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Preparation and Biological Activity Studies of Octenyl Succinic Anhydride Starch-Based Emulsions Containing Natural Essential Oils and Their Components

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Abstract: In this study, clove essential oil (CL), eugenol (EU), and cinnamaldehyde (CI) were immobilized in starch sodium octenyl succinate (SSO) using an emulsification method. The main characteristics, stability, and biological properties of the prepared emulsions were established. Particle size analyses using dynamic light scattering showed that the smaller droplets were characteristic of emulsions containing CI (205–218 nm) and EU (181–236 nm), while the largest droplets were determined for CL emulsions (293–348 nm). Moreover, the highest antioxidant activity was determined for CL (79–83%) and EU (80–88%) emulsions, while CI emulsions showed the greatest antibacterial activity. The obtained emulsions were applied to the paper sheets and the bioactive properties of coated paper were studied. Evaluation of antioxidant properties revealed that high antioxidant activity reaching 76–92% and 87–91% was characteristic of coatings containing CL and EU, respectively. Meanwhile, coatings containing CI showed quite low antioxidant activity (4–9%) but demonstrated the greatest antimicrobial effect on Gram-negative and Gram-positive bacteria as well as yeasts. Hence, CL and EU emulsions could be used as effective natural antioxidants, whereas CI emulsions could be applied as an antimicrobial agent on cellulose-based substrates for a wide range of human health protection applications.

Keywords: clove oil; eugenol; cinnamaldehyde; starch sodium octenyl succinate; emulsion; antioxidant; antimicrobial

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1. Introduction

Currently, the use of materials from natural sources rather than conventional synthetic substances has increased considerably. Essential oils (EOs) are secondary metabolites of plants with biological characteristics such as anti-inflammatory, antibacterial, antioxidant, and antitumor and have been used for many years as spices, pharmaceuticals, and raw materials for perfumes and other cosmetic products [1].

Clove essential oil consists of more than 30 compounds and can have a significant biological effect on human health, including antimicrobial, antioxidant, and anticancer activity [2,3]. Depending on the botanical origin and extraction method, the proportions of identified compounds in the essential oil can be different. Eugenol is the main compound and comprises 50% to 90% of clove essential oil. Meanwhile, the remaining compounds are mainly eugenyl acetate, β–caryophyllene, and α–humulene [2]. Eugenol is a phenolic compound characterized by antioxidant, antimicrobial, anticancer, and anti-inflammatory properties [4]. However, eugenol has relatively low chemical stability; therefore, additional efforts are required to preserve its activity.

Among the EOs, remarkable antimicrobial and antibiotic-potentiating activities have been highlighted for cinnamaldehyde abundant in the essential oils of *Cinnamomum* spp. [5]. Cinnamaldehyde is food-compatible, economically attractive, and can be used in a wide range of applications as an antimicrobial agent against pathogenic microorganisms [6].

In general, EOs have been shown to have a great inhibitory effect on bacteria, mold, fungi, viruses, and microbial toxin synthesis, as well as restricting the development of oxidative deterioration [7–9]. However, the application of free EOs could be restricted due to poor solubility in water, low chemical stability, and high volatility, which leads to a reduction in long-lasting bioactivity [10]. Encapsulation is a promising tool to overcome the various limitations of EO formulations, improve their functionality, and protect them from external environmental conditions [11]. Natural biopolymers are nontoxic and biodegradable substances and can be used as carrier materials in the encapsulation of bioactive compounds [12]. Polysaccharides such as starch, chitosan, carrageenan, and cellulose can be used as matrixes for the various biologically active substances for the development of different forms such as emulsions [13], powders [14], coatings [15], films [16], patches [10], and wound dressings [17]. For the past decade, materials based on biopolymers with immobilized essential oils have been developed and applied in various areas such as the food industry [18], active packaging technologies [19], medical devices [20], cosmetics, and personal care products [10].

The main issues in the food safety and quality area are microbial contamination and oxidation of lipids/proteins, increasing the risk of foodborne illness [21,22]. In recent years, there has been growing concern about active food packaging containing synthetic materials and additives that adversely affect human health and/or make packaging unsustainable in terms of recycling [23]. Research reports have shown that EOs can be combined with different cellulose-based substrates. In one study, paper-based materials containing free coriander essential oil were investigated against Gram-positive (*S. aureus*, *B. subtilis*) and Gram-negative (*E. coli*, *P. aeruginosa*) bacteria, yeast (*C. albicans*), and fungal strain (*A. brasiliensis*), and such materials demonstrated a high fungicidal effect [24]. In another study, a paper substrate with free lavender essential oil was developed and the antifungal properties of the treated paper samples have been found to be more pronounced than antibacterial ones [25]. In addition, new packaging systems have been developed, such as active cardboard trays coated with emulsions containing encapsulated EOs such as carvacrol, oregano, and cinnamon [26]. In the research conducted by other authors [27], cellulose-based paper impregnated with encapsulated cumin seed essential oil demonstrated good antioxidant efficiency and antimicrobial activity against *E. coli* and *S. aureus*.

In terms of medical applications, various studies related to cellulose substrates with modified surfaces reported anti-inflammatory and antibacterial activities with a wide range of effects [10,28–30]. Cellulose-based materials were impregnated or coated with various emulsions containing essential oils such as peppermint [28], savory [29], rose, and sage [30] to increase therapeutic effectiveness and release time. Other authors [10] designed cosmetic polysaccharide-based emulsion formulations with encapsulated lavender essential oil and applied them to obtain skin-friendly cellulose support patches with antimicrobial and moisturizing properties.

The aim of this work was to prepare and evaluate the emulsions containing essential oils or their components and their applicability for the development of bioactive coatings on cellulose substrate. For the preparation of emulsions, ecofriendly materials such as octenyl succinic anhydride modified starch and various amounts of clove essential oil, eugenol, and cinnamon aldehyde were used. The obtained emulsions were cast on paper sheets and the bioactive properties of the coatings were investigated.

2. Materials and Methods

2.1. Materials

Clove essential oil, eugenol (≥98 of purity), and *trans*-cinnamaldehyde (≥98 of purity), 2,2-diphenyl-1-picrylhydrazyl were purchased from Sigma-Aldrich (St. Louis, MO,

USA). Starch sodium octenylsuccinate was purchased from Ingredion GmbH (Hamburg, Germany). Ethanol was received from AB Vilniaus degtine (Vilnius, Lithuania). Nutrient media were used for the cultivation of microorganisms and the determination of bacterial antimicrobial resistance and were purchased from Liofilchem (Roseto degli Abruzzi, Italy). Strains of *Staphylococcus aureus* (ATCC 9144), *Bacillus cereus* (ATCC 6051), *Listeria monocytogenes* (ATC35152), *Escherichia coli* (ATCC 8739), and *Candida albicans* (ATCC 14053) were purchased from Liofilchem (Roseto degli Abruzzi, Italy).

2.2. Preparation of Emulsions

The aqueous emulsions consisting of 20% (*w*/*w*) starch sodium octenyl succinate (SSO) and 2.5, 5, and 10% (*w*/*w*) of clove essential oil (CL), eugenol (EU), or *trans*-cinnamaldehyde (CI) were prepared by using a rotor-stator homogenizer (Ultra-Turrax T25 digital, IKA, Staufen, Germany) at 12,000 rpm for 5 min. The conditions and quantities of the materials for the emulsion preparation were chosen based on the results of our previous experiments.

2.3. Particle Size and Zeta Potential Measurements

The particle size of emulsions was measured according to the cumulative distribution of intensity by using a Delsa^{TM} Nano C particle size meter (Beckman Coulter, Malvern, UK), which uses photon correlation spectroscopy to determine particle size by measuring the rate of fluctuations in the laser light intensity scattered by particles. The non-negative least-squares (NNLS) algorithm was used to analyze dynamic light scattering data for the particle size distribution. All scattered light measurements were made at an angle of 165°. Zeta potential measurements were performed using the same equipment. For particle size and zeta potential measurements, the emulsions were diluted with distilled water to reach a concentration of 1% *w*/*v* to avoid multiple scattering effects. The particle size and zeta potential measurements were performed at 20 °C temperature. Measurements were carried out in triplicates and data were averaged.

2.4. pH Measurements

Measurements of emulsions' pH were performed with a Microprocessor pH 211 m (Hanna Instruments Ltd., Leighton Buzzard, UK) at 20 °C temperature.

2.5. Viscosity Measurements

The viscosity of the emulsions was measured by using a rotational viscometer Smart L (Fungilab S.A, Barcelona, Spain) at 20 °C temperature. The samples were placed into a 50 mL glass container (10 cm × 2.5 cm) and viscosity measurements were performed using the L3 spindle at 100 rpm.

2.6. Evaluation of Emulsions Stability

A total of 10 mL of emulsion was placed in a 15 mL volume centrifuge tube and kept for 14 days at temperatures of 5, 20, and 40 °C. Parameters such as particle size (see Section 2.3), pH (see Section 2.4), sedimentation index (SI), and creaming index (*CRI*) of the emulsion were determined after 2, 7, 10, and 14 days. SI and *CRI* parameters were calculated as follows:

$$CRI = \frac{H_C}{H_T} \cdot 100\% \quad (1)$$

where H_T—the total height of the emulsion in the tube (mm), and H_C—the height of the serum layer in the tube (mm).

$$SI = \frac{h_C}{h_T} \cdot 100\% \quad (2)$$

where: h_T—the total height of the emulsion in the tube (mm), and h_C—the height of precipitate in the tube (mm).

2.7. Evaluation of Antioxidant Activity of Emulsions

The antioxidant activity of the emulsions was assessed by measuring the disappearance of the purple color of the ethanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). A total of 5 mg of emulsion was mixed with 10 mL of 0.04 mg/ml of DPPH ethanol solution and kept in the dark for 30 min while stirring at 200 rpm at room temperature. Subsequently, the samples were filtrated and the absorbance of the filtrate was measured at 517 nm using a UV–Vis spectrophotometer (T60, PG Instruments, Great Britain). A DPPH solution without emulsion was used as the control sample. The antioxidant activity of the emulsion was expressed as radical scavenging activity (%) and calculated using the following equation:

$$Radical\ scavenging = \frac{A_0 - A_S}{A_0} \cdot 100\% \quad (3)$$

where A_0—the absorbance of the initial DPPH solution (control sample), and A_S—the absorbance of DPPH solution with a sample.

2.8. Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Emulsions

The bacteria strains of Gram-positive cocci *Staphylococcus aureus* (ATCC 9144) and Gram-positive sporogenic rods *Bacillus cereus* (ATCC 6051), *Listeria monocytogenes* (ATC35152), Gram-negative rods *Escherichia coli* (ATCC 8739), and yeast *Candida albicans* (ATCC 14053) were used for the study of antibacterial properties. Antibacterial properties of the aqueous emulsions consisting of 2.5, 5, and 10% (*w*/*w*) of CL, EU, or CI were evaluated according to bacteriostatic and bactericidal activities on the tested strains of bacteria employing the determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The MIC was evaluated by the broth dilution method, and the MBC was evaluated by plating [31]. The tests were carried out twice.

2.9. Preparation of Coated Paper

Active coatings were obtained by casting emulsions on paper sheets. Emulsions containing 2.5, 5, and 10% (*w*/*w*) of CL, EU, or CI were prepared as detailed in Section 2.2. A total of 2 mL of each emulsion was applied on paper by using casting equipment (RK Print Coat Instruments, Royston, UK) and a wired bar with a wire diameter of 0.64 mm.

2.10. FT-IR Spectroscopy Measurements

FT-IR spectra of coated paper, essential oils, and their components were recorded by using a Perkin–Elmer Frontier spectrophotometer with a single reflectance horizontal ATR (Attenuated Total Reflectance) cell equipped with a diamond crystal. The data were recorded in the spectral range from 550 to 4000 cm^{-1} by accumulating 5 scans with a resolution of 4 cm^{-1}.

2.11. Evaluation of Antioxidant Activity of Coated Paper

The antioxidant activity of the coatings was evaluated using the DPPH method. A total of 2 sheets of each sample (1 cm × 1 cm) were placed in a glass beaker and 10 mL of 0.04 mg/mL of DPPH ethanol solution was added. The assessment was performed as described in Section 2.7.

2.12. Determination of Antimicrobial Activity of Coated Paper

The disk diffusion method was used to evaluate the antibacterial activity of paper samples coated with emulsions containing 2.5, 5, and 10% (*w*/*w*) CL, EU, or CI against the bacteria strains of Gram-positive cocci *Staphylococcus aureus* (ATCC 9144) and Gram-positive sporogenic rods *Bacillus cereus* (ATCC 6051), *Listeria monocytogenes* (ATC35152), Gram-negative rods *Escherichia coli* (ATCC 8739), and yeast *Candida albicans* (ATCC 14053).

The microorganism's inoculum of the selected strains was used for the study of antibacterial properties and spread over an agar plate. The microorganism's inoculum

was adjusted to 0.5 MacFarland and the volume of 0.1 mL containing 1.5×10^7 bacteria was used for an inoculation onto the entire surface of a Mueller-Hinton agar (MHA) Petri plate using a sterile cotton-tipped swab to form an even lawn. Coated paper discs of 6 mm diameter were placed on the surface of each MHA plate. Cultures were grown on agar at 37 °C for 24 h. The "zone of inhibition" was measured using a digital Vernier caliper. The experiments were performed in duplicates and mean values of inhibition zones and standard deviations were determined.

2.13. Statistical Analyses

Data were statistically handled by the one-way analysis of variance (ANOVA for Excel, version 2.2). All analyses were carried out in triplicates unless it is specified otherwise, and the results were expressed as mean value ± standard deviation. Duncan's multiple-range test was applied for the calculation of the significant differences among the values of characteristic parameters at probability level $p < 0.05$.

3. Results and Discussion

3.1. Preparation and Characterization of Emulsions

Emulsions are one of the most widely used encapsulation systems for essential oils that increase their solubility in water, reduce volatility, and improve the stability of active substances [32]. Compared to conventional emulsions, Pickering emulsions are stabilized with solid particles that include good stability against coalescence, higher loading capacity, and lower release rate [33]. The particles used in polysaccharide-based Pickering emulsions involve starch, chitosan, β-cyclodextrin, cellulose derivatives, etc. [34]. Starch modified with octenyl succinic anhydride is recognized as safe and approved as a food additive [35] and could be one most recommended for the preparation of Pickering emulsions [33,34].

In this study, hydrophobically modified waxy maize starch such as starch sodium octenyl succinate (SSO) was used to immobilize clove essential oil (CL), eugenol (EU), and cinnamaldehyde (CI). The aqueous emulsions consisting of 20% (*w*/*w*) of SSO and 2.5, 5, and 10% (*w*/*w*) of CL, EU, or CI were prepared by using the rotor-stator homogenization method. The main characteristics of the prepared emulsions such as droplet size, polydispersity index, zeta potential, pH, and viscosity were determined (Table 1).

Table 1. Characteristics of prepared emulsions containing CL, EU, or CI.

Emulsion	Droplet Size (nm)	PI	Zeta Potential (mV)	pH	Viscosity (mPa·s)
EM-CL-2.5	293 ± 1^{a}	0.18 ± 0.04^{b}	-16.1 ± 2.1^{c}	3.58 ± 0.02	14.4 ± 0.7^{a}
EM-CL-5	326 ± 8^{c}	0.18 ± 0.04^{ab}	-20.0 ± 1.2^{a}	3.51 ± 0.02	18.0 ± 0.9^{b}
EM-CL-10	348 ± 8^{c}	0.16 ± 0.03^{ab}	-19.9 ± 0.5^{abc}	3.45 ± 0.02	31.9 ± 1.6^{c}
EM-EU-2.5	181 ± 9^{ab}	0.31 ± 0.01^{b}	-13.9 ± 2.1^{abc}	3.54 ± 0.02	22.0 ± 1.1^{a}
EM-EU-5	233 ± 10^{ab}	0.24 ± 0.02^{ab}	-12.5 ± 0.4^{c}	3.41 ± 0.02	25.1 ± 1.3^{a}
EM-EU-10	236 ± 7^{b}	0.23 ± 0.00^{ab}	-15.7 ± 0.4^{a}	3.34 ± 0.02	37.5 ± 1.9^{b}
EM-CI-2.5	205 ± 16^{ab}	0.32 ± 0.04^{b}	-14.5 ± 0.8^{a}	3.41 ± 0.02	14.3 ± 0.7^{a}
EM-CI-5	211 ± 9^{ab}	0.28 ± 0.02^{ab}	-9.1 ± 2.2^{c}	3.39 ± 0.02	19.8 ± 1.0^{b}
EM-CI-10	218 ± 11^{b}	0.23 ± 0.01^{ab}	-11.6 ± 0.9^{abc}	3.21 ± 0.02	31.2 ± 1.6^{c}

[a–c]: the different letters within the column for the emulsion with the same essential oil show that the results are significantly different ($p < 0.05$; Duncan test).

The droplet size of the emulsion depended on the active components used and their concentration in the formulation. The smallest droplets with diameters of 293, 181, and 205 nm were obtained using 2.5% of CL, EU, and CI in emulsions preparations, respectively. The size of the emulsion droplets slightly increased with an increase in the oil phase concentration. Significant differences in droplet size were observed when comparing the emulsion samples with the lowest and highest concentrations of essential oils in the preparations. The smaller droplets were characteristic of emulsions containing CI

(205–218 nm) and EU (181–236 nm) while the largest droplets were found in the case of CL emulsions (293–348 nm). The differences in droplet size using the same emulsifier and different essential oils or their components mainly depend on variations in chemical composition and interactions with the emulsifier [36]. Different essential oils have distinct chemical compositions and varying surface tensions. Essential oils also interact differently with the emulsifiers used in the formulation. Some essential oils may have stronger interactions with certain emulsifiers, leading to smaller droplets, while others may not form as stable emulsions and result in larger droplets [37].

The polydispersity index (PI) represents the distribution of oil droplets in the emulsion. PI values closer to zero indicate homogeneous distribution and great emulsion stability [38]. In addition, the zeta potential characteristics can also play a role in the prediction of the emulsion stability. Emulsions with zeta potential values greater than ±30 mV can be regarded as stable due to electrostatic repulsion at the surface, whereas emulsions with lower zeta potential values are considered less stable [39]. Values of zeta potentials greater than ±30 mV represent high stability. Approx. ±20 mV indicates short-term stability, and values below ±5 mV show low stability due to rapid aggregation of particles [40]. As can be seen in Table 1, the zeta potential values of the emulsion droplets were negative and did not significantly depend on the concentration of EO. The highest and lowest negative values of the zeta potential were detected in the CL and CI emulsions, respectively.

The pH value of the emulsions was below 3.58 and slightly decreased with increasing amount of essential oil or active component. The viscosity of the emulsions at a shear rate of 100 rpm depended on the essential oil or active component used and its amount. A similar viscosity in the range of 14.4–31.9 mPa·s and 14.3–31.2 mPa·s was characteristic of emulsions containing CL and CI, respectively, while EU emulsions had slightly higher viscosity ranging from 22.0 to 37.5 mPa·s.

3.2. Study of Emulsions' Stability

Emulsions are known to be thermodynamically unstable due to phase separation, leading to loss of functionality of the system [41]. The instability of emulsions can be explained by migration phenomena (creaming and sedimentation) and size variations. The creaming and sedimentation can be generated by gravitational or centrifugal forces. In terms of droplet size variation, different mechanisms such as flocculation, coalescence, and Oswald ripening were described in the literature [33].

In this work, the stability of the emulsions was assessed by following the changes in droplet size, pH, sedimentation index (SI), and creaming index (CRI) during the 14 days of storage at different temperatures. Table 2 represents the data of the droplet size and the polydispersity index of the emulsions after storage at 5, 20, and 40 °C for 14 days. The temperature of 40 °C for the storage of the emulsion was chosen for the accelerated stability testing. When comparing the characteristics of freshly prepared emulsions (Table 1) and those after 14 days of storage (Table 2), the droplet size of all emulsions increased during the storage period. Furthermore, storage conditions had an impact on changes in droplet size. When the emulsions were kept at a temperature of 5 °C, they were more stable. For instance, the droplet size of the EM-CL-10 emulsion was 463, 628, and 891 nm when the samples were stored at temperatures of 5, 20, and 40 °C, respectively.

Figure 1 shows the droplet size of the emulsions during different time intervals (0, 2, 7, 10, and 14 days) when the samples were kept at a temperature of 5 °C. The droplet size of all emulsions gradually increased; however, samples containing 2.5% of essential oil or active components were more stable compared to those with the higher concentration of bioactive compounds.

According to the literature, when the droplet size of the emulsion does not change significantly during a period of observation, it is considered that the emulsion system is stable [42]. In addition, prepared emulsions with droplets of smaller size usually remain quite stable for a certain time. This is explained by the fact that, with a smaller droplet size, destabilization processes such as coalescence or flocculation of droplets occur more

slowly [34]. Regarding long-term stability, various strategies could be used to increase emulsion stability. For instance, thickening agents and stabilizers provide additional viscosity and steric stabilization, respectively. Also, the addition of electrolytes or salts can improve stability by increasing the repulsive forces between the droplets [33,41].

Table 2. Droplet size and polydispersity index of emulsions stored at different temperatures for 14 days.

Emulsion	Droplet Size (nm)			Polydispersity Index		
	5 °C	20 °C	40 °C	5 °C	20 °C	40 °C
EM-CL-2.5	389 ± 11 ^{a}	421 ± 9 ^{c}	422 ± 14 ^{c}	0.24 ± 0.01 ^{c}	0.28 ± 0.02 ^{a}	0.25 ± 0.01 ^{abc}
EM-CL-5	440 ± 17 ^{a}	511 ± 12 ^{c}	500 ± 25 ^{c}	0.23 ± 0.03 ^{ab}	0.25 ± 0.03 ^{ab}	0.27 ± 0.01 ^{a}
EM-CL-10	463 ± 18 ^{a}	628 ± 32 ^{b}	891 ± 29 ^{c}	0.25 ± 0.03 ^{a}	0.32 ± 0.03 ^{abc}	0.34 ± 0.01 ^{c}
EM-EU-2.5	254 ± 13 ^{ab}	254 ± 12 ^{ab}	400 ± 56 ^{b}	0.30 ± 0.04 ^{ab}	0.32 ± 0.01 ^{b}	0.26 ± 0.05 ^{ab}
EM-EU-5	323 ± 12 ^{a}	416 ± 42 ^{c}	462 ± 26 ^{c}	0.28 ± 0.01 ^{b}	0.28 ± 0.08 ^{ab}	0.27 ± 0.01 ^{ab}
EM-EU-10	450 ± 7 ^{a}	508 ± 19 ^{a}	908 ± 40 ^{b}	0.26 ± 0.03 ^{ab}	0.30 ± 0.02 ^{ab}	0.32 ± 0.04 ^{b}
EM-CI-2.5	274 ± 7 ^{a}	333 ± 30 ^{abc}	371 ± 18 ^{c}	0.28 ± 0.03 ^{ab}	0.27 ± 0.03 ^{ab}	0.30 ± 0.01 ^{b}
EM-CI-5	310 ± 15 ^{a}	402 ± 21 ^{b}	597 ± 135 ^{c}	0.30 ± 0.02 ^{ab}	0.28 ± 0.04 ^{ab}	0.30 ± 0.02 ^{b}
EM-CI-10	413 ± 36 ^{a}	487 ± 31 ^{a}	723 ± 58 ^{b}	0.27 ± 0.05 ^{a}	0.27 ± 0.05 ^{a}	0.27 ± 0.05 ^{a}

$^{a–c}$: the different letters for the droplet size and polydispersity index parameters of the same sample within the row show that the results are significantly different ($p < 0.05$; Duncan test).

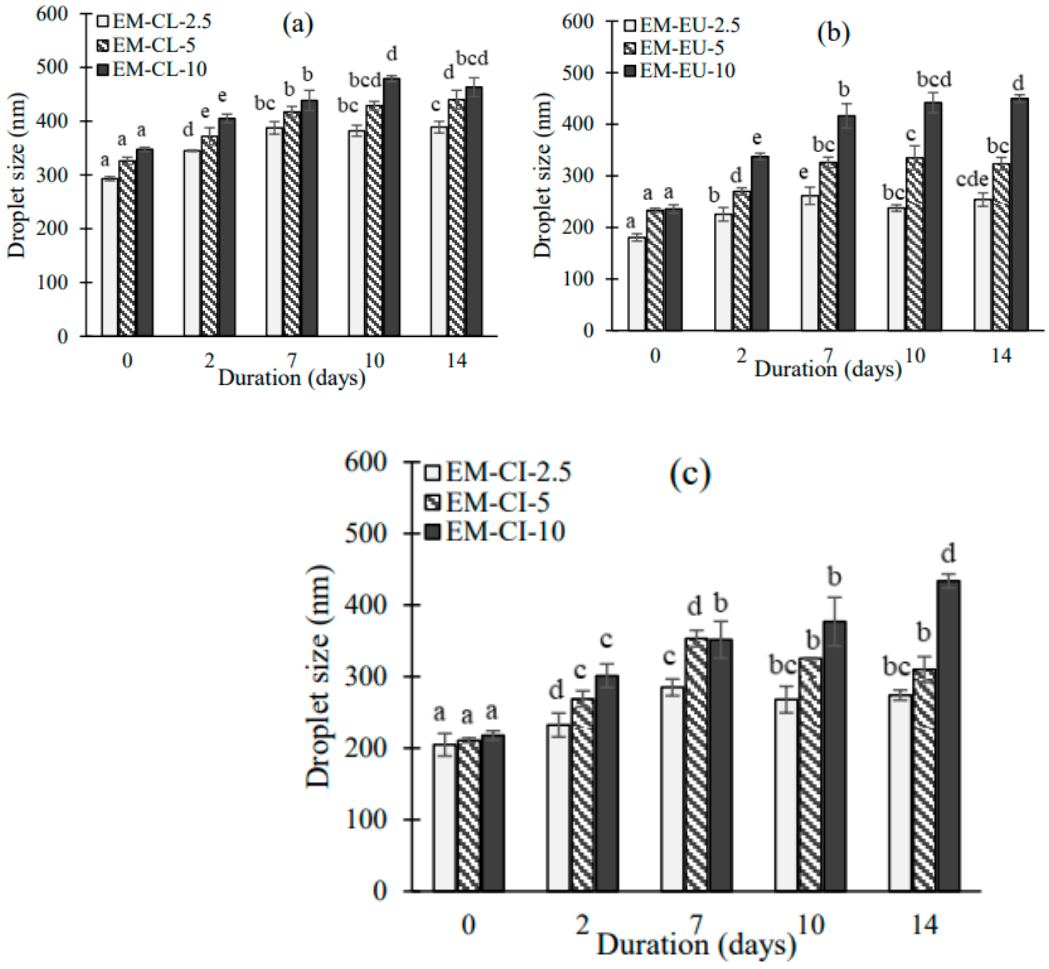

Figure 1. Changes in emulsion droplet size under different storage times at 5 °C temperature: (**a**)—CL emulsions; (**b**)—EU emulsions; (**c**)—CI emulsions; $^{a–e}$: the different letters for the same sample during the storage time show that the results are significantly different ($p < 0.05$; Duncan test).

The visual evaluation of the stability was carried out by observing sedimentation and creaming. At the same time, both destabilization processes of the emulsion, such as droplet coalescence/floculation, and solid sedimentation processes, can occur [43,44]. In this work, storage stability was evaluated by calculating the sedimentation and creaming index after 2, 7, 10, and 14 days (Table 3). The sedimentation of the particles was observed after 2 days in all samples. When the samples were kept at 20 °C, the sedimentation index (6%) did not change during the storage period. Furthermore, the highest sedimentation was determined for emulsions kept at 40 ° C and consisting of 5 and 10% active substances. The creaming of the emulsions was observed only after 10 days of storage at a temperature of 40 ° C. The highest creaming index was determined for EM-EU samples, the CRI values ranged from 10 to 18%.

Table 3. The sedimentation index and the creaming index of the emulsions stored at different temperatures on Days 2, 7, 10, and 14.

Emulsion	Sedimentation Index (%) on Day 2/7/10/14			Creaming Index (%) on Day 2/7/10/14		
	5 °C	20 °C	40 °C	5 °C	20 °C	40 °C
EM-CL-2.5	4/4/6/6	6/6/6/6	6/6/6/6	0/0/0/0	0/0/0/0	0/0/4/8
EM-CL-5	4/4/6/6	6/6/6/6	6/6/10/10	0/0/0/0	0/0/0/0	0/0/4/8
EM-CL-10	4/4/6/6	6/6/6/6	6/6/8/12	0/0/0/0	0/0/0/0	0/0/4/8
EM-EU-2.5	6/6/6/6	6/6/6/6	6/6/6/6	0/0/0/0	0/0/0/0	0/0/6/10
EM-EU-5	6/6/6/6	6/6/6/6	6/6/6/8	0/0/0/0	0/0/0/0	0/0/6/12
EM-EU-10	6/6/6/6	6/6/6/6	6/6/8/10	0/0/0/0	0/0/0/0	0/0/10/18
EM-CI-2.5	4/4/6/6	6/6/6/6	6/6/6/6	0/0/0/0	0/0/0/0	0/0/6/8
EM-CI-5	4/4/6/8	6/6/6/6	6/6/8/8	0/0/0/0	0/0/0/0	0/0/6/8
EM-CI-10	4/4/6/8	6/6/6/6	6/6/8/8	0/0/0/0	0/0/0/0	0/0/8/10

pH is a very important parameter because a significant change in its value can indicate chemical changes in the components present in the formulation [45]. The pH changes of emulsions on the 14th day of storage at different temperatures are presented in Figure 2. The pH value of all emulsions decreased with increasing amounts of essential oils or active components during the storage. However, the pH values of CL and EU emulsions only slightly decreased, and no significant changes were observed during the storage time. Significant changes in pH were observed in the CI emulsions when the samples were stored for 14 days at a temperature of 40 °C. In this case, the pH values changed from 3.11–3.21 to 2.87–2.75 depending on the amount of CI in the sample.

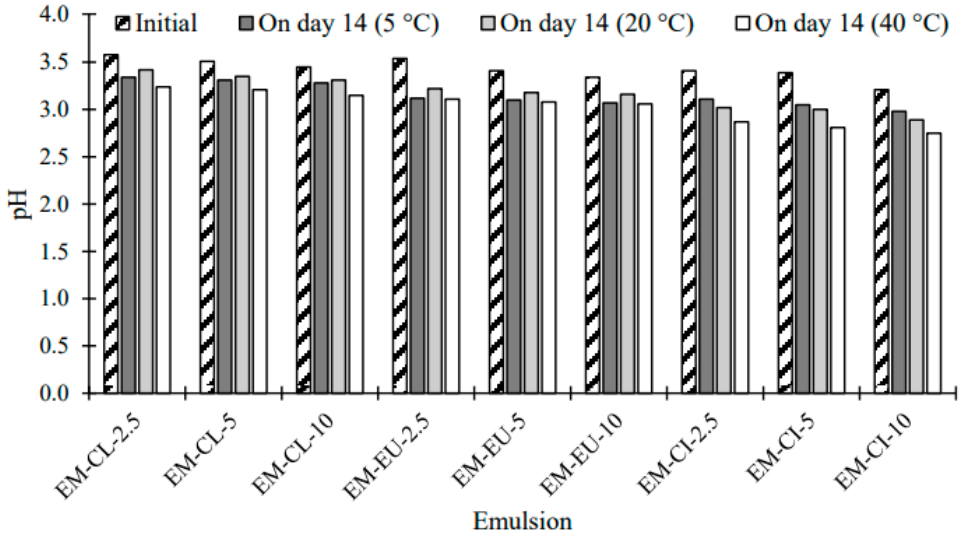

Figure 2. pH values of emulsions stored at different temperatures for 14 days.

3.3. Biological Activity of Emulsions

Emulsions containing essential oils exhibit a range of biological activities depending on the chemical composition and properties of both the essential oils and the emulsion system. Research has shown that clove essential oil represents a high antioxidant activity in vitro and in vivo. Eugenol is the main component of clove essential oil and the main contributor to the biological effects of clove essential oil [46]. Clove essential oil can inhibit lipid peroxidation in foods, reduce oxidative stress, and is considered safe for use in food, cosmetics, and pharmaceutical applications for a wide range of beneficial health promotion activities [47]. Meanwhile, cinnamaldehyde is already known for its high antimicrobial action [6]. It can be used in pharmaceuticals, cosmetics, personal care, and cleaning products in the European Union [48]. Moreover, clove essential oil, eugenol, and cinnamaldehyde are generally recognized as safe and approved by the Food and Drug Administration of the United States [49].

In this work, the antioxidant properties of the emulsions were investigated using the DDPH method, and the obtained results were expressed as radical scavenging activity (%). As can be seen from Figure 3, free radical scavenging activity depended on the active component used in the emulsion. The highest activity was determined for the EM-CL (79–83%) and EM-EU (80–88%) emulsions. Meanwhile, samples containing CI did not show significant antioxidant properties, and the radical scavenging activity varied from 1 to 4% only. Mainly, the chemical structure of compounds influences the capacity of antioxidant activity. Phenolic compounds including eugenol are known for their strong antioxidant properties because the hydroxyl group can donate hydrogen atoms to free radicals, thereby neutralizing them [50]. On the other hand, cinnamon aldehyde contains an aldehyde functional group which may also contribute to its antioxidant activity through different mechanisms; however, with relatively low radical scavenging activity compared to emulsions containing eugenol or clove oil [51].

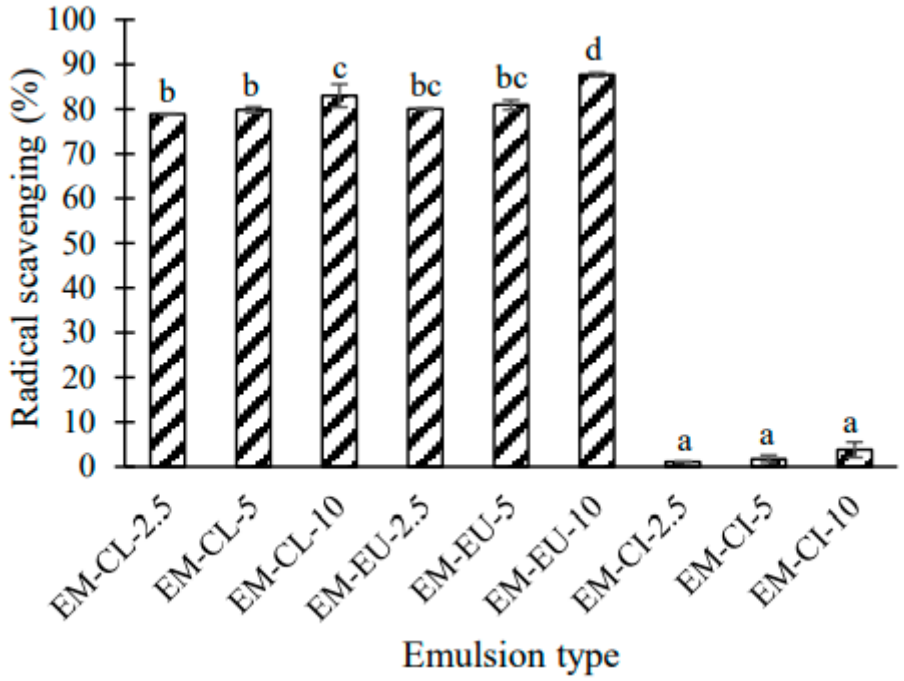

Figure 3. The radical scavenging activity (%) of various emulsions. $^{a–d}$: the different letters for different emulsions show that the results are significantly different ($p < 0.05$; Duncan test).

The emulsions containing 10% of CL, EU, or CI were tested for their antimicrobial activity against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*) bacteria as well as yeasts (*Candida albicans*) and MIC and MBC were established (Table 4). The establishment of MIC and MBC values

demonstrated that emulsions containing 10% of CL, EU, or CI had bacteriostatic and bactericidal activity on the tested bacteria strains as was shown by the inhibition of the growth and the killing of ≥99.9% bacteria at certain concentration (Table 4).

Table 4. MIC and MBC of emulsions containing 10% of CL, EU, or CI against different microorganisms.

Microorganism	EM-CL-10		EM-EU-10		EM-CI-10	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
E. coli	0.50	1.00	1.00	2.00	0.50	0.50
S. aureus	0.50	4.00	0.125	2.00	0.25	0.50
L. monocytogenes	0.50	2.00	1.00	2.00	0.25	0.50
B. cereus	0.50	-	0.50	-	0.50	25.00
C. albicans	1.00	2.00	0.50	1.00	0.125	0.25

The lowest MIC (0.125 mg/mL) was characteristic of the emulsion containing EU for *S. aureus*. Meanwhile, the lowest MIC (0.125 mg/mL) and MBC (0.25 mg/mL) values of emulsion containing CI were for eukaryotic cell—*Candida albicans*.

The comparison of MBC and MIC values for Gram-positive bacteria *S. aureus* showed that the MBC/MIC ratio was > 4 for EM-CL-10 and EM-EU-10. Such an effect was considered bacteriostatic. Meanwhile, the MBC/MIC ratio determined for the emulsions against *E. coli* and *L. monocytogenes* was ≤4, indicating the bactericidal effect.

3.4. Preparation and Characterization of Coated Paper

The coated paper containing different amounts of clove essential oil, eugenol, and cinnamon aldehyde was prepared by casting emulsions on paper. The coated paper sheets (see Figure 4) were characterized using FT-IR spectroscopy. The FT-IR spectra of pure essential oils (CL, EU, CI), paper, and coated paper (C-CL, C-EU, C-CI) are presented in Figure 5.

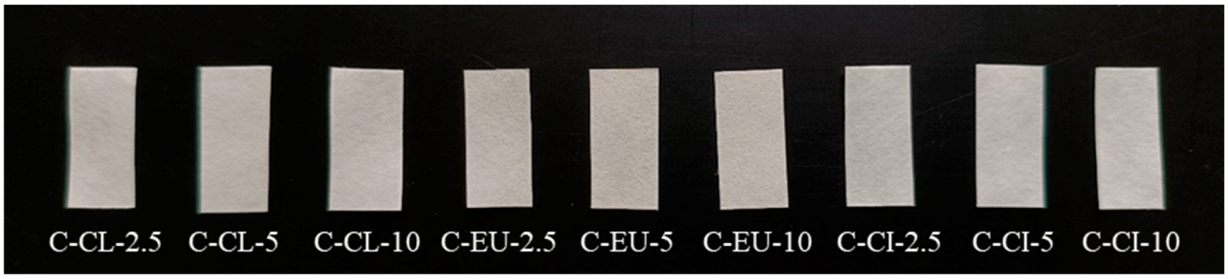

Figure 4. The coated paper sheets obtained using different emulsions.

As can be seen in Figure 5a,b, the FT-IR spectra of CL and EU are uniform due to the composition of clove oil, which contains a substantial amount (40–90%) of eugenol [46]. The characteristic peaks (A) of EU at 1636, 1607, and 1512 cm^{-1} were found to be attributed to the stretching C=C of the aromatic moiety [52]. Other peaks at 912, 816, and 795 cm^{-1} were found from eugenol, which could be due to the presence of CH_2 deformation vibration, stretching vibration of the C–O bond, and bonding vibrations of C–H, respectively [53]. As can be seen in Figure 5c, the FT-IR spectra of CI showed a characteristic peak (B) at 1670 cm^{-1}, corresponding to the R-CHO stretching vibration. Furthermore, there were some peaks at 1450, 746, and 688 cm^{-1}, which can be attributed to the phenyl group of CI [53]. In the FT-IR spectra of the coated paper samples, the main characteristic peaks, A and B, can be attributed to CL, EU, and CI, respectively, which were also visible; however, these were at a lower intensity. In addition, the intensity of peaks was higher for the coatings with higher quantities of active substances. Meanwhile, the bands at 3290 and

2895 cm^{-1} in the spectra of the samples containing paper are assigned to the characteristic stretching vibrations of –OH and $–CH_2$ in cellulose, respectively.

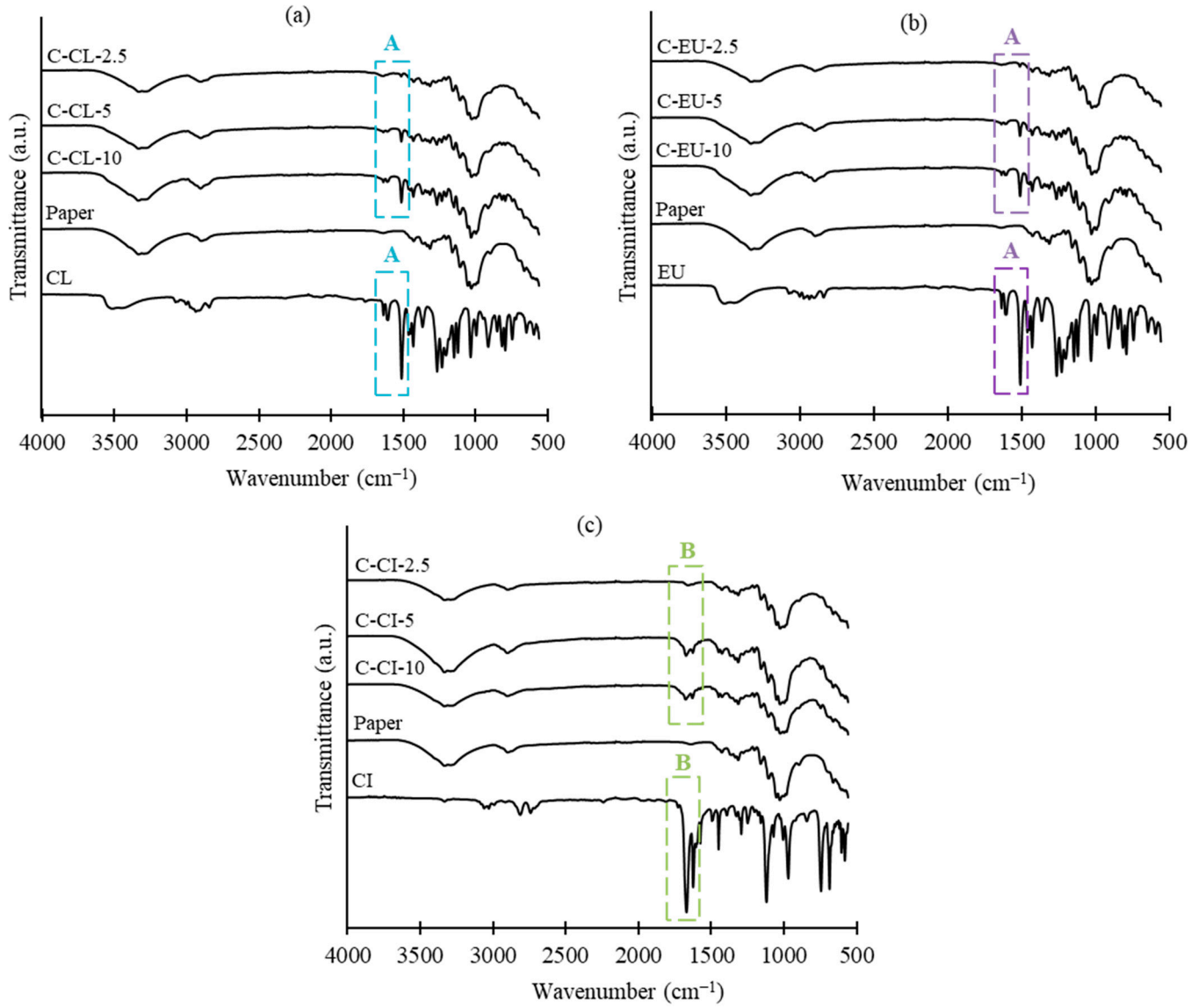

Figure 5. FT-IR spectra of samples: (**a**) clove essential oil (CL), paper and coated paper sheets (C-CL); (**b**) eugenol (EU), paper and coated paper sheets (C-EU); (**c**) cinnamaldehyde (CI), paper and coated paper sheets (C-CI). The dashed line boxes and letters show the groups of the absorption peaks: A—1636, 1607, and 1512 cm^{-1}; B—1670 cm^{-1}.

3.5. Biological Activity of Coatings

The antioxidant and antimicrobial activities of coatings were investigated by the DPPH method and disk diffusion assays, as demonstrated by the data provided in Tables 5 and 6, respectively. The biological activities of the coatings correlated with the amount of EO in the coating. Studies of the antioxidant properties of coated paper revealed that high antioxidant activity was characteristic of the clove essential oil emulsion coatings (76–92%) and the eugenol emulsion coatings (87–91%), while the coatings containing cinnamon aldehyde showed quite low antioxidant activity (4–9%). Furthermore, the antioxidant activity of the coatings was determined on Day 28 during the storage at ambient temperature. After this period, the antioxidant activity of the coatings was reduced; however, samples still exhibited relatively high activity, especially at higher quantities of EO in the coatings.

Table 5. The antioxidant activity of the coatings on Day 1 and Day 28.

Coating	Amount of Coating (mg/cm^2)	Radical Scavenging on Day 1 (%)	Amount of Coating (mg/cm^2)	Radical Scavenging on Day 28 (%)
C-CL-2.5	3.83 ± 0.5	76 ± 1.9 ^{a}	2.80 ± 0.2	45.5 ± 0.8 ^{a}
C-CL-5	4.28 ± 1.1	90 ± 0.1 ^{c}	3.05 ± 0.2	74.4 ± 1.7 ^{b}
C-CL-10	4.23 ± 0.7	92 ± 0.3 ^{c}	3.10 ± 0.2	83.2 ± 0.9 ^{c}
C-EU-2.5	4.43 ± 0.7	87 ± 0.4 ^{a}	2.95 ± 0.1	60.3 ± 1.5 ^{a}
C-EU-5	4.45 ± 0.3	91 ± 0.4 ^{c}	3.00 ± 0.1	78.2 ± 1.4 ^{c}
C-EU-10	4.28 ± 0.3	91 ± 0.5 ^{c}	2.75 ± 0.1	79.0 ± 0.5 ^{c}
C-CI-2.5	3.65 ± 0.1	4 ± 0.5 ^{ab}	3.05 ± 0.0	0.30 ± 0.2 ^{a}
C-CI-5	4.13 ± 0.5	5 ± 2.2 ^{ab}	2.70 ± 0.1	2.50 ± 1.4 ^{abc}
C-CI-10	3.48 ± 0.2	9 ± 3.0 ^{b}	2.75 ± 0.3	5.10 ± 0.2 ^{c}

$^{a–c}$: the different letters within the column for the coating containing the same essential oil show that the results are significantly different ($p < 0.05$; Duncan test).

The antimicrobial activities of coatings against various microorganisms such as Gram-negative, Gram-positive, and yeast are presented in Table 6.

The disk diffusion method was sensitive in showing the antimicrobial effectiveness of paper samples coated with emulsions containing different quantities of CL, EU, or CI against Gram-positive and Gram-negative bacteria and yeast.

Antibacterial activity test results for the Gram-positive bacteria *S. aureus*, *L. monocytogenes*, *B. cereus*, *Gram-negative E. coli*, and *C. albicans* indicated that the size of inhibition zone diameters (IZDs) correlated with the different percentages of CL, EU, or CI in the coating.

The inhibitory effect of the paper samples coated with emulsions containing 2.5% of CL was not detected against Gram-negative and Gram-positive bacteria and yeast *C. albicans*. The results of this study showed that all tested coatings exhibited the highest activity when 10% of essential oil was used in the emulsion preparation. Furthermore, this study showed that the most effective coating was the CI coating. It was determined that *E. coli* was resistant to C-CI-2.5 and IZD increased to 18.70 ± 0.01 mm when C-CI-10 was used. Also, the increase of IZDs was demonstrated after the treatment of *B. cereus* and *C. albicans* with the paper coated with emulsions containing different percentages of CI. The size of the IZDs was greater for *C. albicans*, and formed 10.85 ± 0.04 mm, 13.60 ± 0.02, and 15.49 ± 0.02 mm when using 2.5, 5, and 10% of CI in the coating formulation, respectively. The same was observed for *B. cereus* when the IZDs were 7.59 ± 0.02 mm, 17.10 ± 0.02, and 18.34 ± 0.03 mm using 2,5, 5, and 10% of CI, respectively.

Table 6. Inhibition zones of coated paper samples against Gram-positive and Gram-negative bacteria by disk diffusion method.

Coating	Inhibition Zone (mm) *				
	C. albicans	*S. aureus*	*E. coli*	*B. cereus*	*L. monocytogenes*
C-CL-2.5	NI	NI	NI	NI	NI
C-CL-5	NI	NI	NI	8.02 ± 0.06 ^{a}	NI
C-CL-10	8.37 ± 0.03	NI	9.36 ± 0.11	8.74 ± 0.06 ^{b}	NI
C-EU-2.5	NI	NI	NI	8.02 ± 0.01 ^{a}	NI
C-EU-5	NI	NI	8.73 ± 0.05 ^{a}	8.50 ± 0.02 ^{b}	NI
C-EU-10	10.79 ± 0.02	NI	9.91 ± 0.04 ^{b}	10.45 ± 0.05 ^{c}	NI
C-CI-2.5	10.85 ± 0.04 ^{a}	NI	NI	7.59 ± 0.02 ^{a}	NI
C-CI-5	13.60 ± 0.02 ^{b}	NI	13.23 ± 0.04 ^{a}	17.10 ± 0.02 ^{b}	7.39 ± 0.04 ^{a}
C-CI-10	15.49 ± 0.02 ^{c}	10.81 ± 0.01	18.70 ± 0.01 ^{b}	18.34 ± 0.03 ^{c}	11.16 ± 0.02 ^{b}

* NI = No inhibition; $^{a–c}$: the different letters within the column for coating containing same essential oil show that the results are significantly different ($p < 0.05$; Duncan test).

4. Conclusions

This study was designed to obtain emulsions by combining clove essential oil, eugenol, and cinnamaldehyde with hydrophobically modified starch and applying these formulations on paper substrates to provide antioxidant and antimicrobial properties. First, it was important to evaluate the main characteristics and stability of the prepared emulsions. The largest droplets were measured in emulsions with clove essential oil (293–348), and the smaller ones were characteristics of emulsions containing eugenol (181–236 nm) and cinnamaldehyde (205–218 nm). The storage conditions of the emulsions had an impact on the changes in the size of the droplets and pH value during the 14 days of storage time. When the emulsions were kept at a temperature of 5 °C, the emulsions were more stable compared to the samples kept at 20 and 40 °C. A slight sedimentation of the modified starch particles was observed after 2 days in all samples, while the creaming of the emulsions was detected only after 10 days of storage at 40 °C. Important changes in pH were observed for CI emulsions when samples were stored at a temperature of 40 °C. However, additional studies of emulsion such as long-term stability tests and the addition of stabilizers or other essential oils and biopolymers could be suggested for further studies.

This study of the biological activity of the emulsions confirmed that the formulations with clove essential oil and eugenol represented a high antioxidant action, while the emulsions containing cinnamaldehyde had the highest antimicrobial effect. The emulsions were cast on paper and sheets were characterized by using FT-IR. The results of biological activities testing of the coatings emphasize that emulsions enriched with clove essential oil, eugenol, and cinnamaldehyde could potentially be used as natural antioxidant and antibacterial coatings, respectively, combined with cellulose-based substrates. Also, further studies concerning the different techniques of the coating formation, e.g., spray coating, various printing techniques, etc., could be suggested.

In terms of the economic feasibility of emulsions and active coating production, the actual costs of production would depend on various factors, including the availability of raw materials, the scale of production, application areas, etc. Emulsions based on starch sodium octenyl succinate and essential oils have become increasingly popular compared to synthetic substances because of their non-toxicity, biocompatibility, natural abundance, and the relatively low cost of raw materials. Furthermore, the application of emulsions in areas such as paper coatings requires low amounts of emulsion formulations to obtain products with high biological activity. However, additional studies that focus on the areas of specific applications of the products should be performed.

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References

1. Ni, Z.J.; Wang, X.; Shen, Y.; Thakur, K.; Han, J.; Zhang, J.G.; Hu, F.; Wei, Z.J. Recent updates on the chemistry, bioactivities, mode of action, and industrial applications of plant essential oils. *Trends Food Sci. Technol.* **2021**, *110*, 78–89. [CrossRef]
2. Haro-González, J.N.; Castillo-Herrera, G.A.; Martínez-Velázquez, M.; Espinosa-Andrews, H. Clove Essential Oil (*Syzygium aromaticum* L. Myrtaceae): Extraction, Chemical Composition, Food Applications, and Essential Bioactivity for Human Health. *Molecules* **2021**, *26*, 6387. [CrossRef] [PubMed]

3. Haro-González, J.N.; Schlienger de Alba, B.N.; Martínez- Velázquez, M.; Castillo-Herrera, G.A.; Espinosa-Andrews, H. Optimization of Clove Oil Nanoemulsions: Evaluation of Antioxidant, Antimicrobial, and Anticancer Properties. *Colloids Interfaces* **2023**, *7*, 64. [CrossRef]
4. Ulanowska, M.; Olas, B. Biological Properties and Prospects for the Application of Eugenol—A Review. *Int. J. Mol. Sci.* **2021**, *22*, 3671. [CrossRef]
5. Usai, F.; Di Sotto, A. *trans*-Cinnamaldehyde as a Novel Candidate to Overcome Bacterial Resistance: An Overview of In Vitro Studies. *Antibiotics* **2023**, *12*, 254. [CrossRef]
6. Doyle, A.A.; Stephens, J.C. A review of cinnamaldehyde and its derivatives as antibacterial agents. *Fitoterapia* **2019**, *139*, 104405. [CrossRef] [PubMed]
7. Pandey, V.K.; Tripathi, A.; Srivastava, S.; Dar, A.H.; Singh, R.; Farooqui, A.; Pandey, S. Exploiting the bioactive properties of essential oils and their potential applications in food industry. *Food Sci. Biotechnol.* **2023**, *32*, 885–902. [CrossRef] [PubMed]
8. Villalobos-Delgado, L.H.; Nevárez-Moorillon, G.V.; Caro, I.; Quinto, E.J.; Mateo, J. Natural antimicrobial agents to improve foods shelf life. In *Food Quality and Shelf Life*; Galanakis, C.M., Ed.; Academic Press: Cambridge, MA, USA, 2019; pp. 159–200. [CrossRef]
9. Prakash, B.; Kedia, A.; Mishra, P.K.; Dubey, N.K. Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agri-food commodities—Potentials and challenges. *Food Control* **2015**, *47*, 381–391. [CrossRef]
10. Danila, A.; Muresan, E.I.; Ibanescu, S.A.; Popescu, A.; Danu, M.; Zaharia, C.; Türkoğlu, G.C.; Erkan, G.; Staras, A.I. Preparation, characterization, and application of polysaccharide-based emulsions incorporated with lavender essential oil for skin-friendly cellulosic support. *Int. J. Biol. Macromol.* **2021**, *191*, 405–413. [CrossRef]
11. Yammine, J.; Chihib, N.E.; Gharsallaoui, A.; Ismail, A.; Karam, L. Advances in essential oils encapsulation: Development, characterization and release mechanisms. *Polym. Bull.* **2023**, *81*, 3837–3882. [CrossRef]
12. Gheorghita Puscaselu, R.; Lobiuc, A.; Sirbu, I.O.; Covasa, M. The Use of Biopolymers as a Natural Matrix for Incorporation of Essential Oils of Medicinal Plants. *Gels* **2022**, *8*, 756. [CrossRef] [PubMed]
13. Majeed, U.; Majeed, H.; Liu, X.; Shafi, A.; Liu, T.; Ye, J.; Meng, Q.; Luo, Y. Succinylated starch emulsified Eugenol and Carvacrol nanoemulsions improved digestive stability, bio-accessibility and Salmonella typhimurium inhibition. *Int. J. Biol. Macromol.* **2024**, *259*, 129230. [CrossRef] [PubMed]
14. Mandura Jarić, A.; Haramustek, L.; Nižić Nodilo, L.; Vrsaljko, D.; Petrović, P.; Kuzmić, S.; Jozinović, A.; Aladić, K.; Jokić, S.; Šeremet, D.; et al. A Novel Approach to Serving Plant-Based Confectionery—The Employment of Spray Drying in the Production of Carboxymethyl Cellulose-Based Delivery Systems Enriched with *Teucrium montanum* L. Extract. *Foods* **2024**, *13*, 372. [CrossRef]
15. Karami-Eshkaftaki, Z.; Saei-Dehkordi, S.; Albadi, J.; Moradi, M.; Saei-Dehkordi, S.S. Coated composite paper with nano-chitosan/cinnamon essential oil-nanoemulsion containing grafted CNC@ZnO nanohybrid; synthesis, characterization and inhibitory activity on *Escherichia coli* biofilm developed on grey zucchini. *Int. J. Biol. Macromol.* **2024**, *258*, 128981. [CrossRef] [PubMed]
16. Wang, K.; Wang, Y.; Cheng, M.; Wang, Y.; Zhao, P.; Xi, X.; Lu, J.; Wang, X.; Han, X.; Wang, J. Preparation and characterization of active films based on oregano essential oil microcapsules/soybean protein isolate/sodium carboxymethyl cellulose. *Int. J. Biol. Macromol.* **2024**, *258*, 128985. [CrossRef] [PubMed]
17. Moradi, M.; Barati, A.; Moradi, S.; Zarinabadi, E. Synthesis and characterization of starch-based hydrogels containing myrtus oil nanoemulsion for wound dressings. *Polym. Bull.* **2024**, *81*, 3043–3062. [CrossRef]
18. Zhang, W.; Ezati, P.; Khan, A.; Assadpour, E.; Rhim, J.W.; Jafari, S.M. Encapsulation and delivery systems of cinnamon essential oil for food preservation applications. *Adv. Colloid Interface Sci.* **2023**, *318*, 102965. [CrossRef] [PubMed]
19. Tian, B.; Liu, J.; Yang, W.; Wan, J.B. Biopolymer Food Packaging Films Incorporated with Essential Oils. *J. Agric. Food Chem.* **2023**, *71*, 1325–1347. [CrossRef] [PubMed]
20. Lemnaru, G.-M.; Motelica, L.; Trusca, R.D.; Ilie, C.I.; Croitoru, A.-M.; Ficai, D.; Oprea, O.; Stoica-Guzun, A.; Ficai, A.; Ditu, L.-M.; et al. Antimicrobial Wound Dressings based on Bacterial Cellulose and Independently Loaded with Nutmeg and Fir Needle Essential Oils. *Polymers* **2023**, *15*, 3629. [CrossRef]
21. Hosseini, H.; Tajian, Z.; Jafari, S.M. Improving the shelf-life of food products by nano/micro-encapsulated ingredients. In *Food Quality and Shelf Life*; Galanakis, C.M., Ed.; Academic Press: Cambridge, MA, USA, 2019; pp. 159–200. [CrossRef]
22. Tropea, A. Microbial Contamination and Public Health: An Overview. *Int. J. Environ. Res. Public Health* **2022**, *19*, 7441. [CrossRef]
23. Sharma, S.; Barkauskaite, S.; Duffy, B.; Jaiswal, A.K.; Jaiswal, S. Characterization and Antimicrobial Activity of Biodegradable Active Packaging Enriched with Clove and Thyme Essential Oil for Food Packaging Application. *Foods* **2020**, *9*, 1117. [CrossRef]
24. Kostova, L.; Lasheva, V.; Georgieva, D.; Damyanova, S.; Fidan, H.; Stoyanova, A.; Gubenia, O. Characterization of Active Paper Packaging Materials with Coriander Essential Oil (*Coriandrum Sativum* L.). *J. Chem. Technol. Metall.* **2020**, *55*, 2085–2093.
25. Todorova, D.; Yavorov, N.; Lasheva, V.; Damyanova, S.; Kostova, I. Lavender Essential Oil as Antibacterial Treatment for Packaging Paper. *Coatings* **2023**, *13*, 32. [CrossRef]
26. Buendía-Moreno, L.; Soto-Jover, S.; Ros-Chumillas, M.; Antolinos, V.; Navarro-Segura, L.; Sánchez-Martínez, M.J.; Martínez-Hernández, G.B.; López-Gómez, A. Innovative cardboard active packaging with a coating including encapsulated essential oils to extend cherry tomato shelf life. *LWT* **2019**, *116*, 108584. [CrossRef]

27. Hemmatkhah, F.; Zeynali, F.; Almasi, H. Encapsulated Cumin Seed Essential Oil-Loaded Active Papers: Characterization and Evaluation of the Effect on Quality Attributes of Beef Hamburger. *Food Bioprocess Technol.* **2020**, *3*, 533–547. [CrossRef]
28. Rosu, G.; Muresan, E.I.; Spac, A.F.; Diaconu, M.; Ciolacu, D.E.; Danila, A.; Tita, C.; Muresan, A. Aromatherapeutic and Antibacterial Properties of Cotton Materials Treated with Emulsions Containing Peppermint Essential Oil (*Menthae piperitae aetheroleum*). *Polymers* **2023**, *15*, 2348. [CrossRef] [PubMed]
29. Štular, D.; Šobak, M.; Mihelčič, M.; Šest, E.; German Ilić, I.; Jerman, I.; Simončič, B.; Tomšič, B. Proactive Release of Antimicrobial Essential Oil from a "Smart" Cotton Fabric. *Coatings* **2019**, *9*, 242. [CrossRef]
30. Stan, M.S.; Chirila, L.; Popescu, A.; Radulescu, D.M.; Radulescu, D.E.; Dinischiotu, A. Essential Oil Microcapsules Immobilized on Textiles and Certain Induced Effects. *Materials* **2019**, *12*, 2029. [CrossRef]
31. Balouiri, M.; Sadiki, M.; Ibnsouda, S.K. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal.* **2016**, *6*, 71–79. [CrossRef] [PubMed]
32. Ding, M.; Zhang, T.; Zhang, H.; Tao, N.; Wang, X.; Zhong, J. Gelatin molecular structures affect behaviors of fish oil-loaded traditional and Pickering emulsions. *Food Chem.* **2020**, *309*, 125642. [CrossRef]
33. Nhouchi, Z.; Watuzola, R.; Pense-Lheritier, A.M. A review on octenyl succinic anhydride modified starch-based Pickering-emulsion: Instabilities and ingredients interactions. *J. Texture Stud.* **2022**, *53*, 581–600. [CrossRef] [PubMed]
34. Yang, Y.; Fang, Z.; Chen, X.; Zhang, W.; Xie, Y.; Chen, Y.; Liu, Z.; Yuan, W. An Overview of Pickering Emulsions: Solid-Particle Materials, Classification, Morphology, and Applications. *Front. Pharmacol.* **2017**, *8*, 287. [CrossRef] [PubMed]
35. Apostolidis, E.; Stoforos, N.G.; Mandala, I. Starch physical treatment, emulsion formation, stability, and their applications. *Carbohydr. Polym.* **2023**, *305*, 120554. [CrossRef]
36. Linke, A.; Weiss, J.; Kohlus, R. Factors determining the surface oil concentration of encapsulated lipid particles: Impact of the emulsion oil droplet size. *Eur. Food Res. Technol.* **2020**, *246*, 1933–1943. [CrossRef]
37. Ho, T.M.; Razzaghi, A.; Ramachandran, A.; Mikkonen, K.S. Emulsion characterization via microfluidic devices: A review on interfacial tension and stability to coalescence. *Adv. Colloid Interface Sci.* **2022**, *299*, 102541. [CrossRef]
38. Sun, H.; Li, S.; Chen, S.; Wang, C.; Liu, D.; Li, X. Antibacterial and antioxidant activities of sodium starch octenylsuccinate-based Pickering emulsion films incorporated with cinnamon essential oil. *Int. J. Biol. Macromol.* **2020**, *159*, 696–703. [CrossRef]
39. Krishnan, R.; Mohan, K.; Ragavanab, K.V.; Nisha, P. Insights into headway in essential oil-based Pickering emulsions for food applications. *Sustain. Food Technol.* **2023**, *1*, 363. [CrossRef]
40. Soltanzadeh, M.; Peighambardoust, S.H.; Ghanbarzadeh, B.; Mohammadi, M.; Lorenzo, J.M. Chitosan nanoparticles encapsulating lemongrass (*Cymbopogon commutatus*) essential oil: Physicochemical, structural, antimicrobial and in-vitro release properties. *Int. J. Biol. Macromol.* **2021**, *192*, 1084–1097. [CrossRef]
41. Agama-Acevedo, E.; Bello-Pérez, L.A. Starch as an emulsions stability: The case of octenyl succinic anhydride (OSA) starch. *Curr. Opin. Food Sci.* **2017**, *6*, 78–83. [CrossRef]
42. Wu, X.; Xu, N.; Cheng, C.; McClements, D.J.; Chen, X.; Zou, L.; Liu, W. Encapsulation of hydrophobic capsaicin within the aqueous phase of water-in-oil high internal phase emulsions: Controlled release, reduced irritation, and enhanced bioaccessibility. *Food Hydrocoll.* **2021**, *123*, 107184. [CrossRef]
43. Błaszczyk, M.M.; Przybysz, Ł. Sedimentation of Microparticles in Highly Concentrated Non-Newtonian Emulsions. *Appl. Sci.* **2022**, *12*, 10442. [CrossRef]
44. Wang, D.; Huang, X.; Wang, Y. Managing the Phase Separation in Double Emulsion by Tuning Amphiphilicity via a Supramolecular Route. *Langmuir* **2014**, *48*, 14460–14468. [CrossRef]
45. Campelo, P.H.; Junqueira, L.A.; Resende, J.V.; Zacarias, R.D.; Barros Fernandes, R.V.; Botrel, D.A.; Borges, S.V. Stability of lime essential oil emulsion prepared using biopolymers and ultrasound treatment. *Int. J. Food Prop.* **2017**, *20*, S564–S579. [CrossRef]
46. Kumar Pandey, V.; Shams, R.; Singh, R.; Dar, A.H.; Pandiselvam, R.; Rusu, A.V.; Trif, M. A comprehensive review on clove (*Caryophyllus aromaticus* L.) essential oil and its significance in the formulation of edible coatings for potential food applications. *Front. Nutr.* **2022**, *9*, 987674. [CrossRef] [PubMed]
47. Tomas, A.; Maroyi, A.; Cheikhyoussef, N.; Hussein, A.A.; Cheikhyoussef, A. Health-promoting activities of clove (*Syzygium aromaticum*) extracts. In *Clove (Syzygium aromaticum)*; Ramadan, M.F., Ed.; Academic Press: Cambridge, MA, USA, 2022; pp. 619–637. [CrossRef]
48. Commission Implementing Regulation (EU) No 872/2012, 2012. List of Flavouring Substances Provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council. Available online: https://eur-lex.europa.eu/eli/reg_impl/2012/872/oj (accessed on 9 May 2024).
49. Jackson-Davis, A.; White, S.; Kassama, L.S.; Coleman, S.; Shaw, A.; Mendonca, A.; Cooper, B.; Thomas-Popo, E.; Gordon, K.; London, L. A Review of Regulatory Standards and Advances in Essential Oils as Antimicrobials in Foods. *J. Food Prot.* **2023**, *86*, 100025. [CrossRef] [PubMed]
50. Gülçin, İ. Antioxidant activity of eugenol: A structure-activity relationship study. *J. Med. Food* **2011**, *14*, 975–985. [CrossRef]
51. Chen, X.; Shang, S.; Yan, F.; Jiang, H.; Zhao, G.; Tian, S.; Chen, R.; Chen, D.; Dang, Y. Antioxidant Activities of Essential Oils and Their Major Components in Scavenging Free Radicals, Inhibiting Lipid Oxidation and Reducing Cellular Oxidative Stress. *Molecules* **2023**, *28*, 4559. [CrossRef] [PubMed]

52. Tarhan, I. A robust method for simultaneous quantification of eugenol, eugenyl acetate, and β-caryophyllene in clove essential oil by vibrational spectroscopy. *Phytochemistry* **2021**, *191*, 112928. [CrossRef]
53. Zhang, J.; Guo, Z.; Chen, S.; Dong, H.; Zhang, X.; Qin, Y.; Yao, C.; Xu, F. High-barrier, strong, and antibacterial paper fabricated by coating acetylated cellulose and cinnamaldehyde for food packaging. *Cellulose* **2021**, *28*, 4371–4384. [CrossRef]