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Unraveling the antimicrobial activity of nutmeg and turmeric essential oils against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* spp.

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Abstract

The consumer demand for a reduction in the use of synthetic additives in food has been providing a greater search and incentive for the food industries to use new alternatives for food preservation. Among them, there is the initiative to use essential oils (EOs) due to their antimicrobial properties, coming from specific compounds in their compositions. However, in view of limitations related to the use of EOs, as well as their susceptibility to oxidation and degradation, the possibility arises of employing protection methods such as microencapsulation to minimize the impairment of the benefits associated with the application of EOs. This study aimed to examine the antimicrobial effect of turmeric and nutmeg EOs against microbial strains of *Staphylococcus aureus, Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* spp., as well as the microparticles of the EOs involved. Analyses of the minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC), and the developed microparticles, as well as the verification of the synergistic inhibitory action between these oils, were carried out. For free oils, antimicrobial action was evidenced against the vast majority of microorganisms tested, with free nutmeg EO having a better antimicrobial effect than free turmeric oil. In contrast, for encapsulated oils, only antimicrobial action was noted against strains of *Listeria*. Furthermore, the synergism of free oils did not potentiate the antimicrobial action. Regarding the alternative of microencapsulation of EOs, it was obtained that the results in which chitosan was used as wall material were more promising than when gelatin was used as wall material.

Keywords: minimum inhibitory concentration; essential oils; microencapsulation.

Practical Application: Unpublished results on the antimicrobial effect of turmeric and nutmeg essential oils.

1 INTRODUCTION

Various food preservation methods can be used to prevent diseases transmitted by food (DTAs), including chemical agents. Despite the effectiveness of this method, there is an increasing demand for the use of natural compounds as antimicrobial agents, mainly due to the interest in reducing synthetic and or artificial preservatives (Khorshidian et al., 2018). Adding alternative antimicrobials, whose properties are equivalent to those of artificial preservatives, is an initiative for a healthier lifestyle based on food. Nevertheless, there are obstacles to the widespread use of natural compounds, as they must have antimicrobial properties and food compatibility simultaneously. In this context, new antimicrobial agents of natural origin have been discovered, such as essential oils (EOs) from aromatic medicinal plants. EO complex mixtures can contain about 20–80 individual components (Laranjo et al., 2017) and various volatile plant compounds, essentially terpenoids and phenolic compounds (Fokou et al., 2020; Mendonca et al., 2018). Therefore, EOs are known for their antioxidant activity, generally attributed to phenolic compounds. In addition, EOs have great potential for antifungal and antibacterial activities, acting against a wide range of microorganisms (Khorshidian et al., 2018; Laranjo et al., 2017), and they can be used in many types of processing during food production, such as thermal, non-thermal, and high-pressure processes to inhibit microorganisms (Taylor, 2018).

Among the various compounds that can be used to produce EOs, *Curcuma longa* (*Zingiberaceae* family) and nutmeg (*Myristica fragrans*) are compounds of interest for this study. *Curcuma longa* is popularly known as turmeric/saffron, and the main component is curcumin, a yellowish bioactive pigment with anti-inflammatory and antioxidant potential. This compound can be used against viruses, bacteria, and fungi (Grasso et al., 2017). Therefore, turmeric is widely used in the food, pharmaceutical, cosmetic, traditional, and herbal medicine industries and is mainly used to prevent and combat cancer, malaria, diabetes, arthritis, hepatitis, triglycerides, and cholesterol and as a healing agent (Grasso et al., 2017). In addition,

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nutmeg EO (*Myristica fragrans*) is also shown for its medicinal and therapeutic properties as a fungicide, carminative, digestive, anti-inflammatory, and antioxidant (Akbar, 2020). The EO can be extracted from the leaf, mace, kernel, and seed of *Myristica fragrans* (Ashokkumar et al., 2022).

The effectiveness of EOs has been previously demonstrated against various food-borne pathogens such as *Staphylococcus aureus, Vibrio cholerae,* and *Candida albicans*. Its performance as an antioxidant is evident when used in the dosage range of 0.01–10 $mg.mL^{-1}$ in cellular models (Valdivieso-Ugarte et al., 2019). However, limitations associated with the biological properties of EOs are known, including high volatility, photosensitivity, and degradation upon exposure to temperature and light, leading to a reduction in bioavailability and low miscibility with water (Garcia et al., 2021).

Given this, microencapsulation is being investigated and used as an effective technique to protect EOs from evaporation and oxidation. In this way, it is possible to control the respective biological properties of the oils, improving water solubility and bioavailability of lipophilic compounds, which makes this technique practical and promising (Garcia et al., 2021). This method facilitates product handling by allowing controlled release of active compounds and reducing their reactivity. However, the efficacy and stability of microparticles depend on the encapsulant, the microencapsulation technique, and the release mechanism used (Martins et al., 2021). The complex coacervation method emerges as a viable alternative to prolong the helpful life of oils with functional appeal, retaining their original properties, providing protection against adverse conditions, masking undesirable flavors and aromas, and further promoting the controlled release of the active agent (Agibert, 2018). This process combines two hydrocolloid solutions with opposite charges, resulting in the interaction and precipitation of complex polymers. This process is responsible for forming microcapsules, small particles capable of ejecting contents under certain conditions and at certain speeds.

Therefore, this study investigated the antimicrobial activity of turmeric and nutmeg EOs against some strains of *S. aureus*, *Listeria monocytogenes*, *Escherichia coli,* and *Salmonella* spp. The oils effect was studied individually and in combination with other oils (synergism). In addition, we investigated the antimicrobial activity of microparticles containing such microencapsulated oils (isolated and combined) obtained by complex coacervation.

2 MATERIALS AND EXPERIMENTAL PROCEDURES

Nutmeg (*Myristica fragrans*) (NEO) and turmeric (*Curcuma longa*) EOs (TEO) from the commercial brands Quinarí®, Brazil, and Terraflor, Brazil, were used.

Standard strains of *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *L. monocytogenes* (ATCC 7644), and *Salmonella* Tiphymurium (ATCC 14028) were used. In addition, two wild-type strains of each bacteria isolated from food in previous studies (da Rosa et al., 2022; da Silva Cândido et al., 2020; Morasi et al., 2022) were stored at the Food Microbiology Laboratory II (UNICAMP) with the following coding: *S. aureus* A1*, S. aureus* 94*, E. coli* 46*, E. coli* 116*, Listeria innocua* 4445*, L. innocua* 4422*, Salmonella* 1R, and *Salmonella* 2R.

The wall materials used in the production of the microparticles were Type-B gelatin, bovine skin (GE; gel strength 244 bloom, LF 21502/04, Gelita South America, São Paulo, SP, Brazil), chitosan (CHI; Polymar Ciência e Nutrição, 20100210, Degree of Deacetylation = 89%, PM = 69,000 $g.moL^{-1}$, Fortaleza, CE, Brazil), and gum Arabic (GA; IRX49345, supplied by Colloids Naturels Brasil Comercial Ltda., São Paulo, SP, Brazil).

2.1 Characterization of the essential oils

Chromatographic analysis of EOs and microparticles was carried out using an HP-6890 gas chromatograph (GC) coupled to an HP-5975 selective mass detector under the following conditions: HP capillary column-5MS (30 μm × 0.25 mm × 0.25 μm); injector temperature: 220°C; column temperature: 60°C, 3°C/min, 240°C; detector temperature: 250°C; injected volume: 1.0 mL; carrier gas flow rate (He): 1.0 $mL.min^{-1}$; and split ratio: 40:1.

The identification was carried out by calculating the retention rates of the analytes, using the co-injection of a mixture of hydrocarbon standards (C8–C24), comparison with the electronic library of the equipment (NIST-11), and with data from the literature (Adams, 2007).

2.2 Determination of minimum inhibitory concentration

This study investigated the MIC determination in 96-well cell culture plates, and each well contained 200 μL of Tryptone Soy Broth (TSB) and 0.5% of Tween 80 and the EO at their respective concentration. Bacterial suspensions were standardized, resulting in a final concentration of 10^5 $CFU.mL^{-1}$ in each well. The plates were incubated at 37°C for 24 h at the end. After incubation, 50 μL of 0.01% resazurin solution was added, and staining indicative of microbial growth was performed according to the previously reported study by de Almeida et al. (2023) with adaptations.

2.3 Determination of minimum bactericidal concentration

For the identification of MBC, 10 μL was taken from the MIC indicator well and from three previous wells, which were inserted in plates with Nutrient Agar using the microdrop technique (de Almeida et al., 2023; Knezevic et al., 2016).

2.4 Synergism

The synergistic was determined from binary combinations (blends) between the oils using the microdilution technique (de Almeida et al., 2023; Ouedrhiri et al., 2017). The concentration in the first well was half the MIC of each oil to be combined (20 μL of each oil), following the same methodology for dilution and evaluation of growth, inhibition with resazurin, or bactericidal effect through MBC. The results were performed using the mathematical formula (Equation 1):

$$\Sigma FICI = FIC\ (A) + FIC\ (B) \quad (1)$$

where:

$FIC\ (A) = \frac{MIC\ (A)\ in\ combination}{MIC\ (A)\ alone}$;

$FIC\ (B) = \frac{MIC\ (B)\ in\ combination}{MIC\ (B)\ alone}$.

Thus, the FICI values are as follows: ≤ 0.5 = synergism; 0.5–0.75 = partial synergism; 0.76–1.0 = additive; 1.0–4.0 indifferent (non-interactive); and > 4.0 = antagonism.

2.5 Production of particles loaded with essential oils

The particles were prepared by the complex coacervation method (Gonçalves et al., 2018; Prata & Grosso, 2015). The formulations used to produce the capsules are shown in Supplementary Table 1. The oil phase consists of 3 g. However, 1.5 g of each oil was used for particles with two oils. The emulsifying phase (50°C) was homogenized for 3 min at 14,000 rpm (Ultra Turrax T-18 Homogenizer, IKA Works, Inc., USA). Then, the complexed phase was added, still at 50°C, and the pH of the system was adjusted to the coacervation pH of the polymeric pair (pH = 4.0). The systems were cooled to 10°C and remained in this condition until the moment of the other analyses.

2.6 Particle characterization

2.6.1 Humidity of particles

The percentage of water present in the particles was determined from the difference between the known mass of the wet sample and mass after drying in an oven at 70°C for 24 h. The humidity content of the particles was calculated from the following formula (Equation 2):

$$\%\ U = \frac{MU - MS}{MU} \quad (2)$$

where:

%U: humidity present in the particles;

MU: mass of wet particles;

MS: mass of dry particles.

2.6.2 Loading capacity

The determination of encapsulation efficiency was performed as described by Prata and Grosso (2015). Initially, the content of the particles was extracted from the dry material, obtained in the determination of the humidity of the particles by macerating a known mass of dry particles in a mortar with Ethanol Absolute P.A. (99.5%) of the known final volume. Next, the samples resuspended in ethanol were submitted to chromatographic analysis, allowing the identification of the analytes of the microparticles. In this way, using the area and

Table 1. Analytes detected in the TEO and NEO by gas chromatograph (GC).

Peak	Retention time (min)	RI*	Identification	% Relative
Turmeric (TEO)				
1	5.66	934	α-Pinene	9.58
2	6.71	974	Sabinene	6.54
3	6,82	978	β-Pinene	4.06
4	7.62	1,006	α-Phelandrene	1.38
5	7.8	1,011	δ-3-Carene	0.47
6	8.27	1,025	Para-cymen	1.66
7	8.4	1,029	Limonene	1.23
8	8.49	1,031	1,8-Cineol (eucalyptol)	0.85
9	14.11	1,179	4-Terpineol	733
10	14.64	1,192	α-Terpineol	2.33
11	18.67	1,287	Isobornyl acetate	2.58
12	21.28	1,350	α-Terpenyl acetate	0.98
13	22.36	1,376	α-Copaene	1.75
14	23.62	1,406	Methyleugenol	2.46
15	24.15	1,419	Trans-caryophyllene	2.74
16	25.19	1,445	*M* = 202	0.95
17	26.59	1,480	ϒ-Curcumene	1.08
18	26.74	1,484	α-Curcumene	3.06
19	27.25	1,496	α-Zingiberene	4
20	27.75	1,509	β-Bisabolene	0.64
21	28.34	1,525	β-Sesquiphelandrene	2.77
22	31.46	1,606	*M* = 216	1
23	33.52	1,662	*M* = 218	8.2
24	33.8	1,669	AR-turmerone	14.27
25	33.94	1,673	Tumerone	13.22
26	35.03	1,703	Curlone	4.09
27	37.58	1,775	*M* = 218	0.77
Nutmeg (NEO)				
1	5.69	935	α-Pinene	11.84
2	6.74	975	Sabinene	9.33
3	6.84	979	β-Pinene	6.05
4	7.61	1,006	α-Phelandrene	0.63
5	7.79	1,011	δ-3-Carene	0.64
6	8.27	1,025	Para-cymen	1.63
7	8.41	1,029	Limonene	1.74
8	8.49	1,031	1,8-Cineol (eucalyptol)	0.63
9	14.2	1,181	4-Terpineol	13.71
10	14.7	1,193	α-Terpineol	4.25
11	14.93	1,199	ϒ-Terpineol	0.45
12	17.36	1,257	*M* = 204	1.38
13	18.7	1,288	Lavandulyl acetate	4.13
14	21.3	1,351	α-Terpenyl acetate	1.79
15	22.39	1,377	α-Copaene	3.48
16	22.75	1,385	*M* = 202	0.44
17	23.68	1,408	Methyleugenol	4.28
18	24.19	1,420	Trans-caryophyllene	4.62
19	28.3	1,523	Myristicin	1.66
20	31.1	1,596	AR-dihydro-turmerone	4.71
21	31.69	1,612	*M* = 216	12.55
22	31.81	1,615	*M* = 216	7.73
23	33.34	1,657	*M* = 216	2.36

RI*: retention index; NEO: nutmeg essential oils; TEO: turmeric essential oils.

relative percentage data referring to the analytes identified in the samples that underwent chromatography, it is possible to determine the percentage of total charge of each analyte present in the samples, comparing them with each other about the levels found and relating them with the wall materials used for the microparticles and with the microencapsulated oils in each case, and thus, say how the encapsulation took place, simplistically.

2.6.3 Statistical analysis

All statistics were calculated in the Sisvar 5.6 software. Data were tested for the mean, standard deviation, analysis of variance (ANOVA), and test of means (Tukey's method, $p \leq 0.05$) (Ferreira, 2019).

3 RESULTS AND DISCUSSION

3.1 Determination of essential oil compounds of TEOs and NEOs

EOs can have chemical variations that influence biological activity against microorganisms (Hu et al., 2017). Thus, determining compounds present in EOs is of great importance for understanding how the correlation with their properties occurs because, in most cases, the antimicrobial effect of EOs is attributed to their respective major compound. Therefore, the chromatographic analysis of each oil was carried out, as well as the identification of the analytes present in the oils, as shown in Supplementary Figures 1A and 1B.

A total of 27 compounds were identified for TEO, while 23 compounds were identified for NEO (Table 1).

In the case of TEO, the compound found mostly was AR-turmerone (14.27%). Studies have shown that the AR-turmerone and curlone compounds in TEO were active against some microorganisms, such as *S. aureus* (Singh et al., 2011). On the contrary, Ali et al. (2023) showed that zingiberene (33.16%) was the principal constituent, followed by tumerone (20.93%) and β-sesquiphelandrene (16.49%). In another study, the most common compounds identified were turmerone (40%), curlone (34%), zingiberene (8%), and benzene (6%) (26). These variations are justifiable since ecological and geographic conditions, plant age, and harvest time can affect the chemical profile (Hu et al., 2017).

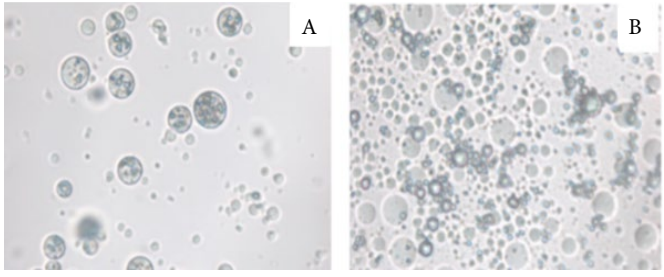

Figure 1. (A) Optical microscopy of microparticles of GE-GA loaded with essential oil of nutmeg and turmeric. (B) Optical microscopy of microparticles of CHI-GA loaded with essential oil of nutmeg and turmeric.

Despite this, reports in the literature indicate that this TEO has antioxidant potential (Kutti Gounder & Lingamallu, 2012; Ali et al., 2023), probably due to a synergistic action between its major components that contribute to eliminating free radicals (Ferreira et al., 2013). According to Lee et al. (2003) and Negi et al. (2005), in an attempt to determine the antimicrobial action of turmeric oleoresin microcapsules, the fraction of TEO with the highest percentage of AR-turmerone showed the most increased antimicrobial activity.

The main compounds identified in this study for NEO were 4-terpineol (13.71%), α-pinene (11.84%), and sabinene (9.33%). Furthermore, it is noted that there are three analytes, whose retention times are t_R = 1.69; t_R = 31.81, and t_R = 33.34 min, which have mass spectra practically identical to that of AR-turmerone, found in turmeric oil. However, it was impossible to identify which analytes these are because they are not present in the GC-MS library (NIST-11) nor in the literature used for identification (Adams, 2007).

Ashokkumar et al. (2022) obtained from leaf oil the sabinene (17.17%), β-pinene (6.44%), d-limonene (5.03%), and b-myrcene (4.74%) (monoterpenes). They also showed the presence of eugenol (16.60%) and myristicin (9.12%), both part of the phenylpropene group (Ashokkumar et al., 2022). In another study with NEO from seeds, the major components were monoterpenes and sesquiterpenes (e.g., sabinene, α- and β-pinene, d-limonene, γ-terpinene, α-thujene, camphene, α-terpinolene, γ-amorphene, α-bergamotene, isogermacrene D, α-copaene, and isoterpinolene) (Mickus et al., 2021).

3.2 Determination of the MIC and MBC of the oils individually

3.2.1 Escherichia coli

Three *E. coli* strains (i.e., ATCC 25922, *E. coli* 46, and 116) were tested to evaluate the antimicrobial activity of the EOs, which presented different MIC values against each of the strains. According to our data, NEO was highly efficient against *E. coli* ATCC 25922, whose inhibition occurred with a concentration of 2.5% (0.01 mL). As for *E. coli* 46, inhibition occurred with 5% (0.02 mL) of NEO. Oppositely, TEO against *E. coli* ATCC 25922 and *E. coli* 46 were obtained with MIC > 10% oil, which means that it was impossible to determine the MIC, as there was growth in all wells (MIC > 10% oil). On the contrary, *E. coli* 116 was inhibited with 10% (0.04 mL mg) of NEO and 10% (0.04 mL) of TEO. The antibacterial effect of NEO against *E. coli* has already been shown by other authors (Ansory et al., 2020; dos Santos, 2016; Özkan et al., 2018). Likewise, the absence of an inhibitory effect of TEO on *E. coli* has also been demonstrated in other studies (de Araújo et al., 2015; Franco et al., 2007).

3.2.2 Staphylococcus aureus

The MIC results obtained from NEO against all *S. aureus* (ATCC 25923, *S. aureus* A1, and *S. aureus* 94) corresponded to the concentration of 10% oil (0.04 mL). For TEO, the results were the same against *S. aureus* ATCC 25923 and *S. aureus* 94,

with inhibition at 10% oil (0.04 mL). However, for *S. aureus* A1, it was obtained if MIC > 10%. The antimicrobial effect of NEO against *S. aureus* has been previously demonstrated (Thileepan et al., 2017). Furthermore, compounds from nutmeg, such as trimyristin, myristic acid, and myristicin, are some of those responsible for antimicrobial activity against *S. aureus* and *E. coli* (Narasimhan and Dhake, 2006). Recently, the effect has been applied NEO as efflux pump inhibitors (EPIs) in methicillin-resistant *S. aureus* (MRSA) (Franco et al., 2007). Regarding the impact of TEO against *S. aureus,* components such as αr-tumerone and curlone can have microbial activity against *S. aureus* (Singh et al., 2011).

3.2.3 Salmonella *spp.*

NEO was able to inhibit all strains of *Salmonella* Tiphymurium, including ATCC 14028 with a MIC of 2.5% oil (0.01 mL), *Salmonella* 1R with a MIC of 3.3% (0.013 mL), and *Salmonella* 2R with a MIC was 10% oil (0.04 mL). Regarding TEO, the MIC was 10% oil (0.04 mL) for *Salmonella* Tiphymurium ATCC 14028 and *Salmonella* 1R and MIC > 10% oil for *Salmonella* 2R. The effectiveness of NEO against *E. coli, Salmonella typhi, and S. aureus* has already been demonstrated previously (Omoruyi & Emefo, 2012). The antimicrobial activity is due to active components, such as monoterpene hydrocarbons (α-pinene, β-pinene, and sabinene), oxygenated monoterpenes, and aromatic ethers (myristicin, elemicin, and safrole) (Jansen & Westphal, 1999; Thileepan et al., 2017). In contrast, TEO has also been pointed out as without antimicrobial activity against *Salmonella choleraesuis* (Franco et al., 2007).

3.2.4 Listeria *sp.*

The MIC of NEO against *Listeria* was 8.3% NEO (0.03 mL) for *L. monocytogenes* ATCC 7644 and 10% oil (0.04 mL) for *L. innocua* 4445 *and L. innocua* 4422. Regarding TEO, the MIC was 10% oil (0.04 mL) against *L. monocytogenes* ATCC 7644 and *L. innocua* 4445, whereas the MIC of TEO against *L. innocua* 4422 was mainly higher than 10%. Therefore, our data on the effectiveness of NEO against *Listeria* resemble the previously reported (Smith-Palmer et al., 1998). The performance of the NEO against *Listeria* may be due to the release of inhibitory volatiles at certain temperatures (Smith-Palmer et al., 1998). Our work also demonstrates that, similar to the literature data, TEO does not show antimicrobial activity against *Listeria* (Antunes et al., 2012). On the contrary, Ahmed et al. (2012) reported a more significant antimicrobial action of TEO against Gram-positive than Gram-negative bacteria. This divergence may be due to the lack of standardization of the techniques used, resulting in substantial variations in MICs and MBCs even when oils of similar composition are used.

3.2.5 MBC

Concerning MBC, there was microbial growth in the vast majority of cases, and in some cases, the growth was more notable, with large and visible colonies. In other cases, it is possible to notice some minimal increase, which is evidenced by tiny colonies.

Thus, it can be said that there was a predominance of bacteriostatic rather than bactericidal effect on the part of the oils used because most of the petri dish sessions exhibited microbial growth, which indicates that the oils could inhibit the microorganisms tested. In a few sessions, it was noticed that there was no growth.

3.3 Synergism

Combinations were performed between NEO and TEO, allowing the evaluation of the synergistic against microorganisms. The concentration of oil used was 10% of total oil, with 5% of NEO (0.02 mL) and 5% of TEO (0.02 mL) against *E. coli* ATCC 25922*, L. monocytogenes* ATCC 7644*, S. aureus* ATCC 25923*, E. coli* 116, *S. aureus* 94*, Salmonella* 2R, and *Salmonella* 1R. The value of *Salmonella* Tiphymurium ATCC 14028*, L. innocua* 4445, and *E. coli* 46 was 8.3% (0.017 of NEO and 0.017 mL of TEO), while the value of *L. innocua* 4422 was 6.7% (0.013 mL of TEO and 0.013 mL of nutmeg oil). Finally, for *S. aureus* A1, there is predominance, resulting in a MIC > 10% of oil. The evaluation of the synergism between the oils is shown in Table 2. However, it was not possible to evaluate the synergistic effect against *L. innocua* 4422*, E. coli* 46*, E. coli* ATCC 25922*, Salmonella* 2R, and *S. aureus* A1, because the mathematical formula (presented in Section 2.4) could not be applied, as it requires the MIC values of the oils tested isolated too, which were higher than 10% for turmeric oil (MIC > 10%, indeterminate to TEO) not allowing us to determine how the synergism took place against these bacteria mentioned.

This result was somewhat unexpected because most of the combined oils were indifferent and had no synergism. Thus, the oils did not enhance the antimicrobial effect; in some cases, antagonism even occurred. Also, it can be observed that NEO proved to be more effective than TEO, in general, as smaller amounts of oil used (%) were able to inhibit microorganisms.

Table 2. Evaluation of the synergism.

Strains	MIC (mL)		MIC (mL)		SY
	NEO	TEO	NEO + TEO		
L.innocua 4445	0.040	0.040	0.017	0.017	NI (1.6)
L.innocua 4422	0.040	> 0.040	0.013	0.013	–
L. monocytogenes ATCC 7644	0.030	0.040	0.020	0.020	NI (2.2)
E. coli 116	0.040	0.040	0.020	0.020	NI (2.0)
E. coli 46	0.020	> 0.040	0.017	0.017	–
E. coli ATCC 25922	0.010	> 0.040	0.020	0.020	–
Salmonella 1R	0.013	0.040	0.020	0.020	AN (4.0)
Salmonella 2R	0.040	> 0.040	0.020	0.020	–
S. Tiphymurium ATCC 14028	0.010	0.040	0.017	0.017	AN (4.2)
S. aureus 94	0.040	0.040	0.020	0.020	NI (2.0)
S. aureus A1	0.040	> 0.040	> 0.020	> 0.020	–
S. aureus ATCC 25923	0.040	0.040	0.020	0.020	NI(2.3)

MIC: minimal inhibitory concentration; SY: synergistic; NI: no interaction; AN, antagonism.

3.4 MIC and MBC determination of microencapsulated oils

3.4.1 Determination of MIC and MBC using Gelatin and Chitosan microparticles with NEO and TEO synergism

NEO and TEO encapsulated with GA-GE and GA-CHI were ineffective against any of the strains evaluated, as microbial growth occurred in all wells. NEO (GA-CHI) was an exception for *L. innocua* 4422*, L. innocua* 4445, and *L. monocytogenes* ATCC 7644. As for MBC, microbial growth was observed in all Petri dishes cultured with the respective MIC and wells.

According to Wang et al. (2009), an inhibitory effect of the microcapsule curcumin (from turmeric) was found with different sensitivity of one species compared with the others. This effect was demonstrated against *Y. enterocolitica*, *B. subtilis*, *Bacillus cereus*, *A. niger, P. notatum*, and *S. cerevisiae* because the microcapsule curcumin retained the broad-spectrum inhibitory effect of free curcumin after the microencapsulation and spray-drying processes. In addition, Hammoud (2015) noticed that Gram-negative pathogens appeared more resistant than Gram-positive ones, possibly due to the outer phospholipid membrane that alters cell permeability. In this specific research, curcumin's MBC and MIC values had a low antimicrobial effect on foodborne pathogens, including *S. aureus*, *E. coli*, *Salmonella* spp., and *L. monocytogenes*. Therefore, curcumin at low concentrations cannot be considered an antimicrobial agent.

In addition, Arshad et al. (2018) assessed the antimicrobial activity of nutmeg microcapsules against five bacterial food pathogens, including *Vibrio parahemolyticus*, *Vibrio alginolyticus*, *L. monocytogenes*, *B. cereus*, and *E. coli*. It was reported to have an inhibitory effect against *E. coli* and *B. cereus*, while no effect was evidenced against *V. parahemolyticus*, *V. alginolyticus*, and *L. monocytogenes*. Takikawa et al. (2002) reported nutmeg's antimicrobial activity against *E. coli* and found that the bacteria was susceptible to β-pinene. Also, α-pinene and β-pinene, which are significant components in nutmeg EOEO, have been demonstrated to have antimicrobial activity and are supposed to disrupt cellular membranes by the lipophilic compounds (Dorman & Deans, 2000).

The synergistic effect (MIC) of microparticles containing TEO and NEO was observed against strains *E. coli* 46*, E. coli* 116*, E. coli* ATCC 25922*, Salmonella* 1R*, Salmonella* 2R, and *Salmonella* Tiphymurium ATCC 14028. Microbial growth was obtained in all wells in all cases for both GE and CHI particles. When GE particles were used, *S. aureus* 94 grew in all concentrations, whereas when CHI particles were used, the MIC was achieved in the third well in one of the cases and in the second well in the other cases. For *S. aureus* A1, growth occurred in all concentrations when GE particles were used, whereas when CHI particles were used, the MIC happened in the first well in one of the cases and in the second well in the other cases. For *S. aureus* ATCC 25923, growth occurred at all concentrations when the GE particles were used, whereas when the CHI particles were used, the MIC was in the third well in all cases. Against *L. innocua* 4422 and *L. innocua* 4445*,* both strains grew at all concentrations when using the GE particles, and the MIC occurred in the fifth well in all cases for both strains when using the CHI particles. Finally, for *L. monocytogenes* ATCC 7644, growth was observed at all concentrations when using the GE particles, but with CHI particles, the MIC occurred in the sixth well in one case and in the seventh well in the other cases.

Regarding MBC, microbial growth was present in all Petri dishes cultured with the respective MIC and wells.

Thus, the microparticle results were similar for the two oils, with antimicrobial activity detected only against *L. innocua* 4422, *L. innocua* 4445, and *L. monocytogenes* ATCC 7644. In addition, lower MIC values (higher efficacy) were observed for the encapsulated NEO compared with TEO. The particles containing GE as wall material did not show efficient results, as they did not exhibit antimicrobial activity (for both tested oils). Moreover, the particles containing CHI could inhibit the three mentioned strains. For microparticles containing a combination of oils (synergism), the antimicrobial activity against the same three *Listeria* strains was remarkable, and the MIC results were even lower. In addition, inhibition of *S. aureus* ATCC 25923, *S. aureus* A1, and *S. aureus* 94 was also observed.

Notably, different wall materials, including EOs, configure other release profiles in the medium (Paulo et al., 2019). Under this bias, studies such as that by No et al. (2002) reported that chitosan acetate showed a more substantial antibacterial effect against Gram-positive bacteria than against Gram-negative ones, probably due to differences in the structure of the wall of microorganisms that may facilitate or hinder the entry of the substance into the cell.

The optical microscopy analysis of the particles allows the visualization of the oil dispersion in the coacervates. According to the study by de Almeida et al. (2023) and Gonçalves et al. (2018), the microparticles containing CHI of wall material formed mononuclear systems, while those containing GE were polynuclear systems. Figures 1A and 1B shows the exposure.

The morphological difference between the coacervates can be explained by the interfacial effect that both biopolymers exert differently in each case because they have different properties and are present in different concentrations in the coacervates. Therefore, this difference in dispersion in the medium and the morphology of microparticles is known to have a direct relationship and influence on the release profile and, consequently, on the MIC obtained for each situation (de Almeida et al., 2023).

Thus, it can be said that the most efficient wall material was CHI. The particles containing encapsulated NEO had lower MIC results (greater efficacy) than the particles containing only TEO, and the MIC results of the particles containing synergism against the same strains (*Listeria*) were even lower than the results of the microparticles containing the individually encapsulated oils. In addition to their contribution to antimicrobial activity against the three strains of *S. aureus*, the individually encapsulated oils were not able to produce inhibition.

3.5 Analysis of humidity of particles

To determine the humidity of the particles, the moisture content was weighed before drying, and the contents determined after drying the samples in the oven were used. In this way, the analysis was performed for both the individually encapsulated

oils and the synergism of the oils for the two bases used, GE and CHI, combined with gum Arabic (Table 3).

The humidity percentages of the tested microparticles were considerably high, all above 90% and very close to the maximum value. Gonçalves et al. (2018) prepared microparticles by the complex coacervation method and obtained humidity contents of 86.3% in GE-GA particles and 72.8% humidity in CHI-GA particles.

3.6 Analysis of the loading capacity

The encapsulation efficiency of microparticles with the encapsulated EOs was analyzed using the constituents identified in the commercial oils and the samples containing microparticles. The total amount of compounds in the samples and encapsulation was evaluated through the dry mass of the particles and the relative percentage of analytes identified by chromatography (see Table 4).

Six samples containing a wet mass of particles were separated, and their profile was analyzed from the chromatographic analyses. The identification of each sample follows as shown in Table 4, each referring to each isolated and combined oil containing the respective wall materials. The dry mass of particles, obtained after drying in an oven, was resuspended in a specific volume of Ethanol P.A, as explained in the methodology.

Thus, based on the chromatograms obtained for the tested samples, it was possible to assemble Tables 5 and 6, allowing the comparison of the areas and relative percentages of six identified analytes, three of which refer to NEO (i.e., α-pinene, β-phellandrene, and 4-terpineol) and three related to TEO (i.e., AR-tumerone, tumerone, and curlone).

About Samples A and B (synergism of TEO and NEO), it is notable that the areas of the analytes in the GE microparticles are much larger than in the CHI one. This is also true for Samples E and F (NEO only). As for Samples C and D (TEO only), the

Table 3. Moisture content and load of microparticles and standard deviation.

	NEO		TEO		SY	
Microparticles	GA-GE	GA-CHI	GA-GE	GA-CHI	GA-GE	GA-CHI
Moisture content (%)	91.52 ± 0.01	99.41 ± 0.00	99.56 ± 0.00	99.04 ± 0.00	99.92 ± 0.01	98.83 ± 0.00
Load (g/g of dry particle)	1.15 ± 0.03	0.08 ± 0.01	0.06 ± 0.00	0.13 ± 0.01	0.49 ± 0.01	0.14 ± 0.00

NEO: nutmeg essential oil; TEO: turmeric essential oil; SY: synergism/combination of NEO and TEO; GE: gelatin; CHI: chitosan; GA: gum Arabic.

Table 4. Wet mass samples used containing microparticles, subjected to chromatography.

	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
Oil	SY	SY	TEO	TEO	NEO	NEO
Wall material (+ G.A)	CHI	GE	GE	CHI	GE	CHI
Dry mass (g)	0.11	0.3	0.06	0.13	1.15	0.08
Ethanol P.A (mL)	2.2	6	1.2	2.6	23	1.6

*G.A: gum arabic; CHI: chitosan; GE: gelatina; NEO: nutmeg essential oil; TEO: turmeric essential oil; SY: synergism.

Table 5. Data regarding the analytes found in NEO.

tR (min)		**5.65**		**6.70**		**14.10**	
		α-pinene		**β-phellandrene**		**4-Terpineol**	
Sample		**Area**	**% Relative**	**Area**	**% Relative**	**Area**	**% Relative**
A	SY-CHI	375336	3.66	n.d.	n.d.	n.d.	n.d.
B	SY-GE	1111129	2.63	1008119	2.39	6262718	1.48
C	TEO-GE	–	–	–	–	–	–
D	TEO-CHI	–	–	–	–	–	–
E	NEO-GE	5592990	21.34	3415042	13.03	2795877	10.67
F	NEO-CHI	4129722	18.45	825523	3.69	n.d.	n.d.
Data regarding the analytes found in TEO							
tR (min)		**33.78**		**33.92**		**35.10**	
		Ar-tumerone		**Tumerone**		**Curlone**	
Sample		**Area**	**% Relative**	**Area**	**% Relative**	**Area**	**% Relative**
A	SY-CHI	2839304	27.68	2219765	21.64	666081	6.50
B	SY-GE	106414695	25.18	74795384	17.70	27203295	6.44
C	TEO-GE	5606403	39.66	1325233	9.37	846344	5.99
D	TEO-CHI	126568718	49.73	23825211	9.36	18142458	7.13
E	NEO-GE	–	–	–	–	–	–
F	NEO-CHI	–	–	–	–	–	–

n.d.: not detected; CHI: chitosan; GE: gelatina; NEO: nutmeg essential oil; TEO: turmeric essential oil; SY: synergism.

Table 6. Total charge of components obtained in samples containing microparticles.

Samples	NEO α-pinene (%)	NEO β-phellandrene (%)	NEO 4-terpineol (%)	TEO Ar-tumerone (%)	TEO Tumerone (%)	TEO Curlone (%)
A (SY-CHI)	33.27	n.d	n.d	251.64	196.73	59.09
B (SY-GE)	8.77	7.97	11.38	83.93	59.0	21.47
C (TEO-GE)	–	–	–	661.0	156.17	99.83
D (TEO-CHI)	–	–	–	382.54	72.0	54.85
E (NEO-GE)	18.56	11.33	9.28	–	–	–
F (NEO-CHI)	230.63	46.13	n.d	–	–	–

SY: synergism of oils; TEO: turmeric essential oil; NEO: nutmeg essential oil; GE: gelatin (wall material); CHI: chitosan (wall material); n.d: not detected.

behavior is the opposite; the areas in the CHI base are more significant than in the GE base. It is also noted that some analytes were not detected for Samples A and F (n.d.). This occurred due to their low concentration in the sample (small amount of oil extracted and/or incorporated).

Therefore, it is possible to calculate the total percentage of each analyte found in the samples containing the microparticles and express their full charge, as shown Table 6, using the dry mass of the samples and the relative percentage of the identified analytes.

Thus, it can be noted that, for most cases, the analytes are found in a higher percentage when the wall material used was CHI, except for Samples C and D, in which the percentages of the analytes were higher when the wall material used was GE. Considering that the MIC results were better using CHI as the wall material of the microparticles, it makes sense to have found that the identified analytes are in more significant amounts in the samples containing CHI (in general) because the major components present in EOs are responsible for conferring their antimicrobial properties.

Singh et al. (2011) reported that the major components found in TEO were αr-tumerone and curlone, which showed activity against *S. aureus*, for example. The authors justify the action as a function of the high concentration of αr-tumerone. Furthermore, according to Bauer (1985), about 80% of the compounds in nutmeg EO are represented by terpenes such as α- and β-pinene, sabinene, limonene, and 4-terpineol. Other important components are eugenol, elemicin, and safrole, in addition to myristicin.

Finally, a brief comparison can be made between the results obtained using free EOs and microencapsulated oils. For free oils, antimicrobial action was evidenced against most microorganisms tested. In contrast, for encapsulated oils, only antimicrobial activity was noted against strains of *Listeria* (and only in the case of particles with synergism, there was inhibition of strains of *S. aureus* as well). Thus, free oils proved to be more efficient than when encapsulated against *E. coli* and *Salmonella*, as microencapsulation in these cases did not present antimicrobial action. However, the encapsulated oils were able to reduce the MIC values against some *Listeria* strains, and, in the case of the synergism of the encapsulated oils, there was also inhibition of the *S. aureus* strains. However, individually encapsulated oils could not generate antimicrobial action against *S. aureus* strains.

In short, some factors influence the results and explain possible divergences between the literature and the chemical composition of EOs because the amount of components directly interferes with their antimicrobial potential. Furthermore, the chemical composition itself is influenced by aspects such as the extraction method, parts of the plant used, environmental conditions, and seasonality (Probst, 2012). In addition, regarding the production of microparticles, oil release rates in the medium, the possibility of volatilization of essential compounds, encapsulation efficiency, and other factors that may interfere with the final results must be considered.

3.7 Statistical analysis

Concerning the statistical analysis carried out for the commercial oils tested against the strains used in this study, it was found that, for the strains *Salmonella* 1R *and Salmonella* Tiphymurium ATCC 14028, there was a significant difference between the oils. However, for strains *S. aureus* 94, *S. aureus* ATCC 25923, *L. monocytogenes* ATCC 7644, *L. innocua* 4445, and *E. coli* 116, the percentage of both oils used was the same or very close, resulting in MIC values that do not differ significantly from each other, about used oils, also due to the very similar density that the two oils have compared with each other. Finally, for the strains *E. coli* 46, *E. coli* ATCC 25922, *L. innocua* 4422, *S. aureus* A1, *and Salmonella* 2R, it was not possible to perform the statistical analysis because they resulted in indeterminate MIC values (> 10% oil).

CONCLUSION

It can be observed from the results that, in general, free nutmeg EO showed a better antimicrobial effect than free turmeric oil, as it resulted in lower MIC (the percentage of oil required for effect is lower). Furthermore, such antimicrobial action was more effective against Gram-negative microorganisms (*E. coli* and *Salmonella*). Beyond that, the synergism of free oils did not potentiate the antimicrobial action, as they resulted in indifferent and antagonistic effects.

Regarding the alternative of microencapsulation of EOs, it was obtained that the results in which chitosan was used as wall material were more promising than when gelatin was used as wall material. In addition, the antimicrobial action was only evidenced against Gram-positive microorganisms (when using the synergism of oils), but especially against all strains of *Salmonella*.

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REFERENCES

Adams, R. P. (2007). *Identification of essential oil components by gas chromatography/mass spectrometry* (4th Ed.). Allured Publishing Corporation.

Agibert, S. A. C. (2018). *Adição de óleo de amendoim alto oleico encapsulado em chocolate amargo*. Universidade de São Paulo.

Ahmed, S. A., Jabbar, I. I., & Abdul, H. E. (2012). Study the Antibacterial Activity of *Zingiber officinale* roots against Some of Pathogenic Bacteria. *Al-Mustansiriyah Journal of Science*, *23*(3), 63-70.

Akbar, S. (2020). *Handbook of 200 Medicinal Plants: A Comprehensive Review of Their Traditional Medical Uses and Scientific Justifications*. Springer.

Ali, M. S., Haq, M., Roy, V. C., Ho, T. C., Park, J. S., Han, J. M., & Chun, B.-S. (2023). Development of fish gelatin/carrageenan/zein bio-nanocomposite active-films incorporated with turmeric essential oil and their application in chicken meat preservation. *Colloids Surfaces B Biointerfaces*, *226*, 113320. https://doi.org/10.1016/j.colsurfb.2023.113320

Ansory, H. M., Fitriani, I. N., Nilawatii, A. (2020). Chemical Separation and Antibacterial Activity of Nutmeg seed Essential Oil against *Shigella* sp. and *Escherichia coli* ATCC 25922. *IOP Conference Series: Materials Science and Engineering*, *846*(1), 012005. https://doi.org/10.1088/1757-899X/846/1/012005

Antunes, S. A., Robazza, S., Schittler, L., & Gomes, A. (2012). Essential Oil Against Pathogenic Bacteria. *Ciência e Tecnologia de Alimentos*, *32*(3), 525-530. https://doi.org/10.1590/S0101-20612012005000082

Arshad, H., Ali, T. M., Abbas, T., & Hasnain, A. (2018). Effect of Microencapsulation on Antimicrobial and Antioxidant Activity of Nutmeg Oleoresin Using Mixtures of Gum Arabic, OSA, and Native Sorghum Starch. *Starch*, *70*(7-8), 1700320. https://doi.org/10.1002/star.201700320

Ashokkumar, K., Vellaikumar, S., Muthusamy, M., Dhanya, M. K., & Aiswarya, S. (2022). Compositional variation in the leaf, mace, kernel, and seed essential oil of nutmeg (*Myristica fragrans Houtt.*) from the Western Ghats, India. *Natural Product Research*, *36*(1), 432-435. https://doi.org/10.1080/14786419.2020.1771713

Bauer, K. (1985). *Common fragrance and flavor materials: preparation, properties and uses*. VCH Verlagsgesellschaft.

da Rosa, M. C., Iacuzio, R., Barbosa, G. R., Pereira, R. C. L., Cruzado-Bravo, M., Rall, V. L. M., Vallim, D. C., & Silva, N. C. C. (2022). Detection of *Listeria innocua* in the dairy processing chain: resistance to antibiotics and essential oils. *Food Science and Technology*, *42*, e81421. https://doi.org/10.1590/fst.81421

da Silva Cândido, T. J., da Silva, A. C., de Matos, L. G., da Silva do Nascimento, M., Camargo, C. H., Zanella, R. C., Rall, V. L. M., & Silva, N. C. C. (2020). Enterotoxigenic potential and molecular typing of *Staphylococcus sp.* isolated from organic and conventional fresh Minas cheese in the state of São Paulo, Brazil. *International Dairy Journal*, *102*, 104605. https://doi.org/10.1016/j.idairyj.2019.104605

de Almeida, J. M., Crippa, B. L., de Souza, V. V. M. A., Alonso, V. P. P., da Motta, E. S. J., Picone, C. S. F., Prata, A. S., & Silva, N. C. C. (2023). Antimicrobial action of Oregano, Thyme, Clove, Cinnamon, and Black pepper essential oils free and encapsulated against foodborne pathogens. *Food Control*, *144*, 109356. https://doi.org/10.1016/j.foodcont.2022.109356

de Araújo, R. G. M., Assis, D., Lemes, S. R., de Melo-Reis, P. R., de Araújo, L. A., de Paiva, E. S., & Silva, C. B. (2015). Estudo de caso: Avaliação da atividade antimicrobiana do óleo essencial do açafrão (*Curcuma longa*). *Revista EVS - Revista de Ciências Ambientais e Saúde*, *42*(4), 425-431. https://doi.org/10.18224/est.v42i4.4361

Dorman, H., & Deans, S. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, *88*(2), 308-316. https://doi.org/10.1046/j.1365-2672.2000.00969.x

dos Santos, S. M. (2016). *Filmes ativos comestíveis elaborados com óleos essenciais aplicados em maçãs minimamente processadas* (Vol. 147). Universidade Federal do Triângulo Mineiro - UFTM Programa.

Ferreira, D. F. (2019). SISVAR: A computer analysis system for fixed effects split-plot type designs. *Revista Brasileira de Biometria*, *37*(4), 529-535. https://doi.org/10.28951/rbb.v37i4.450

Ferreira, F. D., Mossini, S. A. G., Ferreira, F. M. D., Arrotéia, C. C., Costa, C. L. Da, Nakamura, C. V., & Machinski, M. Jr. (2013). The inhibitory effects of *Curcuma longa* L. essential oil and curcumin on *Aspergillus flavus* Link growth and morphology. *Scientific World Journal*, *2013*, 343804. https://doi.org/10.1155/2013/343804

Fokou, J. B. H., Dongmo, P. M. J., & Boyom, F. F. (2020). Essential oil's chemical composition and pharmacological properties. In H. A. El-Shemy (ed.) *Essential oils-oils of nature* (p. 13). IntechOpen.

Franco, A. L. P., Oliveira, T. B., Ferri, P. H., Bara, M. T. F., & de Paula, J. R. (2007). Avaliação da composição química e atividade antibacteriana dos óleos essenciais de *Aloysia gratissima* (Gillies & Hook) Tronc. (Alfazema), *Ocimum gratissimum* L. (Alfavaca-Cravo) E *Curcuma longa* L. (Açafrão). *Revista Eletrônica de Farmácia*, *4*(2), 208-220. https://doi.org/10.5216/ref.v4i2.3063

Garcia, L. G. S., da Rocha, M. G., Lima, L. R., Cunha, A. P., de Oliveira, J. S., de Andrade, A. R. C., Ricardo, N. M. P. S., Pereira-Neto, W. A., Sidrim, J. J. C., Rocha, M. F. G., Vieira, R. S., & Brilhante, R. S. N. (2021). Essential oils encapsulated in chitosan microparticles against *Candida albicans* biofilms. *International Journal of Biological Macromolecules*, *166*, 621-632. https://doi.org/10.1016/j.ijbiomac.2020.10.220

Gonçalves, N. D., Grosso, C. R. F., Rabelo, R. S., Hubinger, M. D., & Prata, A. S. (2018). Comparison of microparticles produced with combinations of gelatin, chitosan, and gum Arabic. *Carbohydrate Polymers*, *196*, 427-432. https://doi.org/10.1016/j.carbpol.2018.05.027

Grasso, E. D. C., Aoyama, E. M., & Furlan, M. R. (2017). Ação Anti-inflamatória de *Curcuma longa* L. (*Zingiberaceae*). *Revista Eletrônica Thesis*, *14*(28), 117-129.

Hammoud, C. J. (2015). *Investigating the inhibitory effects of free and encapsulated curcumin on foodborne pathogens*. American University of Beirut.

Hu, Y., Zhang, J., Kong, W., Zhao, G., & Yang, M. (2017). Mechanisms of antifungal and anti-aflatoxigenic properties of essential oil derived from turmeric (*Curcuma longa*) on *Aspergillus flavus*. *Food Chemistry*, *220*, 1-8. https://doi.org/10.1016/j.foodchem.2016.09.179

Jansen, P. C. M., & Westphal, E. (Eds.). (1999). *Plant Resources of South-East Asia* (Vol. 1). Backhuys Publishers.

Khorshidian, N., Yousefi, M., Khanniri, E., & Mortazavian, A. M. (2018). Potential application of essential oils as antimicrobial preservatives in cheese. *Innovative Food Science & Emerging Technologies*, *45*, 62-72. https://doi.org/10.1016/j.ifset.2017.09.020

Knezevic, P., Aleksic, V., Simin, N., Svircev, E., Petrovic, A., & Mimica-Dukic, N. (2016). Antimicrobial activity of *Eucalyptus camaldulensis* essential oils and their interactions with conventional antimicrobial agents against multi-drug resistant *Acinetobacter baumannii*. *Journal of Ethnopharmacology*, *178*, 125-136. https://doi.org/10.1016/j.jep.2015.12.008

Kutti Gounder, D., & Lingamallu, J. (2016). Comparison of chemical composition and antioxidant potential of volatile oil from fresh, dried, and cured turmeric (*Curcuma longa*) rhizomes. *Industrial Crops Production*, *38*, 124-131. https://doi.org/10.1016/j.indcrop.2012.01.014

Laranjo, M., Fernández-Léon, A. M., Potes, M. E., Agulheiro-Santos, A. C., & Elias, M. (2017). Use of Essential Oils in Food Preservation. *Antimicrobial Research*, *15*, 71-84.

Lee, H., Choi, K., Cho, K., & Ahn, Y. (2003). Fungicidal activity of ar-turmerone identified in *Curcuma longa* rhizome against six phytopathogenic fungi. *Agricultural Chemistry Biotechnology*, *46*(1), 23-28.

Martins, W. D. S., de Araújo, J. S. F., Feitosa, B. F., Oliveira, J. R., Kotzebue, L. R. V., Agostini, D. L. D. S., de Oliveira, D. L. V., Mazzetto, S. E., Cavalcanti, M. T., & da Silva, A. L. (2021). Lemongrass (*Cymbopogon citratus* DC. Stapf) essential oil microparticles: Development, characterization, and antioxidant potential. *Food Chemistry*, *355*, 129644. https://doi.org/10.1016/j.foodchem.2021.129644

Mendonca, A., Jackson-Davis, A., Moutiq, R., & Thomas-Popo, E. (2018). Use of Natural Antimicrobials of Plant Origin to Improve the Microbiological Safety of Foods. In S. C. Ricke, G. G. Atungulu, C. E. Rainwater, & S. H. Park (ed.). *Food and Feed Safety Systems and Analysis* (p. 249-272). Elsevier.

Mickus, R., Jančiukė, G., Raškevičius, V., Mikalayeva, V., Matulytė, I., Marksa, M., Maciūnas, K., Bernatonienė, J., & Skeberdis, V. A. (2021). The effect of nutmeg essential oil constituents on Novikoff hepatoma cell viability and communication through Cx43 gap junctions. *Biomedicine & Pharmacotherapy*, *135*, 111229. https://doi.org/10.1016/j.biopha.2021.111229

Morasi, R. M., da Silva, A. Z., Nuñez, K. V. M., Dantas, S. T. A., Faganello, C., Juliano, L. C. B., Tiba-Casas, M. R., Pantoja, J. C. F., Amarante, A. F., Júnior, A. F., Rall, V. L. M., & Silva, N. C. C. (2022). Overview of antimicrobial resistance and virulence factors in *Salmonella* spp. isolated in the last two decades from chicken in Brazil. *Food Research International*, *162*(part A), 111955. https://doi.org/10.1016/j.foodres.2022.111955

Narasimhan, B., & Dhake, A. S. (2006). Antibacterial principles from *Myristica fragrans* seeds. *Journal of Medicinal Food*, *9*(3), 395-399. https://doi.org/10.1089/jmf.2006.9.395

Negi, P. S., Chauhan, A. S., Sadia, G. A., Rohinishree, Y. S., & Ramteke, R. S. (2005). Antioxidant and antibacterial activities of various seabuckthorn (*Hippophae rhamnoides* L.) seed extracts. *Food Chemistry*, *92*(1), 119-124. https://doi.org/10.1016/j.foodchem.2004.07.009

No, H. K., Park, N. Y., Lee, S. H., & Meyers, S. P. (2002). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *International Journal of Food Microbiology*, *74*(1-2), 65-72. https://doi.org/10.1016/S0168-1605(01)00717-6

Omoruyi, I. M., & Emefo, O. T. (2012). In vitro evaluation of the antibiogramic activities of the seeds of *Myristica fragrans* on foodborne pathogens. *Malaysian Journal of Microbiology*, *8*(4), 253-258.

Ouedrhiri, W., Mounyr, B., Harki, E. H., Moja, S., & Greche, H. (2017). Synergistic antimicrobial activity of two binary combinations of marjoram, lavender, and wild thyme essential oils. *International Journal of Food Properties*, *20*(12), 3149-3158. https://doi.org/10.1080/10942912.2017.1280504

Özkan, O. E., Olgun, Ç., Güney, B., Gür, M., Güney, K., & Ates, S. (2018). Chemical composition and antimicrobial activity of *Myristica fragrans* & *Elettaria cardamomum* essential oil. *Kastamonu Üniversitesi Orman Fakültesi Derg*, *18*(2), 225-229. https://doi.org/10.17475/kastorman.356765

Paulo, B. B., Schmiele, M., Maximo, G. J., & Prata, A. S. (2019). Carnauba Wax Particles: Investigation of Dripping and Cold-Extrusion Processes. *Journal of American Oil Chemists' Society*, *96*(7), 847-859. https://doi.org/10.1002/aocs.12224

Prata, A. S., & Grosso, C. R. F. (2015). Production of microparticles with gelatin and chitosan. *Carbohydrate Polymers*, *116*, 292-299. https://doi.org/10.1016/j.carbpol.2014.03.056

Probst, I. D. S. (2012). *Atividade antibacteriana de óleos essenciais e avaliação de potencial sinergético*. Universidade Estadual Paulista.

Singh, S., Rajesh, B. S. S., Sahoo, K., Subudhi, E., & Nayak, S. (2011). Chemical Composition of Turmeric Oil (*Curcuma longa* L. cv. Roma) and its Antimicrobial Activity against Eye Infecting Pathogens. *Journal of Essential Oil Research*, *23*(6), 11-18. https://doi.org/10.1080/10412905.2011.9712275

Smith-Palmer, A., Stewart, J., & Fyfe, L. (1998). Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Letters Applied Microbiology*, *26*(2), 118-122. https://doi.org/10.1046/j.1472-765x.1998.00303.x

Takikawa, A., Abe, K., Yamamoto, M., Ishimaru, S., Yasui, M., Okubo, Y., & Yokoigawa, K. (2002). Antimicrobial Activity of Nutmeg against *Escherichia coli 0 157*. *Journal of Bioscience and Bioengineering*, *94*(4), 315-320. https://doi.org/10.1016/S1389-1723(02)80170-0

Taylor, T. M. (2018). Natural Food Antimicrobials: Recent Trends in Their Use, Limitations, and Opportunities for Their Applications in Food Preservation. In X. Fan, H. Ngo, & C. Wu (ed.). *Natural and Bio-Based Antimicrobials for Food Applications* (p. 25-43). American Chemical Society Symposium Series.

Thileepan, T., Thevanesam, V., & Kathirgamanathar, S. (2017). Antimicrobial Activity of Seeds and Leaves of *Myristica fragrans* against Multi-resistant Microorganisms. *Journal of Agricultural Science and Technology*, *7*(5), 302-308. https://doi.org/10.17265/2161-6256/2017.05.002

Valdivieso-Ugarte, M., Gomez-Llorente, C., Plaza-Díaz, J., & Gil, Á. (2019). Antimicrobial, antioxidant, and immunomodulatory properties of essential oils: A systematic review. *Nutrients*, *11*(11), 2786. https://doi.org/10.3390/nu11112786

Wang, Y., Lu, Z., Wu, H., & Lv, F. (2009). Study on the antibiotic activity of microcapsule curcumin against foodborne pathogens. *International Journal of Food Microbiology*, *136*(1), 71-74. https://doi.org/10.1016/j.ijfoodmicro.2009.09.001