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Evaluation of essential oils from *Aloysia polystachya*, *Citrus limon*, *Eucalyptus cinerea*, and *Laurus nobilis*, and (R)-(–)-carvone as natural preservatives in Vienna sausages

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ABSTRACT
Laurus nobilis, *Citrus limon*, *Eucalyptus cinerea* and *Aloysia polystachya* essential oils, and pure (R)-(–)-carvone compound are potential bactericides against the growth of *Leuconostoc mesenteroides* MS1. This strain can alter Vienna sausages by growing under anaerobic conditions, producing gas and causing packages to swell. The objectives of this work were to determine the minimum inhibitory and the minimum bactericidal concentrations of essential oils from *Laurus nobilis*, *Citrus limon*, *Eucalyptus cinerea*, *Aloysia polystachya* essential oils, and (R)-(–)-carvone, using the broth macrodilution technique, and to evaluate their effect on the sensitivity of *Leuconostoc mesenteroides* MS1 in the food matrix during 42 days. Low minimum inhibitory and bactericidal concentrations of the essential oils and pure compound were found, indicating that *Leuconostoc mesenteroides* MS1 is sensitive to them. Furthermore, 0.70 mL of *Aloysia polystachya* and 0.50 mL of *Eucalyptus cinerea*, *Laurus nobilis* and (R)-(–)-carvone were found to maintain viable cells of *Leuconostoc mesenteroides* MS1 at the initial count of 10^3 CFU g^{-1} in 2.5 g of sterile sausage samples.

Keywords: *Leuconostoc mesenteroides* MS1, control of microorganisms in food, antimicrobial activity, sensitivity tests, viable cell count, anaerobic conditions

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RESUMEN
Los aceites esenciales de *Laurus nobilis*, *Citrus limon*, *Eucalyptus cinerea* y *Aloysia polystachya*, junto con el compuesto puro (R)-(–)-carvona, son

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potenciales bactericidas contra el crecimiento de *Leuconostoc mesenteroides* MS1. Esta cepa puede alterar salchichas tipo Viena al crecer en condiciones anaeróbicas, produciendo gas y provocando que los paquetes se hinchen. Los objetivos de este trabajo fueron determinar las concentraciones inhibitorias y bactericidas mínimas de los aceites esenciales de *Laurus nobilis*, *Citrus limon*, *Eucalyptus cinerea*, *Aloysia polystachya,* y del componente puro (R)-(−)-carvona, utilizando la técnica de macrodilución en caldo, y evaluar su efecto sobre la sensibilidad de *Leuconostoc mesenteroides* MS1 en la matriz alimentaria durante 42 días. Se encontraron concentraciones inhibitorias y bactericidas mínimas bajas de los aceites esenciales y del compuesto puro, lo que indica que *Leuconostoc mesenteroides* MS1 es sensible a ellos. Se encontró que 0,70 mL de *Aloysia polystachya* y 0,50 mL de *Eucalyptus cinerea*, *Laurus nobilis* y (R)-(−)-carvona fueron capaces de mantener las células viables de *Leuconostoc mesenteroides* MS1 en el recuento inicial de 10^3 CFU g^{-1} en una muestra de 2,5 g de salchicha estéril.

Palabras clave: *Leuconostoc mesenteroides* MS1, control de microorganismos en alimentos, actividad antimicrobiana, pruebas de sensibilidad, recuento de células viables, condiciones anaeróbicas

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INTRODUCTION

The presence of *Leuconostoc* genus in vacuum-packed cooked sausages has been documented in the literature. This genus has been found in low concentrations in the raw meat and on the surfaces of the processing plant. However, according to Hultman et al. (2015), its concentration increased by almost 98 % in cooked sausages. The microorganisms frequently associated with the spoilage of vacuum-packed sausages are lactic acid bacteria, mainly *Lactobacillus*, *Leuconostoc* and *Oenococcus* (Cejas Farias et al., 2018). In addition, the increase of CO_2 concentration in packages during storage can be attributed to the heterofermentative metabolism of *Lactobacillus* and *Leuconostoc* (Korkeala and Björkroth, 1997).

Padilla-Frausto et al. (2015) identified *Leuconostoc* bacteria in a Vienna sausage processing plant, where the equipment and the conveyor belts were identified as contamination sources. Some *Leuconostoc* strains were isolated from the equipment and the sausage surfaces showed the same genotype.

According to studies conducted by Diez et al. (2009), the species of *Leuconostoc* genus generally grow fast, producing a pH drop (due to the appearance of aldehydes and acids) which results in a milky exudate and sour aroma.

Natural extracts are widely used for their antimicrobial properties (Hammer et al., 1999). Additionally, interest in these types of preservative agents has increased due to consumer demand for healthier food options (Calo et al., 2015). Essential oils are secondary metabolites that participate in processes beyond the primary physiological functions of cell metabolism and reproduction. These secondary metabolites extend their biological effect to organisms other than their source, which

turns them valuable for the pharmaceutical, food and fine chemical industries. In this sense, the importance of finding essential oils to control microbial growth in food, particularly meat-derived products, lies in their potential to act as natural and sustainable preservatives. Essential oils possess antimicrobial properties that can inhibit the growth of pathogenic and spoilage microorganisms, thereby extending the shelf-life of food products and ensuring their safety for consumption. This is especially critical in meat products, which are highly perishable and prone to contamination.

Recent literature demonstrates the effective use of essential oils as potential bactericides in various food matrices (Bermúdez-Vásquez et al., 2019; Enríquez et al., 2024; Ruiz-Navajas et al., 2012; Vignola et al., 2020; Viuda-Martos et al., 2008).

In this context, the application of natural extracts in the inhibition of *Leuconostoc mesenteroides* has been reported by several authors (Fernández-López et al., 2005; Khorsandi et al., 2018; Kivanç et al., 1991; Michelena et al., 2005; Radha Krishnan et al., 2014). In previous studies *Aloysia polystachya*, *Citrus limon, Eucalyptus cinerea* and *Laurus nobilis* essential oils (Serra et al., 2018) and (R)-(−)-carvone (Serra, 2022) proved to be promising in the inhibition of spoilage bacteria in Vienna sausages. Following this assessment, this work had two aims. Firstly, to determine the minimum inhibitory concentrations (MIC) and the minimum bactericidal concentrations (MBC) of *Aloysia polystachya*, *Citrus limon, Eucalyptus cinerea* and *Laurus nobilis* essential oils, and (R)-(−)-carvone pure component. Secondly, to evaluate the effectiveness of *Aloysia polystachya*, *Eucalyptus cinerea* and *Laurus nobilis* essential oils, and (R)-(−)-carvone in the food matrix, against the strain *Leuconostoc mesenteroides* MS1. It is important to note that (R)-(−)-carvone is the main component of *Aloysia polystachya* essential oil and is the only essential oil in this study containing this compound.

MATERIALS AND METHODS

Materials

The essential oils used in this work were obtained by steam stripping from leaves of *Aloysia polystachya*, *Eucalyptus cinerea* and *Laurus nobilis* and peels of *Citrus limon,* using semi-laboratory scale equipment (Figmay, Argentina) for 60 min after boiling started. Before extraction, the vegetal material was ground using a grinder at maximum speed (Moulinex, 750 W) for 2 min. The composition of each essential oil was previously reported by Serra et al. (2018).

(R)-(−)-carvone (Sigma-Aldrich, 98 %), the major compound in *Aloysia polystachya* essential oil, accounts for 90.2 % of its composition. In addition to the essential oils, this pure compound was also evaluated. Bacterial isolation, biochemical and molecular typification of *Leuconostoc mesenteroides* MS1 were described in a previous work (Serra et al., 2018). *Leuconostoc mesenteroides* MS1 is stored as frozen stock (-80 °C) in de Man, Rogosa and Sharpe (MRS) broth (Britania, Buenos Aires, Argentina) using 15 % (v/v) glycerol as cryoprotectant, being routinely reactivated overnight (16–18 h) at 30 °C in MRS broth and counted in MRS agar (after 48 h at 30 °C).

The broth macrodilution technique

A series of screw-cap test tubes containing different volumes of MRS broth was prepared to achieve the required dilutions, into which the antimicrobial agent (essential oil or (R)-(−)-carvone) was added. Given the insolubility between the essential oil and the aqueous culture medium, an emulsion was prepared with each antimicrobial. To achieve this emulsion, the essential oils or (R)-(−)-carvone were mixed with the supernatant of the mixture of soy lecithin in distilled water at 2 % (p/v). The initial concentration of the essential oil or (R)-(−)-carvone was calculated in % w/v. The successive dilutions were prepared considering a minimum final volume required in each tube of 1 mL. In addition, a control tube containing only the culture medium was used.

Then, each tube was inoculated with a standardized suspension of *Leuconostoc mesenteroides* MS1. This inoculation was prepared following the direct suspension of colonies in saline solution (0.85 % NaCl) to obtain a turbidity of 0.50 on the McFarland scale, which is equivalent to 1.50×10^8 CFU mL^{-1} concentration.

Minimum inhibitory and minimum bactericidal concentrations

The minimum inhibitory concentration (MIC) value is defined as the lowest concentration of the antimicrobial agent evaluated that inhibits the visible growth of the microorganism studied. Determination of the minimum bactericidal concentration (MBC) is the most common estimation of bactericidal activity, being defined as the lowest concentration of antimicrobial agent necessary to kill 99.90 % of the final inoculum after incubating each microorganism (Balouiri et al., 2016). Both values were determined by the broth macrodilution technique, reported by

Araújo et al. (2018), with some modifications.

The tests were examined after 48 h of incubation at 30 °C. After that period, the presence or absence of microbial growth in the tubes was observed and the MIC and MBC were determined. When there was no growth and no turbidity was evidenced, a sample was taken from the tube with a hook, and streak plating was then performed. These plates were incubated at 30 °C for 48 h, and the growth or non-growth of the bacteria was observed. From these observations, the MIC and the MBC values were obtained as detailed in Figure 1, which represents a schematic diagram of the macrodilution broth method used.

The tests were carried out in triplicate for each dilution in each essential oil and (R)-(−)-carvone.

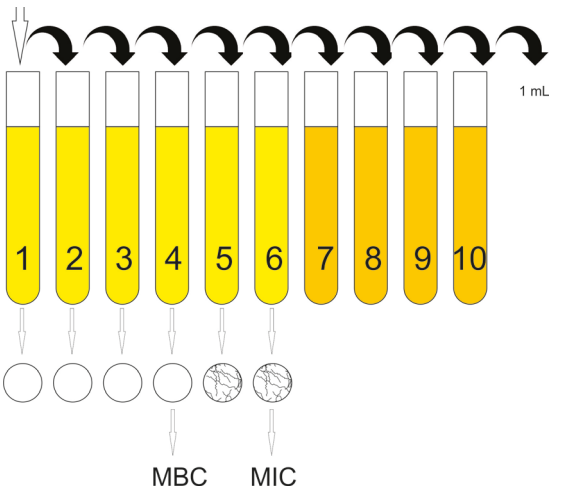


Figure 1. Schematic representation of macrodilution broth method

Sample conditioning of food matrix

The sensitivity tests of essential oils and (R)-(−)-carvone in the food matrix were carried out. The commercial sausages were cut in slices of approximately 2.5 g and sterilized for 15 min at 121 °C. After that, each sample was placed under aseptic conditions in sterile bags. Each bag was inoculated with 0.20 mL of an overnight culture dilution of *Leuconostoc mesenteroides* MS1 (initial concentration of 10^3 CFU g^{-1}) and treated withes 0.10 mL, 0.50 mL or 0.70 mL of each antimicrobial agent (essential oil or (R)-(−)-carvone). As controls, three assays were conducted: (i) the sterile sausage sample, (ii) the sterile sausage sample with antimicrobial agent but without *Leuconostoc mesenteroides* MS1 inoculation, (iii) the sterile sausage sample inoculated with *Leuconostoc mesenteroides* MS1 but without antimicrobial agent. All bags were refrigerated at 4 °C until the microbiological analysis was carried out at 0, 2, 7, 14, 21, 28, 35, and 42 days, according to the procedure proposed by Pesavento et al. (2015).

Each sample was analyzed microbiologically in duplicate on the aforementioned days. To this end, the sample was homogenized for 1 min with 25 mL of peptone solution (hydrolyzed meat peptide, Britania, Buenos Aires, Argentina) and decimal dilutions were plated on MRS agar (Šojić et al., 2015), and incubated for 48 h at 30 °C.

RESULTS AND DISCUSSION

Minimum inhibitory concentration and minimum bactericidal concentration

Different dilutions of the essential oils (*Aloysia polystachya*, *Citrus limon, Eucalyptus cinerea* and *Laurus nobilis*) and the pure (R)-(−)-carvone compound were tested to determine the MIC and MBC values. The values for the essential oils and (R)-(−)-carvone were found in the range of 0.64-1.87 mg/mL and 0.76-2.41 mg/mL, respectively (Table 1). MIC and MBC values of essential oils and (R)-(−)-carvone were low, which means that they are good inhibitors/bactericides against *Leuconostoc mesenteroides* MS1. However, it was observed that the bacteria under study were more sensitive to the essential oils of *Aloysia polystachya*, *Eucalyptus cinerea*, *Laurus nobilis* and the pure (R)-(−)-carvone compound than to the *Citrus limon* essential oil. As expected, the essential oil of *Aloysia polystachya* and the pure (R)-(−)-carvone compound showed similar results, since the latter makes up 90.2 % of the composition of the essential oil (Serra et al., 2018). *Citrus limon* essential oil has lower bactericidal effectiveness compared to other essential oils due to its chemical composition, which is dominated by limonene (Serra et al., 2018), a monoterpene that, although it possesses antimicrobial properties, is not as potent as other compounds found in more effective essential oils (Kačániová et al., 2024; Viuda-Martos et al., 2008; Yazgan et al., 2019).

Antimicrobial activity in food matrix

Table 2 shows the growth of *Leuconostoc mesenteroides* MS1 in 2.5 g of fresh Vienna sausage containing the different essential oils or (R)-(−)-carvone over the successive days of the study. As stated earlier, the initial concentration of the inoculum was in the order of 10^3 CFU g^{-1}. As expected, no bacterial growth was observed in the samples of (i) sterile sausage

Table 1. MIC and MBC values* of different essential oils and (R)-(−)-carvone

	MIC mg/mL	MBC mg/mL
Aloysia polystachya	0.64 ± 0.01	0.76 ± 0.02
Citrus limon	1.87 ± 0.02	2.41 ± 0.10
Eucalyptus cinerea	0.75 ± 0.04	1.10 ± 0.12
Laurus nobilis	0.65 ± 0.02	1.32 ± 0.31
(R)-(−)-carvone	0.66 ± 0.01	0.77 ± 0.02

*The values are expressed as the average ± the standard deviation obtained from the triplicate at each dilution.

and (ii) sterile sausage with antimicrobial aggregate but without inoculation, during all the experiments. On the other hand, the sample (iii) sterile sausage inoculated with *Leuconostoc mesenteroides* MS1 but without antimicrobial addition, showed an increasing count up to 10^8 CFU g^{-1}, which remains until the 42 days (Figure 2).

Figure 2 (a) shows the behavior of the samples treated with 0.10, 0.50 and 0.70 mL of *Aloysia polystachya* essential oil. The samples with 0.1 mL of *Aloysia polystachya* essential oil had a similar development to the inoculated sample used as control, reaching 10^7 CFU g^{-1}. On the other hand, the samples treated with 0.50 mL of *Aloysia polystachya* essential oil showed an increase of *Leuconostoc mesenteroides* MS1 up to 10^4 CFU g^{-1}, decreasing to 10^3 CFU g^{-1} at 42 days. Finally, the addition of 0.70 mL of *Aloysia polystachya* essential oil maintained the bacterial count constant at 10^3 CFU g^{-1} from the beginning to the end of the study. Figure 2 (b) shows the samples with 0.10 and 0.50 mL of (R)-(−)-carvone. The samples with 0.10 mL of (R)-(−)-carvone reached 10^7 CFU g^{-1} at 42 days, while those containing 0.50 mL of (R)-(−)-carvone maintained the initial count concentration (10^3 CFU g^{-1}) until the end of experiment (42 days). Based on the results obtained with *Aloysia polystachya* essential oil and (R)-(−)-carvone, *Laurus nobilis* and *Eucalyptus cinerea* were tested using only 0.50 mL. The count remained at 10^3 CFU g^{-1}, effectively preventing the growth of *Leuconostoc mesenteroides* MS1 as seen in Figure 2 (c) for both essential oils throughout the experiment (42 days).

As reported by Burt (2004), to achieve the same effect observed for the MIC, a higher concentration of the antimicrobial agent must be used when tested in the food matrix. The same results were found in this work, where the concentration used in the food matrix was in the order of fifty times the MIC values using 0.50 mL of Eucalyptus *cinerea, Laurus nobilis* and (R)-(−)-carvone and 75 times the MIC value using 0.70 mL of *Aloysia polystachya*.

The bactericidal activity of the essential oils and (R)-(−)-carvone used in this work was previously reported in the literature. For example, Akgül et al. (1989) found antimicrobial activity in *Laurus nobilis* essential oil against *Enterobacter aerogenes* and *Streptococcus faecalis,* while Biondi et al. (1993) reported the effectiveness of this agent against *Streptococcus faecalis*, *Staphylococcus aureus*, *Micrococcus luteus, Bacillus subtilis, Escherichia coli*, *Hafnia alvei* and *Proteus vulgaris*. Regarding the antimicrobial activity of *Citrus limon,* Fisher and Phillips (2006) found this essential oil effective against *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* O157 and *Campylobacter jejuni*. On the other hand, Settanni et al. (2012) reported the effectiveness of this agent against *Listeria monocytogenes* and *Staphylococcus aureus* and no antimicrobial activity against *Salmonella*.

Based on the available literature, no antimicrobial activity against bacteria has been previously reported for *Aloysia polystachya* essential oil. Regarding *Eucalyptus* spp. essential oil, its antibacterial, fungicidal, antiseptic and pest control properties have been demonstrated (Batish et al., 2008). Finally, Aggarwal et al. (2002) reported activity in gram-positive and gram-negative

Table 2. Time-course of *Leuconostoc Mesenteroides* MS1 growth in fresh Vienna sausage containing the different essential oils or (R)-(−)-carvone

Day	w/o EO	*A. Polystachya*			(R)-(−)-carvone		*L. Nobilis*	*E. Cinerea*
		0.10 mL	0.50 mL	0.70 mL	0.10 mL	0.50 mL	0.50 mL	0.50 mL
	CFU g^{-1}*		CFU g^{-1}*		CFU g^{-1}*		CFU g^{-1}*	CFU g^{-1}*
0	3.63×10^3	4.07×10^3	2.75×103^3	1.65×10^3	6.16×103	3.30×10^3	1.10×10^3	2.20×10^3
2	3.85×10^5	3.52×10^5	7.59×10^3	1.52×10^3	9.24×104	3.06×10^3	1.37×10^3	3.52×10^3
7	8.14×10^6	2.48×10^7	2.35×10^5	1.10×10^3	1.20×10^6	3.14×10^3	1.79×10^3	3.85×10^3
14	1.00×10^8	6.00×10^7	1.00×10^5	1.28×10^3	3.05×10^6	3.01×10^3	1.23×10^3	2.75×10^3
21	1.00×10^8	4.85×10^6	8.00×10^4	1.08×10^3	5.06×10^6	1.84×10^3	9.90×10^2	2.08×10^3
28	6.49×10^7	3.85×10^6	6.40×10^4	1.06×10^3	1.35×10^6	1.38×10^3	1.01×10^3	1.34×10^3
35	1.03×10^8	2.59×10^6	5.28×10^4	9.57×10^2	4.23×10^6	1.09×10^3	1.05×10^3	1.29×10^3
42	5.50×10^7	3.63×10^6	9.90×10^3	8.25×10^2	2.87×10^6	9.13×10^2	9.68×10^2	1.14×10^3

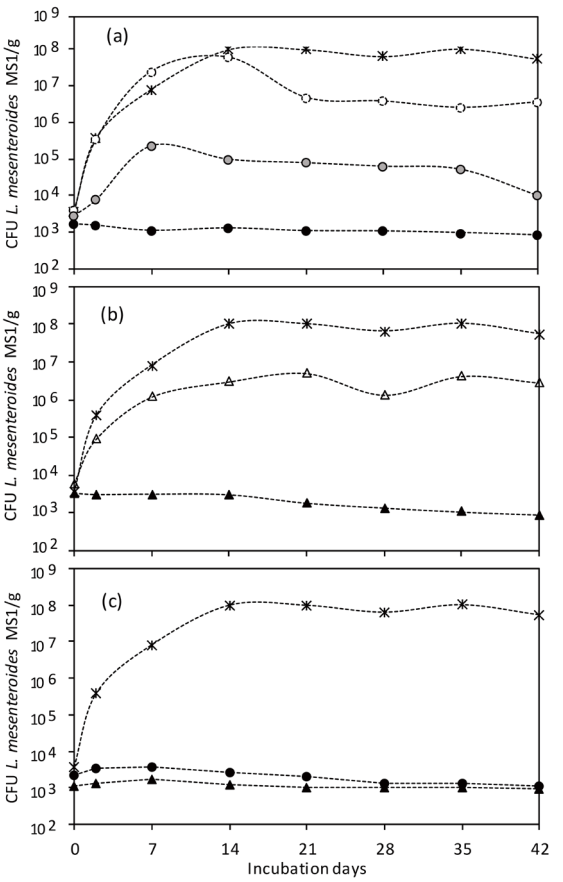


Figure 2. Growth of *Leuconostoc mesenteroides* MS1 in Vienna sausage samples treated with different antimicrobial agents: (a) ○ 0.10, ◍ 0.50, ● 0.70 mL of *Aloysia polystachya* essential oil; (b) Δ 0.10, ▲ 0.50 mL of (R)-(−)-carvone; (c) ▲0.50 mL of *Laurus nobilis* or ● *Eucalyptus cinerea*. The control ✳ correspond to the sterile sausage sample inoculated with *Leuconostoc mesenteroides* MS1 without antimicrobial addition.

bacteria for (R)-(−)-carvone.

CONCLUSIONS

The minimum inhibitory and minimum bactericidal concentrations of *Aloysia polystachya*, *Eucalyptus cinerea*, *Laurus nobilis* and *Citrus limon* essential oils and (R)-(−)-carvone were evaluated against the growth of *Leuconostoc mesenteroides* MS1. This strain acts as a spoilage microorganism in vacuum-packed Vienna sausages, causing packaging swelling after two to three weeks of refrigerated storage. Low values of minimum inhibitory concentrations and minimum bactericidal concentrations of the essential oils and of the pure compound evaluated have been found, which means that the bacteria under study are sensitive to them. It was found that *Leuconostoc mesenteroides* MS1 is more sensitive to *Aloysia polystachya*, *Laurus nobilis*, *Eucalyptus cinerea* essential oils and pure (R)-(−)-carvone compound than to *Citrus limon.*

The assays in the sausage matrix of 2.5 g showed a constant bacterial count, in the order of 10^3 CFU g^{-1}, using 0.70 mL of *Aloysia polystachya* or 0.50 mL of *Eucalyptus cinerea*, *Laurus nobilis,* or (R)-(−)-carvone. Approximately 50 and 75 times the MIC values were needed to achieve the antimicrobial activity desired in the food matrix system studied, using 0.50 mL of *Eucalyptus cinerea, Laurus nobilis* and (R)-(−)-carvone, and using 0.70 mL of *Aloysia polystachya,* respectively.

Regarding the *Leuconostoc mesenteroides* MS1 counts during the experiment, the results obtained were in line with expectations based on the concentrations and types of essential oils used. A significant reduction in microorganisms was observed in the samples treated with essential oils, particularly at higher concentrations, confirming their antimicrobial potential. However, in samples with lower concentrations of essential oil or (R)-(−)-carvone, *Leuconostoc mesenteroides* MS1 counts were higher (10^8 CFU g^{-1}), suggesting that longer exposure times or higher concentrations may be necessary to achieve effective control, particularly in meat products.

Aloysia polystachya, *Eucalyptus cinerea, Laurus nobilis* essential oils and (R)-(−)-carvone represent an alternative to the common chemical-synthesis food preservatives against common spoilage bacteria found in Vienna sausages, particularly *Leuconostoc mesenteroides* MS1.

A potential application of the essential oils or pure (R)-(−)-carvone compound evaluated in this study lies in their incorporation into active packaging systems, particularly designed for Vienna sausages and other meat products. This approach represents an innovative solution to extend the shelf-life of the product by inhibiting the growth of spoilage microorganisms such as *Leuconostoc mesenteroides* MS1 under refrigerated storage conditions. Integrating these antimicrobial compounds into polymeric matrices (encapsulation technologies), biodegradable films, or edible coatings could not only improve the microbiological quality of the food but also reduce the need for conventional chemical additives, addressing consumer demands for more natural and safer food products. Additionally, the incorporation of essential oils into active packaging systems could enable the gradual release of active compounds, maintaining their efficacy throughout the product

shelf-life. This feature is particularly relevant for highly sensitive products, such as vacuum-packed sausages, which are prone to swelling caused by heterofermentative lactic bacteria.

In the long term, this strategy could contribute to the development of a more sustainable food supply chain by promoting the use of natural compounds and reducing reliance on synthetic preservatives. Therefore, further studies aimed at optimizing concentrations, encapsulation techniques, and sensory evaluation of treated products represent key steps toward translating these findings into practical and viable applications in the food industry.

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REFERENCES

Aggarwal, K. K., Khanuja, S. P. S., Ahmad, A., Santha Kumar, T. R., Gupta, V. K., and Kumar, S. (2002). Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of Mentha spicata and Anethum sowa. *Flavour and Fragrance Journal*, *17*, 59–63. https://doi.org/10.1002/ffj.1040

Akgül, A., Kivanç, M., and Bayrak, A. (1989). Chemical composition and antimicrobial effect of Turkish laurel leaf oil. *Journal of Essential Oil Research*, *1*(6), 277–280. https://doi.org/10.1080/10412905.1989.9697798

Araújo, M. K., Gumiela, A. M., Bordin, K., Luciano, F. B., and Macedo, R. E. F. (2018). Combination of garlic essential oil, allyl isothiocyanate, and nisin Z as bio-preservatives in fresh sausage. *Meat Science*, *143*, 177–183. https://doi.org/10.1016/j.meatsci.2018.05.002

Balouiri, M., Sadiki, M., and Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Batish, D. R., Singh, H. P., Kohli, R. K., and Kaur, S. (2008). Eucalyptus essential oil as a natural pesticide. *Forest Ecology and Management*, *256*, 2166–2174. https://doi.org/10.1016/j.foreco.2008.08.008

Bermúdez-Vásquez, M. J., Granados-Chinchilla, F., and Molina, A. (2019). Chemical composition and antimicrobial activity of the essential oil of Psidium guajava and Cymbopogon citratus. *Agronomía Mesoamericana*, *30*, 147–163. https://doi.org/10.15517/am.v30i1.33758

Biondi, D., Cianci, P., Geraci, C., Ruberto, G., and Piattelli, M. (1993). Antimicrobial activity and chemical composition of essential oils from sicilian aromatic plants. *Flavour and Fragrance Journal*, *8*(6), 331–337. https://doi.org/10.1002/ffj.2730080608

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods–a review. *International Journal of Food Microbiology*, *94*(3), 223–53. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Calo, J. R., Crandall, P.G., O'Bryan, C. A., and Ricke, S. C. (2015). Essential oils as antimicrobials in food systems – A review. *Food Control*, 54, 111–119. https://doi.org/10.1016/j.foodcont.2014.12.040

Cejas Farias, T. K., Navarro Villarruel, C. L., Avila Novoa, M. G., Gutiérrez Lomelí, M., and Frausto, J. P. (1st, 2nd and 3rd of November, 2018). Parámetros biocinéticos de BAL tras la reproducción del deterioro de salchichas empacadas al vacío, in: XX Congreso Internacional - Inocuidad de Alimentos - XXXV Reunión Nacional de Microbiología, Higiene y Toxicología de Los Alimentos, Nuevo Vallarta, México.

Diez, A. M., Jaime, I., and Rovira, J. (2009). The influence of different preservation methods on spoilage bacteria populations inoculated in morcilla de Burgos during anaerobic cold storage. *International Journal of Food Microbiology*, *132*(2-3), 91–99. https://doi.org/10.1016/j.ijfoodmicro.2009.03.019

Enríquez, M., Serrano, G., Cuadrado, D., and Ricaurte, P. (2024). Efecto de los aceites esenciales de plantas aromáticas en la conservación de embutidos. *Revista de la Sociedad Científica del Paraguay*, *29*, 196–225. https://doi.org/10.32480/rscp.2024.29.1.196

Fernández-López, J., Zhi, N., Aleson-Carbonell, L., Pérez-Álvarez, J. A., and Kuri, V. (2005). Antioxidant and antibacterial activities of natural extracts: Application in beef meatballs. *Meat Science*, *69*(3), 371–380. https://doi.org/10.1016/j.meatsci.2004.08.004

Fisher, K. and Phillips, C. A. (2006). The effect of lemon, orange and bergamot essential oils and their components on the survival of *Campylobacter jejuni, Escherichia coli O157, Listeria monocytogenes, Bacillus cereus* and *Staphylococcus aureus* in vitro and in food systems. *Journal of Applied Microbiology*, *101*(6), 1232–1240. https://doi.org/10.1111/j.1365-2672.2006.03035.x

Hammer, K. A., Carson, C. F., and Riley, T. V. (1999). Antimicrobial activity of essential oils and other

plant extracts. *Journal of Applied Microbiology*, *86*(6), 985–990. https://doi.org/10.1046/j.1365-2672.1999.00780.x

Hultman, J., Rahkila, R., Ali, J., Rousu, J., and Björkroth, K. J. (2015). Meat processing plant microbiome and contamination patterns of cold-tolerant bacteria causing food safety and spoilage risks in the manufacture of vacuum-packaged cooked sausages. *Applied and Environmental Microbiology*, *81*(20), 7088–7097. https://doi.org/10.1128/AEM.02228-15

Kačániová, M., Čmiková, N., Vukovic, N. L., Verešová, A., Bianchi, A., Garzoli, S., Ben Saad, R., Ben Hsouna, A., Ban, Z., and Vukic, M. D. (2024). Citrus limon Essential Oil: Chemical Composition and Selected Biological Properties Focusing on the Antimicrobial (In Vitro, In Situ), Antibiofilm, Insecticidal Activity and Preservative Effect against Salmonella enterica Inoculated in Carrot. *Plants*, *13*(4), 524. https://doi.org/10.3390/plants13040524

Khorsandi, A., Ziaee, E., Shad, E., Razmjooei, M., Hadi Eskandari, M., and Aminlari, M. (2018). Antibacterial Effect of Essential Oils against Spoilage Bacteria from Vacuum-Packed Cooked Cured Sausages. *Journal of Food Protection*, *81*(8), 1386–1393. https://doi.org/10.4315/0362-028X.JFP-17-474

Kivanç, M., Akgül, A., and Doğan, A. (1991). Inhibitory and stimulatory effects of cumin, oregano and their essential oils on growth and acid production of *Lactobacillus plantarum* and *Leuconostoc mesenteroides. International Journal of Food Microbiology*, *13*, 81–85. https://doi.org/10.1016/0168-1605(91)90140-K

Korkeala, H. J. and Björkroth, K. J. (1997). Microbiological Spoilage and Contamination of Vacuum-Packaged Cooked Sausages. *Journal of Food Protection*, *60*(6), 724–731. https://doi.org//10.4315/0362-028X-60.6.724

Michelena, G., Almeida, G., Altuna, B., Eng, F., Legrá, S., Oliveros, M., and Armenteros, S. (2005). Efecto inhibidor del ácido jasmónico sobre el crecimiento de bacterias y hongos. *ICIDCA. Sobre los Derivados de la Caña de Azúcar, 39,* 3–7. https://www.redalyc.org/articulo.oa?id=223120688001

Padilla-Frausto, J. J., Cepeda-Márquez, L. G., Salgado, L. M., Iturriaga, M. H., and Arvizu-Medrano, S. M. (2015). Detection and Genotyping of *Leuconostoc spp.* in a Sausage Processing Plant. *Journal of Food Protection*, *78*(12), 2170–2176. https://doi.org/10.4315/0362-028X.JFP-15-192

Pesavento, G., Calonico, C., Bilia, A. R., Barnabei, M., Calesini, F., Addona, R., Mencarelli, L., Carmagnini, L., Di Martino, M. C., and Lo Nostro, A. (2015). Antibacterial activity of Oregano, Rosmarinus and *Thymus* essential oils against *Staphylococcus aureus* and *Listeria monocytogenes* in beef meatballs. *Food Control*, *54*, 188–199. https://doi.org/10.1016/j.foodcont.2015.01.045

Radha Krishnan, K., Babuskin, S., Azhagu Saravana Babu, P., Sasikala, M., Sabina, K., Archana, G., Sivarajan, M., and Sukumar, M. (2014). Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *International Journal of Food Microbiology*, *171*(3), 32–40. https://doi.org/10.1016/j.ijfoodmicro.2013.11.011

Ruiz-Navajas, Y., Viuda-Martos, M., Sendra, E., Pérez-Álvarez, J. A., and Fernández-López, J. (2012). Chemical characterization and antibacterial activity of *Thymus moroderi* and *Thymus piperella* essential oils, two *Thymus* endemic species from southeast of Spain. *Food Control*, *27*(2), 294–299. https://doi.org/10.1016/j.foodcont.2012.04.005

Serra, M. A., Garnero, J., Nicolau, V., and Andreatta, A. E. (2018). Assessment of natural vegetal extracts in the inhibition of *Leuconostoc mesenteroides MS1. AgriScientia*, *35*(1), 51–62. https://doi.org//10.31047/1668.298x.v1.n35.20454

Serra, M. A. (2022). *Inhibición de bacterias del género* Leuconostoc *a partir del uso de extractos vegetales naturales*. Unpublished doctoral tesis, Universidad Nacional del Litoral, Santa Fe, Argentina.

Settanni, L., Palazzolo, E., Guarrasi, V., Aleo, A., Mammina, C., Moschetti, G., and Germanà, M. A. (2012). Inhibition of foodborne pathogen bacteria by essential oils extracted from citrus fruits cultivated in Sicily. *Food Control*, *26*(2), 326–330. https://doi.org/10.1016/j.foodcont.2012.01.050

Šojić, B., Tomović, V., Kocić-Tanackov, S., Škaljac, S., Ikonić, P., Džinić, N., Živković, N., Jokanović, M., Tasić, T., and Kravić, S. (2015). Effect of nutmeg (*Myristica fragrans*) essential oil on the oxidative and microbial stability of cooked sausage during refrigerated storage. *Food Control*, *54*, 282–286. https://doi.org/10.1016/j.foodcont.2015.02.007

Vignola, M. B., Serra, M. A., and Andreatta, A. E. (2020). Actividad Antimicrobiana de Diversos Aceites Esenciales en Bacterias Benéficas, Patógenas y Alterantes de Alimentos. *Revista Tecnología y Ciencia*, *37*, 92–100. https://doi.org/10.33414/rtyc.37.92-100.2020

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., and Pérez-Álvarez, J. (2008). Antibacterial activity of lemon (*Citrus lemon l.*), mandarin (*Citrus reticulata l.*), grapefruit (*Citrus paradisi l.*) and orange (*Citrus sinensis l.*) essential oils. *Journal of Food Safety*, *28*(4), 567–576. https://doi.org//10.1111/j.1745-4565.2008.00131.x

Yazgan, H., Ozogul, Y., and Kuley, E. (2019). Antimicrobial influence of nanoemulsified lemon essential oil and pure lemon essential oil on food-borne pathogens and fish spoilage bacteria. *International Journal of Food Microbiology*, *306*(2), 108266. https://doi.org/10.1016/j.ijfoodmicro.2019.108266