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Development of thyme essential oil-based coatings and assessment of their antimicrobial activity

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ABSTRACT

In this study, coatings composed of acrylic binder, starch sodium octenylsuccinate and essential oil (EO), as a volatile antibacterial agent were developed and applied to polypropylene films. Among various EOs tested, thymol exhibited the highest antimicrobial activity in agar diffusion assays, resulting in inhibition zone of 12.3 ± 1.2 to 40.0 ± 1.0 mm with *Escherichia coli* and 11.3 ± 0.6 to 88.0 ± 1.0 mm with L. *monocytogenes*. Based on these results, thymol was selected and incorporated in the coatings, and the antimicrobial activity of films with coatings containing various amounts of thyme EO (585 ± 30 – 2159 ± 112 μg/cm^2) were assessed using both agar diffusion and vapor diffusion assays. Films with the highest amount of thyme essential oil (2159 ± 112 μg/cm^2) exhibited significant antimicrobial activity in the agar diffusion test. In vapor diffusion assays the active films demonstrated a significant antibacterial effect, producing a log reduction of 2.3 log *E. coli* and 3.8 for L. *monocytogenes*. Studies on the release of thymol, the main antibacterial component of thyme EO, revealed a continuously release with a maximum concentration of 21.85±2.11 μg/cm^3 achieved after 10 days. The results further indicated that only a very small proportion of the thymol in the coating was released during the storage period.

1. Introduction

Food quality and safety are major concerns for food industries, governments, and consumers. One of the major problems is microbial contamination, which reduces the shelf-life of food and increases the risk of foodborne illnesses (Corrales et al., 2014; Tropea, 2022). *E. coli* and *Listeria monocytogenes* are well-known foodborne bacteria that cause spoilage and pose serious health risks (Gupta & Adhikari, 2022; Ning et al., 2020; Wu et al., 2019). They are widely studied and often serve as model organisms for gram-negative and gram-positive bacteria, respectively (Wu et al., 2019).

Antimicrobial packaging relates to one of the active packaging concepts and can be considered as an extremely challenging technology that could have a significant impact on food safety and shelf-life extension (Quitavalla & Vicini, 2002; Yildirim et at., 2018). One effective approach is the immobilization of antimicrobial compounds on polymer matrices, which can release active agents into the packaging headspace or directly onto food (Commision Regulation EC No., 450/2009; Lacroix, 2011; Pilati et al., 2013). Over the past several years, various antimicrobials, both synthetic and natural, have been incorporated into packaging materials and tested for efficacy. In contrast to chemical antimicrobial additives, natural antimicrobials like essential oils (EOs) can impart bioactive properties to packaging making them more attractive to consumers (Yildirim & Röcker, 2021). EOs possess antibacterial, antifungal, and antiviral activities (Calo et al., 2015; Vilela et al., 2018). They can be incorporated into packaging as polymer additives or coatings (Cha & Chinnan, 2004; Realini & Marcos, 2014). EOs are natural extracts rich in hydrophobic volatile compounds including terpenes, terpenoids, and aromatic constituents (Corrales et al., 2014). Their antimicrobial activity occurs when these compounds are released into the headspace or penetrate the bulk matrix through direct contact (Corrales et al., 2014). Their antimicrobial effects are primarily

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attributed to their hydrophobic volatile compounds, which disrupt bacterial membranes and inhibit cellular processes (Andrade-Ochoa et al., 2021; Matan et al., 2006). In general, EOs are more effective against gram-positive bacteria than against gram-negative bacteria (Kim et al., 1995; Matan et al., 2006; Rios & Recio, 2005). Thyme *(Thymus vulgaris)* EO, commonly used in medical and food applications (Salehi et al., 2018) contains thymol (10–64 %), a phenolic compound with strong antimicrobial properties against both gram-negative and gram-positive bacteria as well as fungi (Burt, 2004; Salehi et al., 2018). Thymol exhibits antimicrobial activity against *E. coli* and L. *monocytogenes* by altering the lipid structure of the bacteria cell, leading to the destruction of cell membrane, cytoplasmic leakage, cell lysis and death (Kavoosi et al., 2013; Texeira et al., 2013; Xu et al., 2008). It also inhibits biofilm formation and induces changes in morphological, metabolic, and genetic changes in bacteria (Al-Kandari et al., 2019). Additionally, thymol inhibits key enzymes involved in bacterial metabolism, impairing growth and survival (Shah et al., 2012). The antimicrobial activity of the EO of thyme, clove, oregano, cinnamon, and basil against several species of bacteria was assessed by direct contact and vapor phase methods (Dobre et al., 2011; Duan et al., 2022; Goñi et al., 2009; Lee et al., 2023; López et al., 2007; Reyes-Jurado et al., 2020; Tullio et al., 2007). The results have shown that thyme and oregano oils possessed significant antibacterial effect against *E. coli* through the vapor phase (Dobre et al., 2011). Use of EO in the vapor phase could have significant advantages including reduced sensory impact on food (Goñi et al., 2009) and reduction of potential chemical irritation, as well as lower dose comparing to the usage of EO in liquid form (Tullio et al., 2007).

The antimicrobial activity of EOs against various foodborne microorganisms in direct contact has been widely documented. However, volatile components present in thyme EO and their antimicrobial effectiveness trough the vapor phase have not been sufficiently investigated. Therefore, this study aimed to develop thyme essential oil based active food packaging, evaluate its antibacterial properties through the vapor phase, and investigate the thymol release from coated films.

2. Materials and methods

2.1. Materials and chemicals

Thyme essential oil *(Thymus vulgaris)*, clove essential oil (*Syzygium aromaticum*), eugenol (≥ 98 %), thymol (≥ 99 %), which has been used as a reference in studies, linalool (≥ 97 %), dimethylsulfoxide, propionic acid, Brain Heart Infusion broth were purchased from Sigma-Aldrich. Starch sodium octenylsuccinate was purchased from Ingredion GmbH (Germany). Premo® Coat BR20 (food contact approved acrylic binder) was supplied by Flint Group (Finland). Corona treated oriented-polypropylene (OPP) film (30 μm) was supplied by SÜDPACK® (Switzerland). Luria-Bertani agar and Brain Heart infusion agar were purchased from Carl Roth. Luria-Bertani broth and Tryptic soy agar was supplied by Merck and Biolife Italiana, respectively. Microbial cultures: *E. coli* (strain ATCC 25,922) and L. *monocytogenes* (ATCC 19,111) were obtained from ETH Zurich and American Type Culture Collection, respectively.

2.2. Antibacterial activity assessment of essential oils by agar disk diffusion assay

The antibacterial activity of essential oils was determined by the agar disk diffusion method. Briefly, 100 μl of overnight bacterial culture (adjusted to 10^6 CFU/mL) suspensions of *E. coli or* L. *monocytogenes* were spread on Luria-Bertani (LB) or brain heart infusion (BHI) agar plates (Greiner Labortechnik Deutschland, Art.-Nr. 633,180, Ø 94 mm, height 16 mm), respectively. Subsequently, filter paper discs (Ø 10 mm; Whatman #1) were placed on the surface of agar plate and impregnated with 10 μL of EO (pure) or EO dimethylsulfoxide (DMSO) solution of different concentration (500 μg/μL; 300 μg/μL; 250 μg/μL; 200 μg/μL; 150 μg/μL; 25 μg/μL). Negative controls were prepared using DMSO only. Propionic acid (PR) was used as a positive control. All Petri dishes were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone, including the diameter of disks.

2.3. Preparation of coated films containing thyme essential oil

The aqueous emulsion consisting of water, 25 % (w/w) starch sodium octenylsuccinate (S) and 25 % (w/w) of thyme essential oil (TH) was prepared by using rotor-stator homogenizer (Polytron® PT 2500 E, disperse element PT-DA 12/2EC-E157, Switzerland) at 15,000 rpm for 2 min. The concentration of starch and the essential oil in the emulsion preparation was chosen aiming to introduce maximum amount of the essential oil by keeping the emulsion stable and was based on results of our preliminary experiments.

The coating compositions were obtained by mixing 34.8 g of emulsion and 65.2 g of acrylic binder (AC). The preparations were cast on corona treated oriented-polypropylene films (OPP) by using a coating machine (Zehntner, ZAA 2300, Switzerland) and profile rods (100 μm or 171 μm thickness of wet film). Thicker samples were prepared by casting the second layer of the coating on the top of the first layer after the drying of the first layer for 30 min. The coated OPP films were dried at room temperature for 2 h and denoted as AC-S-TH and relevant sample number. The coated films were kept in the closed bags for 24 h before further testing.

2.4. Antibacterial activity assessment of coated films by agar diffusion assay

The antibacterial activity of coated OPP films containing thyme essential oil was determined by the agar diffusion method. Briefly, 100 μL of overnight bacterial culture (adjusted to 10^6 CFU/mL) suspensions of *E. coli or* L. *monocytogenes* were spread on Luria-Bertani (LB) or brain heart infusion (BHI) agar plates (Greiner Labortechnik Deutschland, Art.-Nr. 633,180, Ø 94 mm, height 16 mm), respectively. Discs of coated films (Ø 10 mm) were placed on the surface of the inoculated agar plate, with the coated site facing agar. Negative controls were prepared with AC-S coated films without thyme EO. Propionic acid on filter paper was used as a positive control. All Petri dishes were incubated at 37 °C (24 h). Antibacterial activity was evaluated by measuring the diameter of the inhibition zone, including the diameter of discs.

2.5. Antibacterial activity assessment of thyme essential oil and coatings by vapor diffusion assay

The antibacterial activity of AC-S-TH coatings on OPP and pure thyme EO against *E. coli* and L. *monocytogenes* was assessed using a vapor diffusion assay described by Rüegg et al., 2020. An inoculum concentration of 10^4 CFU/mL of *E. coli* or L. monocytogenes in sterile water was adjusted for antibacterial activity test by adding 1 ml of the 10^7 CFU/mL suspension to 1 L of sterile water. Then, 100 mL of sterile water, inoculated with *E. coli or* L. *monocytogenes (*10^7 CFU/L) was sterile filtered through cellulose-nitrate filter (Sartorius Stedim Biotech GmbH, Art.-Nr. 13,906–47, Ø 47 mm, pore size of 45 μm) using a vacuum filtration system (Millipore, Art.-Nr. XF54 230 50) to adjust the initial bacteria load to 10^6 CFU/ filter. The inoculated filters were then placed on Tryptic soy agar (TSA) (Biolife, Italy) plates (Eppendorf, Germany, Ø 60 mm). Sterile paper discs with a diameter of 55 mm (Whatmann, Art.-Nr. 1002–055) or AC-S-TH coated OPP films of the same diameter containing various amounts of thyme EO (585 ± 30 μg/cm^2; 1056 ± 17 μg/cm^2; 2159 ± 112 μg/cm^2) were fixed in the lid of each Petri dish, in order not to have direct contact with the bacteria. The headspace volume was adjusted to approximately 14.5 cm^3. Sterile filter paper discs were loaded with 100 μL of thyme EO of different concentration (140 μg/μL; 250 μg/μL; 510 μg/μL) as control samples. The amount of thyme

oil per square centimeter of the paper disk was the same as that in AC-S-TH coatings. Additionally, paper discs were loaded with 100 μL of propionic acid as a positive control. AC-S coatings on OPP without thyme EO (N.C1) and paper discs loaded with 100 μL of DMSO (N.C2) were used as negative controls. Petri dishes were then sealed hermetically by rubber ring and wrapped with Parafilm, and incubated for 24 h at 37 °C. After the incubation period, cellulose filters were removed from TSA, transferred into 10-mL BHI broth (L. *monocytogenes*) or LB broth (*E. coli*) and vortexed for 15 min at room temperature. The antimicrobial activity of AC-S-TH coatings, thyme EO and control samples were determined by detecting colony forming units by spread-plate method using BHI agar for L. *monocytogenes* and LB agar for *E. coli* after an incubation at 37 °C for 24 or 48 h, respectively. The microbial results were expressed as logarithms of the number of colony-forming units (CFU) per filter. In addition, the same method was used to determine the initial bacterial load on the cellulose filters after filtration at time t_0. All tests were performed in triplicates.

2.6. Determination thymol release from the coated films

The release of thymol from AC-S-TH coated OPP films into the vapor phase over 21 days was assessed by measuring the remaining thymol in the AC-S-TH coating using GC and calculating the amount released. Coated film samples were cut into the 1 cm x 5 cm sized strips and each strip was placed into 20 mL volume vials and sealed. The vials were maintained at room temperature for defined periods of time, the coated film samples were taken out from the vials, dissolved in 5 mL of pyridine and the amount of thymol remaining in the coatings was established by analyzing pyridine solution using gas chromatograph GC2010 Plus with flame ionization detector (Shimadzu Technologies, Kyoto, Japan). Analyte separation was performed using a Rxi-5 MS (Restek Corporation, Bellefonte, PA, USA), capillary column (film thickness: 0,25 μm, 30 m long, 0,25 mm internal diameter) column. The stationary phase contained 5 % diphenyl and 95 % polydimethylsiloxane. The 99.999 % purity helium was used as carrier gas with the flow rate of 1.19 mL/min. The injection port temperature was maintained at 290 °C and volume injected was 1 μL, split ratio 1:10. Flame ionization detector temperature was 330 °C. The oven temperature was programmed at 70 °C for 2 min, then increased to 250 °C at 20 °C/min and maintained for 2 min. The experiments were carried out in triplicates. The quantitative determination of thymol in test solutions was carried out using an external standard and a calibration curve. The linear calibration curve (peak area vs. concentration, R^2>0.999) was formed by processing a series of calibration solutions in the range from 15.57 to 1993.00 μg per milliliter.

2.7. Statistical analyses

Data were statistically handled by the one-way analysis of variance (ANOVA for Excel, vers. 2.2). All experiments were carried out in triplicate, and the results were expressed as Mean ± SD. Duncan's multiple-range test was applied for the calculation of the significant differences among the values of characteristic parameters at probability level $P < 0.05$.

3. Results and discussion

3.1. Antibacterial activity of selected essential oils

To develop an effective antibacterial packaging material, it is essential to first evaluate and determine the unique antibacterial properties and effective concentrations of each essential oil. Thus, first the antibacterial activity of EOs or active compounds such as thyme, clove, linalool, and eugenol was assessed by using agar disk diffusion assay. Different concentrations of EO (1000; 500; 300; 250; 200; 150; 25 μg/μL) were prepared in dimethylsulfoxide (DMSO) and applied onto filter paper discs, which were then placed on LB or BHI agar plates inoculated with *E. coli* and L. *monocytogenes*, respectively. The inhibition zones, indicating the antibacterial activities, were measured after 24 h (see Table 1 and Table 2). Propionic acid (PR) applied onto filter paper was used as a positive control. As shown in Table 1, thyme oil exhibited the highest antibacterial activity against *E. coli*. It also showed the highest antibacterial activity against L. *monocytogenes*, while eugenol was the second most effective EO fallowed by clove oil and linalool (see Table 2). Based on the antibacterial activity results obtained by the agar diffusion method, thyme oil was selected for further experiments as the most effective essential oil against *E. coli* and L. *monocytogenes*. Other authors (Hoda et al., 2022; Rota et al., 2008) have also assessed antibacterial activity of thyme EO by measuring minimum inhibitory concentration, minimal bactericidal concentration, and inhibition zones by agar diffusion assay, and revealed that thyme EO is particularly effective against L. *monocytogenes* as in our studies.

The USFDA classifies EOs as GRAS and includes oils such as thyme, clove, oregano, nutmeg, basil, mustard, and cinnamon (USFDA, 1997). However, there are regulatory limitations on the accepted daily intake. In Europe, EO fall under Regulation 1334/2008 on natural flavourings (Regulation (EC) No 1334, 2008).

3.2. Development of coatings for packaging films

An effective approach to developing antibacterial packaging materials is by coating the packaging films with formulations containing EO with antibacterial properties. In this method, natural, modified or synthetic polymers can be used as coating binders (Bastarrachea et al., 2015). Moreover, a wide range of packaging films can be used, including synthetic options such as polyethylene, polypropylene, polystyrene, as well as natural biodegradable polymers such as starch, cellulose, and proteins (Bastarrachea et al., 2015; Corrales et al., 2014; Cutter et al., 2006).

In this study, active coatings containing acrylic binder, starch sodium octenylsuccinate and thyme EO were prepared and applied at varying thicknesses onto corona treated oriented polypropylene films to achieve different thymol loadings. Table 3 presents the key characteristics of the prepared coatings, including the amount of coating on the films and the quantities of thyme EO and its main antibacterial component, thymol, per square centimetre of the film. The application of different coating

Table 1
Antibacterial activity of EO or active compounds against *E. coli* by the agar diffusion method.

EO or active compound	Concentration (μg/μL)						
	1000	500	300	250	200	150	25
	Inhibition zone (mm)						
Thyme EO	40.0 ± 1.0^{cF}	32.3 ± 2.5^{bE}	27.0 ± 2.0^{bD}	21.7 ± 1.5^{cC}	19.7 ± 0.6^{dBC}	19.0 ± 1.7^{cB}	12.3 ± 1.2^{cA}
Linalool	21.3 ± 1.2^{bD}	17.7 ± 0.6^{aC}	15.7 ± 0.6^{aB}	16.3 ± 0.6^{bB}	16.0 ± 0.0^{cB}	15.3 ± 0.6^{bB}	0^{aA}
Clove EO	16.7 ± 0.6^{aD}	16.0 ± 1.0^{aD}	14.3 ± 0.6^{aE}	13.0 ± 1.0^{aB}	12.7 ± 0.6^{aB}	12.0 ± 0.0^{aB}	0^{aA}
Eugenol	20.0 ± 0.0^{bF}	16.3 ± 0.6^{aE}	15.0 ± 0.0^{aC}	14.3 ± 0.6^{abBC}	14.0 ± 0.0^{bB}	12.7 ± 0.6^{aD}	10.8 ± 0.3^{bA}
PR	29.7 ± 0.6	29.7 ± 0.6	29.7 ± 0.6	29.7 ± 0.6	29.7 ± 0.6	29.7 ± 0.6	29.7 ± 0.6
DMSO	0	0	0	0	0	0	0

$^{a–d}$: the different lowercase letters within the same column for different sample show that the results are significantly different ($P < 0.05$; Duncan test).
$^{A–F}$: the different uppercase letters within the same row for each sample concentration show that the results are significantly different ($P < 0.05$; Duncan test).

Table 2
Antibacterial activity of active compounds against L. *monocytogenes* by the agar diffusion method.

EO or active compound	Concentration (μg/μL)						
	1000	500	300	250	200	150	25
	Inhibition zone (mm)						
Thyme EO	88.0 ± 1.0^{cF}	42.7 ± 2.5^{cE}	32.7 ± 0.6^{cD}	23.3 ± 2.9^{cC}	21.7 ± 2.9^{dBC}	18.7 ± 1.5^{cB}	11.3 ± 0.6^{cA}
Linalool	17.7 ± 1.5^{aD}	13.7 ± 0.6^{aC}	11.7 ± 0.6^{aB}	11.0 ± 0.6^{aB}	11.0 ± 0.0^{bB}	11.0 ± 0.0^{bB}	0^{aA}
Clove EO	19.3 ± 0.6^{abC}	16.7 ± 1.2^{abB}	15.3 ± 1.2^{bB}	15.3 ± 0.6^{bB}	0^{aA}	0^{aA}	0^{aA}
Eugenol	20.0 ± 0.0^{bD}	19.7 ± 0.6^{bD}	16.0 ± 1.0^{bB}	17.0 ± 2.0^{bB}	15.3 ± 0.6^{cB}	12.7 ± 1.5^{bE}	10.5 ± 0.5^{bA}
PR	29.7 ± 0.6	29.7 ± 0.6	29.7 ± 0.6	29.7 ± 0.6	29.7 ± 0.6	29.7 ± 0.6	29.7 ± 0.6
DMSO	0	0	0	0	0	0	0

$^{a–d}$: the different lowercase letters within the same column for different sample show that the results are significantly different ($P < 0.05$; Duncan test).
$^{A–F}$: the different uppercase letters within the same row for each sample concentration show that the results are significantly different ($P < 0.05$; Duncan test).

Table 3
The characteristics of AC-S-TH coatings.

Sample	Amount of coating (μg/cm^2)	Amount of thyme EO* (μg/cm^2)	Amount of thymol** (μg/cm^2)
AC-S-TH-1	2925 ± 150	585 ± 30	242 ± 7
AC-S-TH-2	5280 ± 85	1056 ± 17	587 ± 29
AC-S-TH-3	10,795 ± 560	2159 ± 112	930 ± 4

* The amount of thyme EO was calculated based on the dry coating weight, using the known theoretical proportions of each component (acrylic binder, modified starch, and thyme EO) in the formulation.
** amount of thymol in the coating determined using GC.

thicknesses resulted in varying amounts of coatings on the films, ranging from 2925 ± 150 to 10,795 ± 560 μg/cm^2, which corresponds to thyme EO amounts from 585 ± 30 to 2159 ± 112 μg/cm^2. Thymol content was quantified using gas chromatography (GC) analysis, with values ranged from 242 ± 7 to 930 ± 4 μg/cm^2. The differences in thymol percentage relative to total EO are due to theoretical EO calculations versus GC-measured thymol content, which varied slightly based on coating uniformity and application.

3.3. Antibacterial activity of coated films

The antimicrobial activity of oriented polypropylene films coated with AC-S-TH was evaluated using the agar diffusion assay. To assess the effect of coating in particular its role in the release of thyme essential oil, the results were directly compared with those obtained from filter paper samples loaded with the same amount of thyme EO (Table 4). The results show that the antibacterial activity of AC-S-TH coatings and filter paper discs, both containing same amounts of thyme oil, differed significantly against *E. coli* and L. *monocytogenes*. AC-S-TH-1 and AC-S-TH-2 did not show any antibacterial activity against both bacteria. Filter paper containing same amounts of thyme EO on the other hand resulted in an inhibition zone of 12.17 ± 0.29 and 14.00 ± 0.00 for *E. coli* and 12.50 ± 0.50 and 14.17 ± 0.29 for L. *monocytogenes*, respectively. Films with AC-S-TH-3 coating containing the highest amount of thyme EO (2159 ± 112 μg/cm^2) exhibited 10.67 ± 0.29 and 10.83 ± 0.29 mm inhibition zone against *E. coli* and L. *monocytogenes*, respectively. Filter paper containing same amount of thyme oil (TH-3) resulted in an inhibition zone of 18.67 ± 0.58 and 19.00 ± 0.00 mm against those two bacteria. The difference in antibacterial activity of AC-S-TH coatings and thyme oil on paper discs can be attributed to variations in the release of essential oil from the solid coating and the impregnated filter paper. Thyme oil is released much faster from the filter paper, while the low release rates from the coating limited its activity. Only the coatings with the highest amount of thyme EO content exhibited antibacterial activity.

Table 4
Antibacterial activity of AC-S-TH coatings or TH on filter paper against *E. coli* and L. *monocytogenes*.

Sample	Inhibition zone (mm)	
	E. coli	L. *monocytogenes*
AC-S-TH-1	0	0
AC-S-TH-2	0	0
AC-S-TH-3	10.67 ± 0.29	10.83 ± 0.29
TH-1	12.17 ± 0.29	12.50 ± 0.50
TH-2	14.00 ± 0.00	14.17 ± 0.29
TH-3	18.67 ± 0.58	19.00 ± 0.00

Since that most food products are typically packaged without direct contact with the top layer of packaging material, the antibacterial properties of coated films and the non-immobilized active agent (thyme EO) were investigated through the vapor phase in tightly sealed Petri dishes using vapor diffusion method (described in Section 2.5). Films with active coatings or filter paper impregnated with thyme EO were affixed to the inside of the Petri dish lids, which were then placed over a Petri dish containing solid agar medium inoculated with microorganisms, thereby preventing direct contact with the active sample. The antibacterial activity of both the coated films and the non-immobilized EO against *E. coli* and L. *monocytogenes* was evaluated. The amounts of thyme EO applied onto a filter paper were equivalent to those present in the coatings. The antibacterial activity results against *E. coli* and L. *monocytogenes*, obtained by using the vapor diffusion method, are presented in Figs. 1 and 2, respectively. As shown in Figs. 1a and 1b, when negative control samples without essential oil (N.C1 and N.C2) were used, *E. coli* grew from 5.5 log CFU per filter to 10.3 log CFU per filter and 10.4 log CFU per filter, respectively. Coated films with the least amount of thyme oil (AC-S-TH-1 with 585 ± 30 μg/cm^2 thyme oil) did not show any significant antibacterial activity comparing to N.C1. On the other hand, coated films containing a higher amount of thyme oil (1056 ± 17 μg/cm^2) showed a significant antibacterial effect, with a 2.3 log reduction compared to control samples. A further increase in thyme oil in the coating to 2159 ± 112 μg/cm^2 did not result in a further increase in antibacterial activity. Unlike coated samples, thyme oil in filter paper showed a significant antibacterial effect at the lowest concentration (585 ± 30 μg/cm^2), resulting in a log reduction of 1.5. Increasing in thyme oil concentration to 1056 ± 17 μg/cm^2 and 2159 ± 112 μg/cm^2 further enhanced the antibacterial effect, showing log reduction of 2.2 and 3.1, respectively. Positive controls with propionic acid reduced the number of *E. coli* to 0 CFU per filter in approximately 24 h.

The antibacterial activity of AC-S-TH coatings and non-immobilized TH samples against L. *monocytogenes* by vapor diffusion assay is presented in Fig. 2. After 24 h of incubation the number of L. *monocytogenes* bacteria increased from 5.42 log (0 h control) to 9.79 and 9.84 log (control samples without essential oil). The positive control with propionic acid on the other hand reduced the number of L. *monocytogenes* to 0 CFU per filter in approximately 24 h. When coated films AC-S-TH were used an increase in thyme oil concentration in the coating from 585 ± 30 to 2159 ± 112 μg/cm^2 resulted in an increase in antibacterial showing log reductions of 0.94 and 3.84, respectively. Use of non-immobilized TH samples showed a similar antibacterial effect; i.e., an increase in thyme oil concentration resulted in an increase in antibacterial activity. However, the antibacterial activities were higher (1.88 to

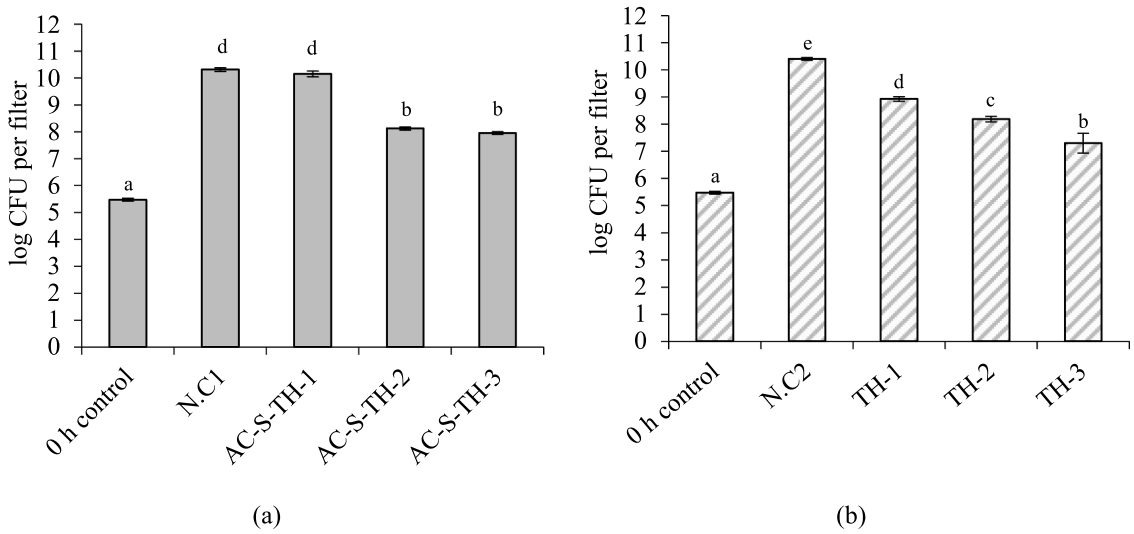

Fig. 1. The antibacterial activity of AC-S-TH coatings (a) and non-immobilized TH (b) against *E. coli* by vapor diffusion assay. Amount of thyme oil in coatings: AC-S-TH-1 – 585 ± 30 μg/cm^2; AC-S-TH-2 – 1056 ± 17 μg/cm^2; AC-S-TH-3 – 2159 ± 112 μg/cm^2. Amount of TH in non-immobilized TH samples was the same as in the coatings. a–e: the different lowercase letters for within different sample show that the results are significantly different ($P < 0.05$; Duncan test).

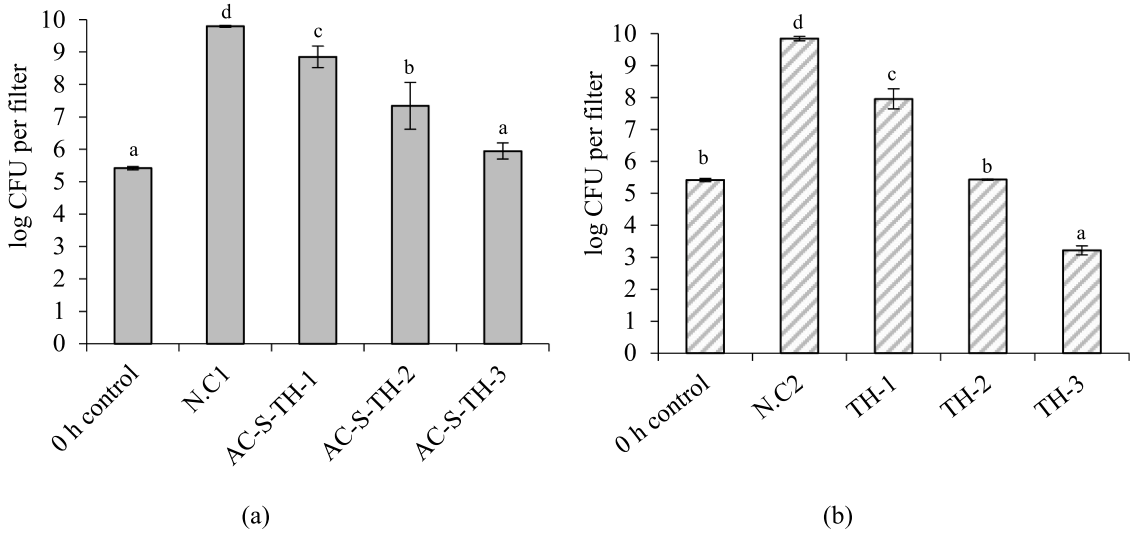

Fig. 2. The antibacterial activity of AC-S-TH coatings (a) and non-immobilized TH (b) against L. *monocytogenes* by vapor diffusion assay. Amount of TH in the coatings: AC-S-TH-1 – 585 ± 30 μg/cm^2; AC-S-TH-2 – 1056 ± 17 μg/cm^2; AC-S-TH-3 – 2159 ± 112 μg/cm^2. Amount of TH in non-immobilized TH samples was the same as in the coatings. a–d: the different lowercase letters for within different sample show that the results are significantly different ($P < 0.05$; Duncan test).

6.63 log CFU per filter) with the same amount of thyme oil compared to coated film samples. Similar results were observed in other study of Kang (Kang, 2022) which reported a 2.5 and 3.3 log-reduction in the number of the *E. coli* and L. *monocytogenes* respectively, on romaine lettuce surface, compared to the control samples, when using emulsified TH.

It can be concluded that the antibacterial activity of coated films against *E. coli* and L. *monocytogenes* depended on the amount of immobilized TH. AC-S-TH coatings with highest amount of TH (2159 ± 112 μg/cm^2) showed the highest antibacterial activity through the vapor phase against *E. coli* and L. *monocytogenes*. In general thyme EO on filter paper showed higher antibacterial activities compared to a coated film samples which is probably due to the faster release of thyme oil from the filter paper. Antibacterial activity of thyme oil in coatings or on filter paper were higher against L. *monocytogenes* than *E. coli* in both agar disc diffusion assay and vapor diffusion assay. The obtained results were in agreement with the findings of other studies which indicated that thyme essential oil (*Thymus vulgaris*) exhibits stronger activity against L. *monocytogenes* compared to *E. coli* (Hoda et al., 2022; Rota et al., 2008; Kang, 2022).

3.4. Thymol release studies

The chemical composition of thyme EO was previously evaluated by other authors (Teixeira et al., 2013). Thyme EO is mainly composed of oxygenated monoterpenes and the major component of this group is thymol which is a phenolic compound with strong antimicrobial properties. In this study, selected thyme EO was found to contain 42.8 % thymol (determined by calculating the peak areas of the processed chromatogram, see Suppl. Fig. 1). In order to understand the release of thymol from the AC-S-TH coated OPP films containing different amount of thyme EO, they were stored in a sealed container for 21 days. The release of thymol from the coating into the headspace of sealed container was assessed by measuring the remaining thymol in the AC-S-TH coating using GC and calculating the amount released. Pyridine was selected as a solvent in order to dissolve coatings components such

as acrylic binder and modified starch in which EO was immobilized. A representative chromatogram of the coating solution, showing all detected peaks including the solvent (retention time is approx. 3 min.) and thymol (retention time approx. 7.16 min.), is presented in Fig. 3. It was further noted that all principal components were consistently present in the chromatograms of all tested samples throughout the storage period (Suppl. Fig. 2), indicating sufficient stability of EO constituents in the coatings. The chromatographic profile of the representative component thymol in the coated film sample (AC-S-TH-3) during storage time is given in Fig. 4. The chromatogram shows a decrease in the intensity of the signal characteristic of the thymol remaining in the AC-S-TH-3 sample after 0, 2, 7 and 21 days of the storage (Fig. 4) indicating a continuous release of thymol during the storage period.

As shown in the diagrams in Fig. 5, the amount of released thymol was gradually increasing for all samples during the storage period. During the first 7 days, the amounts of thymol released from the AC-S-TH-1, AC-S-TH-2 and AC-S-TH-3 coatings reached levels of 3.71–10.15 μg/cm^3, 2.76–11.21 μg/cm^3, 3.99–13.65 μg/cm^3, respectively. However, no statistically significant differences were observed between the coatings. After 10 days, the amount of thymol released from AC-S-TH-2 and AC-S-TH-3 increased substantially, reaching maximum levels of 19.63 ± 2.32 μg/cm^3 and 21.85 ± 2.11 μg/cm^3, respectively. In contrast, the thymol released from AC-S-TH-1 coating at day 10 was 11.19 μg/cm^3 approximately half the amount released from the other two samples. From day 10 to day 21, no further significant increase in thymol release was detected. The release rate of thymol can vary depending on the immobilization method and the properties of the matrix. Previous studies have shown that thymol exhibits a faster release rate than carvacrol when embedded in maltodextrin and soy protein matrices (Ulloa et al., 2017). Additionally, the release of oregano essential oil from chitosan matrices was slower than that observed for thymol in comparable conditions (Ma et al., 2022). These findings are consistent with our results. In our study, the comparison of the released amount of thymol (Fig. 5a) and amount of thymol remaining in the coating (Fig. 5b) reveals that only a very small portion of the thymol in the coating is released during storage period. This suggest that the coating formulation can be further optimized to achieve a faster thymol release, thereby enhancing antibacterial activity and potentially reducing the total amount of thyme essential oil required. A dynamic equilibrium is reached after 10 days and no significant increase in the thymol concentrations is observed afterwards.

4. Conclusions

In this study, we have demonstrated that an acrylic binder with starch sodium octenylsuccinate can be successfully used to immobilize thyme essential oil. The resulting coatings, applied to polypropylene films, exhibited a gradual release of main active component - thymol. This resulted in antibacterial activity which was successfully quantified using vapor diffusion assay. The coated films exhibited significant antibacterial activity trough the vapor phase, reducing the growth of *E. coli* and L. *monocytogenes* cells by 2.3 log CFU per filter and 3.8 CFU per filter, respectively, compared to control samples without thyme oil. The continuous release of thymol from the coatings into the headspace during a storage of 10 days shows that it may have potential for use of such coatings for food packaging applications especially for those that are susceptible to bacterial growth and has a short shelf life. Thus, the use of thymol in active food packaging has the potential to extend shelf life and enhance food safety by providing antimicrobial protection while reducing the need for synthetic additives. It is important to note that the release of thymol in a sealed headspace vial does not fully replicate real-world food packaging conditions. Therefore, future studies should evaluate release kinetics and antimicrobial performance under more practical scenarios, including interactions with food matrices and packaging permeability.

Ethical statement

Herewith I confirm that the research presented does not involve any animal or human study. It does not also include sensory evaluation and customer survey.

Prof. Dr. Selcuk Yildirim

CRediT authorship contribution statement

Vesta Navikaite-Snipaitiene: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ramune Rutkaite:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Liudas Ivanauskas:** Writing – review & editing,

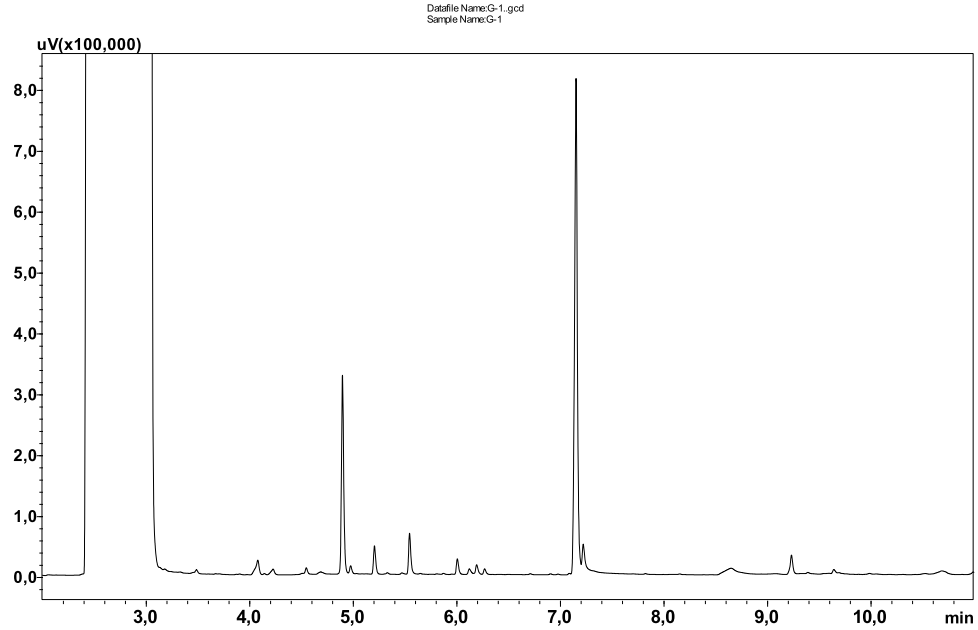

Fig. 3. The representative gas chromatogram of AC-S-TH coating solution in pyridine.

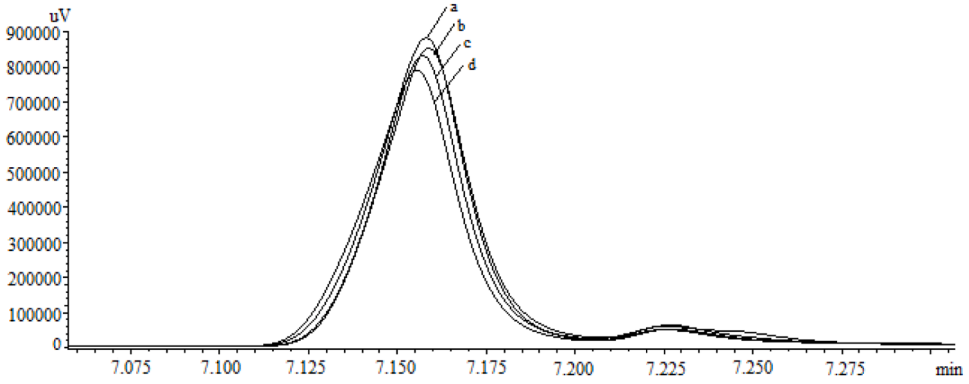

Fig. 4. The thymol gas chromatography profile of AC-S-TH-3 sample during the storage: a – day 0 (initial sample); b – day 2; c – day 7; d – day 21.

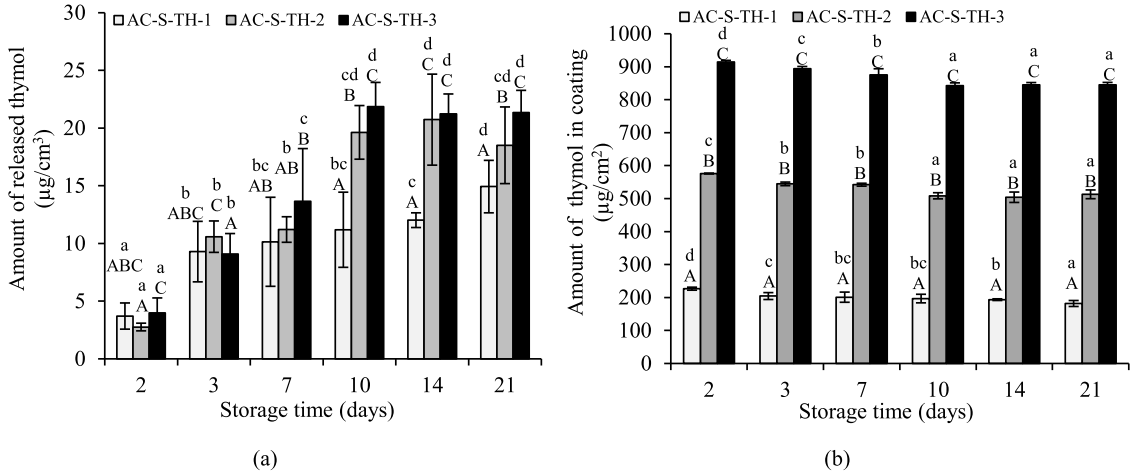

Fig. 5. Amount of released thymol (a) and thymol remaining in the coatings (b) during the storage of the samples. a–d: the different lowercase letters for the same sample within the storage time show that the results are significantly different ($P < 0.05$; Duncan test).; A–C: the different uppercase letters for different sample with the same storage time show that the results are significantly different ($P < 0.05$; Duncan test).

Data curation. **Valdas Jakstas:** Writing – review & editing, Data curation. **Lars Fieseler:** Supervision, Methodology. **Nadine Rüegg:** Writing – review & editing, Methodology. **Selçuk Yildirim:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2025.101367.

Data availability

Data will be made available on request.

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