved in an appropriate solvent (*e.g.*, ethanol) to release the encapsulated oil. The solution was centrifuged to separate the supernatant containing the dissolved oil. The concentration of the oil in the supernatant was measured using a spectrophotometer. Standard solutions of known concentrations were used to create a calibration curve for accurate quantification. The encapsulation efficiency was calculated using the formula;

$$EE(\%) = \left(\frac{Weight\ of\ encapsulated\ oil}{Weight\ of\ initial\ oil\ used}\right) x100 \quad (1)$$

The yield of microcapsules is the percentage of the initial materials that form the final microcapsules. After drying, the final microcapsules were weighed. The yield was calculated using the formula;

$$Yield\ (\%) = \left(\frac{Weight\ of\ final\ microcapsules}{Weight\ of\ initial\ materials\ (wall\ materials+oils)}\right) \quad (2)$$

3. Results and Discussions

3.1. GC-MS Analysis of Essential Oils

The components of lemon and eucalyptus oils by GC–MS are given in Tables 1 and 2, as well as in Figures 4 and 5.

Limonene was found to be the major constituent (64 %), followed by β-pinene (13,25 %), γ-terpinene (10 %), α-pinene (2.25 %), sabinene (2.25 %), neral (0.90 %), beta-bisabolene (0.87 %). Although similar results are obtained in the literature, the amount of limonene, the main component, varies from region to region [20, 21, 22].

Table 1 *Chemical composition of the lemon essential oil*

Components	**%**
Neryl acetate	0.33
Alpha pinene	2.25
Alpha terpineol	0.20
Alpha thujene	0.35
Beta bisabolene	0.87
Beta pinene	13.25
Gamma terpinene	10.00
Limonene	64.00
Neral	0.90
Paracymene	0.20
Sabinene	2.25
Geranyl acetate	0.55

The major constituents of the eucalyptus oil were 1,8-cineole (73.687%), followed by alpha-Pinene (16.891%), Alloaromadendrene (1.621%), trans-Pinocarveol (1.336%), Alpha-Terpinolene (1.154%) and Ledene (0.792%) [23, 24]. The most substantial peak in the GC chromatogram of lemon oil is limonene [25]. Eucalyptus oil contains the main element 1.8-cineole, followed by alpha pinene, which is consistent with the literature [26]**.**

Table 2 *Chemical composition of the eucalyptus essential oil*

Components	**%**
alpha-Pinene	16.891
beta-Pinene	0.364
beta.-Myrcene	0.100
l-Phellandrene	0.154
1,8-Cineole	73.687
gamma.-Terpinene	0.309
p-cymene	1.330
Isovaleric acid, isopentyl ester	0.132
2-Ethylfuran	0.411
trans-Pinocarveol	1.336
alpha.-Terpineol	0.599
Alloaromadendrene	1.621
Alpha-Terpinolene	1.154
Aromadendrene	0.314
4-terpineol	0.262
trans-p-Mentha-1(7),8-dien-2-ol	0.131
alpha gurjunene	0.258
Ledene	0.792
Viridiflorol	0.160
Terpenyl acetate	nd
Limonene	nd

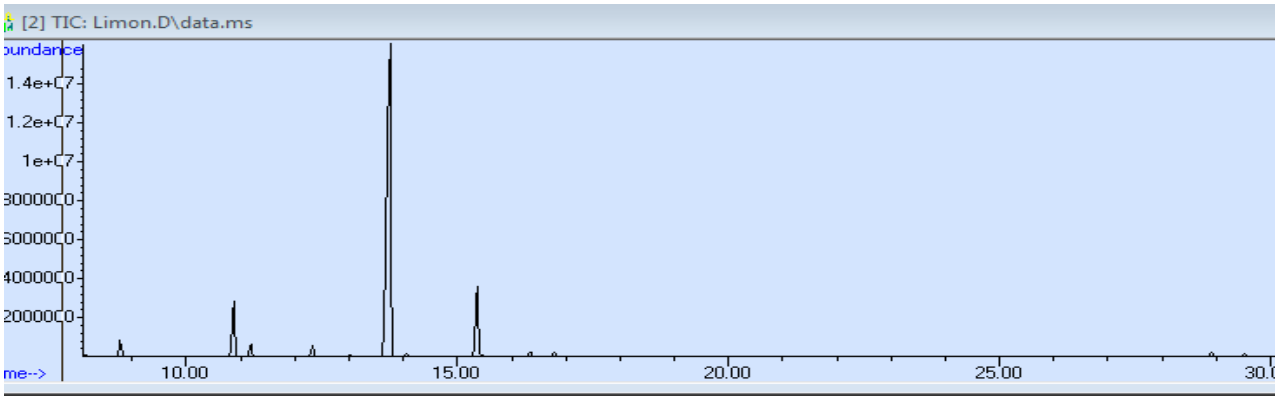

Figure *4. GC-MS chromatogram of Lemon essential oil*

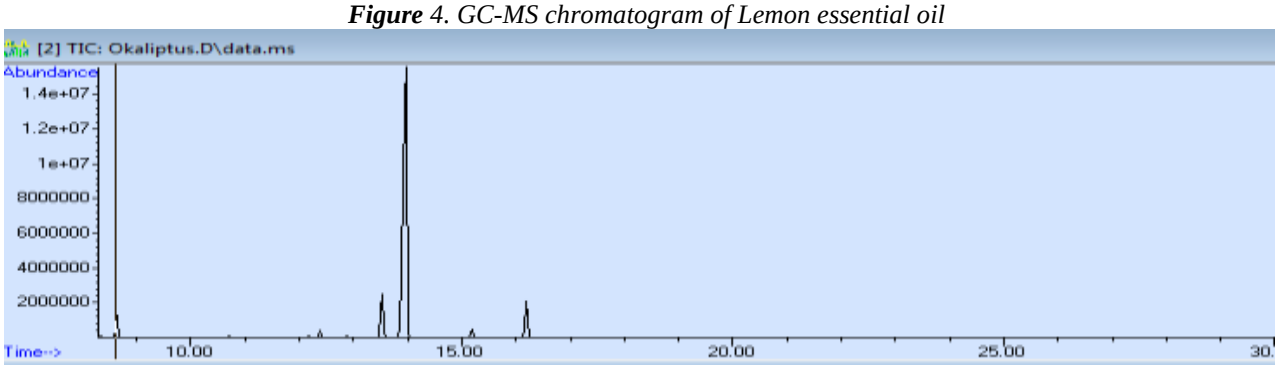

Figure 5. *GC-MS chromatogram of Eucalyptus Globulus essential oil*

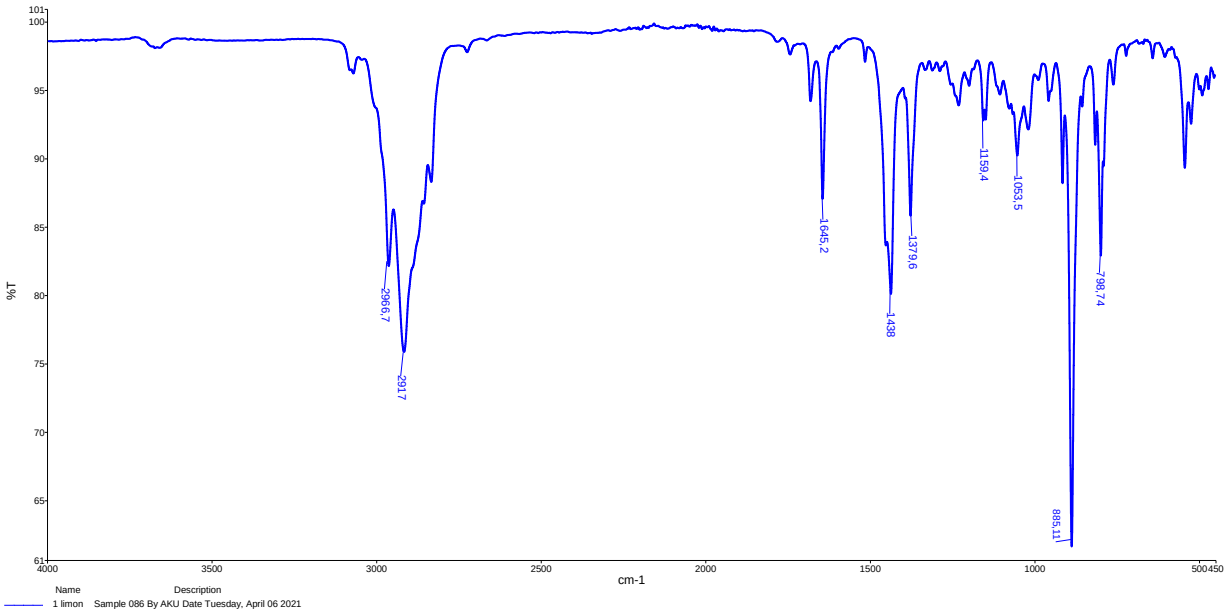

Figure 6 *FT-IR analysis of lemon essential oil*

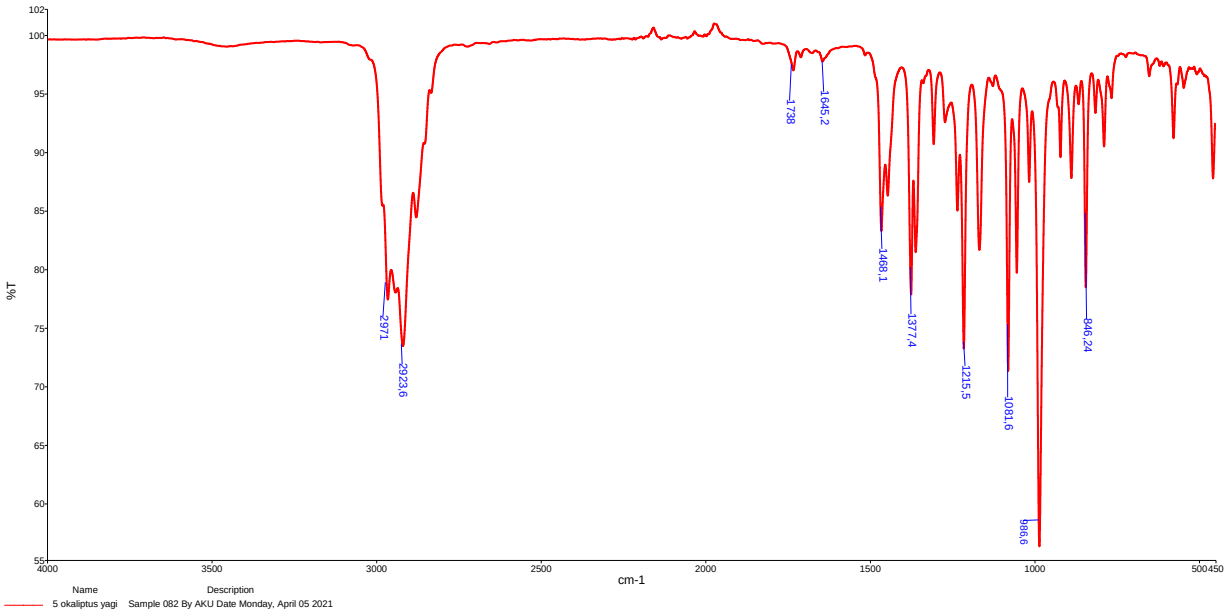

Figure 7 *FT-IR analysis of eucalyptus essential oil*

3.2. FTIR Analysis of Essential Oils

The FTIR spectrum of lemon oil is given in Figure 8. From the spectrum of lemon oil, the characteristic C-H stretching at 2966 and 2919 cm^{-1} and C=O stretching vibration at 1645 cm^{-1} were observed. Aromatic C-H and C-C bending vibration peaks were observed at 886 and 798 cm^{-1} , respectively [27, 28, 29].

In the FTIR spectra of Eucalyptus essential oil, the peaks at 2971 and 2923 cm^{-1} indicate the presence of CH_3 asymmetric and symmetric stretching. The C=O stretching vibration was determined at 1738 cm^{-1}. A band at 1645 cm^{-1} can be allocated to stretching vibrations in C=C bonds. The bond at 1468 cm^{-1} is corresponded to -C-H deformation bending. The C-O vibration was determined at 1377 cm^{-1}. In the infrared spectrum of the sample, the C-O carbonyl band was observed at 1215 cm^{-1}. The absorption peak at 986 cm^{-1} indicated CH_2 vibration [30, 31, 32].

3.3. DTA/TG Analysis of Essential Oils

TG curve for lemon oil shows a decomposition stage at 160 ºC, suggesting the decomposition of ethano

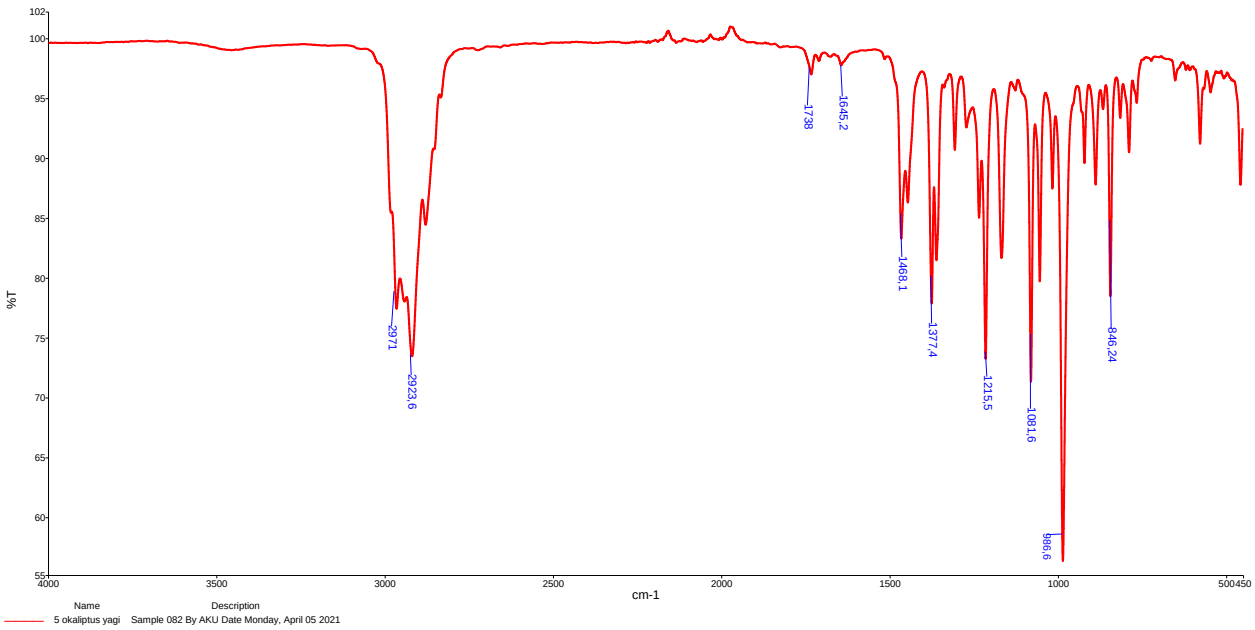

Figure 7 *FT-IR analysis of eucalyptus essential oil*

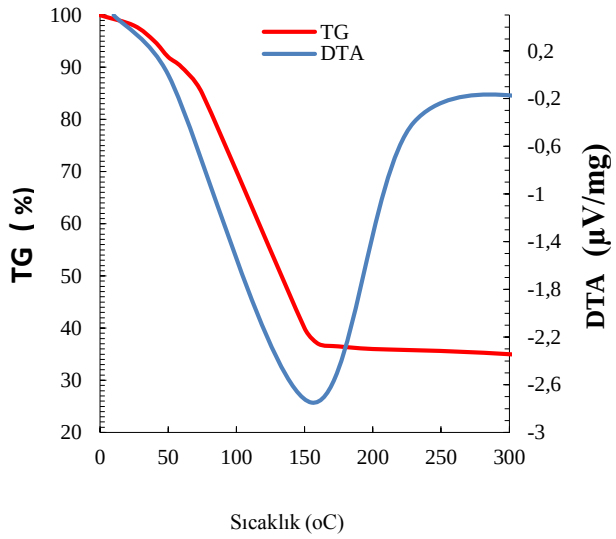

Figure 8 *DTA/TG analysis of lemon essential oil*

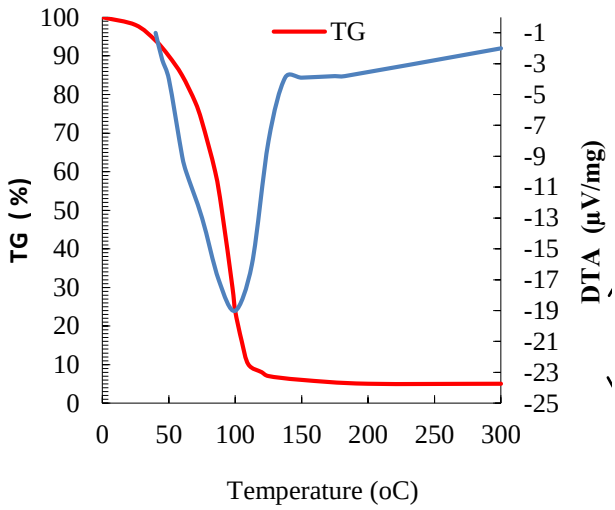

Figure 9 *DTA/TG analysis of eucalyptus essential oil*

l and essential oil. As can be seen, %67 mass loss was observed up to 300 ºC. The DTA curve for the lemon essential oil shows a single endothermic peak, with a peak temperature of 155°C. This may be related to the composition of the essential oils. In TG curves, Figure 9, the eucalyptus essential oil showed % 90 total mass loss up to 110ºC. The DTA curve for the eucalyptus essential oil shows a single endothermic peak, with a peak temperature of 100

3.4. Digital Microscope Images of Encapsulated essential Oils

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Optical micrographs of the produced samples show °C. Furthermore, similar results were obtained in the literature [33, 34, 35] that the encapsulated products are polydisperse, spherical, uniformly and homogeneously dispersed as small droplets surrounded by the wall material; this is clearly observed in Figure 10,11,12,13 [36].

3.5. SEM Images of Encapsulated Essential Oils

A SEM technique was used to evaluate the morphology of the microcapsules that had been

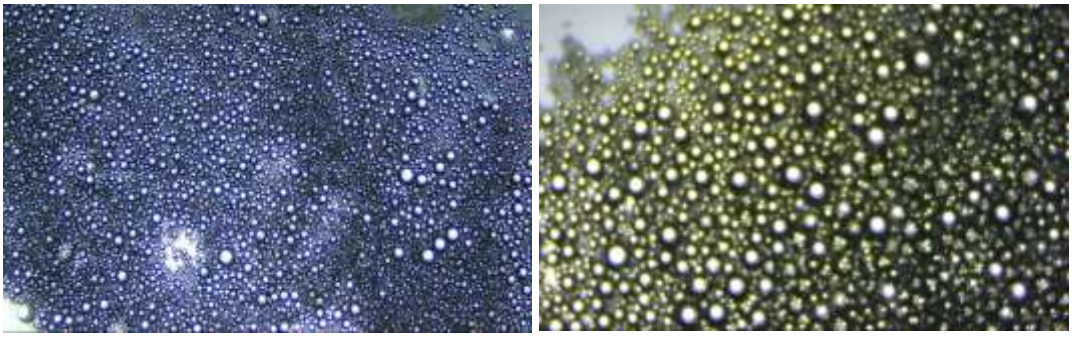

Figure 10. *Digital microscopy images of the encapsulated lemon oil*

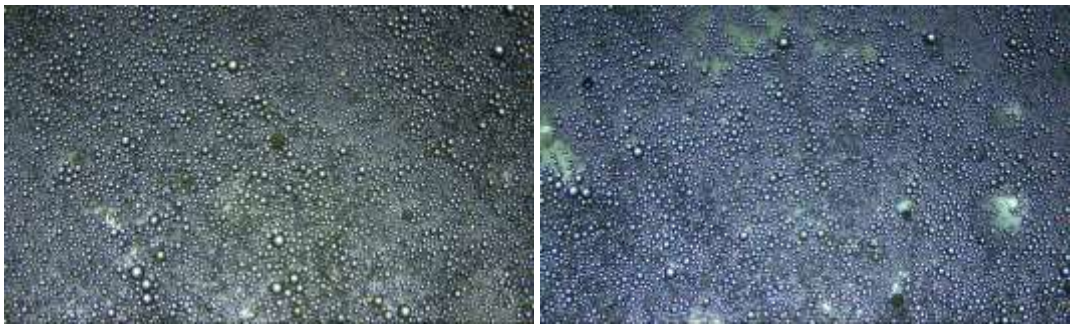

Figure 11. *Digital microscopy images of the encapsulated eucalyptus oil*

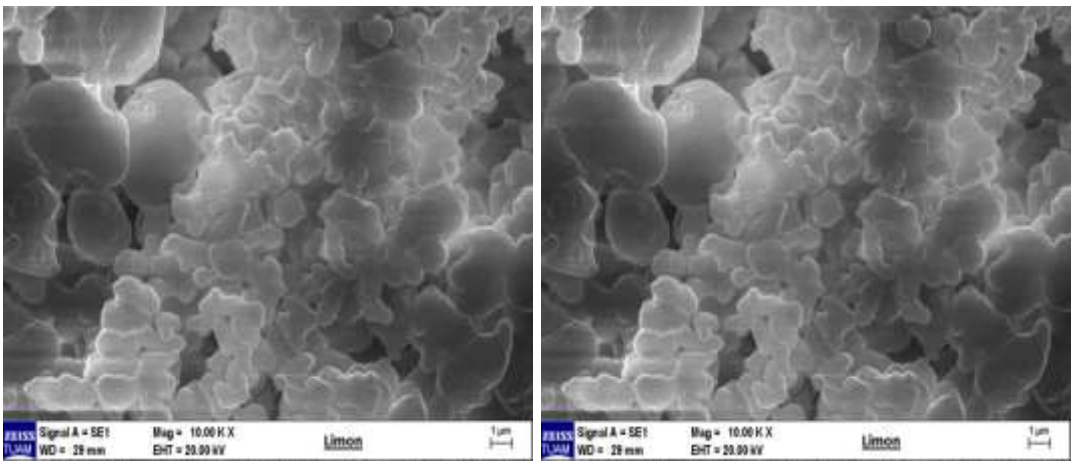

Figure 12. *SEM images of the encapsulated lemon oil*

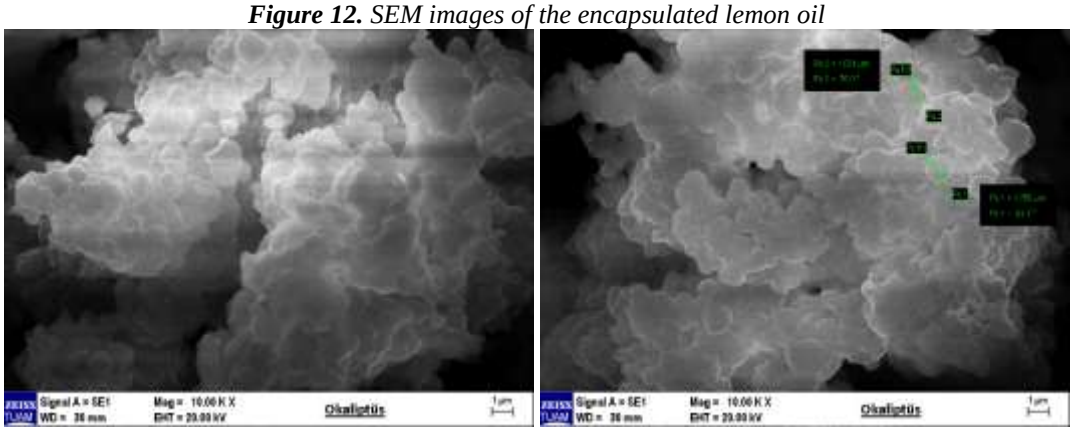

Figure 13. *SEM images of the encapsulated eucalyptus oil*

produced. Firstly, the specimens were coated with a layer of carbon using a magnetron sputter. SEM weas used to analyze the shape, particle size, and distribution of encapsulated lemon and eucalyptus. All microcapsules were magnified 10X magnifications. SEM images show that the lemon and eucalyptus capsules are 1 µm or less in size and close to spherical shape [36, 37, 38].

3.6. Encapsulation Efficiency (EE) and Yield of Microcapsules

Encapsulation efficiency data for both lemon and eucalyptus oil are shown in Table 3. The present study shows that both essential oils can encapsulate with an encapsulation efficiency of around 75%. As shown in Table 3, the microencapsulation of lemon oil using gelatin, Arabic gum, and chitosan yielded 74%, which was more significant than eucalyptus oil (70%). It is seen that the encapsulation efficiency for eucalyptus oil gives similar results (68-70%), while higher values (93-95%) were obtained for lemon oil [18, 39].

Tablo 3 *Encapsulation efficiency (EE%)*

No	Essential oil	EE %	Yield (%)
1	Lemon	78	74
2	Eucalyptus	75	70

4. Conclusions

Essential oils are volatile compounds widely used by various industries and are easily degradable, limiting their application. This study studied the encapsulation of lemon and eucalyptus essential oils using the complex coacervation method, and microcapsules were successfully obtained.

Gelatin, gum Arabic, and chitosan were used as shell materials, and glutaral aldehyde was used as a cross-linker. The microcapsules were filtered and dried at ambient temperature. Their physical and morphological properties characterized the encapsulated EOs.

The purity of the essential oils used was confirmed by FT-IR analysis and compared with the data in the device library. FT-IR results were found to be consistent with the literature. In the same way, gas chromatography of the essential oils was also performed to reveal the active ingredients, which showed similarity with the literature.

SEM characterized the morphology of the capsules. The microcapsules were observed to have an average diameter of 1-2 µm. Considering the microcapsules in the literature, it can be said that the ones obtained in the project are small in diameter. However, SEM photographs also observed that agglomeration occurred during drying.

One of the critical evaluation criteria of the encapsulation process is encapsulation efficiency, and in our study, 75-78% efficiency was obtained depending on the type of essential oil used.

Microencapsulated essential oils allow the fragrance of essential oils to remain longer. The material around the capsule dissolves slowly, which helps the fragrance to provide a longer-lasting effect. This feature will find its way into many applications, from perfumes to cleaning products.

Author Statements:

- **Ethical approval:** The conducted research is not related to either human or animal use.
- **Conflict of interest:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper
- **Acknowledgement:** The authors would like to thank Art de Huile Co, Istanbul, Türkiye for providing the essential oils.
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- **Data availability statement:** The data that support the findings of this study are available on request from the corresponding author.

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