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Application of antimicrobial packaging based on modified calcium carbonate and EOs for RTE meat products

Nadine Rüegg [a], Stephanie Rosa Teixeira [a], Barbara Maria Beck [a], Fabien Wilhelm Monnard [b], Rico Menard [c], Selçuk Yildirim [a,*]

[a] Zurich University of Applied Sciences, Department of Life Sciences and Facility Management, Campus Reidbach, 8820 Waedenswil, Switzerland
[b] Omya International AG, Baslerstrasse, 4665 Oftringen, Switzerland
[c] Micarna SA, Neue Industriestrasse, 9602 Bazenheid, Switzerland

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ABSTRACT

Highly porous modified calcium carbonate (MCC) powder has been successfully integrated into packaging material as a coating and the coated packaging films were loaded with 5, 10 or 30 wt % thyme and rosemary essential oil (EO). Resulting MCC labels were applied as labels and showed antimicrobial activities against *L. innocua* in *in vitro* test. After 6 days MCC labels with 10 and 30 wt % thyme EO showed significant reductions in *in vitro* tests (2.9 and > 8.5 log CFU/filter). When MCC labels with rosemary EO were used, only 30 wt % loading showed a significant reduction (1.6 log CFU/filter). Subsequently, the antimicrobial activity of MCC labels with 30 wt % EOs against *L. innocua* on ready to eat meat product were studied under normal atmosphere and modified atmosphere (MA). Use of MCC labels with 30 wt % thyme EO loading combined with MA packaging showed a significant microbial reduction of 1.2 log CFU/g on cooked ham after 21 days (compared to untreated MCC labels packaged under MA). On the other hand, use of MCC labels loaded with 30 wt % rosemary EO (with MA) showed significant reductions of *L. innocua* on sliced cooked chicken breast (2.6 log CFU/g) as well as cooked ham (1.3 log CFU/g).

1. Introduction

Food packaging plays an important role in preservation of the quality of food and ensuring its safety. For most products this role is fulfilled with the barrier function of the packaging, where packaging acts as a barrier between the products and the environment for gases, light, chemicals and microorganisms. This preservation function has been enhanced with the active packaging concepts developed during the last decades (Yildirim et al., 2018). Such active packaging systems are designed to release active substances into the headspace or to the product or can remove substances from the food or from the headspace of the packaging and by doing so can extend the shelf life of the food while maintaining its quality, safety and integrity (Sharma, Barkauskaite, Jaiswal, & Jaiswal, 2021; Vilela et al., 2018). Among the active packaging systems antimicrobial packaging is particularly important to preserve the microbial quality of the food and ensure its safety (Yildirim et al., 2018). Antimicrobial agents that are released from the packaging can interact with biological molecules and therefore affect the growth of various spoilage microorganisms, as well as food pathogens (Brockgreitens & Abbas, 2016; Ju et al., 2019; Souza, Ferreira, Paula, Mitra, & Rosa, 2021).

Within antimicrobial packaging systems, those containing essential oils (EOs) are of particular interest, as they can release the volatile EOs into the headspace and have antimicrobial effect without having direct contact with food (Ahmed et al., 2020; Ribeiro-Santos, Andrade, de Melo, & Sanches-Silva, 2017; Yildirim & Röcker, 2021). EOs are volatile secondary metabolites extracted from different parts of plants such as flowers, fruits, leaves, roots, stems and barks (Ju et al., 2019; Nikmaram et al., 2018; Ríos, 2016). They possess a complex variety of major and minor components, which could have different mode of action for antimicrobial activity in the cell (Bhavaniramya, Vishnupriya, Al-Aboody, Vijayakumar, & Baskaran, 2019; Ju et al., 2019; Maisanaba et al., 2017; Pateiro et al. 2021; Sharma et al., 2021). The antimicrobial activity of AP containing EOs has been extensively studied in *in vitro* studies and in food tests (Maisanaba et al., 2017; Pateiro et al., 2021; Ribeiro-Santos et al., 2017; Shin, Harte, Ryser, & Selke, 2010; Sirocchi

* Corresponding author.
E-mail address: selcuk.yildirim@zhaw.ch (S. Yildirim).

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et al., 2017; Skandamis & Nychas, 2002; Vilela et al., 2018). Effectivity of EOs may vary depending on used EOs, carrier material, food product, microflora as well as the environmental conditions (Burt, 2004; Silva, Domingues, & Nerín, 2018).

An important potential application for AP systems with EOs are packaged ready-to-eat (RTE) meat products, as these are susceptible to microbial spoilage and are often consumed without further heat treatment by consumer (Kramer, Wunderlich, & Muranyi, 2019). After heat treatment at production, these products are prone to cross-contamination with spoilage microorganisms or pathogenic bacteria *via* equipment such as slicers or cutting boards (Bērziņš, Hellström, Siliņš, & Korkeala, 2010). Especially psychrotrophic and facultative anaerobic pathogens like *Listeria monocytogenes* are of concern, since they are able to grow under refrigeration temperatures in products packaged under vacuum or modified atmosphere (MA) and cause serious diseases (Kramer et al., 2019; Rød, Hansen, Leipold, & Knøchel, 2012). In 2017, 0.48 listeriosis cases per 100′000 population were reported in the EU member states with a fatality rate of 13.8 % (EFSA & ECDC, 2018).

One of the major challenges in the use of EOs in AP is to integrate them into the packaging (film or coating) in such a way that they remain chemically stable and do not lose their effectiveness (Fernández-López & Viuda-Martos, 2018). In addition, a suitable inert carrier system is needed to avoid direct contact of EOs with food and enable a controlled release of EOs into the headspace of the package to maintain and ensure antimicrobial activity throughout the shelf life of the food (Ribeiro-Santos et al., 2017; Silva et al. 2018; Vasile & Baican, 2021). In a previous study we have demonstrated that the highly porous modified calcium carbonate (MCC) powder can be used to integrate the EOs and release them in a controlled way and therefore has a potential to be used in antimicrobial packaging systems for food (Rüegg et al., 2020). Antimicrobial activity of MCC powders containing EOs against *L. innocua* have been studied in *in vitro* tests and in food tests under normal atmosphere in petri dishes. In the next step we went further and integrated the MCC powder into the packaging material to develop antimicrobial packaging labels. Thereby MCC powder was integrated in a polyacrylate-based coating and the coating formulation was used to coat polyethylene terephthalate (PET) films. It has been shown that these PET/MCC films allow a controlled release of EOs into the gas atmosphere where release rates are dependent on the EO loading and the ambient temperature (Hettmann et al. 2022). In this study the PET films containing MCC coatings were loaded with thyme and rosemary EO and their antimicrobial activity against *L. innocua* was evaluated in *in vitro* tests. Furthermore, the antimicrobial activities of the PET/MCC labels containing EOs were investigated against *L. innocua* in RTE meat products (sliced cooked chicken breast and sliced cooked ham) packaged under normal atmosphere or modified atmosphere (50 % CO_2 and 50 % N_2) and stored at 7 °C.

2. Material and methods

2.1. Essential oils and MCC carrier

Rosemary EO Morocco (*Rosmarinus officinalis leaf oil*) and thyme red EO Hungary (*Thymus vulgaris flower/Leaf oil*) were purchased from Bernardi Group (France). Both EOs were stored in the dark at 21 ± 1 °C and utilised before the expiration date.

Modified calcium carbonate (MCC) having a median particle size of 6.6 μm, a top cut (d_{98}) of 14.5 μm, a BET specific surface area of 60 m^2/g was used as the porous filler. This mineral was provided by Omya International AG (Switzerland).

2.2. Preparation of the MCC labels

Sodium neutralized polyacrylate dispersing agent (9.8 g, 42 % solid content) was dispersed in 284 mL water and 205.8 g of modified calcium carbonate (MCC) was added step by step. The pH of the polyacrylate binder Acronal 500D (45.6 g, 46 wt % solid content) was adjusted to 9 with NaOH 30 wt % and the binder was added to the previous solution to obtain the coating formulation. Before use, the coating composition was stirred for 5 min to achieve a homogeneous distribution of the mineral in the coating formulation. Finally, the coating composition was applied with a coating table RK303 multicoater (Erichsen) onto a PET film Hostaphan RN 100 μm (PützFolie, Germany) and dried with a S-Dryer machine (Durrer, Switzerland) to obtain a coating weight of 50 g/m^2.

2.3. Coating porosimetry measurement

The specific pore volume was measured using a Micromeritics Autopore V 9620 mercury porosimeter having a maximum applied pressure of mercury 414 MPa (60 000 psi), equivalent to a Laplace throat diameter of 0.004 μm (~ 4 nm). The equilibration time used at each pressure step is 20 s. The sample material is sealed in a 5 cm^3 chamber powder penetrometer for analysis. The data are corrected for mercury compression, penetrometer expansion and sample material compression using the software Pore-Comp (Gane, Kettle, Matthews, & Ridgway, 1996).

2.4. Preparation of inoculum for antimicrobial activity tests

Gram-positive bacteria *Listeria innocua* (ATCC 33039, a *Listeria monocytogenes* surrogate) were selected for the evaluation of the antimicrobial activity of MCC labels loaded with EOs in *in vitro* and in food tests. The preparation of the inoculum was done in accordance with Rüegg et al. (2020). The final concentrations were 10^7 and 10^3 colony forming units (CFU)/mL for *in vitro* and food test, respectively. To confirm the counted CFU/mL the microbial load was additional determined with spread-plate method on Brain Heart Infusion (BHI) agar.

2.5. In vitro antimicrobial activity tests of MCC labels loaded with EOs

For the detection of the antimicrobial activity of active MCC labels in agar plates the *in vitro* method described by Rüegg et al. (2020) was used. Therefore, 100 mL of sterile water, inoculated with *L. innocua* (10^4 cfu/mL) was sterile filtered through a cellulose nitrate filter with a pore size of 0.45 μm (Sartorius Stedim Biotech GmbH, Germany) for every test to adjust the initial concentration to 10^6 cfu/ filter. Afterwards, cellulose nitrate filters were transferred in sterile plastic petri dishes with 60 mm diameter (Eppendorf, Germany) on Tryptone Soya Agar (TSA) (Oxoid, UK). MCC labels (0.002 m^2) were loaded homogenously with 5 wt %, 10 wt % and 30 wt % (based on dry weight MCC) EOs (thyme or rosemary) by application of a dispensing system (E2-EUR Series, Nordson Switzerland). These and untreated MCC labels (negative control) were placed in the lid of each petri dish with a headspace volume of approximately 20 cm^3. Petri dishes were stored at 7.5 ± 0.9 °C for 6 days. After storage the antimicrobial activity of MCC labels loaded with EOs were determined by detecting colony forming units by spread-plate method using BHI agar after an incubation of approximately 24°h at 37 °C. Microbiological counts were expressed as logarithms of the number of CFU per filter (log CFU/ filter). All tests were conducted in fivefold performance. At each time point five different petri dishes were analysed. Microbial load reductions were calculated comparing microbial load of the individual samples and the untreated MCC labels at the same day.

2.6. Antimicrobial activity tests of MCC labels loaded with EOs with RTE meat under real packaging conditions

To evaluate the antimicrobial activity of MCC labels under real packaging conditions 56.7 g ± 0.29 g sliced cooked chicken breast (chicken breast meat, nitrite salting mix, seasoning mix, glucose syrup, glucose, maltodextrin, sugar, yeast extract, thickening agent: E407a,

locust bean gum, stabiliser: E450, antioxidant: E301, aroma; a_w: 0.980, pH: 6.18) (Optigal Pouletbrust, Micarna SA, Switzerland) or 100 g ± 0.85 g sliced ham (pork, nitrite salting mix (table salt, preservative: E 250), seasoning mix, maltodextrin, glucose, yeast extract, stabiliser: E 451, antioxidant: E 301, aroma; a_w: 0.981, pH: 6.04) (M-Budget Hinterschinken, Micarna SA, Switzerland) were packaged in packaging trays (PS-EVOH-PE with peel, 0.5 mm, 204 × 147 × 14 mm, Stäger & Co AG, Switzerland). Headspace volume of sliced cooked chicken breast packages was 281.14 cm^3 (product/ headspace ratio 1:4) and for ham 221.45 cm^3 (product/headspace ratio 1:2.2). MCC labels (0.018 m^2) were loaded homogenously with 30 wt % thyme or rosemary EO using a dispensing system (E2-EUR Series, Nordson Switzerland). The active MCC label was then glued on a high barrier lidding film (Ecoweb M-Pap 57 AF, 57 μm, Südpack, Germany) prior to sealing. Afterwards trays were immediately packaged under normal and modified atmosphere (50 % CO_2, 50 % N_2) using a tray sealer (T 200, Multivac, Switzerland) in order to minimize losses of EOs. As negative control sliced cooked chicken breast and ham were packaged with MCC labels without EO loading. Then the samples were inoculated with *L. innocua*. Therefore, each top slice of the cooked chicken breast or ham was inoculated with 0.1 mL of the inoculum containing 10^3 CFU/mL *L. innocua* using a syringe through an airtight septum. The initial *L. innocua* load and the recovery rate of inoculated bacteria was detected 1 h after sampling preparation (t0). Additionally, the initial bacterial load of *L. innocua* was determined in non-inoculated samples. All samples were stored at 6.89 ± 0.4 °C for 21 days. Microbial analyses were carried out after 6, 12 and 21 days of storage. For this reason, the top slice of the meat sample was diluted 1:10 with Half Fraser Broth (Biokar Diagnostics, France) and homogenised for 120 s at 300 rpm using a stomacher (Seward Stomacher 400 circulator). After serial dilution of the samples the spread-plate method was used to determine the microbial load of *L. innocua* on Agar Listeria acc. to Ottaviani & Agosti (ALOA) (Biolife, Italy) after 24 h at 37 °C. Microbiological counts were expressed as logarithms of the number of CFU per gram (log CFU/g). All tests were conducted in fivefold performance. At each time point samples from five independent packages have been analysed. Microbial load reductions were calculated comparing microbial load of the individual samples and the untreated MCC labels at the same day.

2.7. Statistical analyses

For microbiological analyses all results are expressed as means ± standard deviation (SD). The data were analysed by one-factorial analysis of variance (ANOVA) with statistical software package R, version 3.6.1. In order to detect differences between specific factor levels, a post-hoc analysis with error inflation correction following Tukey HSD was applied. If data were not normally distributed Kruskal-Wallis, a pairwise Wilcoxon test was performed. Statistically significant differences were assumed if $P < 0.05$.

3. Results and discussion

3.1. Optimisation of essential oil loading on MCC labels

Polyethylene terephthalate (PET) films (100 μm) was chosen as a substrate to apply the coating formulation containing modified calcium carbonate to have a better handling and stability. The coating was characterized by mercury intrusion porosimetry resulting in a total intra particle intruded specific pore volume of 0.301 cm^3/g (applied pore size range: 0.004 – 0.67 μm).

In order to evaluate the effect of the amount of EO loaded onto MCC labels on the antimicrobial activity, MCC labels were homogeneously loaded with 5, 10 or 30 wt % thyme or rosemary EO. The selection of the EOs is based on their antimicrobial activities against *L. innocua* studied in a previous study (Rüegg et al. 2020). Subsequently, the *in vitro* antimicrobial activity of MCC labels was tested through the vapour phase (no direct contact) against *L. innocua* under cold storage conditions at 7 °C on TSA. Cold storage conditions were selected because many perishable foods prone to microbiological growth are stored at refrigeration temperatures.

When untreated MCC labels were used, *L. innocua* grew from 5.7 log CFU/filter to 6.4 log CFU/g after 1 day and to 9.5 log CFU/filter after 6 days (Fig. 1). This growth behaviour of *L. innocua* under cold storage conditions is in line with results previously published by Rüegg et al. (2020). By using MCC labels with 5 wt % thyme EO loading, an increase in microbial load occurred similar to the untreated MCC label and no significant reduction in microbial load was observed within the 6 days of storage. Increasing the thyme EO loading to 10 wt % slightly reduced the microbial load to 5.5 log CFU/filter on day 1 resulting in a reduction of 1.3 log CFU/filter compared to the control samples at the same day. Microbial load increased afterwards to 6.6 log CFU/filter on day 6, which are also significantly lower compared to the microbial load with untreated MCC label (reduction of 2.9 log CFU/filter). Further increasing the thyme EO loading to 30 wt % resulted in similar antimicrobial effect against *L. innocua* compared to the 10 wt % after 1 day. However, after 6 days microbial load was below the detection limit of 1 log CFU/filter resulting in a reduction of > 8.5 log CFU/filter.

In contrary, MCC labels loaded with 5 or 10 wt % rosemary EO showed no reduced growth of *L. innocua* within the 6 days and the microbial load was similar to the untreated MCC label. Increasing the loading of rosemary EO to 30 wt % resulted in significant reduction of 0.6 and 1.6 log CFU/filter on day 1 and day 6 compared to the control samples with untreated MCC labels.

These results confirm the previous findings of Rüegg et al. (2020) that MCC loaded with thyme EO have a higher *in vitro* antimicrobial effect against *L. innocua* compared to samples with rosemary EO loading. This result is independent of the application of MCC as a powder or as a coating on a label. MCC labels loaded with 30 wt % EO showed the highest antimicrobial activity in the *in vitro* tests against *L. innocua* over the storage time of 6 days. It is known that the complexity of the food matrix has a negative effect on the antimicrobial activity of EOs, due to binding of volatile EO components with the food (Aminzare, Hashemi, Hassanzadazar, & Hejazi, 2016; Burt, 2004; Fisher & Phillips, 2006; Otero et al. 2014; Ribeiro-Santos et al., 2017). Wang, Heising, Fogliano, and Dekker (2020) also showed that a higher fat content in ground beef led to a reduced antimicrobial activity of carvacrol (main component of *e.g.* oregano EO), which they attributed to the partitioning of carvacrol in the fat phase. Therefore, higher concentrations of EOs are needed to inhibit the growth of microorganisms in food compared to *in vitro* tests (Ward, Delaquis, Holley, & Mazza, 1998). Therefore 30 wt % EO loading was selected for further evaluation of the antimicrobial activity of thyme and rosemary EO in food tests with RTE meat products. Although thyme EO showed a higher antimicrobial activity than rosemary EO in *in vitro* tests, the food tests are performed with both EOs, as the previous study by Rüegg et al. (2020) showed that the latter EO showed a higher antimicrobial activity in preliminary tests with sliced cooked chicken breast.

3.2. Antimicrobial activity of MCC labels loaded with thyme or rosemary EO in food tests under real packaging conditions

Subsequent to the previous *in vitro* test, the antimicrobial activity of MCC labels loaded with 30 wt % thyme or rosemary EO was studied in food tests with sliced cooked chicken breast and sliced cooked ham (ready to eat food). Samples were packaged either under normal atmosphere (NA) and modified atmosphere (MA) containing 50 % CO_2 and 50 % N_2 and afterwards inoculated with *L. innocua* and stored at 7 °C for 21 days. A lower loading of *L. innocua* was chosen compared to the previous *in vitro* test, since the bacterial load of *Listeria* spp. in food is usually very low.

No *Listeria* could be detected in any non-inoculated sliced cooked chicken breast samples over the entire storage period of 21 days (data

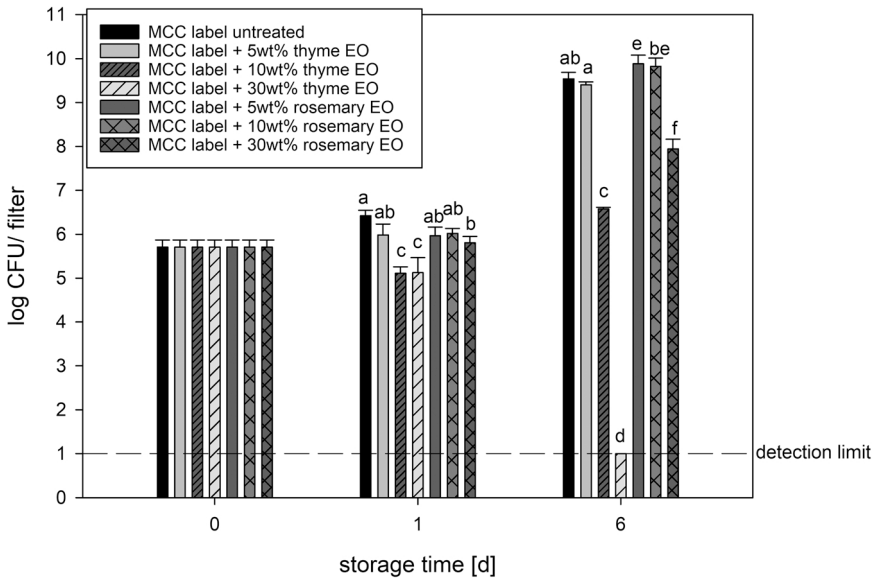

Fig. 1. Antimicrobial activity of modified calcium carbonate (MCC) labels loaded with 5, 10 or 30 wt % thyme or rosemary essential oil (EO) or untreated MCC labels on the growth of Listeria innocua (ATCC 33090) on tryptone soy agar (TSA) plates at 7 °C. Results are expressed as mean (log CFU/filter) ± standard deviation (n = 5). Same letters within a time point indicate that the results are not statistically significantly different ($p \geq 0.05$).

not shown). Growth of *L. innocua* from detection limit (1.0 log CFU/g) to 4.9 log CFU/g could be detected on inoculated sliced cooked chicken breast under normal atmosphere within the first 12 days with untreated MCC labels (Fig. 2). Afterwards, no significant growth or reduction of *L. innocua* could be detected in these samples by the end of the storage period of 21 days.

The use of MCC labels with 30 wt % thyme EO loading did not significantly reduce microbial growth in the NA packaged samples during the whole storage time. On the other hand, the use of MCC labels with 30 wt % rosemary EO resulted in a microbial load of 3.4 log CFU/g after 21 days, which is equivalent to a significant reduction of 1.2 log CFU/g.

In our previous study by Rüegg et al. (2020), where EO loaded MCC powders were used, we also observed a higher antimicrobial activity with rosemary EO against *L. innocua* on sliced cooked chicken breast compared to thyme EO. In both studies, growth could be reduced by rosemary EO, leading to significantly lower microbial loads compared to the untreated MCC.

No growth of *L. innocua* was observed with untreated MCC labels in combination with MA containing 50 % CO_2 until day 6. Afterwards, the microbial load significantly increased from detection limit (1.0 log CFU/g) to 3.2 and 3.9 log CFU/g at 12 and 21 days, respectively. At the end of the storage time, there was no longer any significant difference in the microbial load between NA and MA packaged sliced cooked chicken breast samples. Conclusively, it can be said that the intrinsic factors of the sliced cooked chicken breast alone, in combination with cold storage at 7 °C and use of MA with 50 % CO_2 is not enough to reduce the growth of *L. innocua* after 21 days as *Listeria* spp. have a high resistance to environmental stress (*e.g.* acid, salt, temperature) (Ryser & Marth, 2007). A study on turkey roll slices inoculated with *Listeria monocytogenes* also showed that the use of 30–50 % CO_2 in MA was insufficient to inhibit the microbial growth over a storage time of 30 days. At the end of storage, comparable bacteria concentrations to the NA packaged control were achieved (Farber & Daley, 1994).

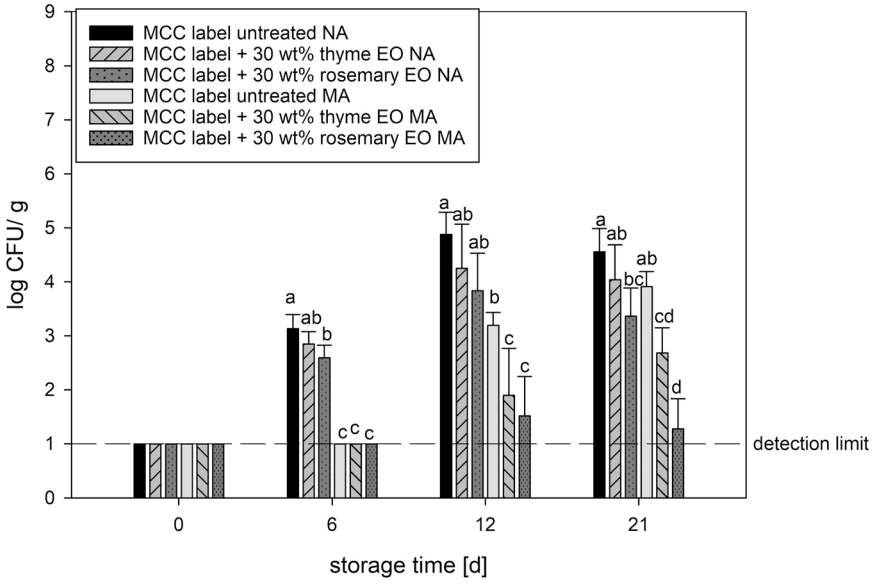

Fig. 2. Antimicrobial activity of MCC labels with 30 wt % thyme or rosemary EO loading and untreated MCC labels (negative control) in food tests on the growth of L. innocua (ATCC 33090) on sliced cooked chicken breast packaged under normal atmosphere (NA) or modified atmosphere (MA) (50 % CO_2/ 50 % N_2) at 7 °C. Results are expressed as mean (log CFU/filter) ± standard deviation (n = 5). Same letters within a time point indicate that the results are not statistically significantly different ($p \geq 0.05$).

Similar to the untreated MCC label samples, no growth of *L. innocua* was observed with the MCC labels with thyme EO loading after day 6. However, a significant decrease in microbial load to 1.9 and to 2.7 log CFU/g was observed on day 12 and 21, which corresponds to a reduction of 1.3 and 1.2 log CFU/g, respectively.

When MCC labels with rosemary EO loading were combined with MA, the microbial growth could also significantly be reduced, resulting in a microbial load of 1.3 log CFU/g after 21 days. Compared to the samples packaged with untreated MCC labels (under MA), this led to a microbial load reduction of 1.7 log CFU/g on day 12 to 2.6 CFU/g on day 21. The maximum achieved microbial load reduction by combination of MA and MCC labels loaded with thyme EO and rosemary EO (compared to untreated MCC label with NA) was 3.0 log CFU/g and 3.4 log CFU/g at day 12, respectively.

Additional to the sliced cooked chicken breast the antimicrobial activity of the newly developed MCC labels loaded with EOs were also studied with sliced cooked ham made of pork (packaged under NA or MA).

Growth of *L. innocua* from detection limit (1.0 log CFU/g) to 3.7 log CFU/g, 6.6 log CFU/g and 8.2 log CFU/g could be detected on inoculated sliced cooked ham packaged under NA with untreated MCC labels on day 6, 12 and 21, respectively (Fig. 3). Compared to the sliced cooked chicken breast, the growth was more pronounced in the packaged ham. The a_w and pH values for both products are very similar. Therefore, the difference in the growth of *Listeria* could be due to differences in the composition of the products. Verheyen et al. (2020) showed that the fat content in the food can reduce the lag phase of the Listeria monocytogenes. Sliced cooked ham used in this study had a higher fat content (5 wt %) compared to sliced cooked chicken breast samples (1 wt %). No Listeria was detected in the non-inoculated samples of this product (data not shown).

The use of MCC labels loaded with thyme EO and packaged under NA did not significantly inhibit or reduce the growth of *L. innocua* on sliced cooked ham and after 21 days, the microbial load increased to 7.6 log CFU/g.

In contrast, the use of MCC labels with rosemary EO resulted in a significantly lower microbial load of *L. innocua* (5.1 log CFU/g) on day 12, which corresponds to a reduction of 1.5 log CFU/g. However, after 21 days microbial load reached to 7.1 log CFU/g which was not significantly different compared to the untreated MCC labels.

Use of MA with untreated MCC labels resulted in a delay of *L. innocua* growth on sliced cooked ham until day 6 for all samples tested, which is comparable with the sliced cooked chicken breast samples. Afterwards, *L. innocua* grew with a lower growth rate and microbial load increased to 2.3 log CFU/g at day 12 and 3.3 log CFU/g at day 21, which are significantly lower than those of the NA packaged samples at each time point. Therefore, use of MA resulted in a microbial load reduction of 4.3 log CFU/g und 4.9 log CFU/g after 12 and 21 days, respectively. Compared to the sliced cooked chicken breast samples, the use of MA inhibited the growth of bacteria in ham more effectively. This could be due to the fact, that the solubility of CO_2 in sliced cooked ham is higher than in sliced cooked chicken breast due to the higher fat content (Jakobsen & Bertelsen, 2002).

Combination of MCC labels loaded with 30 wt % thyme EO and MA reduced the growth of *L. innocua* after day 6 and microbial load increased to 1.2 log CFU/g after 12 days resulting in a significant reduction of 1.1 log CFU/g compared to untreated MCC labels. *L. innocua* grew afterwards further and reached to 3.0 log CFU/g after 21 days, but the microbial reduction was no longer significant.

Similar growth of *L. innocua* was observed with MCC labels loaded with 30 % rosemary EO and packaged under MA compared to samples loaded with thyme EO. After 21 days a significant microbial reduction of 1.3 log CFU/g was achieved compared to untreated MCC labels with MA.

Although thyme EO showed higher antimicrobial activity in *in vitro* tests, in food tests the antimicrobial activities of both EOs (thyme or rosemary) against *L. innocua* were similar (no statistical difference) during the whole storage time. This might be due to the fact that thymol, and carvacrol, the main components of thyme EO, are phenols which can bind to amino or hydroxylamine groups of proteins and therefore cannot form the complex with the bacterial membrane to have an antimicrobial effect (Juven, Kanner, Schved, & Weisslowicz, 1994). On the other hand, eucalyptol, the main component of the rosemary EO, is a hydrocarbon monoterpene and not a phenol. Furthermore Gaglio et al. (2021) demonstrated that carvacrol showed antimicrobial activity against *L. monocytogenes* on melon and pumpkin slices, but not in protein containing ham and salmon. Different studies also showed that the complexity of the food matrix, the interactions with food ingredients (*e.g.* proteins, fats, salts, carbohydrates) as well as storage and packaging conditions can negatively influence the antimicrobial activity of EOs or their main components (Atarés & Chiralt, 2016; Gaglio et al., 2021; Gutierrez, Barry-Ryan, & Bourke, 2008; Higueras, López-Carballo, Hernández-Muñoz, Catalá, & Gavara, 2014).

A direct comparison of the antimicrobial activity achieved within this study with the literature data is not possible, because of the differences in experimental setups (selected food (recipe), selected active

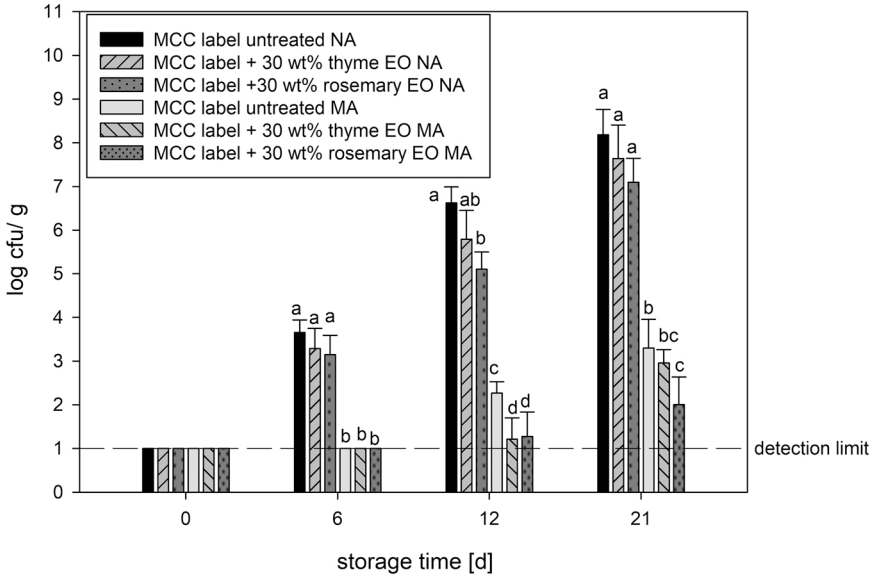

Fig. 3. Antimicrobial activity of MCC labels with 30 wt % thyme or rosemary EO loading and untreated MCC labels (negative control) in food tests on the growth of L. innocua (ATCC 33090) on sliced cooked ham packaged under normal atmosphere (NA) or modified atmosphere (MA) (50 % CO_2/ 50 % N_2) at 7 °C. Results are expressed as mean (log CFU/filter) ± standard deviation (n = 5). Same letters within a time point indicate that the results are not statistically significantly different ($p \geq 0.05$).

packaging designs (EO type, EO concentration, EO integration, packaging atmosphere, product quantity and product/headspace ratio), storage temperature and inoculation (type of microorganisms and concentration)). It is known that EOs that are added directly to the food or come into direct contact with food begin to degrade rapidly (Sharma et al., 2021). Therefore, lower doses of EOs are required when they are incorporated into packaging materials (Avila-Sosa, Palou, & López-Malo, 2016) and released afterwards over the headspace to the food surface where the microbial contamination often occurs (Quesada, Sendra, Navarro, & Sayas-Barberá, 2016). Despite this, most of the studies evaluated the antimicrobial activity of EOs by direct addition of the EOs to the food or placing the antimicrobial labels containing EOs on the food products (Blanco-Lizarazo, Betancourt-Cortés, Lombana, Carrillo-Castro, & Sotelo-Díaz, 2017; Boskovic et al., 2017; Giarratana et al., 2016; Irkin & Esmer, 2010; Lee, Lee, Yang, & Song, 2016; Souza et al. 2019). Furthermore, antimicrobial activity of EOs in RTE meat products are often evaluated under NA (Ruiz-Navajas et al. 2015; Sharma, Mendiratta, Agarwal, & Gurunathan, 2020), although these products are industrially packaged under MA or vacuum to control microbial safety.

4. Conclusion

In this study it has been shown that active packaging labels with highly porous MCC coating loaded with EOs (thyme and rosemary oil) can reduce the growth of *L. innocua* in *in vitro* tests. However, the results showed that the *in vitro* results cannot be transferred to food test. Use of MCC labels loaded with EOs showed lower antimicrobial activity against *L. innocua* in sliced cooked chicken breast and sliced cooked ham packaged under normal atmosphere compared to *in vitro* test. It has been also demonstrated that the combination of modified atmosphere with 50 % CO_2 and MCC labels loaded with EOs can significantly reduce the growth of *L. innocua* in the RTE meat products tested in this study. With regard to industrial application, active MCC labels releasing EOs should be tested with the targeted food products under real conditions (packaging atmosphere, product amount, product/headspace ratio, activity through the headspace and storage conditions). Additionally, as the EOs released from the labels may influence the organoleptic properties of the food, it would be also necessary to carry out sensory tests.

CRediT authorship contribution statement

Nadine Rüegg: Methodology, Investigation, Visualization, Project administration, Writing – original draft, Writing – review & editing. **Stephanie Rosa Teixeira:** Investigation. **Barbara M. Beck:** Investigation. **Fabien W. Monnard:** Investigation, Supervision, Funding acquisition. **Rico Menard:** Methodology, Investigation. **Selçuk Yildirim:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Selcuk Yildirim reports financial support was provided by Innosuisse Swiss Innovation Agency.

Data Availability

The authors are unable or have chosen not to specify which data has been used.

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References

Yildirim, S., & Röcker, B. (2021). Sustainable antimicrobial packaging technologies. *Sustainable food packaging technology* (pp. 323–348). John Wiley & Sons.

Ahmed, S., Sameen, D. E., Lu, R., Li, R., Dai, J., Qin, W., & Liu, Y. (2020). Research progress on antimicrobial materials for food packaging. *Critical Reviews in Food Science and Nutrition*, 1–14. https://doi.org/10.1080/10408398.2020.1863327

Aminzare, M., Hashemi, M., Hassanzadazar, H., & Hejazi, J. (2016). The USe Of Herbal Extracts And Essential Oils As A Potential Antimicrobial In Meat And Meat Products; A review. *Journal of Human, Environment, and Health Promotion, 1*(2), 63–74. https://doi.org/10.29252/jhehp.1.2.63

Atarés, L., & Chiralt, A. (2016). Essential oils as additives in biodegradable films and coatings for active food packaging. *Trends in Food Science & Technology, 48*, 51–62. https://doi.org/10.1016/j.tifs.2015.12.001

Avila-Sosa, R., Palou, E., & López-Malo, A. (2016). Chapter 15 - essential oils added to edible films. In V. R. Preedy (Ed.), *Essential oils in food preservation, flavor and safety* (pp. 149–154). San Diego: Academic Press.

Bhavaniramya, S., Vishnupriya, S., Al-Aboody, M. S., Vijayakumar, R., & Baskaran, D. (2019). Role of essential oils in food safety: Antimicrobial and antioxidant applications. *Grain & Oil Science and Technology, 2*(2), 49–55. https://doi.org/10.1016/j.gaost.2019.03.001

Blanco-Lizarazo, C. M., Betancourt-Cortés, R., Lombana, A., Carrillo-Castro, K., & Sotelo-Díaz, I. (2017). Listeria monocytogenes behaviour and quality attributes during sausage storage affected by sodium nitrite, sodium lactate and thyme essential oil. *Food Science and Technology International, 23*(3), 277–288. https://doi.org/10.1177/1082013216686464

Boskovic, M., Djordjevic, J., Ivanovic, J., Janjic, J., Zdravkovic, N., Glisic, M., & Baltic, M. (2017). Inhibition of Salmonella by thyme essential oil and its effect on microbiological and sensory properties of minced pork meat packaged under vacuum and modified atmosphere. *International Journal of Food Microbiology, 258*, 58–67. https://doi.org/10.1016/j.ijfoodmicro.2017.07.011

Brockgreitens, J., & Abbas, A. (2016). Responsive food packaging: recent progress and technological prospects. *Comprehensive Reviews in Food Science and Food Safety, 15*(1), 3–15. https://doi.org/10.1111/1541-4337.12174

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods - a review. *International Journal of Food Microbiology, 94*(3), 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Bērziņš, A., Hellström, S., Siliņš, I., & Korkeala, H. (2010). Contamination patterns of listeria monocytogenes in cold-smoked pork processing. *Journal of Food Protection, 73*(11), 2103–2109. https://doi.org/10.4315/0362-028x-73.11.2103

EFSA (European Food Safety Authority), & ECDC (European Centre for Disease Prevention and Control). (2018). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA Journal, 16*(12), Article 262. https://doi.org/10.2903/j.efsa.2018.5500

Farber, J. M., & Daley, E. (1994). Fate of listeria monocytogenes on modified-atmosphere packaged Turkey roll slices. *Journal of Food Protection, 57*(12), 1098–1100. https://doi.org/10.4315/0362-028x-57.12.1098

Fernández-López, J., & Viuda-Martos, M. (2018). Introduction to the special issue: Application of essential oils in food systems. *Foods, 7*(4), 56. https://doi.org/10.3390/foods7040056

Fisher, K., & 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

Gaglio, R., Botta, L., Garofalo, G., Miceli, A., Settanni, L., & Lopresti, F. (2021). Carvacrol activated biopolymeric foam: An effective packaging system to control the development of spoilage and pathogenic bacteria on sliced pumpkin and melon. *Food Packaging and Shelf Life, 28*, Article 100633. https://doi.org/10.1016/j.fpsl.2021.100633

Gane, P. A., Kettle, J. P., Matthews, G. P., & Ridgway, C. J. (1996). Void space structure of compressible polymer spheres and consolidated calcium carbonate paper-coating formulations. *Industrial & Engineering Chemistry Research, 35*(5), 1753–1764. https://doi.org/10.1021/ie950413m

Giarratana, F., Muscolino, D., Ragonese, C., Beninati, C., Sciarrone, D., Ziino, G., … Panebianco, A. (2016). Antimicrobial activity of combined thyme and rosemary essential oils against Listeria monocytogens in Italian mortadella packaged in modified atmosphere. *Journal of Essential Oil Research, 28*(6), 467–474. https://doi.org/10.1080/10412905.2016.1165744

Gutierrez, J., Barry-Ryan, C., & Bourke, P. (2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *International Journal of Food Microbiology, 124*(1), 91–97. https://doi.org/10.1016/j.ijfoodmicro.2008.02.028

Hettmann, K., Monnard, F.W., Melo Rodriguez, G., Hilty, F.M., Yildirim, S., Schoelkopf, J. , 2022. Porous Coatings to Control Release Rates of Essential Oils to Generate an Atmosphere with Botanical Actives. 15(6), 2155. Retrieved from https://www.mdpi.com/1996–1944/15/6/2155.

Higueras, L., López-Carballo, G., Hernández-Muñoz, P., Catalá, R., & Gavara, R. (2014). Antimicrobial packaging of chicken fillets based on the release of carvacrol from

chitosan/cyclodextrin films. *International Journal of Food Microbiology, 188*, 53–59. https://doi.org/10.1016/j.ijfoodmicro.2014.07.018

Irkin, R., & Esmer, O. K. (2010). Control of listeria monocytogenes in ground chicken breast meat under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of bay essential oil at 4°C. *Food Science and Technology Research, 16*(4), 285–290. https://doi.org/10.3136/fstr.16.285

Jakobsen, M., & Bertelsen, G. (2002). The use of CO_2 in packaging of fresh red meats and its effect on chemical quality changes in the meat: a review. *Journal of Muscle Foods, 13*(2), 143–168. https://doi.org/10.1111/j.1745-4573.2002.tb00326.x

Ju, J., Chen, X., Xie, Y., Yu, H., Guo, Y., Cheng, Y., … Yao, W. (2019). Application of essential oil as a sustained release preparation in food packaging. *Trends in Food Science & Technology, 92*, 22–32. https://doi.org/10.1016/j.tifs.2019.08.005

Juven, B. J., Kanner, J., Schved, F., & Weisslowicz, H. (1994). Factors that interact with the antibacterial action of thyme essential oil and its active constituents. *Journal of Applied Bacteriology, 76*(6), 626–631. https://doi.org/10.1111/j.1365-2672.1994.tb01661.x

Kramer, B., Wunderlich, J., & Muranyi, P. (2019). Inactivation of Listeria innocua on packaged meat products by pulsed light. *Food Packaging and Shelf Life, 21*, Article 100353. https://doi.org/10.1016/j.fpsl.2019.100353

Lee, K.-Y., Lee, J.-H., Yang, H.-J., & Song, K. B. (2016). Production and characterisation of skate skin gelatin films incorporated with thyme essential oil and their application in chicken tenderloin packaging. *International Journal of Food Science & Technology, 51*(6), 1465–1472. https://doi.org/10.1111/ijfs.13119

Maisanaba, S., Llana-Ruiz-Cabello, M., Gutiérrez-Praena, D., Pichardo, S., Puerto, M., Prieto, A. I., … Cameán, A. M. (2017). New advances in active packaging incorporated with essential oils or their main components for food preservation. *Food Reviews International, 33*(5), 447–515. https://doi.org/10.1080/87559129.2016.1175010

Nikmaram, N., Budaraju, S., Barba, F. J., Lorenzo, J. M., Cox, R. B., Mallikarjunan, K., & Roohinejad, S. (2018). Application of plant extracts to improve the shelf-life, nutritional and health-related properties of ready-to-eat meat products. *Meat Science, 145*, 245–255. https://doi.org/10.1016/j.meatsci.2018.06.031

Otero, V., Becerril, R., Santos, J. A., Rodríguez-Calleja, J. M., Nerín, C., & García-López, M.-L. (2014). Evaluation of two antimicrobial packaging films against Escherichia coli O157:H7 strains in vitro and during storage of a Spanish ripened sheep cheese (Zamorano). *Food Control, 42*, 296–302. https://doi.org/10.1016/j.foodcont.2014.02.022

Pateiro, M., Munekata, P. E. S., Sant'Ana, A. S., Domínguez, R., Rodríguez-Lázaro, D., & Lorenzo, J. M. (2021). Application of essential oils as antimicrobial agents against spoilage and pathogenic microorganisms in meat products. *International Journal of Food Microbiology, 337*, Article 108966. https://doi.org/10.1016/j.ijfoodmicro.2020.108966

Quesada, J., Sendra, E., Navarro, C., & Sayas-Barberá, E., 2016. Antimicrobial Active Packaging including Chitosan Films with Thymus vulgaris L. Essential Oil for Ready-to-Eat Meat. 5(3), 57. Retrieved from https://www.mdpi.com/2304–8158/5/3/57.

Ribeiro-Santos, R., Andrade, M., de Melo, N. R., & Sanches-Silva, A. (2017). Use of essential oils in active food packaging: Recent advances and future trends. *Trends in Food Science & Technology, 61*, 132–140. https://doi.org/10.1016/j.tifs.2016.11.021

Ríos, J.-L. (2016). Essential oils: what they are and how the terms are used and defined. In V. R. Preedy (Ed.), *Essential oils in food preservation, flavor and safety* (pp. 3–10). San Diego: Academic Press.

Rüegg, N., Beck, B. M., Monnard, F. W., Hilty, F. M., Wicht, A., Schoelkopf, J., & Yildirim, S. (2020). Evaluation of the potential of functionalised calcium carbonate as carrier for essential oils with regard to antimicrobial packaging applications. *Packaging Technology and Science, 33*(8), 333–343. https://doi.org/10.1002/pts.2508

Ruiz-Navajas, Y., Viuda-Martos, M., Barber, X., Sendra, E., Perez-Alvarez, J. A., & Fernández-López, J. (2015). Effect of chitosan edible films added with Thymus moroderi and Thymus piperella essential oil on shelf-life of cooked cured ham. *Journal of Food Science and Technology, 52*(10), 6493–6501. https://doi.org/10.1007/s13197-015-1733-3

Ryser, E.T., Marth, E.H., 2007. Listeria, Listeriosis, and Food Safety (3rd ed.): CRC Press.

Rød, S. K., Hansen, F., Leipold, F., & Knøchel, S. (2012). Cold atmospheric pressure plasma treatment of ready-to-eat meat: Inactivation of Listeria innocua and changes in product quality. *Food Microbiology, 30*(1), 233–238. https://doi.org/10.1016/j.fm.2011.12.018

Sharma, H., Mendiratta, S. K., Agarwal, R. K., & Gurunathan, K. (2020). Bio-preservative effect of blends of essential oils: natural anti-oxidant and anti-microbial agents for the shelf life enhancement of emulsion based chicken sausages. *Journal of Food Science and Technology, 57*(8), 3040–3050. https://doi.org/10.1007/s13197-020-04337-1

Sharma, S., Barkauskaite, S., Jaiswal, A. K., & Jaiswal, S. (2021). Essential oils as additives in active food packaging. *Food Chemistry, 343*, Article 128403. https://doi.org/10.1016/j.foodchem.2020.128403

Shin, J., Harte, B., Ryser, E., & Selke, S. (2010). Active packaging of fresh chicken breast, with Allyl Isothiocyanate (AITC) in combination with Modified Atmosphere Packaging (MAP) to control the growth of pathogens. *Journal of Food Science, 75*(2), M65–M71. https://doi.org/10.1111/j.1750-3841.2009.01465.x

Silva, F., Domingues, F. C., & Nerín, C. (2018). Trends in microbial control techniques for poultry products. *Critical Reviews in Food Science and Nutrition, 58*(4), 591–609. https://doi.org/10.1080/10408398.2016.1206845

Sirocchi, V., Devlieghere, F., Peelman, N., Sagratini, G., Maggi, F., Vittori, S., & Ragaert, P. (2017). Effect of Rosmarinus officinalis L. essential oil combined with different packaging conditions to extend the shelf life of refrigerated beef meat. *Food Chemistry, 221*, 1069–1076. https://doi.org/10.1016/j.foodchem.2016.11.054

Skandamis, P. N., & Nychas, G.-J. E. (2002). Preservation of fresh meat with active and modified atmosphere packaging conditions. *International Journal of Food Microbiology, 79*(1), 35–45. https://doi.org/10.1016/S0168-1605(02)00177-0

Souza, A. G., Ferreira, R. R., Paula, L. C., Mitra, S. K., & Rosa, D. S. (2021). Starch-based films enriched with nanocellulose-stabilized Pickering emulsions containing different essential oils for possible applications in food packaging. *Food Packaging and Shelf Life, 27*, Article 100615. https://doi.org/10.1016/j.fpsl.2020.100615

Souza, V. G. L., Pires, J. R. A., Vieira, É. T., Coelhoso, I. M., Duarte, M. P., & Fernando, A. L. (2019). Activity of chitosan-montmorillonite bionanocomposites incorporated with rosemary essential oil: From in vitro assays to application in fresh poultry meat. *Food Hydrocolloids, 89*, 241–252. https://doi.org/10.1016/j.foodhyd.2018.10.049

Vasile, C., & Baican, M. (2021). Progresses in food packaging, food quality, and safety—controlled-release antioxidant and/or antimicrobial packaging. *Molecules, 26*(5), 1263. https://doi.org/10.3390/molecules26051263

Verheyen, D., Bolívar, A., Pérez-Rodríguez, F., Baka, M., Skåra, T., & Van Impe, J. F. (2020). Isolating the effect of fat content on Listeria monocytogenes growth dynamics in fish-based emulsion and gelled emulsion systems. *Food Control, 108*, Article 106874. https://doi.org/10.1016/j.foodcont.2019.106874

Vilela, C., Kurek, M., Hayouka, Z., Röcker, B., Yildirim, S., Antunes, M. D. C., … Freire, C. S. R. (2018). A concise guide to active agents for active food packaging. *Trends in Food Science & Technology, 80*, 212–222. https://doi.org/10.1016/j.tifs.2018.08.006

Wang, L., Heising, J., Fogliano, V., & Dekker, M. (2020). Fat content and storage conditions are key factors on the partitioning and activity of carvacrol in antimicrobial packaging. *Food Packaging and Shelf Life, 24*, Article 100500. https://doi.org/10.1016/j.fpsl.2020.100500

Ward, S. M., Delaquis, P. J., Holley, R. A., & Mazza, G. (1998). Inhibition of spoilage and pathogenic bacteria on agar and pre-cooked roast beef by volatile horseradish distillates. *Food Research International, 31*(1), 19–26. https://doi.org/10.1016/S0963-9969(98)00054-4

Yildirim, S., Röcker, B., Pettersen, M. K., Nilsen-Nygaard, J., Ayhan, Z., Rutkaite, R., … Coma, V. (2018). Active packaging applications for food. *Comprehensive Reviews in Food Science and Food Safety, 17*(1), 165–199. https://doi.org/10.1111/1541-4337.12322