

Article

Chitosan-Based Films with Essential Oil Components for Food Packaging

Magdalena Woźniak ¹, Joanna Młodziejewska ¹, Karolina Stefanowska ¹, Lucyna Mrówczyńska ², Anna Sip ³, Renata Dobrucka ^{4,*} and Izabela Ratajczak ¹

- ¹ Department of Chemistry, Faculty of Forestry and Wood Technology, Poznan University of Life Sciences, Wojska Polskiego 75, 60625 Poznań, Poland; magdalena.wozniak@up.poznan.pl (M.W.); joakoz2@outlook.com (J.M.); karolina.stefanowska@up.poznan.pl (K.S.); izabela.ratajczak@up.poznan.pl (I.R.)
- ² Department of Cell Biology, Faculty of Biology, Adam Mickiewicz University, Uniwersytetu Poznańskiego 6, 61614 Poznań, Poland; lucyna.mrowczynska@amu.edu.pl
- ³ Department of Biotechnology and Food Microbiology, Faculty of Food Science and Nutrition, Poznan University of Life Sciences, Wojska Polskiego 48, 60627 Poznań, Poland; anna.sip@up.poznan.pl
- ⁴ Department of Industrial Products and Packaging Quality, Institute of Quality Science, Poznań University of Economics and Business, Al. Niepodległości 10, 61875 Poznań, Poland
- * Correspondence: renata.dobrucka@ue.poznan.pl

Abstract: Chitosan-based films show great potential in terms of application in food preservation and are also promising carriers of biologically active ingredients. This paper presents the potential use of chitosan-based films with the addition of essential oil components, e.g., carvacrol, eugenol, and isoeugenol, intended for food packaging. The characteristics of the obtained films were determined, including antibacterial, mechanical, barrier, and structural parameters. In addition, the antibacterial and antioxidant effects of the essential oil components were assessed. Eugenol (44.41%) and isoeugenol (43.56%) showed high antiradical activities, similar to the activity of Trolox (44.33%), which is used as a standard antioxidant. In turn, carvacrol was characterized by the strongest effect against the examined strains of bacteria, both Gram-positive and Gram-negative. The chitosan film with carvacrol showed the most valuable antibacterial and mechanical properties (tensile strength and elongation at break). The antibacterial activities of the chitosan–carvacrol films were higher than that of the carvacrol solution. The inhibition zones of the chitosan–carvacrol films were in the range 29–41 mm (except for *Enterococcus faecalis*, with an inhibition zone of 15 mm) compared to the inhibition zones of the carvacrol solution (28 mm). The results showed that chitosan is an effective carrier of fragrance compounds, mainly carvacrol. However, all the tested chitosan-based films with the addition of fragrance compounds showed appropriate parameters (biological, mechanical, and barrier), which makes them an ecological alternative to plastics intended for food packaging.

Keywords: eugenol; carvacrol; isoeugenol; human erythrocytes; antibacterial activity



Citation: Woźniak, M.; Młodziejewska, J.; Stefanowska, K.; Mrówczyńska, L.; Sip, A.; Dobrucka, R.; Ratajczak, I. Chitosan-Based Films with Essential Oil Components for Food Packaging. *Coatings* **2024**, *14*, 830. <https://doi.org/10.3390/coatings14070830>

Academic Editors: Ana Luisa Fernando and Victor Gomes Lauriano Souza

Received: 27 May 2024

Revised: 27 June 2024

Accepted: 28 June 2024

Published: 3 July 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The increasing degradation of the natural environment and numerous legal regulations (e.g., Directive 2019/904 of the European Parliament and Council) regarding the use of plastic packaging have resulted in the search for new environmentally friendly materials that could replace commonly used plastic packaging. Therefore, scientists and the food industry are developing toward the use of renewable, abundant, and natural alternatives that can replace traditional and non-renewable plastics. Bio-based plastic packaging materials are one of the potential materials which use can reduce the enormous volume of plastic waste and reduce CO₂ emissions [1,2]. Natural materials used in the production of food packaging materials include polysaccharides (cellulose, chitosan, alginate, pectins, agar, or starch), proteins (casein, gelatin, collagen, or soy protein), and lipids (beeswax or vegetables oils) [3]. In the past few years, special attention has been paid to packaging

systems with biological properties, mainly antimicrobial effects, which may extend the shelf life of packaged food products [4,5]. However, for the food packaging to have an antibacterial effect, it must contain an antibacterial agent, and to minimize the impact on the natural environment, the best solution seems to be the use of antimicrobial agents of natural origin.

Essential oils, obtained from various parts of aromatic plants, owing to their multifaceted properties, including antimicrobial, antioxidant, anticancer, and neuroprotective effects, are used in various applications [6]. The use of essential oils continues to grow, mainly in cosmetology, pharmaceuticals, healthcare, and the food industry [7–9]. Essential oils are known for their complex bouquet of fragrance compounds, also called aroma compounds, which are chemical compounds with a wide range of odors [10]. The constituents of essential oils are monoterpenes, phenolic acids, a variety of terpene hydrocarbons, alkaloids, isoflavones, and flavonoids [11,12]. The biological activities of essential oils depend on their chemical profiles. However, oils obtained from the same plant, but from a different source (from a varied geographical region), have been characterized by different chemical compositions and, consequently, distinct biological activities, which may be related to various proportions of volatile compounds found in essential oils [13]. Moreover, biological properties of essential oils may be attributed to the action of individual compounds, their synergistic effect, or both [14,15]. Terpenes and terpenoids are the main bioactive components of essential oils, exhibiting antimicrobial, antioxidant, and anticancer activities [12,16–20]. They play a crucial role in medicine, particularly in antimicrobial research [7,13]. Phenolic compounds are also important components of essential oils with pharmacological effects [21].

Eugenol (4-allyl-2-methoxyphenol) belongs to phenolic compounds found in essential oils of many plant species, mainly occurring in clove oil [22]. It shows multidirectional effects, including antioxidant, analgesic, and anti-inflammatory activities [22,23]. Eugenol has been shown to be active against a wide range of pathogens, including fungi, bacteria, as well as viruses [23,24]. Because of its pharmacological effects, eugenol has many applications, including cosmetology (fragrances in perfumes or soaps), medicine, and pharmacology [25]. Isoeugenol (2-methoxy-4-[(E)-prop-1-enyl]phenol) is an isomer of eugenol and has some similar biological properties to it. Isoeugenol exhibits antimicrobial and antioxidant activities and possesses the ability to protect DNA against damage [26,27]. It occurs naturally in oils from cloves, cinnamon, and nutmeg [27]. Carvacrol (2-methyl-5-propan-2-ylphenol) belongs to a phenolic monoterpene family and is a constituent of essential oils produced from various aromatic plants, including oregano, thyme, wild bergamot, and pepperwort [28]. It demonstrates therapeutic properties, such as antimicrobial, anticancer, antioxidant, antiaging, and diabetes prevention [28–30]. Because of its beneficial properties, carvacrol is used to preserve or improve food quality as well as human health.

The biological effects of essential oils and their components makes them suitable to be used as bioactive ingredients in various matrices to obtain functional food packaging materials [31–33]. Essential oils and their fragrance constituents have also been used as additions to the chitosan matrix, mainly to improve the antimicrobial activities and other biological properties, such as the antioxidant activities, of the final materials [34–37].

However, the introduction of bioactive substances to chitosan not only improved the biological properties of the obtained films but also influenced other parameters, including mechanical and barrier [34,38]. Essential oil components, such as eugenol and carvacrol, have been used as additives in packaging materials, as described in the literature [39–41], but often these studies have only focused on the characterization of chitosan-based films with the addition of a single essential oil component, or materials containing essential oil components with other biologically active substances, and not on comparing the properties of films with different fragrance compounds. Therefore, the goal of this research was to compare the antibacterial activities, mechanical parameters, and barrier properties of chitosan films with the addition of eugenol, isoeugenol, and carvacrol (Figure 1) to determine their application potentials as ecological and bioactive food packaging. Moreover,

the biological activities of these essential oil components, including antibacterial and antiradical activities and the cytotoxic effects of these compounds on human red blood cells (RBCs) as a cell model, were assessed. In addition, by comparing the antibacterial effects of fragrance compounds with the activities of films with their addition, the potential of chitosan as a carrier of biomolecules with antagonistic activity against bacteria was assessed.

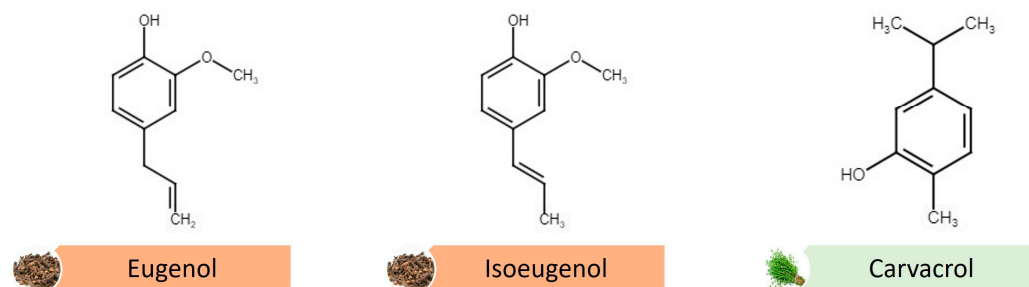


Figure 1. The chemical structures of the essential oil components.

2. Materials and Methods

2.1. Materials

Carvacrol, eugenol, isoeugenol, Tween 20, DPPH· (2,2-diphenyl-1-picrylhydrazyl), Trolox, and phosphate-buffered saline (PBS) were purchased from Merck KGaA (Darmstadt, Germany). Ethanol at a 70% concentration was used as a solvent of the compounds. Ethanol and acetic acid were purchased from Avantor Performance Materials (Gliwice, Poland), while chitosan (flakes, 86% deacetylated) was purchased from ChitoLytic (Toronto, Canada). Muller–Hinton agar (OXOID CM 0337) was purchased from Graso (Poland).

2.2. Production of Chitosan-Based Films

The chitosan-based solution was obtained by dissolving chitosan (3 g) in 3% acetic acid (300 mL). The solution was next homogenized and transferred to Teflon-covered Petri dishes and dried at room temperature to obtain pure chitosan films (CHT). Chitosan-based films with the addition of fragrance compounds were prepared by adding these compounds (3.05 mL) to the chitosan solution (300 mL) to obtain final concentrations of essential oil components of 1%. Additionally, Tween 20 (2.0 mL) was added to each solution. After homogenizing the solutions, pouring them onto plates, and drying them at room temperature, three different films were obtained: chitosan–carvacrol (CHT-CAR), chitosan–eugenol (CHT-EUG), and chitosan–isoeugenol (CHT-ISOEUG).

2.3. Antibacterial Activities

The antibacterial activities of the carvacrol, eugenol, isoeugenol (10 mg/mL), and chitosan films were assessed using the agar diffusion method against the following bacterial strains: *Bacillus cereus* (ATCC 10876), *Bacillus megaterium* (5tk soil isolate), *Enterococcus faecalis* (54jk cheese isolate), *Enterococcus faecium* (25g cheese isolate), *Escherichia coli* (ATCC 10536), *Listeria innocua* (ATCC 30090), *Listeria monocytogenes* (ATCC 15313), *Pseudomonas aeruginosa* (ATCC 15443), *Salmonella* Enteritidis (05/07 isolate from the collection of the Department of Biotechnology and Food Microbiology), *Salmonella* Paratyphi (ATCC 9150), *Salmonella* Typhimurium (ATCC 13311), *Staphylococcus aureus* (ATCC 25923), and *Yersinia enterocolitica* (ATCC 9610).

The Petri dishes containing the Muller–Hinton agar were inoculated with a standardized inoculum of indicator strains at 10^5 CFU/mL. In the next step, 10 μ L of each fragrance compound was dropped onto the surface of the agar medium. In the case of the chitosan films, each film sample (10 mm in diameter) was placed on a Petri dish containing Mueller–Hinton agar inoculated with 10^6 CFU/mL of the indicator strains. The Petri dishes were incubated at $35 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ for 24 h. The results were presented as the diameters of the

inhibition zones formed around the film samples or compound solutions and measured in millimeters using a computer-scanning system (MultiScaneBase v14.02).

2.4. Antiradical Activities

An ethanolic solution (200 μL) of DPPH $\cdot$ (at a 0.1 mM concentration) was mixed with the solution of each tested compound (200 μL) or Trolox, which was used as the standard antioxidant (the positive control). The ethanolic solution (200 μL) of DPPH $\cdot$ mixed with the PBS buffer (200 μL) was used as the negative control. All the samples were vortexed (Bio Vortex V1, Biosan, Riga, Latvia) and were incubated for 30 min in the absence of light at room temperature ($\sim 22^\circ\text{C}$). Following incubation, the absorbance (Abs) of every solution was measured at 517 nm using a BioMate™ 160 UV–Visible spectrophotometer (Thermo Scientific, Waltham, MA, USA). The percentage DPPH-scavenging activities of the compounds tested and reference compound (Trolox) were calculated using the following equation:

$$\text{DPPH-scavenging activity(\%)} = \frac{\text{Abs}_0 - \text{Abs}_1}{\text{Abs}_0} \cdot 100$$

where Abs_0 is the absorbance of the negative control solution, and Abs_1 is the absorbance of the examined compound or Trolox solution.

Every sample tube with the tested or reference compound was prepared in triplicate, and every experiment was repeated three times. The results are presented as the average value obtained from all the experiments performed.

2.5. Hemolytic Activities

The human red blood cells (RBCs) used in this study were purchased as concentrates ($\sim 65\%$ hematocrit) from the blood bank in Poznań, according to the bilateral agreement (No. ZP/2867/D/21) signed between the Regional Center for Blood Donation and Hematology in Poznań and Adam Mickiewicz University in Poznań. The RBC concentrates were centrifuged ($960 \times g$; 10 min; 4°C) (Sigma 3-30K Sartorius AG, Göttingen, Germany) three times in phosphate-buffered saline (PBS) (pH = 7.4) supplemented with 10 mM glucose to obtain an RBC suspension of 1.65×10^9 cells/mL (15% hematocrit). After washing, the RBCs (1.65×10^8 cells/mL; 1.5% hematocrit) were incubated in PBS buffer supplemented with 10 mM glucose and containing the tested compounds at a concentration equal to 0.1 mg/mL for 60 min at 37°C , under gently shaking (BioSan Thermo-Shaker TS-100C, Biosan, Riga, Latvia). The positive controls were RBCs incubated in ice-cold deionized water without the addition of the tested compounds. The negative controls were RBCs incubated in PBS buffer supplemented with 10 mM glucose without the addition of the tested compounds. Each sample was prepared in triplicate, and the experiments were repeated three times with RBCs obtained from different donors. Following incubation, all the sample tubes were centrifuged (Sigma 3-30K, Sartorius AG, Göttingen, Germany) ($960 \times g$; 10 min; 4°C), and the degree of hemolysis was estimated by measuring the absorbance (Abs) of the supernatants with a BioMate™ 160 UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA) at 540 nm. The hemolytic activity (%) of the compounds tested was calculated using the following equation:

$$\text{Hemolytic activity (\%)} = \frac{\text{Abs}_t}{\text{Abs}_{pc}} \cdot 100$$

where Abs_t is the absorbance of the supernatant with tested samples, and Abs_{pc} is the absorbance of the supernatant of the positive control sample.

A hemolysis degree of $\leq 5\%$ indicates no hemolytic activity of the tested compound at the concentration used and means it is biocompatible. The results are presented as the average values obtained from three independent experiments.

2.6. Mechanical Parameters of Films

The tensile strengths and elongations at break of the tested films were determined according to ASTM D882-12 [42] using an Instron universal testing machine (Model 5965, Instron, Norwood, MA, USA). Before the analysis, the samples (1.5 × 10 cm) were conditioned at 23 °C and 50% relative humidity for 48 h.

2.7. Water Vapor Transmission Rates

The water vapor transmission rates (WVTRs) of the tested films were determined in accordance with ISO 2528:2017 [43]. This test was conducted in a container at a relative humidity of 65% and a temperature of 23 °C and containing anhydrous calcium chloride (Avantor Performance Materials, Gliwice, Poland). Samples of films were placed in the container, which was then closed tightly.

2.8. Infrared Spectroscopy

The spectra of the films were recorded using attenuated total reflectance Fourier-transform infrared spectroscopy (Nicolet iS5, ATR-FTIR, Thermo Fisher Scientific, Waltham, MA, USA). The spectra were recorded at 4000–600 cm^{−1} at a resolution of 4 cm^{−1}, recording 32 scans.

2.9. Statistical Analysis

Statistical analysis—factorial one-way ANOVA followed by Tukey’s honest significant difference (HSD) test at $\alpha = 0.05$ —was performed using TIBCO Software Inc.’s Statistica version 13.3 (Palo Alto, CA, USA).

Graphs presenting the results were prepared using the Chiplot program (<https://www.chiplot.online/>, accessed on 25 April 2024).

3. Results

3.1. Biological Activities of Fragrance Compounds

The initial stage of this research was the assessment of the biological activities of carvacrol, eugenol, and isoeugenol. The antibacterial and antioxidant effects of the tested compounds and their cytotoxic effects on the membranes of human red blood cells were compared.

3.1.1. Antibacterial Activities

The antibacterial effects of the tested compounds were evaluated against both Gram-positive and Gram-negative bacterial strains. The results, quantified as the diameter of the inhibition growth zone, are presented in Table 1.

Table 1. The antibacterial activities of the tested compounds.

Bacterial Strain	Carvacrol	Eugenol	Isoeugenol
	Inhibition Zone (mm)		
	Gram-positive bacteria		
<i>B. cereus</i>	28	16	20
<i>B. megaterium</i>	28	18	20
<i>E. faecalis</i>	28	16	22
<i>E. faecium</i>	28	16	18
<i>L. innocua</i>	28	16	20
<i>L. monocytogenes</i>	28	18	18
<i>S. aureus</i>	26	14	20
Gram-negative bacteria			
<i>E. coli</i>	>28	20	28
<i>P. aeruginosa</i>	28	16	16

Table 1. Cont.

Bacterial Strain	Carvacrol	Eugenol	Isoeugenol
	Inhibition Zone (mm)		
<i>S. Enteritidis</i>	28	16	16
<i>S. Typhimurium</i>	28	18	>28
<i>S. Paratyphi</i>	28	16	20
<i>Y. enterocolitica</i>	28	16	20

Inhibition zones (average diameters (in mm) of bacterial growth inhibition areas): 5–10 mm—weak activity; 11–14 mm—medium activity; >14 mm—strong activity.

Carvacrol exhibited the highest activities against the tested bacterial strains among all the tested compounds. It showed high activities against all the tested bacterial strains, with inhibition zones of 26–28 mm. Isoeugenol showed higher activities, against most tested bacterial strains, than its isomer. It demonstrated particularly strong activities against two Gram-negative bacteria—*E. coli* and *S. Typhimurium*, with an inhibition zone of 28 mm.

3.1.2. Antioxidant Activities

The antioxidant potentials of the tested essential oil components were evaluated using the DPPH· radical scavenging assay. The results of the antiradical abilities of the tested compounds are presented in Figure 2.

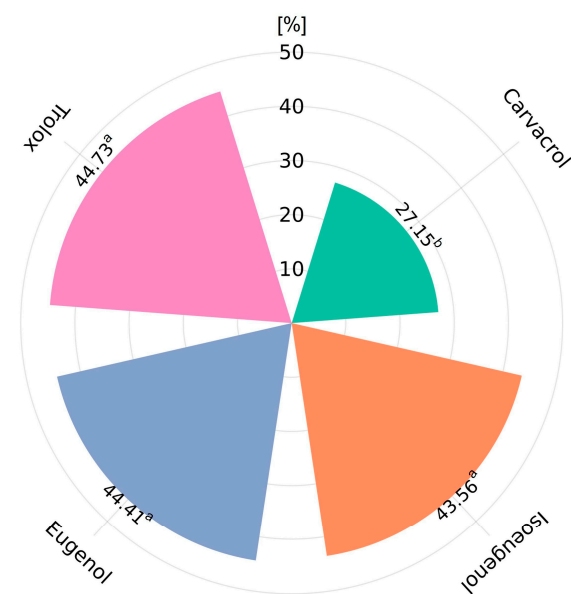


Figure 2. The antiradical activities of the tested compounds. Trolox was used as a standard antioxidant. Identical superscripts next to the results mean no significant difference (for $p < 0.05$) between the mean values according to Tukey's post hoc HSD test.

The DPPH· free radical scavenging activities of the tested compounds were in the range from 27.15% (carvacrol) to 44.41% (eugenol). Eugenol and isoeugenol showed high activities comparable to that of the reference antioxidant (Trolox), which was confirmed by statistical analysis (Supplementary Materials). Carvacrol exhibited a moderate DPPH·-scavenging ability, which was approximately 60% of the activity of the Trolox.

3.1.3. Hemolytic Activities

The hemolytic activities of the essential oil components, at a 0.1 mg/mL concentration, were evaluated using RBCs as a cell model, and the results are presented in Figure 3.

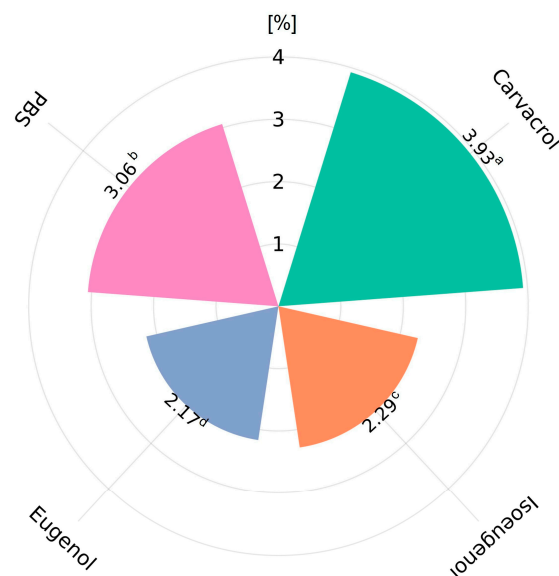


Figure 3. The hemolytic activities of the tested compounds. Identical superscripts next to the results mean no significant difference (for $p < 0.05$) between the mean values according to Tukey's post hoc HSD test.

The tested compounds exhibited varying hemolytic activities, ranging from 3.93% (carvacrol) to 2.17% (eugenol). The activity of the carvacrol was statistically higher than that calculated for RBCs incubated in the PBS buffer; however, all the tested fragrant compounds with a hemolytic activity of $\leq 5\%$ are considered as non-cytotoxic (biocompatible) compounds, namely, without any detrimental effects on the molecular structure and function of the cell membrane [44].

3.2. Characterization of Chitosan-Based Films with Fragrance Compounds

In the second stage of this research, chitosan-based films with the additions of eugenol, isoeugenol, and carvacrol were analyzed for antimicrobial activities and assessment of mechanical and barrier parameters.

3.2.1. Antimicrobial Activities

As shown in the data presented in Table 2, all the tested films showed antibacterial activities, while the chitosan–carvacrol film had the highest antimicrobial activity. The largest inhibition zones (≥ 40 mm) for this film were observed for three strains of Gram-positive bacteria—*B. cereus*, *B. megaterium*, and *L. monocytogenes*, and one Gram-negative bacterium—*E. coli*. The chitosan films with the additions of eugenol and isoeugenol showed comparable and lower antibacterial activities than the CHT-CAR film. The chitosan–isoeugenol film showed higher activities, especially in the case of Gram-negative bacteria, than the CHT-EUG film. In turn, the pure chitosan film did not show any antibacterial action.

Table 2. The antibacterial activities of the tested films.

Bacterial Strain	Chitosan– Carvacrol (CHT-CAR)	Chitosan– Eugenol (CHT-EUG)	Chitosan– Isoeugenol (CHT-ISOEUG)	Chitosan (CHT)
	Inhibition Zone (mm)			
Gram-positive bacteria				
<i>B. cereus</i>	40	19	24	0
<i>B. megaterium</i>	40	25	20	0
<i>E. faecalis</i>	15	12	0	0

Table 2. Cont.

Bacterial Strain	Chitosan–Carvacrol (CHT-CAR)	Chitosan–Eugenol (CHT-EUG)	Chitosan–Isoeugenol (CHT-ISOEUG)	Chitosan (CHT)
Inhibition Zone (mm)				
<i>E. faecium</i>	32	0	0	0
<i>L. innocua</i>	38	23	23	0
<i>L. monocytogenes</i>	41	21	22	0
<i>S. aureus</i>	33	21	19	0
Gram-negative bacteria				
<i>E. coli</i>	40	10	17	0
<i>P. aeruginosa</i>	36	19	19	0
<i>S. Enteritidis</i>	36	11	22	0
<i>S. Typhimurium</i>	36	17	21	0
<i>S. Paratyphi</i>	29	12	0	0
<i>Y. enterocolitica</i>	31	12	12	0

Inhibition zones (average diameters (in mm) of bacterial growth inhibition areas): 5–10 mm—weak activity; 11–14 mm—medium activity; >14 mm—strong activity.

3.2.2. Mechanical and Barrier Parameters

The chitosan-based films with the additions of fragrance compounds were characterized by varied mechanical parameters, namely, tensile strength (TS) and elongation at break (EB), as presented in Figure 4.

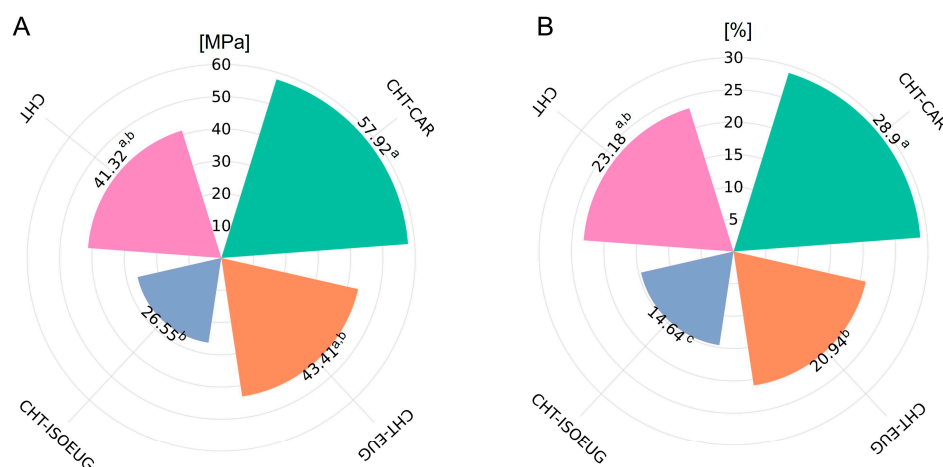


Figure 4. The tensile strengths (A) and elongations at break (B) of the chitosan-based films (CHT—pure chitosan film; CHT-CAR—chitosan-carvacrol film; CHT-EUG—chitosan-eugenol film; CH-ISOEUG—chitosan-isoeugenol film). Identical superscripts next to the results mean no significant difference (for $p < 0.05$) between the mean values according to Tukey's post hoc HSD test.

The TS values determined for the tested films ranged from 26.55 (CHT-ISOEUG) to 57.92 MPa (CHT-CAR). The addition of the isoeugenol to the chitosan matrix resulted in a decrease in the TS value of the film, while the addition of the carvacrol increased the tensile strength of the obtained film, which is confirmed by statistical analysis. A similar dependence was also observed for the EB values determined for the tested films—the lowest value was obtained for CHT-ISOEUG and the highest for CHT-CAR.

The additions of the essential oil components to the chitosan matrix resulted in higher WVTR values (Figure 5) for films with fragrance compounds compared to the film produced only from chitosan, which was confirmed by statistical analysis. The additions of the fragrance compounds to the chitosan influenced not only the strength parameters but also the barrier parameters of the films.

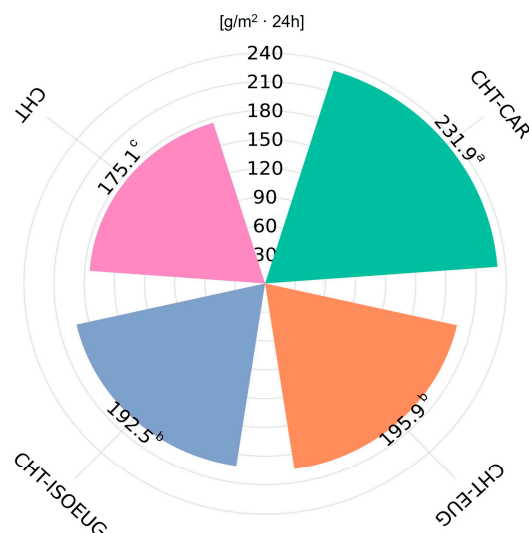


Figure 5. The water vapor transmission rates (WVTRs) determined for the tested chitosan-based films (CHT—pure chitosan film; CHT-CAR—chitosan-carvacrol film; CHT-EUG—chitosan-eugenol film; CH-ISOEUG—chitosan-isoeugenol film). Identical superscripts next to the results mean no significant difference (for $p < 0.05$) between the mean values according to Tukey's post hoc HSD test.

3.2.3. Structural Characteristics

The FTIR spectra of the chitosan film and chitosan–aromatic compound films, presented in Figure 6, indicated changes in the structures of the tested films after the incorporation of these compounds into the chitosan matrix.

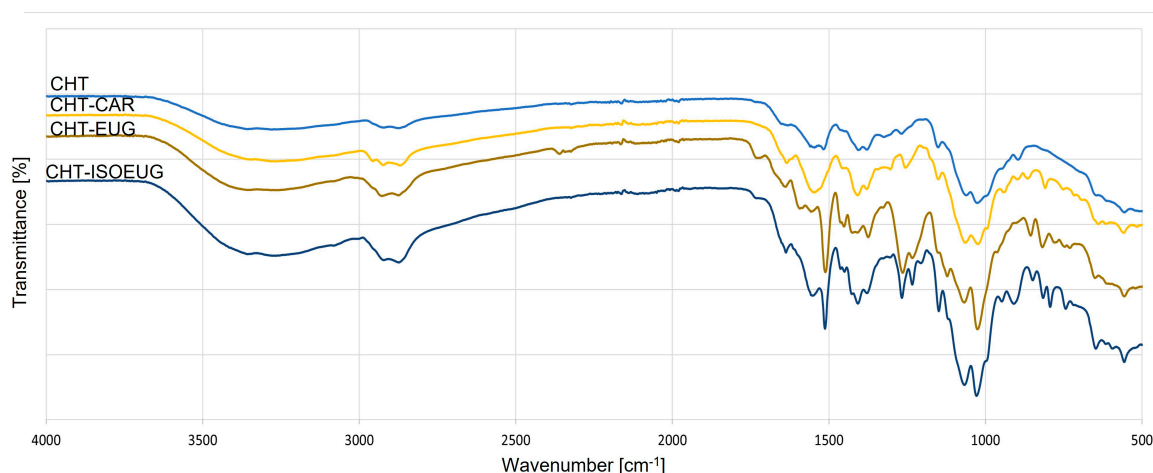


Figure 6. The ATR-FTIR spectra of the chitosan-based films (CHT—pure chitosan film; CHT-CAR—chitosan-carvacrol film; CHT-EUG—chitosan-eugenol film; CH-ISOEUG—chitosan-isoeugenol film).

The spectrum of chitosan (CHT) contains characteristic bands for this polymer, including the bands at 3257 cm^{-1} (O-H and N-H stretching vibrations), 1641 cm^{-1} (C=O stretching from amide I), 1550 cm^{-1} (N-H bending from amide II), and the bands at 1263, 1149, 1023, and 893 cm^{-1} , correspond to the saccharide structure (C-N stretching, C-O-C symmetric stretching, and C-O stretching) [45,46]. The spectra of the chitosan films with eugenol (CHT-EUG), isoeugenol (CHT-ISOEUG), and carvacrol (CHT-CAR) indicated some differences in the structures of these two-component films compared to the CHT film; however, some of the bands originating from the aroma compounds were overlapped with bands characteristic of the chitosan. These differences were mainly related to the increase in the transmittance intensity of the bands in the IR spectra of the films with fragrance compounds and result from the functional groups and the presence of

an aromatic ring in these compounds. The most important changes were observed in the transmittance intensities of the bands at 1511 cm^{-1} and 1636 cm^{-1} , attributed to the C=C stretching aromatic ring, which were particularly visible in the cases of the films with eugenol and isoeugenol. Moreover, in the FTIR spectra of the chitosan–fragrance compounds, changes (in most cases, increases) in transmittance intensities were observed for the bands at 1451 cm^{-1} and 1376 cm^{-1} ($-\text{CH}_3$ bending), 1267 cm^{-1} (the stretching vibration of the C–O bond of the hydroxyl-bound carbon), 1234 cm^{-1} (C–C stretching vibrations), and 816 cm^{-1} (CH_2 bending vibrations) [40,46,47]. The changes in the FTIR spectra of the chitosan–fragrance compound films indicated that all the tested essential oil components were effectively incorporated into the polymer matrix.

4. Discussion

4.1. Biological Activities of Fragrance Compounds

Microbial growth and lipid oxidation are the main factors of food spoilage, causing the loss of the nutritional and sensory values of food and, as a result, contributing to its waste [48,49]. Therefore, the first stage of this research was to determine the antibacterial and antioxidant effects of the carvacrol, eugenol, and isoeugenol.

4.1.1. Antimicrobial Activities

Carvacrol was characterized by high activities against all the tested bacterial strains in this study, with inhibition zones of $>28\text{ mm}$ (*S. aureus*—inhibition zone = 26 mm) (Table 1). The results of Bryan et al. [50] confirmed the antibacterial efficiencies of carvacrol against *S. aureus*, *E. coli*, *Klebsiella pneumonia*, and *Streptococcus pneumonia* with inhibition zones, at an $800\text{ }\mu\text{g/mL}$ concentration, in the range $23\text{--}26\text{ mm}$. The results by these authors indicated that carvacrol, at $800\text{ }\mu\text{g/mL}$, had no activity against *P. aeruginosa*, while in the present study, it showed activity against this pathogen. Moreover, carvacrol exhibited activities against *S. aureus*, *S. epidermidis*, *B. cereus*, *F. faecalis*, *S. Typhimurium*, and *Pseudomonas fluorescens* [29,51,52]. The antibacterial actions of carvacrol can be involved with various mechanisms, including damaging the bacterial cell membrane [53,54], depleting intracellular ATP by a reduction in its synthesis or by an increase in its hydrolysis [55,56], inducing reactive oxygen species [57], and inhibiting efflux pumps [58]. The antibacterial effects of carvacrol can be attributed to its influence on the structural and functional properties of the cell membrane. According to the mechanism presented by Xu et al. [53], carvacrol, because of its hydrophobic nature, interacts with the lipid bilayer of the cell membrane and inserts between fatty acid chains, resulting in the destabilization of the membrane structure and increasing its fluidity and permeability. Khan et al. [28] demonstrated the antimicrobial activity of carvacrol against *E. coli* through its severe deleterious effect on the bacterial membrane disruption, including its depolarization, leading to bacterial cell disintegration, and its ability to disrupt the integrity of the bacterial cell membrane, causing the release of the cellular contents.

In this study, eugenol and isoeugenol exhibited high antibacterial activities; however, they were lower than those of carvacrol (Table 1). Isoeugenol demonstrated the highest activity against *S. Typhimurium*, with an inhibition zone exceeding 28 mm . The inhibiting action of isoeugenol against *S. Typhimurium*, with an MIC equal to $312.5\text{ }\mu\text{g/mL}$, was confirmed by Zhang et al. [26]. Eugenol displayed lower antibacterial activities against both Gram-positive and Gram-negative bacteria in comparison to the activities of its isomer—iseoeugenol, as presented in Table 1. According to the data presented in a paper by Zhang et al. [26], eugenol exhibited lower activities against Gram-positive bacteria, including *S. aureus*, *B. subtilis*, and *L. monocytogenes* (MIC = $625\text{ }\mu\text{g/mL}$) than isoeugenol (MIC = $312.5\text{ }\mu\text{g/mL}$). In case of the activities against Gram-negative bacteria, they showed the same effects (MIC = $312.5\text{ }\mu\text{g/mL}$) against *E. coli* and *Shigella dysenteriae*, while isoeugenol exhibited a higher activity (MIC = $312.5\text{ }\mu\text{g/mL}$) against *S. Typhimurium* than eugenol (MIC = $625\text{ }\mu\text{g/mL}$) [26]. In turn, Resende et al. [59] noticed that eugenol exhibited lower minimal bactericidal concentrations (MBC = 0.75%) against *E. coli*, *S. aureus*, *L. monocytogenes*, and *S. Enteritidis* than isoeugenol (MBC = 1.5%). A study by da Silva et al. [60]

indicated that eugenol exhibited higher activities against *P. aeruginosa*, *K. pneumoniae*, and *B. cereus* than isoeugenol, while isoeugenol showed a higher activity against *S. aureus*. The results obtained in a study by Muniz et al. [61] demonstrated that isoeugenol has a direct antibacterial effect against the *S. aureus* strain, while eugenol inhibits the growth of bacteria. Based on the results obtained in this study (Table 1) and the data presented in the literature, it can be concluded that the antibacterial effects of eugenol and isoeugenol are related to the species of pathogens used in testing their activities. However, both isomers were characterized by antibacterial activities against various species of bacteria, which confirmed the data contained in Table 1 and the literature data [25,26]. The mechanism of the antibacterial activities of both eugenol and isoeugenol is based on their ability to change the bacterial cell membrane [62]. Eugenol enhanced the cell membrane permeability, causing the leakage of the intracellular ATP and macromolecules, ultimately leading to the death of bacterial cells [63,64]. Hyldgaard et al. [27] presented the mechanism of the antibacterial action of isoeugenol, which involves its interaction with the membrane in a reversible and non-invasive way, causing its destabilization. Moreover, isoeugenol caused an increase in the membrane fluidization, and this effect was enhanced by the presence of a phenolic hydroxyl group in the isoeugenol molecule [27,65].

The research results presented in this work, as well as the literature data, indicate that the components of the essential oils possess antibacterial activities, but they vary depending on the tested chemical compound, its concentration, and the bacterial species or even strains.

4.1.2. Antiradical and Hemolytic Activities

Eugenol and isoeugenol exhibited the highest antiradical effects, comparable to the activity determined for the standard (Trolox). The high and comparable radical scavenging activities of the eugenol and isoeugenol were confirmed by Ito et al. [66]. In turn, the results presented by Zhang et al. [26] showed that isoeugenol exhibited a higher ability to scavenge DPPH· free radicals ($EC_{50} = 17.1 \mu\text{g/mL}$) than eugenol ($EC_{50} = 22.6 \mu\text{g/mL}$), but both isomers had lower activities than that of Trolox ($EC_{50} = 13.5 \mu\text{g/mL}$). The results by Horvathova et al. [67] indicated that eugenol and carvacrol were capable for DPPH· free radical scavenging, but eugenol showed a higher activity. Also, the higher antiradical activity of eugenol ($EC_{50} = 9 \mu\text{g/mL}$) in comparison to carvacrol ($EC_{50} = 267 \mu\text{g/mL}$) was confirmed by Mastelić et al. [68]. The moderate free radical scavenging activity of carvacrol was confirmed by the results presented in Figure 2 and by the research of Yildiz et al. [69], who demonstrated that the DPPH-antiradical activity of carvacrol, at a 1000 ppm concentration, was 18.3% compared to the activity of α -tocopherol (94.9%).

The antioxidant properties of the essential oil components depend on various factors, including the chemical structures of the compounds (phenols have higher antioxidant activities compared to sesquiterpene hydrocarbons and non-isoprenoid components, which have weak antiradical activities), concentration (their antioxidant activities are expressed only in a rather narrow range of concentrations), the type of antioxidant activity tests used, and the solvents applied to dissolve the compounds [70–74].

Bioactive compounds, for any biomedical applications, should be biocompatible, without any cytotoxic activity or negative effects on the cell membrane structure and function. In food packaging, the cytotoxicities of compounds can be defined as their cytotoxicities toward skin cells, as involved in direct contact with packaging materials. To study the cytotoxicities of bioactive compounds, human RBCs are used as a cell model and a cell membrane model in vitro [75,76]. Hemolytic assays using human RBCs are also intended to assess the cytotoxicities of compounds used in food packaging [77]. Hemolytic activities of compounds or their mixtures exceeding 5% is a sign of their cytotoxicities at given concentrations and excludes them from further evaluation for biomedical applications. None of the tested compounds used in this study (eugenol, isoeugenol, and carvacrol) showed any negative effects on the RBC membrane structure or function, with hemolytic activities lower than 5% (Figure 3). Therefore, it can be concluded that these essential oil

components are biocompatible and can be applied as compounds, without any associated cytotoxicity, in the food industry.

The first stage of this research confirmed that the tested fragrance compounds have antibacterial and antiradical properties, and at a concentration of 0.1 mg/mL, they do not have a detrimental effect on the structure of the cell membrane. The obtained results suggest that the tested compounds are without any associated cytotoxicity toward human cells and may act as antimicrobial and antioxidant agents in chitosan-based films. Therefore, carvacrol, eugenol, and isoeugenol were applied as bioactive components in chitosan-based films, and their biological and physicochemical parameters were examined to determine the possibility of their use in food packaging.

4.2. Characterization of Chitosan-Based Films with Fragrance Compounds

The addition of the essential oil components to the chitosan resulted in the antibacterial effects of the chitosan-based films. The film produced only from chitosan had no effect against the bacterial strains, which is confirmed by the literature data [37,78]. The CHT-CAR film showed the strongest antibacterial effect, which is consistent with the results of the antimicrobial activities of the essential oil components—carvacrol was characterized by a greater ability to limit bacterial growth than eugenol and isoeugenol. However, it can be noticed that the inhibition zone caused by the CHT-CAR film was larger than in the case of the zone caused by the action of the carvacrol solution, which may indicate a synergistic effect between chitosan and carvacrol or a reduction in the volatility of the compound that was cross-linked in the chitosan matrix. The effects of the films consisting of chitosan and carvacrol against *E. coli*, *S. aureus*, and *S. Typhimurium* have already been confirmed in the literature [39,79]. The chitosan–carvacrol film was characterized by good mechanical parameters as a potential food-packaging material. It showed the highest values of the tensile strength and elongation at break compared to the other samples. The introduction of carvacrol to the chitosan matrix can decrease the interaction between its chains by reducing the intermolecular forces and internal hydrogen bonds between the chains, resulting in greater mobility of the polymer chains and, therefore, more flexible films. The results by Yuan et al. [79] showed that the introduction of carvacrol to the chitosan matrix resulted in a reduction in the TS (from 22.23 to 8.54 MPa) and EB (from 31.51 to 17.37%) compared to the pure chitosan film. Also, Lopez-Mata et al. [39] showed in their research that the addition of carvacrol at concentrations of 0.5–1.5% to chitosan caused decreases in the mechanical parameters (TS and EB). The addition of carvacrol to chitosan resulted in increased WVTR values compared to the pure chitosan film. The opposite relationship has been described in the literature [39,79]. Differences in the results of the mechanical and barrier parameters of films based on chitosan and carvacrol obtained by other authors from those presented in this paper may result from the use of different types of chitosan (different degrees of deacetylation), different chitosan and carvacrol concentrations, and the addition of glycerin.

The antibacterial effects of chitosan films with eugenol and isoeugenol, compared to the effects of the fragrance compound solutions, depended on the tested pathogen strain. In the case of the eugenol, the chitosan–eugenol film (CHT-EUG) showed higher activities than the eugenol solution against Gram-positive bacteria—*B. megaterium*, *L. innocua*, and *S. aureus*. On the other hand, the CHT-EUG film showed significantly lower activities compared to the eugenol solution against strains of Gram-negative bacteria—*E. coli* and *S. Enteritidis*—and no activity against the Gram-positive bacterial strain *E. faecium*. In turn, the chitosan film with the addition of isoeugenol (CHT-ISOEUG) was characterized by higher activities than the isoeugenol solution against *B. cereus* and *E. Enteritidis*, while this film showed no activities against *E. faecalis*, *E. faecium*, and *S. Paratyphi*, and isoeugenol limits the development of these pathogens. The antibacterial effects of eugenol and its isomer have been used to obtain food packaging materials with various matrices, not only chitosan ones [80,81]. The addition of isoeugenol to chitosan resulted in decreases in the mechanical and barrier parameters of the obtained film compared to the pure chitosan film. Differences in the mechanical, barrier, and structural parameters of the obtained films

may result from differences in the chemical structures of the essential oil components and different interactions with the chitosan matrix. The incorporation of compounds with a hydrophobic character in chitosan may have a negative impact on the attractive forces between chitosan molecules and may increase segmental movements between them and, thus, affect the mechanical and physical parameters of the obtained films [82].

5. Conclusions

The tested components of the essential oils, namely, eugenol, isoeugenol, and carvacrol, were characterized by antibacterial, antioxidant, and hemolytic activities, which, however, depended on the chemical structures of the tested compounds. The antibacterial and antioxidant properties of these compounds indicate their potential use in food packaging as bioactive and biocompatible (non-cytotoxic) components to reduce food spoilage. Carvacrol was characterized by a significantly greater ability to inhibit bacterial growth than eugenol and its isomer—iseugenol. The strong antimicrobial effect of the carvacrol was preserved when it was incorporated into a chitosan matrix—the chitosan–carvacrol film showed strong antimicrobial effects against all the tested pathogens. Moreover, the film composed of chitosan and carvacrol was characterized by good mechanical parameters and moderate barrier properties. The stronger antibacterial effect of the chitosan–carvacrol film than the film with eugenol and its isomer may be caused by more effective cross-linking in the chitosan matrix, which resulted in a reduction in its volatility. Also, differences in the mechanical and barrier properties of the tested films may result from variations in their interactions with the chitosan matrix. Based on the obtained test results, it can be assumed that the biological activities, as well as the mechanical and barrier parameters, of the films with the additions of the fragrance compounds depend on the properties of these compounds (even within isomeric compounds) and their interactions with the chitosan matrix. However, the obtained results indicated that the films based on chitosan and essential oil components (eugenol, isoeugenol, and carvacrol) were characterized by appropriate mechanical, barrier, and biological parameters to be considered as potential natural and ecological materials for food packaging. In further research, we plan to use the developed chitosan-based films with the additions of fragrance compounds as packaging materials for fresh market products—fresh fruits and vegetables—and to assess the parameters of packaged food products.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/coatings14070830/s1>: Table S1: The antiradical and hemolytic activity of tested compounds with statistical analysis; Table S2: The tensile strength (TS) and elongation at break (%) for chitosan-based films; Table S3: The water vapor transmission rate of chitosan-based films.

Author Contributions: Conceptualization, M.W. and I.R.; methodology, M.W., L.M., A.S. and R.D.; formal analysis, M.W., J.M., K.S., L.M., A.S. and R.D.; writing—original draft preparation, M.W.; writing—review and editing, J.M., K.S., L.M., A.S., R.D. and I.R.; visualization, M.W.; supervision, I.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

- Andrade, M.S.; Ishikawa, O.H.; Costa, R.S.; Seixas, M.V.S.; Rodrigues, R.C.L.B.; Moura, E.A.B. Development of Sustainable Food Packaging Material Based on Biodegradable Polymer Reinforced with Cellulose Nanocrystals. *Food Packag. Shelf Life* **2022**, *31*, 100807. [\[CrossRef\]](#)
- Mendes, A.C.; Pedersen, G.A. Perspectives on Sustainable Food Packaging: Is Bio-Based Plastics a Solution? *Trends Food Sci. Technol.* **2021**, *112*, 839–846. [\[CrossRef\]](#)
- Teixeira-Costa, B.E.; Andrade, C.T. Natural Polymers Used in Edible Food Packaging—History, Function and Application Trends as a Sustainable Alternative to Synthetic Plastic. *Polysaccharides* **2021**, *3*, 32–58. [\[CrossRef\]](#)
- Mousavi Khaneghah, A.; Hashemi, S.M.B.; Limbo, S. Antimicrobial Agents and Packaging Systems in Antimicrobial Active Food Packaging: An Overview of Approaches and Interactions. *Food Bioprod. Process.* **2018**, *111*, 1–19. [\[CrossRef\]](#)
- Fadji, T.; Rashvand, M.; Daramola, M.O.; Iwarere, S.A. A Review on Antimicrobial Packaging for Extending the Shelf Life of Food. *Processes* **2023**, *11*, 590. [\[CrossRef\]](#)
- Bolouri, P.; Salami, R.; Kouhi, S.; Kordi, M.; Asgari Lajayer, B.; Hadian, J.; Astatkie, T. Applications of Essential Oils and Plant Extracts in Different Industries. *Molecules* **2022**, *27*, 8999. [\[CrossRef\]](#) [\[PubMed\]](#)
- Verdeguer, M.; Sánchez-Moreiras, A.M.; Araniti, F. Phytotoxic Effects and Mechanism of Action of Essential Oils and Terpenoids. *Plants* **2020**, *9*, 1571. [\[CrossRef\]](#) [\[PubMed\]](#)
- Sharma, S.; Barkauskaite, S.; Jaiswal, A.K.; Jaiswal, S. Essential Oils as Additives in Active Food Packaging. *Food Chem.* **2021**, *343*, 128403. [\[CrossRef\]](#) [\[PubMed\]](#)
- Liu, T.; Liu, L. Fabrication and Characterization of Chitosan Nanoemulsions Loading Thymol or Thyme Essential Oil for the Preservation of Refrigerated Pork. *Int. J. Biol. Macromol.* **2020**, *162*, 1509–1515. [\[CrossRef\]](#) [\[PubMed\]](#)
- De Melo Pereira, G.V.; Medeiros, A.B.P.; Camara, M.C.; Magalhães Júnior, A.I.; De Carvalho Neto, D.P.; Bier, M.C.J.; Soccol, C.R. Production and Recovery of Bioaromas Synthesized by Microorganisms. In *The Role of Alternative and Innovative Food Ingredients and Products in Consumer Wellness*; Academic Press: Cambridge, MA, USA, 2019; pp. 315–338. [\[CrossRef\]](#)
- 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. [\[CrossRef\]](#)
- Masyita, A.; Mustika Sari, R.; Dwi Astuti, A.; Yasir, B.; Rahma Rumata, N.; Emran, T.B.; Nainu, F.; Simal-Gandara, J. Terpenes and Terpenoids as Main Bioactive Compounds of Essential Oils, Their Roles in Human Health and Potential Application as Natural Food Preservatives. *Food Chem. X* **2022**, *13*, 100217. [\[CrossRef\]](#) [\[PubMed\]](#)
- Walasek-Janusz, M.; Grzegorzczak, A.; Malm, A.; Nurzyńska-Wierdak, R.; Zalewski, D. Chemical Composition, and Antioxidant and Antimicrobial Activity of Oregano Essential Oil. *Molecules* **2024**, *29*, 435. [\[CrossRef\]](#) [\[PubMed\]](#)
- Chouhan, S.; Sharma, K.; Guleria, S. Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives. *Medicines* **2017**, *4*, 58. [\[CrossRef\]](#) [\[PubMed\]](#)
- Gavaric, N.; Mozina, S.S.; Kladar, N.; Bozin, B. Chemical Profile, Antioxidant and Antibacterial Activity of Thyme and Oregano Essential Oils, Thymol and Carvacrol and Their Possible Synergism. *J. Essent. Oil Bear. Plants* **2015**, *18*, 1013–1021. [\[CrossRef\]](#)
- Costa, C.A.R.D.A.; Kohn, D.O.; De Lima, V.M.; Gargano, A.C.; Flório, J.C.; Costa, M. The GABAergic System Contributes to the Anxiolytic-like Effect of Essential Oil from *Cymbopogon Citratus* (Lemongrass). *J. Ethnopharmacol.* **2011**, *137*, 828–836. [\[CrossRef\]](#) [\[PubMed\]](#)
- Guimarães, A.C.; Meireles, L.M.; Lemos, M.F.; Guimarães, M.C.C.; Endringer, D.C.; Fronza, M.; Scherer, R. Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils. *Molecules* **2019**, *24*, 2471. [\[CrossRef\]](#)
- Mutlu-Ingok, A.; Devcioglu, D.; Dikmetas, D.N.; Karbancioglu-Guler, F.; Capanoglu, E. Antibacterial, Antifungal, Antimycotoxic, and Antioxidant Activities of Essential Oils: An Updated Review. *Molecules* **2020**, *25*, 4711. [\[CrossRef\]](#) [\[PubMed\]](#)
- Pandey, A.K.; Mohan, M.; Singh, P.; Palni, U.T.; Tripathi, N.N. Chemical Composition, Antibacterial and Antioxidant Activity of Essential Oil of *Eupatorium Adenophorum* Spreng. from Eastern Uttar Pradesh, India. *Food Biosci.* **2014**, *7*, 80–87. [\[CrossRef\]](#)
- Koyama, S.; Heinbockel, T. The Effects of Essential Oils and Terpenes in Relation to Their Routes of Intake and Application. *Int. J. Mol. Sci.* **2020**, *21*, 1558. [\[CrossRef\]](#) [\[PubMed\]](#)
- Dhifi, W.; Bellili, S.; Jazi, S.; Bahloul, N.; Mnif, W.; Nahar, L.; Basar, N.; Sarker, S.D. Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. *Medicines* **2016**, *3*, 25. [\[CrossRef\]](#) [\[PubMed\]](#)
- Nisar, M.F.; Khadim, M.; Rafiq, M.; Chen, J.; Yang, Y.; Wan, C.C. Pharmacological Properties and Health Benefits of Eugenol: A Comprehensive Review. *Oxid. Med. Cell. Longev.* **2021**, *2021*, 2497354. [\[CrossRef\]](#) [\[PubMed\]](#)
- Marchese, A.; Barbieri, R.; Coppo, E.; Orhan, I.E.; Daglia, M.; Nabavi, S.F.; Izadi, M.; Abdollahi, M.; Nabavi, S.M.; Ajami, M. Antimicrobial Activity of Eugenol and Essential Oils Containing Eugenol: A Mechanistic Viewpoint. *Crit. Rev. Microbiol.* **2017**, *43*, 668–689. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zari, A.T.; Zari, T.A.; Rehman Hakeem, K.; Ferreira de Oliveira, P.; Ribeiro, D.; Ascenso, A.; Santos, C. Anticancer Properties of Eugenol: A Review. *Molecules* **2021**, *26*, 7407. [\[CrossRef\]](#) [\[PubMed\]](#)
- Ulanowska, M.; Olas, B. Biological Properties and Prospects for the Application of Eugenol—A Review. *Int. J. Mol. Sci.* **2021**, *22*, 3671. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zhang, L.L.; Zhang, L.F.; Xu, J.G.; Hu, Q.P. Comparison Study on Antioxidant, DNA Damage Protective and Antibacterial Activities of Eugenol and Isoeugenol against Several Foodborne Pathogens. *Food Nutr. Res.* **2017**, *61*, 1353356. [\[CrossRef\]](#) [\[PubMed\]](#)

27. Hyldgaard, M.; Mygind, T.; Piotrowska, R.; Foss, M.; Meyer, R.L. Isoeugenol Has a Non-Disruptive Detergent-like Mechanism of Action. *Front. Microbiol.* **2015**, *6*, 154666. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Khan, I.; Bahuguna, A.; Kumar, P.; Bajpai, V.K.; Kang, S.C. Antimicrobial Potential of Carvacrol against Uropathogenic *Escherichia coli* via Membrane Disruption, Depolarization, and Reactive Oxygen Species Generation. *Front. Microbiol.* **2017**, *8*, 282618. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Cacciatore, I.; Di Giulio, M.; Fornasari, E.; Di Stefano, A.; Cerasa, L.S.; Marinelli, L.; Turkez, H.; Di Campi, E.; Di Bartolomeo, S.; Robuffo, I.; et al. Carvacrol Codrugs: A New Approach in the Antimicrobial Plan. *PLoS ONE* **2015**, *10*, e0120937. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Sharifi-Rad, M.; Varoni, E.M.; Iriti, M.; Martorell, M.; Setzer, W.N.; del Mar Contreras, M.; Salehi, B.; Soltani-Nejad, A.; Rajabi, S.; Tajbakhsh, M.; et al. Carvacrol and Human Health: A Comprehensive Review. *Phyther. Res.* **2018**, *32*, 1675–1687. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Alkan Tas, B.; Sehit, E.; Erdinc Tas, C.; Unal, S.; Cebeci, F.C.; Menciloglu, Y.Z.; Unal, H. Carvacrol Loaded Halloysite Coatings for Antimicrobial Food Packaging Applications. *Food Packag. Shelf Life* **2019**, *20*, 100300. [\[CrossRef\]](#)
32. Calva-Estrada, S.J.; Jiménez-Fernández, M.; Lugo-Cervantes, E. Protein-Based Films: Advances in the Development of Biomaterials Applicable to Food Packaging. *Food Eng. Rev.* **2019**, *11*, 78–92. [\[CrossRef\]](#)
33. Huang, X.; Ge, X.; Zhou, L.; Wang, Y. Eugenol Embedded Zein and Poly(Lactic Acid) Film as Active Food Packaging: Formation, Characterization, and Antimicrobial Effects. *Food Chem.* **2022**, *384*, 132482. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Stefanowska, K.; Woźniak, M.; Dobrucka, R.; Ratajczak, I. Chitosan with Natural Additives as a Potential Food Packaging. *Materials* **2023**, *16*, 1579. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Motelica, L.; Fica, D.; Fica, A.; Truşcă, R.D.; Ilie, C.I.; Oprea, O.C.; Andronescu, E. Innovative Antimicrobial Chitosan/ZnO/Ag NPs/Citronella Essential Oil Nanocomposite—Potential Coating for Grapes. *Foods* **2020**, *9*, 1801. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Yuan, G.; Chen, X.; Li, D. Chitosan Films and Coatings Containing Essential Oils: The Antioxidant and Antimicrobial Activity, and Application in Food Systems. *Food Res. Int.* **2016**, *89*, 117–128. [\[CrossRef\]](#)
37. Foster, L.J.; Butt, J. Chitosan Films Are NOT Antimicrobial. *Biotechnol. Lett.* **2011**, *33*, 417–421. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Barik, M.; BhagyaRaj, G.V.S.; Dash, K.K.; Shams, R. A Thorough Evaluation of Chitosan-Based Packaging Film and Coating for Food Product Shelf-Life Extension. *J. Agric. Food Res.* **2024**, *16*, 101164. [\[CrossRef\]](#)
39. López-Mata, M.A.; Ruiz-Cruz, S.; Silva-Beltrán, N.P.; Ornelas-Paz, J.D.J.; Zamudio-Flores, P.B.; Burrueal-Ibarra, S.E. Physicochemical, Antimicrobial and Antioxidant Properties of Chitosan Films Incorporated with Carvacrol. *Molecules* **2013**, *18*, 13735–13753. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Bonilla, J.; Poloni, T.; Lourenço, R.V.; Sobral, P.J.A. Antioxidant Potential of Eugenol and Ginger Essential Oils with Gelatin/Chitosan Films. *Food Biosci.* **2018**, *23*, 107–114. [\[CrossRef\]](#)
41. Basumatary, I.B.; Mukherjee, A.; Kumar, S. Chitosan-Based Composite Films Containing Eugenol Nanoemulsion, ZnO Nanoparticles and Aloe Vera Gel for Active Food Packaging. *Int. J. Biol. Macromol.* **2023**, *242*, 124826. [\[CrossRef\]](#) [\[PubMed\]](#)
42. ASTM D882-12; Standard Test Method for Tensile Properties of Thin Plastic Sheeting. ASTM International: West Conshohocken, PA, USA, 2014.
43. ISO 2528:2017; Sheet Materials—Determination of Water Vapour Transmission Rate (WVTR)—Gravimetric (Dish) Method. International Organization for Standardization: Geneva, Switzerland, 2017.
44. Woźniak, M.; Sip, A.; Mrówczyńska, L.; Broniarczyk, J.; Waśkiewicz, A.; Ratajczak, I. Biological Activity and Chemical Composition of Propolis from Various Regions of Poland. *Molecules* **2023**, *28*, 141. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Costa, S.M.; Ferreira, D.P.; Teixeira, P.; Ballesteros, L.F.; Teixeira, J.A.; Figueiro, R. Active Natural-Based Films for Food Packaging Applications: The Combined Effect of Chitosan and Nanocellulose. *Int. J. Biol. Macromol.* **2021**, *177*, 241–251. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Jung, B.O.; Chung, S.J.; Lee, S.B. Preparation and Characterization of Eugenol-Grafted Chitosan Hydrogels and Their Antioxidant Activities. *J. Appl. Polym. Sci.* **2006**, *99*, 3500–3506. [\[CrossRef\]](#)
47. Tarhan, İ. A Robust Method for Simultaneous Quantification of Eugenol, Eugenyl Acetate, and β -Caryophyllene in Clove Essential Oil by Vibrational Spectroscopy. *Phytochemistry* **2021**, *191*, 112928. [\[CrossRef\]](#)
48. Snyder, A.B.; Martin, N.; Wiedmann, M. Microbial Food Spoilage: Impact, Causative Agents and Control Strategies. *Nat. Rev. Microbiol.* **2024**, *ahead of print*. [\[CrossRef\]](#)
49. Gómez-Estaca, J.; López-de-Dicastillo, C.; Hernández-Muñoz, P.; Catalá, R.; Gavara, R. Advances in Antioxidant Active Food Packaging. *Trends Food Sci. Technol.* **2014**, *35*, 42–51. [\[CrossRef\]](#)
50. Abass Bryn, I.; Tariq Abid, A.; Naji Obied, H. Antibacterial Activity of Carvacrol against Different Types of Bacteria. *J. Nat. Sci. Res.* **2014**, *4*, 13–17.
51. Miladi, H.; Zmantar, T.; Chaabouni, Y.; Fedhila, K.; Bakhrouf, A.; Mahdouani, K.; Chaieb, K. Antibacterial and Efflux Pump Inhibitors of Thymol and Carvacrol against Food-Borne Pathogens. *Microb. Pathog.* **2016**, *99*, 95–100. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Ben Arfa, A.; Combes, S.; Preziosi-Belloy, L.; Gontard, N.; Chaliel, P. Antimicrobial Activity of Carvacrol Related to Its Chemical Structure. *Lett. Appl. Microbiol.* **2006**, *43*, 149–154. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Xu, J.; Zhou, F.; Ji, B.P.; Pei, R.S.; Xu, N. The Antibacterial Mechanism of Carvacrol and Thymol against *Escherichia coli*. *Lett. Appl. Microbiol.* **2008**, *47*, 174–179. [\[CrossRef\]](#) [\[PubMed\]](#)

54. Wang, L.H.; Wang, M.S.; Zeng, X.A.; Zhang, Z.H.; Gong, D.M.; Huang, Y.B. Membrane Destruction and DNA Binding of *Staphylococcus Aureus* Cells Induced by Carvacrol and Its Combined Effect with a Pulsed Electric Field. *J. Agric. Food Chem.* **2016**, *64*, 6355–6363. [[CrossRef](#)] [[PubMed](#)]
55. Nostro, A.; Papalia, T. Antimicrobial Activity of Carvacrol: Current Progress and Future Prospectives. *Recent Pat. Antiinfect. Drug Discov.* **2012**, *7*, 28–35. [[CrossRef](#)]
56. Shen, S.; Zhang, T.; Yuan, Y.; Lin, S.; Xu, J.; Ye, H. Effects of Cinnamaldehyde on *Escherichia coli* and *Staphylococcus aureus* Membrane. *Food Control* **2015**, *47*, 196–202. [[CrossRef](#)]
57. Khan, S.T.; Khan, M.; Ahmad, J.; Wahab, R.; Abd-Elkader, O.H.; Musarrat, J.; Alkhathlan, H.Z.; Al-Kedhairi, A.A. Thymol and Carvacrol Induce Autolysis, Stress, Growth Inhibition and Reduce the Biofilm Formation by *Streptococcus Mutans*. *AMB Express* **2017**, *7*, 49. [[CrossRef](#)]
58. Chueca, B.; Pérez-Sáez, E.; Pagán, R.; García-Gonzalo, D. Global Transcriptional Response of *Escherichia coli* MG1655 Cells Exposed to the Oxygenated Monoterpenes Citral and Carvacrol. *Int. J. Food Microbiol.* **2017**, *257*, 49–57. [[CrossRef](#)] [[PubMed](#)]
59. Resende, D.B.; de Abreu Martins, H.H.; de Souza, T.B.; Carvalho, D.T.; Piccoli, R.H.; Schwan, R.F.; Dias, D.R. Synthesis and in Vitro Evaluation of Peracetyl and Deacetyl Glycosides of Eugenol, Isoeugenol and Dihydroeugenol Acting against Food-Contaminating Bacteria. *Food Chem.* **2017**, *237*, 1025–1029. [[CrossRef](#)]
60. da Silva, F.F.M.; Monte, F.J.Q.; de Lemos, T.L.G.; do Nascimento, P.G.G.; de Medeiros Costa, A.K.; de Paiva, L.M.M. Eugenol Derivatives: Synthesis, Characterization, and Evaluation of Antibacterial and Antioxidant Activities. *Chem. Cent. J.* **2018**, *12*, 34. [[CrossRef](#)]
61. Muniz, D.F.; dos Santos Barbosa, C.R.; de Menezes, I.R.A.; de Sousa, E.O.; Pereira, R.L.S.; Júnior, J.T.C.; Pereira, P.S.; de Matos, Y.M.L.S.; da Costa, R.H.S.; de Moraes Oliveira-Tintino, C.D.; et al. In Vitro and in Silico Inhibitory Effects of Synthetic and Natural Eugenol Derivatives against the NorA Efflux Pump in *Staphylococcus aureus*. *Food Chem.* **2021**, *337*, 127776. [[CrossRef](#)] [[PubMed](#)]
62. Angane, M.; Swift, S.; Huang, K.; Butts, C.A.; Quek, S.Y. Essential Oils and Their Major Components: An Updated Review on Antimicrobial Activities, Mechanism of Action and Their Potential Application in the Food Industry. *Foods* **2022**, *11*, 464. [[CrossRef](#)] [[PubMed](#)]
63. Ashrafudoulla, M.; Mizan, M.F.R.; won Ha, A.J.; Park, S.H.; Ha, S. Do Antibacterial and Antibiofilm Mechanism of Eugenol against Antibiotic Resistance *Vibrio parahaemolyticus*. *Food Microbiol.* **2020**, *91*, 103500. [[CrossRef](#)] [[PubMed](#)]
64. Qian, W.; Sun, Z.; Wang, T.; Yang, M.; Liu, M.; Zhang, J.; Li, Y. Antimicrobial Activity of Eugenol against Carbapenem-Resistant *Klebsiella pneumoniae* and Its Effect on Biofilms. *Microb. Pathog.* **2020**, *139*, 103924. [[CrossRef](#)] [[PubMed](#)]
65. Gharib, R.; Najjar, A.; Auezova, L.; Charcosset, C.; Greige-Gerges, H. Interaction of Selected Phenylpropenes with Dipalmitoylphosphatidylcholine Membrane and Their Relevance to Antibacterial Activity. *J. Membr. Biol.* **2017**, *250*, 259–271. [[CrossRef](#)] [[PubMed](#)]
66. Ito, M.; Murakami, K.; Yoshino, M. Antioxidant Action of Eugenol Compounds: Role of Metal Ion in the Inhibition of Lipid Peroxidation. *Food Chem. Toxicol.* **2005**, *43*, 461–466. [[CrossRef](#)] [[PubMed](#)]
67. Horvathova, E.; Navarova, J.; Galova, E.; Sevcovicova, A.; Chodakova, L.; Snahtkanova, Z.; Melusova, M.; Kozics, K.; Slamenova, D. Assessment of Antioxidative, Chelating, and DNA-Protective Effects of Selected Essential Oil Components (Eugenol, Carvacrol, Thymol, Borneol, Eucalyptol) of Plants and Intact *Rosmarinus Officinalis* Oil. *J. Agric. Food Chem.* **2014**, *62*, 6632–6639. [[CrossRef](#)] [[PubMed](#)]
68. Mastelić, J.; Jerković, I.; Blažević, I.; Poljak-Blaži, M.; Borović, S.; Ivančić-Baće, I.; Smrčeki, V.; Žarković, N.; Brčić-Kostić, K.; Vikić-Topić, D.; et al. Comparative Study on the Antioxidant and Biological Activities of Carvacrol, Thymol, and Eugenol Derivatives. *J. Agric. Food Chem.* **2008**, *56*, 3989–3996. [[CrossRef](#)] [[PubMed](#)]
69. Yildiz, S.; Turan, S.; Kiralan, M.; Ramadan, M.F. Antioxidant Properties of Thymol, Carvacrol, and Thymoquinone and Its Efficiencies on the Stabilization of Refined and Stripped Corn Oils. *J. Food Meas. Charact.* **2021**, *15*, 621–632. [[CrossRef](#)]
70. 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](#)]
71. Torres-Martínez, R.; García-Rodríguez, Y.M.; Ríos-Chávez, P.; Saavedra-Molina, A.; López-Meza, J.E.; Ochoa-Zarzosa, A.; Garciglia, R.S. Antioxidant Activity of the Essential Oil and Its Major Terpenes of *Satureja macrostema* (Moc. and Sessé Ex Benth.). *Briq. Pharmacogn. Mag.* **2018**, *13*, S875–S880.
72. Baschieri, A.; Ajvazi, M.D.; Tonfack, J.L.F.; Valgimigli, L.; Amorati, R. Explaining the Antioxidant Activity of Some Common Non-Phenolic Components of Essential Oils. *Food Chem.* **2017**, *232*, 656–663. [[CrossRef](#)] [[PubMed](#)]
73. Ruberto, G.; Baratta, M.T. Antioxidant Activity of Selected Essential Oil Components in Two Lipid Model Systems. *Food Chem.* **2000**, *69*, 167–174. [[CrossRef](#)]
74. Shutava, H.G.; Shutava, T.G.; Kavalenka, N.A.; Supichenka, H.N. Antiradical and Antibacterial Activity of Essential Oils from the Lamiaceae Family Plants in Connection with Their Composition and Optical Activity of Components. *Int. J. Second. Metab.* **2018**, *5*, 109–122. [[CrossRef](#)]
75. Mrówczyńska, L.; Hägerstrand, H. Platelet-Activating Factor Interaction with the Human Erythrocyte Membrane. *J. Biochem. Mol. Toxicol.* **2009**, *23*, 345–348. [[CrossRef](#)] [[PubMed](#)]
76. Suvarna, V.; Nair, A.; Mallya, R.; Khan, T.; Omri, A. Antimicrobial Nanomaterials for Food Packaging. *Antibiotics* **2022**, *11*, 729. [[CrossRef](#)] [[PubMed](#)]

77. Sæbø, I.P.; Bjørås, M.; Franzyk, H.; Helgesen, E.; Booth, J.A. Optimization of the Hemolysis Assay for the Assessment of Cytotoxicity. *Int. J. Mol. Sci.* **2023**, *24*, 2914. [[CrossRef](#)]
78. Stanicka, K.; Dobrucka, R.; Woźniak, M.; Sip, A.; Majka, J.; Kozak, W.; Ratajczak, I. The Effect of Chitosan Type on Biological and Physicochemical Properties of Films with Propolis Extract. *Polymers* **2021**, *13*, 3888. [[CrossRef](#)] [[PubMed](#)]
79. Yuan, G.; Lv, H.; Yang, B.; Chen, X.; Sun, H. Physical Properties, Antioxidant and Antimicrobial Activity of Chitosan Films Containing Carvacrol and Pomegranate Peel Extract. *Molecules* **2015**, *20*, 11034–11045. [[CrossRef](#)] [[PubMed](#)]
80. Valencia-Sullca, C.; Ben Messaoud, G.; Sánchez-González, L.; Tehrany, E.A.; Vargas, M.; Atarés, L.; Chiralt, A. Chitosan Films Containing Encapsulated Eugenol in Alginate Microspheres. *Food Hydrocoll.* **2024**, *151*, 109791. [[CrossRef](#)]
81. Krogsgård Nielsen, C.; Kjems, J.; Mygind, T.; Snabe, T.; Schwarz, K.; Serfert, Y.; Meyer, R.L. Antimicrobial Effect of Emulsion-Encapsulated Ioeugenol against Biofilms of Food Pathogens and Spoilage Bacteria. *Int. J. Food Microbiol.* **2017**, *242*, 7–12. [[CrossRef](#)]
82. Kurek, M.; Guinault, A.; Voilley, A.; Galić, K.; Debeaufort, F. Effect of Relative Humidity on Carvacrol Release and Permeation Properties of Chitosan Based Films and Coatings. *Food Chem.* **2014**, *144*, 9–17. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.