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Proceeding Paper

Evaluation of Bacteriostatic Effect of Rosemary and Oregano Essential Oils Against a Non-Pathogenic Surrogate of *Salmonella* spp. (*E. coli* ATCC 9637) †

Theodore John Magtalas and Gerieka Ramos Anapi *

Laboratory of Food Microbiology and Hygiene, Department of Food Science and Nutrition, College of Home Economics, University of the Philippines Diliman, Quezon City M339+HC2, Philippines; tcmagtalas@up.edu.ph

* Correspondence: granapi1@up.edu.ph

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Abstract: Control of bacterial growth in food is essential to maintaining food quality and safety. The use of food additives is one answer raised to address this problem. However, some synthetic antimicrobial additives pose minor to serious health risks to consumers. Natural antimicrobial additives are potential alternatives to synthetic additives that can control microbial growth without significant health risks. This study evaluated the bacteriostatic effect of rosemary essential oil (REO) and oregano essential oil (OEO) against *E. coli* ATCC 9637, a non-pathogenic surrogate of *Salmonella* spp. in culture and in raw chicken breast. Final concentrations of 1.5% REO and 0.15% OEO were added to cultures of *E. coli* ATCC 9637, and the growth rate was evaluated. Raw chicken breast pieces were dipped in *E. coli* ATCC 9637 cultures prior to being dipped in 1.5% REO and 0.15% OEO. The chicken samples were then taken at two-day intervals, and the growth of E. coli ATCC 9637 was analyzed. No growth was observed in the cultures after a 24 h incubation period. The chicken samples treated with 1.5% REO resulted in a 0.69 log reduction compared to the positive control, while those treated with 0.15% OEO resulted in a 0.31 log reduction ($p < 0.05$). This shows that REO and OEO are effective against *E. coli* ATCC 9637 and have promise as natural antimicrobial agents.

Keywords: bacteriostatic; food safety; food additive; essential oils; raw chicken

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1. Introduction

Bacterial growth in meat products is a major concern in the food and meat industry because it can lead to spoilage and foodborne illnesses in consumers [1,2]. This affects the taste, texture, aroma, and overall quality of the meat product. Moreover, it poses a safety concern to buyers and consumers [2]. Controlling bacterial growth in meat products is thus an essential process in maintaining food quality and safety.

Salmonella is a common pathogenic bacteria associated with meat products [1]. *Salmonella* is a Gram-negative rod-shaped bacterium that can cause salmonellosis, of which typhoid fever and enterocolitis are the most common outcomes [1]. Human salmonellosis commonly stems from contaminated egg products, poultry, and pork meats. Salmonellosis symptoms include fever, diarrhea, abdominal pain, nausea, and may result in severe dehydration, death, and chronic sequelae such as arthritis [1]. Salmonellosis cases increased by four thousand between 2022 and 2023 in the Philippines [3]. An investigation of nine wet markets in Metro Manila found that 57.64% out of 720 meat samples were contaminated with *Salmonella* [4]. The Centers for Disease Control and Prevention [5] also estimate that salmonellosis is responsible for around 26,500 hospitalizations and 420 deaths in the USA annually. Due to the danger these microorganisms present, other non-pathogenic microorganisms with similar responses to stimuli may be used in research studies. These surrogate microorganisms allow for less risk for researchers and their facilities while still allowing

for valid and useful results [6]. Some surrogates for *Salmonella* include *E. coli* ATCC 9637, NRRL B-3054 and *E. coli* ATCC 11775, ATCC 25253, and ATCC 25922 [7].

Another potential method to control bacterial growth is the use of food additives. Food additives refer to substances that are not normally consumed as food or as an ingredient in food, which are intentionally added to food in order to control its properties and quality and are then expected to become a component of the food or affect its characteristics [8]. These include antimicrobial agents, which may be natural or synthetic, that mitigate the growth of microorganisms. Synthetic additives are artificially made, while natural additives are obtained and extracted from flora, fauna, and microorganisms. However, synthetic additives have been linked to derivatives that cause negative effects on human health. For example, nitrates and nitrites are known to be involved in the formation of carcinogenic nitrosamines but are still used, as they are considered the only food additive that can inhibit the development of the botulinum toxin, which causes botulism [9]. Some synthetic colorants have also been found to have genotoxic and cytotoxic effects [10]. Due to this reason, natural alternatives are sought after [11,12]. These have been shown to have a negative effect on various intrinsically present microorganisms in meat [11,13–15]. Rosemary (*Rosmarinus officinalis*) and oregano (*Origanum vulgare*) essential oils are common natural antimicrobial agents that have been found to inhibit the growth of bacteria in meat products [16–18]. The antimicrobial activity of rosemary extract comes from terpene derivatives and 1,8-cineole, while that of oregano extract comes from carvacrol, thymol, γ-terpinene, and p-cymene [16–22]. Natural antimicrobial agents may help satisfy the increasing demand to replace the synthetic antimicrobial compounds used in food [11].

The addition of natural preservatives can inhibit bacterial growth. This study aims to evaluate the bacteriostatic effect of rosemary essential oil (REO) and oregano essential oil (OEO) in inhibiting the growth of meat-associated bacteria. Specifically, this study aims to observe the effect of selected concentrations of REO and OEO in inhibiting the growth of a Salmonella surrogate (*E. coli* ATCC 9637) in (1) culture media and (2) raw chicken stored at 4 °C. This research is limited in that only surrogates are used to represent the pathogenic microorganisms.

2. Materials and Methods

2.1. Material

Rosemary (*Rosmarinus officinalis*) and oregano (*Origanum vulgare*) essential oils were procured from a local chemical wholesaler (Asteria Apothecary, Pasig City, Philippines). The manufacturer reported that the rosemary essential oil (REO) was obtained through steam distillation using rosemary leaves from Spain, while the oregano essential oil (OEO) was obtained through steam distillation using oregano leaves from India. The materials were immediately transported to the University of the Philippines-Diliman, College of Home Economics, Laboratory of Food Microbiology and Hygiene (LFMH, UPCHE) to preserve the integrity of the material.

The *E. coli* ATCC 9637 cultures used as surrogates for *Salmonella* spp. were purchased from the Philippine National Collection of Microorganisms (PNCM)-BIOTECH, University of the Philippines Los Baños (BIOTECH-UPLB). The cultures were stored in refrigerated conditions for no more than two weeks and served as the stock cultures until use to maintain the quality of the cultures. The raw chicken breast used for the microbial inoculation studies was procured from Commonwealth Market, Quezon City, and stored in an ice box with a maintained temperature of <7 °C until use.

2.2. Maintenance and Preparation of Microbial Cultures

The maintenance and preparation of the microbial cultures was done following the two-stage protocol of Anapi et al. [23]. Prior to testing the bacteriostatic effect of the essential oils, the meat-associated bacteria underwent a series of activation and enrichment preparation. The refrigerated stock cultures of *E. coli* ATCC 9637 were activated by inoculating a loopful of cells to 10 mL of Nutrient Broth (NB) (HiMedia, Thane, India). The NB

tubes were incubated at 35 °C for 18–24 h. The activated bacterial cultures were enriched by transferring a loopful of cells into NB and then incubated for another 18–24 h at 35 °C.

The working cultures of the test bacteria were prepared by streaking the cells onto pre-solidified nutrient agar (NA) slants (HiMedia Laboratories Pvt. Ltd., Mumbai, India). The NA slants were incubated first at 35 °C for 18–24 h in order for the bacteria to grow, prior to storage at 4 °C until the working culture was further used in the study. Fresh working cultures were prepared every 14 days following the previously described two-stage activation-enrichment protocols. The enriched cultures were serially diluted up to 10^{-7} and spread-plated to obtain a viable bacterial count. Enumeration was conducted in two independent runs with two internal replicates. The bacterial load was adjusted to a final concentration of 1×10^4 CFU/mL prior to use in downstream experiments.

2.3. Preparation of Essential Oil Solutions

REO and OEO solutions were prepared using sterile 0.1% peptone water (PW) (HiMedia, India) containing 0.15% (*w*/*v*) agar as an emulsifying agent [24,25]. For the REO and OEO treatments in culture, 150 µL of REO and 15 µL of OEO were diluted in sterile 0.1% PW containing 0.15% agar to reach a total volume of 5 mL treatment each. For the REO and OEO dipping solutions for raw chicken meat, 1.5 mL of REO and 150 µL of OEO were diluted in sterile 0.1% PW containing 0.15% agar to reach a total volume of 100 mL treatment each.

2.4. Antimicrobial Properties of Rosemary and Oregano Essential Oils in Culture

The procedure of Soyer et al. [26] was adapted in testing the antimicrobial effects of REO and OEO in culture. A volume of 5 mL of REO and OEO solutions, as described in the previous section, were then combined with 5 mL of bacterial culture with a pre-determined count of 2×10^4 CFU/mL for a final bacterial load of 1×10^4 CFU/mL. The bacterial culture without REO or OEO was used as control. The final concentrations for all treatments are listed in Table A1.

The bacterial growth at 35 °C was monitored through spread plate enumeration using pre-solidified nutrient agar plates at 2 h intervals for 6 h and a final enumeration at the 24th hour. At each pre-identified time interval, the inoculated culture media were subjected to serial dilution up to 10^{-5} with 9 mL of 0.1% PW and were spread-plated on nutrient agar plates, incubated at 35 °C for 18–24 h. Microbiological analyses were conducted in two independent runs with two internal replicates and reported as log CFU/mL.

2.5. Antimicrobial Properties of Rosemary and Oregano Essential Oils in Raw Chicken Meat

The procedure of Soyer et al. [26] was adapted in testing the antimicrobial effects of REO and OEO in meat. Raw chicken breasts were cut up into 20 g pieces of similar dimensions and subjected to UV irradiation using five 15-Watt UV-C light sources (Toshiba Lighting and Technology Corp., Kanuma, Japan) for a total of 30 min to reduce inherent surface microflora. The chicken pieces were then dipped into microbial culture with a pre-determined count of 1×10^4 CFU/mL for 10 min. A 30 min waiting period was observed to allow the attachment of bacteria before dipping into 1.5% REO or 0.15% OEO for 10 min. Uninoculated and inoculated chicken samples dipped into sterile 0.1% PW containing 0.15% agar were used as control groups. A 2 min waiting period was observed as the chicken samples were laid onto a sterile stainless steel rack to allow the excess treatment solutions to drip off. All samples were placed in sterile plastic petri dishes (90 mm diameter × 15 mm deep) and stored in sterile zipped plastic bags. Samples were stored for 8 days under refrigerated conditions. This process was performed in duplicate.

Twenty grams (20 g) of chicken breast samples were taken on days 0, 2, 4, 6, and 8. These were homogenized in 180 mL of 0.1% PW using a blender (Hanabishi HJB-115, Beijing, China) and serially diluted up to 10^{-7} prior to spread plating onto MacConkey agar (TM Media, Delhi, India) at appropriate dilutions. The plates were then incubated at

35 °C for 24 h. The microbiological analyses were conducted in two independent runs with two internal replicates and reported as log CFU/g.

2.6. Statistical Analysis

This study followed a completely randomized design (CRD) experimental design. Each sample had an equal chance of receiving a treatment. The means of the cell counts (CFU/g) were obtained from the duplicates of each plating. The statistical analysis was performed using IBM SPSS Statistics 22. The obtained data were subjected to a one-way analysis of variance (ANOVA) and a post hoc Duncan's Multiple Range Test (DMRT) at a 95% confidence level to identify any significant differences.

3. Results and Discussion

3.1. Antimicrobial Properties of Rosemary and Oregano Essential Oils in Cultures

Both individual treatments of rosemary essential oil (REO) and oregano essential oil (OEO) resulted in a growth of <3 log CFU/mL starting at hour 2, with minute survival at hour 6 from the treatment with 1.5% REO. The growth of the microorganisms can be seen in Figure 1.

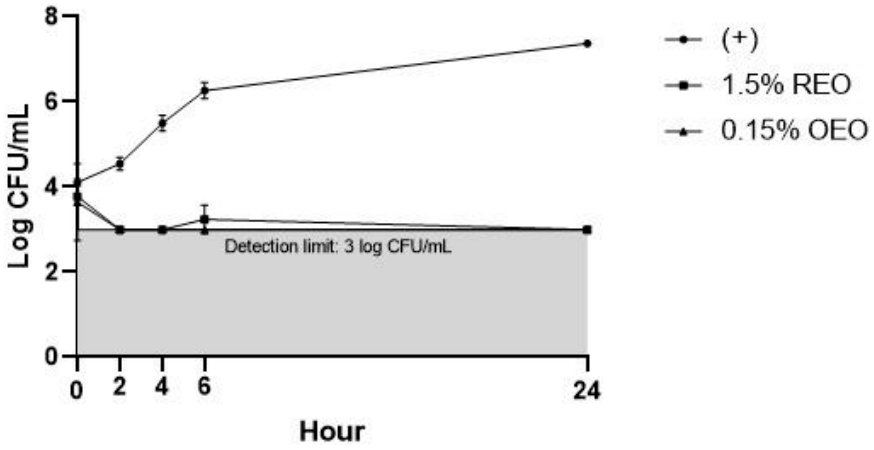

Figure 1. Growth of *E. coli* ATCC 9637 (log CFU/mL) in vitro across treatments.

In the determination of the antimicrobial susceptibility of microorganisms, minimum inhibitory concentration (MIC) is the most commonly used reference value. The MIC is defined as the minimum concentration of an antimicrobial that is able to inhibit the visible growth of a microorganism after an overnight incubation. The minimum bactericidal concentration (MBC) is less commonly used and is defined as the minimum concentration of an antimicrobial that is able to prevent growth after subculturing to a medium without the antimicrobial agent [27].

Rosemary essential oil (REO) has been found to have varying MIC values against different serotypes of *Salmonella*, ranging from 0.5% to 2% *v*/*v* [25,28,29]. Oregano essential oil (OEO) has been observed to have a broad range of MIC values, from 0.0298% to 0.313% *v*/*v* [30–34]. Most of these literature values report steam distillation as the method of extraction and leaves as the source. The method of extraction, plant source, seasonality, and cultivar all affect the chemical composition of the extracted essential oils and therefore affect the MIC and MBC of said essential oils [35]. Similarly, oregano oil composition can be affected by these factors. Figiel et al. [36] found that the total concentration of volatiles in samples differed based on the drying method, ranging from 10.2 to 27.9 g kg^{-1}. De Falco et al. [37] also found that different growth conditions affected the proportions of monoterpene hydrocarbons found in the extracted essential oils.

Due to this literature, this study opted to use rosemary and oregano essential oils, as they have already shown promise as natural antimicrobial agents. Similarly, the concentrations of the essential oils used in this study were based on those literature values, as the

study was unable to perform the MIC determination due to a lack of equipment. Based on the results of this study, 1.5% REO and 0.15% OEO made using this research's sample can be considered well above the MBC, as there was no growth on treatment-free agar plates after incubation for 6 h. Reductions in concentration for future replications of the study may be done in order to obtain an accurate assessment of the MIC and MBC of the REO and OEO samples.

3.2. Antimicrobial Properties of Rosemary and Oregano Essential Oils in Raw Chicken

Slowed growth was observed from microorganisms obtained from treated chicken breast samples. After the eight-day growth period, samples treated with 1.5% REO resulted in a 0.69 log reduction compared to the positive control, while samples treated with 0.15% OEO resulted in a 0.31 log reduction ($p < 0.05$) (Table 1). The growth of the microorganisms throughout the 8-day period can be seen in Figure 2.

Table 1. Microbial counts of *E. coli* ATCC 9637 (log CFU/g) across treatments.

Day	Log CFU/g			
	(−)	**(+)**	**1.5% REO**	**0.15% OEO**
0	4.11 [a]	4.15 [a]	4.08 [a]	4.20 [a]
2	4.66 [ab]	4.81 [b]	4.44 [b]	4.70 [ab]
4	4.98 [a]	5.06 [a]	4.83 [a]	4.74 [a]
6	5.87 [a]	5.99 [a]	5.41 [b]	5.62 [ab]
8	5.89 [b]	6.20 [a]	5.51 [c]	5.89 [b]

[a–c] mean values on the same row followed by the same superscript are not significantly different ($p > 0.05$). Mean values calculated from two independent runs, with two internal replicates [d] based on microbial count at the end of the growth period.

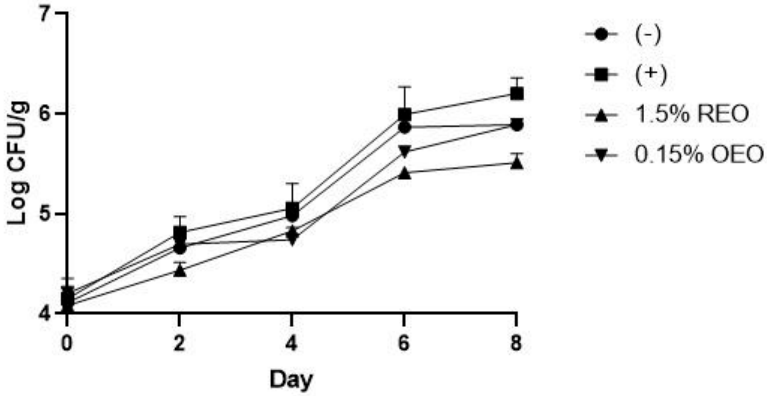

Figure 2. Growth of *E. coli* ATCC 9637 (log CFU/g) on raw chicken breast across treatments. Error bars represent average values and standard deviations calculated from the four data points.

These results show that REO and OEO are effective in reducing the growth of *E. coli* ATCC 9637 in chicken. The discrepancy between the survival and growth of the inoculated bacteria between those in culture and in meat may be due to several factors. Refrigeration of the raw chicken may explain the slowed growth over the course of 8 days compared to the growth rate of the positive control in culture, as refrigerated conditions are far removed from the optimal growth temperature of both *Salmonella* and *E. coli* [38]. Raw chicken breast is a solid mass of porous connective tissue, and it is possible that microorganisms including the inoculant were able to penetrate and grow deeper into the raw chicken than the treatment could reach [39]. A study by Soyer et al. [26] observed that the MIC, three-fold of the MIC, and five-fold of the MIC were found as required concentrations for the reduction of bacterial numbers on dipped meat samples. This was corroborated by Moreira et al. [40]; they found that the reduction of pathogens in food samples required two-, three-, and four-fold of the MIC values of the essential oil they used.

Similar results have been found using REO, OEO, and its constituents against bacterial growth in meat and other food products. Soyer et al. [26] showed that dipping beef in rosemary extract inhibited the growth of *L. monocytogenes*, *Salmonella* Enteritidis, and *E. coli* O157:H7. The major constituents of oregano essential oil, carvacrol and thymol, and combinations of the two were observed to have detrimental effects on *Salmonella* Typhimurium growth in vitro [41]. Carvacrol was able to reduce Salmonella Enteritidis growth in chicken cecal contents by more than 5 log CFU/mL [42]. Burt et al. [43] also observed reduced Salmonella Enteritidis growth in raw chicken pieces after they were exposed to carvacrol vapor.

4. Conclusions

Rosemary and oregano essential oils were found to be effective against *E. coli* ATCC 9637, a surrogate microorganism for *Salmonella* spp., both in vitro and in raw chicken breast samples. Treatment with the essential oils in vitro resulted in no growth at the end of the 24 h growth period, while treatment with REO and OEO in raw chicken resulted in a 0.69 log reduction and 0.31 log reduction, respectively, compared to the positive control. This shows that rosemary and oregano essential oils have promise as natural antimicrobial agents.

There are many avenues upon which the study can be progressed. Potential synergistic effects may be discovered when using REO and OEO in combination. This study may also be replicated against actual *Salmonella* spp. to confirm its effectiveness against the bacteria and reinforce the validity of *E. coli* ATCC 9637 as a surrogate microorganism. Furthermore, the study can be replicated on raw and cooked chicken to determine effectiveness at different stages prior to consumption. Texture profile analysis and sensory evaluation can be performed using uninoculated, treated chicken samples to determine acceptability to consumers. A parallel experiment measuring changes in the physicochemical properties of the chicken samples may reveal data that are able to further rationalize the experimental results. Inherent microflora were observed in the negative control; the identification of these bacteria may reveal interactions with the inoculated *E. coli* ATCC 9637. All of these are potential paths that can be taken in order to advance the study.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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

Appendix A

Table A1. Formulation of treatments for determination of antimicrobial activity.

Treatment	Concentration		
	Culture (Log CFU/mL)	RE (%*v*/*v*)	OEO (%*v*/*v*)
(−)	-	-	-
(+)	4	-	-
1	4	1.5	-
2	4	-	0.15

References

1. Nørrung, B.; Andersen, J.K.; Buncic, S. Main Concerns of Pathogenic Microorganisms in Meat. In *Safety of Meat and Processed Meat*; Toldrá, F., Ed.; Springer: New York, NY, USA, 2009; pp. 3–29. [CrossRef]
2. Saucier, L. Microbial spoilage, quality and safety within the context of meat sustainability. *Meat Sci.* **2016**, *120*, 78–84. [CrossRef]
3. Philippine News Agency. Available online: https://www.pna.gov.ph/articles/1220063 (accessed on 21 November 2024).
4. Santos, P.D.M.; Widmer, K.W.; Rivera, W.L. PCR-based detection and serovar identification of Salmonella in retail meat collected from wet markets in Metro Manila, Philippines. *PLoS ONE* **2020**, *15*, e0239457. [CrossRef] [PubMed]
5. Center for Disease Control. Available online: https://www.cdc.gov/salmonella/general/index.html (accessed on 21 November 2024).
6. Sinclair, R.G.; Rose, J.B.; Hashsham, S.A.; Gerba, C.P.; Haas, C.N. Criteria for selection of surrogates used to study the fate and control of pathogens in the environment. *Appl. Environ. Microbiol.* **2012**, *78*, 1969–1977. [CrossRef] [PubMed]
7. Eblen, D.R.; Annous, B.A.; Sapers, G.M. Studies To Select Appropriate Nonpathogenic Surrogate *Escherichia coli* Strains for Potential Use in Place of *Escherichia coli* O157:H7 and Salmonella in Pilot Plant Studies. *J. Food Prot.* **2005**, *68*, 282–291. [CrossRef] [PubMed]
8. Codex Alimentarius. *General Standard for Food Additives (CXS 192-1995)*; Food Agriculture Organization: Rome, Italy; World Health Organization: Geneva, Switzerland, 2023.
9. Carocho, M.; Morales, P.; Ferreira, I.C.F.R. Natural food additives: Quo vadis? *Trends Food Sci. Technol.* **2015**, *45*, 284–295. [CrossRef]
10. Amchova, P.; Kotolova, H.; Ruda-Kucerova, J. Health safety issues of synthetic food colorants. *Regul. Toxicol. Pharmacol.* **2015**, *73*, 914–922. [CrossRef]
11. Quinto, E.J.; Caro, I.; Villalobos-Delgado, L.H.; Mateo, J.; De-Mateo-Silleras, B.; Redondo-Del-Río, M.P. Food Safety through Natural Antimicrobials. *Antibiotics* **2019**, *8*, 208. [CrossRef] [PubMed]
12. Karpińska-Tymoszczyk, M.; Draszanowska, A.; Danowska-Oziewicz, M. The Effect of Ginger Rhizome Addition and Storage Time on the Quality of Pork Meatloaf. *Foods* **2022**, *11*, 3563. [CrossRef] [PubMed]
13. Oussalah, M.; Caillet, S.; Saucier, L.; Lacroix, M. Antimicrobial effects of selected plant essential oils on the growth of a Pseudomonas putida strain isolated from meat. *Meat Sci.* **2006**, *73*, 236–244. [CrossRef] [PubMed]
14. Radha krishnan, K.; Babuskin, S.; Azhagu Saravana Babu, P.; Sasikala, M.; Sabina, K.; Archana, G.; Sivarajan, M.; Sukumar, M. Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *Int. J. Food Microbiol.* **2014**, *171*, 32–40. [CrossRef] [PubMed]
15. Difonzo, G.; Squeo, G.; Pasqualone, A.; Summo, C.; Paradiso, V.M.; Caponio, F. The challenge of exploiting polyphenols from olive leaves: Addition to foods to improve their shelf-life and nutritional value. *J. Sci. Food Agric.* **2021**, *101*, 3099–3116. [CrossRef]
16. Moreno, S.; Scheyer, T.; Romano, C.S.; Vojnov, A.A. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radic. Res.* **2006**, *40*, 223–231. [CrossRef] [PubMed]
17. Badia, V.; de Oliveira, M.S.R.; Polmann, G.; Milkievicz, T.; Galvão, A.C.; da Silva Robazza, W. Effect of the addition of antimicrobial oregano (*Origanum vulgare*) and rosemary (*Rosmarinus officinalis*) essential oils on lactic acid bacteria growth in refrigerated vacuum-packed Tuscan sausage. *Braz. J. Microbiol.* **2019**, *51*, 289–301. [CrossRef]
18. Oleynikov, V. Antioxidant and antimicrobial properties of oregano extract (*Origani vulgaris herba* L.). *Foods Raw Mater.* **2020**, *8*, 84–90. [CrossRef]
19. Mourey, A.; Canillac, N. Anti-Listeria monocytogenes activity of essential oils components of conifers. *Food Control* **2002**, *13*, 289–292. [CrossRef]
20. Burt, S. Essential oils: Their antibacterial properties and potential applications in foods—A review. *Int. J. Food Microbiol.* **2004**, *94*, 223–253. [CrossRef] [PubMed]
21. Rodriguez-Garcia, I.; Silva-Espinoza, B.A.; Ortega-Ramirez, L.A.; Leyva, J.M.; Siddiqui, M.W.; Cruz-Valenzuela, M.R.; Gonzalez-Aguilar, G.A.; Ayala-Zavala, J.F. Oregano Essential Oil as an Antimicrobial and Antioxidant Additive in Food Products. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 1717–1727. [CrossRef]

22. Nieto, G.; Ros, G.; Castillo, J. Antioxidant and Antimicrobial Properties of Rosemary (*Rosmarinus officinalis* L.): A Review. *Medicines* **2018**, *5*, 98. [CrossRef] [PubMed]
23. Anapi, G.R.; Aba, R.P.M.; Gabriel, A.A. Screening for heat-resistant reference yeast isolate in orange juice. *Food Microbiol.* **2021**, *94*, 103639. [CrossRef]
24. Mann, C.M.; Markham, J.L. A new method for determining the minimum inhibitory concentration of essential oils. *J. Appl. Microbiol.* **1998**, *84*, 538–544. [CrossRef]
25. de Medeiros Barbosa, I.; da Costa Medeiros, J.A.; de Oliveira, K.Á.R.; Gomes-Neto, N.J.; Tavares, J.F.; Magnani, M.; de Souza, E.L. Efficacy of the combined application of oregano and rosemary essential oils for the control of *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* Enteritidis in leafy vegetables. *Food Control* **2016**, *59*, 468–477. [CrossRef]
26. Soyer, F.; Keman, D.; Eroğlu, E.; Türe, H. Synergistic antimicrobial effects of activated lactoferrin and rosemary extract in vitro and potential application in meat storage. *J. Food Sci. Technol.* **2020**, *57*, 4395–4403. [CrossRef]
27. Andrews, J.M. Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemother.* **2001**, *48* (Suppl. S1), 5–16. [CrossRef] [PubMed]
28. Cariri, M.L.; de Melo, A.N.F.; Mizzi, L.; Ritter, A.C.; Tondo, E.; de Souza, E.L.; Valdramidis, V.; Magnani, M. Quantitative assessment of tolerance response to stress after exposure to oregano and rosemary essential oils, carvacrol and 1,8-cineole in *Salmonella* Enteritidis 86 and its isogenic deletion mutants Δdps, ΔrpoS and ΔompR. *Food Res. Int.* **2019**, *122*, 679–687. [CrossRef] [PubMed]
29. Brandt, C.C.; Lobo, V.S.; Fiametti, K.G.; Wancura, J.H.; Oro, C.E.; Oliveira, J.V. Rosemary essential oil microemulsions as antimicrobial and antioxidant agent in tomato paste. *Food Chem. Adv.* **2023**, *2*, 100295. [CrossRef]
30. Hulánková, R.; Bořilová, G. In vitro combined effect of oregano essential oil and caprylic acid against *Salmonella* serovars, *Escherichia coli* O157: H7, *Staphylococcus aureus* and *Listeria monocytogenes*. *Acta Vet. Brno* **2012**, *80*, 343–348. [CrossRef]
31. Mazzarrino, G.; Paparella, A.; Chaves-López, C.; Faberi, A.; Sergi, M.; Sigismondi, C.; Compagnone, D.; Serio, A. Salmonella enterica and Listeria monocytogenes inactivation dynamics after treatment with selected essential oils. *Food Control* **2015**, *50*, 794–803. [CrossRef]
32. Boskovic, M.; Zdravkovic, N.; Ivanovic, J.; Djordjevic, J.; Janjic, J.; Pavlicevic, N.; Baltic, M.Z. Inhibitory effect of thyme and oregano essential oils and some essential oil components on Salmonella Senftenberg and Salmonella Give. *Meat Technol.* **2016**, *57*, 71–75.
33. Scandorieiro, S.; De Camargo, L.C.; Lancheros, C.A.; Yamada-Ogatta, S.F.; Nakamura, C.V.; De Oliveira, A.G.; Andrade, C.G.; Duran, N.; Nakazato, G.; Kobayashi, R.K. Synergistic and additive effect of oregano essential oil and biological silver nanoparticles against multidrug-resistant bacterial strains. *Front. Microbiol.* **2016**, *7*, 760. [CrossRef]
34. Evangelista-Martínez, Z.; Reyes-Vázquez, N.; Rodríguez-Buenfil, I. Antimicrobial evaluation of plant essential oils against pathogenic microorganisms: In vitro study of oregano oil combined with conventional food preservatives. *Acta Univ.* **2018**, *28*, 10–18. [CrossRef]
35. Sadeh, D.; Nitzan, N.; Chaimovitsh, D.; Shachter, A.; Ghanim, M.; Dudai, N. Interactive effects of genotype, seasonality and extraction method on chemical compositions and yield of essential oil from rosemary (*Rosmarinus officinalis* L.). *Ind. Crops Prod.* **2019**, *138*, 111419. [CrossRef]
36. Figiel, A.; Szumny, A.; Gutiérrez-Ortíz, A.; Carbonell-Barrachina, Á.A. Composition of oregano essential oil (*Origanum vulgare*) as affected by drying method. *J. Food Eng.* **2010**, *98*, 240–247. [CrossRef]
37. De Falco, E.; Mancini, E.; Roscigno, G.; Mignola, E.; Taglialatela-Scafati, O.; Senatore, F. Chemical composition and biological activity of essential oils of *Origanum vulgare* L. subsp. *vulgare* L. under different growth conditions. *Molecules* **2013**, *18*, 14948–14960. [CrossRef]
38. Gonthier, A.; Guérin-Faublée, V.; Tilly, B.; Delignette-Muller, M.L. Optimal growth temperature of O157 and non-O157 *Escherichia coli* strains. *Lett. Appl. Microbiol.* **2001**, *33*, 352–356. [CrossRef]
39. Rolfe, C.; Daryaei, H. Intrinsic and extrinsic factors affecting microbial growth in food systems. In *Food Safety Engineering*; Demirci, A., Feng, H., Krishnamurthy, K., Eds.; Springer: Cham, Switzerland, 2020; pp. 3–24. [CrossRef]
40. Moreira, A.C.P.; Lima, E.D.O.; Souza, E.L.D.; Van Dingenen, M.A.; Trajano, V.N. Inhibitory effect of Cinnamomum zeylanicum Blume (Lauraceae) essential oil and beta-pinene on the growth of dematiaceous moulds. *Braz. J. Microbiol.* **2007**, *38*, 33–38. [CrossRef]
41. Zhou, F.; Ji, B.; Zhang, H.; Jiang, H.; Yang, Z.; Li, J.; Li, J.; Yan, W. The antibacterial effect of cinnamaldehyde, thymol, carvacrol and their combinations against the foodborne pathogen *Salmonella* Typhimurium. *J. Food Saf.* **2007**, *27*, 124–133. [CrossRef]
42. Johny, A.K.; Darre, M.J.; Donoghue, A.M.; Donoghue, D.J.; Venkitanarayanan, K. Antibacterial effect of trans-cinnamaldehyde, eugenol, carvacrol, and thymol on *Salmonella* Enteritidis and Campylobacter jejuni in chicken cecal contents in vitro. *J. Appl. Poult. Res.* **2010**, *19*, 237–244. [CrossRef]
43. Burt, S.A.; Fledderman, M.J.; Haagsman, H.P.; van Knapen, F.; Veldhuizen, E.J.A. Inhibition of *Salmonella enterica* serotype Enteritidis on agar and raw chicken by carvacrol vapour. *Int. J. Food Microbiol.* **2007**, *119*, 346–350. [CrossRef] [PubMed]