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Evaluation of the Potential of Functionalised Calcium Carbonate as Carrier for Essential Oils with regard to Antimicrobial Packaging Applications

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Abstract
Functionalised calcium carbonates (FCCs) are inorganic mineral-based particles with a high porosity and extended surface area consisting of hydroxyapatite and calcium carbonate crystal structures. Therefore, FCCs have a high potential to be used as a carrier for active substances such as essential oils (EOs), which are well known for their antimicrobial activities, and control their release in antimicrobial packaging applications. In this study different EOs were loaded on FCCs and their chemical stabilities were evaluated. Afterwards, antimicrobial activities of FCCs containing EOs were studied against *L. innocua* in *in-vitro* tests and in *in-situ* tests using sliced cooked chicken breast. Increase in loading of thyme EO from 5 % to 10 % increased the antimicrobial activity after 1 day. Further increase in the loading of EO to 30% resulted in the same antimicrobial activity as the 10% loading. In further tests FCCs were loaded with 10 % EOs. FCCs loaded with thyme or oregano EO showed the highest reduction in microbial load in *in-vitro* tests at 37°C (≥ 8.6 log cfu/ filter) as well as at 7°C after 6 days (≥ 7.0 log cfu/ filter for thyme EO and 6.5 log cfu/ filter for oregano EO). In *in-situ* tests FCC loaded with either EO did not show any significant antimicrobial activity. FCCs loaded with cinnamon or rosemary EO did not show any significant antimicrobial activity in *in-vitro* tests. However, they showed a significant reduction in microbial load (1.7 log cfu/g for cinnamon and 2 log cfu/g for rosemary) in *in-situ* tests.

Keywords: Functionalised calcium carbonate, essential oils, active packaging, antimicrobial packaging, sliced cooked chicken breast

INTRODUCTION

Food packaging plays an important role in food safety and food quality. Food-related microorganisms can lead to food spoilage but can also cause various food-borne diseases, which is one of the main public health concerns[1]. *Listeria monocytogenes* is one of the most harmful food-borne pathogens and causes listeriosis. In 2016, 0.47 listeriosis cases per 100'000 population was reported in the EU member states with a fatality rate of 16.2 %[2]. The vast majority of listeriosis cases are foodborne (99 %) and are typically linked to ready-to-eat foods (RTE), especially meat products[3-5]. One reason for this is that the surface of the RTE meat products may get contaminated with ubiquitous *L. monocytogenes*, for example during processing by transfer via equipment such as slicers. Even if the product is afterwards stored at refrigeration temperature, *L. monocytogenes* is able to grow[3,6,7]. In general, the control of meat-borne pathogens will continue to be one of the major goals in the future.

One strategy to combat with the pathogens in food would be the use of antimicrobial packaging technologies to inhibit the growth of spoilage microorganisms and pathogens in food. Among the antimicrobial packaging systems, those containing volatile essential oils (EOs) are especially interesting, where the packaging has no direct contact with the food and the antimicrobial activity has to be ensured through the headspace[8-10].

EOs are natural products extracted from plant materials such as flowers, buds, leaves, stem, bark and seeds. They are well known for their antimicrobial activity and their effect on a wide range of microorganisms has been extensively studied in *in-vitro* and in food tests (*in-situ*)[1,8,11,12]. Major components of the EO usually define the antimicrobial activity, but there are often synergies with the minor components. For this reason, their mode of action also includes several targets in the bacterial cell. The lipophilic nature of EOs enables them to partition in the lipids of the cell membrane and mitochondria, make them permeable and lead to the leakage of cell contents[13,14]. One challenge in the use of volatile EOs for antimicrobial packaging is that they have to be integrated into the packaging without losing their efficiency. As well they need a suitable inert carrier system to avoid the direct food contact by flowing around of the EOs within the package and to ensure a controlled release into the packaging gas phase to maintain and ensure the antimicrobial activity during the whole shelf life of the food product[8,15].

In recent years, highly porous particles have been increasingly used to carry active ingredients in the fields of pharmaceutics and food technology[16-18]. Omya International AG has developed a family of inorganic, mineral-based particles (silicate free) with a high porosity and extended surface area by exposing natural ground calcium carbonates in a shear field to conditions of controlled temperature and a non-stable pH in the slightly acidic range. Those particles are referred to as functionalised calcium carbonates (FCC). FCCs have hydrophilic surfaces and consist of hydroxyapatite (HAP) and calcium carbonate crystal structures[19]. They have a size of 5-15 µm and have a highly developed surface and internal structure.

Being used as carriers, they have shown unique effects such as increasing the solubility of hydrophobic actives in water[16], enabling the formulation of very fast releasing systems (oral dispersible tablets)[17] and sustained release applications[18].

In this study, the potential of FCC was uniquely investigated as a carrier system for EOs, towards the development of an antimicrobial packaging. First, EOs, namely rosemary, oregano, cinnamon, clove and thyme EOs were loaded on the FCC powder and their stability were evaluated. Afterwards the effect of loading concentration of EO on FCC powder on the *in-vitro* antimicrobial activity against *L. innocua* was studied. Finally, the *in-vitro* and *in-situ* (using sliced cooked chicken breast) antimicrobial activities of the FCCs loaded with EO against *Listeria innocua* were investigated.

MATERIALS AND METHODS

Essential oils and Carrier

Cinnamon bark EO 65 % Ceylon (*Cinnamomum zeylanicum*), clove bud EO (*Eugenia caryophyllus*), Oreganum 70 % EO Balkans (*Origanum vulgare hirtum*), Rosemary EO Morocco (*Rosmarinus officinalis leaf oil*) and Thyme red EO Hungary (*Thymus vulgaris flower/Leaf oil*) were purchased from Bernardi Group (France). All EOs were stored in the dark at 21 ± 1°C and utilised before the expiration date.

FCC is a calcium carbonate which has undergone partial recrystallisation to form a variable layer of lamellae of calcium phosphate (hydroxyapatite) on the surface of the particles. The FCC used had a median particle size (d50%) of 5.5 µm, a top cut (d98%) of 17 µm and a specific surface area of 110 m^2/g and an intraparticle pore volume of 1.22 cm^3/g as measured by mercury intrusion porosimetry (MIP) as provided by Omya International AG (Oftringen, Switzerland) (Figure 1).

Insert Figure 1 here

Loading of FCC with essential oil

Before loading, the FCC was dried in an oven at 150°C over night. Loading with 5 wt%, 10 wt%, and 30 wt% was achieved using a modified incipient wetness impregnation[16,20]. The EOs were directly added to the carrier without further solvent dilution. The prepared powders were stored under nitrogen atmosphere in the dark at 21±1°C. To evaluate the antimicrobial activity, 0.9 g of the FCC powder loaded with EO was weighed into heat sealable tea bags (55 x 80 mm, Special Tea Company®, USA) directly before testing the antimicrobial activity.

Stability analyses of essential oils loaded on FCC

After the loading procedure, each sample (unloaded material, loaded material and pure oil) was weighed trice (triplicate) in 40 mL clear glass vials with PTFE caps. The loaded porous material's weight corresponded to 0.1 g of EO. The internal standard (IS) solution (min. 20 mL) was prepared on the day of the first measurement (t0) and filled into dark GC vials (one for each measuring date). All vials were stored in a refrigerator. The extraction of the first samples (t0) was performed on the same day as the loading of EOs on FCC. Other samples were stored at 4°C with 80 % humidity in dark conditions for 30 days.

Extraction for GC/MS analysis

At the end of each storage period, 20 mL ethanol were added to the vial. The suspension was stirred for 15 min at room temperature and centrifuged in PTFE tubes (10 min at 12'000 rpm). 1.5 mL of supernatant and 20 µL of IS solution were pipetted into a GC vial. The mixture was analysed by GC/MS. Each replicate was measured twice resulting in six measurements for one sample.

Pure oil preparation for GC/MS analysis

At the end of the storage period, 20 mL ethanol were added to the stored pure oil (110-130 mg). 1.5 mL of solution and 20 µL of IS solution were pipetted into a GC vial. The mixture was analysed by GC/MS. Each replicate was measured twice resulting in six measurements for one sample.

GC/MS parameters

A GC Clarus 680 coupled with an MS Clarus SQ8T from Perkin Elmer was used. An Optima® 5 MS Accent (1.0 µm, 60 m * 0.32 mm ID) from Macherey-Nagel was used. The injector's temperature was set to 300°C. 0.5 µl of sample were injected in the "normal" speed with a split of 30 mL/min. The column's temperature has been held at 80°C for 1.30 min, 30°C/min to 250°C and held for 10.03 min. The pressure was held at 70 kPa. A solvent delay of 4.3 min was used. A full scan was performed between 25 and 400 m/z. The different sets of peaks for each EOs were characterised by means of a NIST library and integrated. The areas were normalized with the one of the IS. Their area-percent were reported.

Preparation of inoculum for *in-vitro* and *in-situ* antimicrobial activity tests

Gram- positive bacteria *Listeria innocua* (ATCC 33039, a *Listeria monocytogenes* surrogate) were selected for the evaluation of the antimicrobial activity of FCCs loaded with EOs in *in-vitro* and *in-situ* tests. For *in-vitro* tests overnight cultures of *L. innocua* were prepared in 10 ml Brain Heart Infusion (BHI) Broth (Biolife, Italy) at 37°C for 12 -18 hours and for *in-situ* tests microorganisms were adapted at 7°C in BHI broth for 5 to 7 days. After cold adaption each culture was centrifuged at 4000 rpm for 2 min at room temperature (Sigma 3-18K). The centrifugation pellets were washed 2 times with 8 mL of 0.1 % peptone water including 0.85 % NaCl (diluting solution) and recentrifuged. After washing and centrifugation, the cultures were resuspendet in diluting solution. The concentrations of microorganisms were determined with a Neubauer improved counting chamber (0.1 mm x 0.0025 mm) and afterwards diluted to a final concentration of 10^7 colony forming units (cfu)/mL for *in-vitro* and *in-situ* tests.

Antimicrobial activity tests of FCCs loaded with essential oil in agar plates

An inoculum concentration of 10^4 cfu/mL *L. innocua* in sterile water was adjusted for every *in-vitro* antimicrobial activity test. 100 mL of the inoculum 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). The loaded EO FCCs and untreated FCC (negative control) were placed in the lid of each petri dish to avoid direct contact. The headspace volume was adjusted to approximately 14.5 cm^3. Then petri dishes were sealed with a rubber ring, wrapped with Parafilm and packed individually in PET ax/PE high barrier bags (Wipf AG, Switzerland) with a volume of max. 160 cm^3 for incubation at 37°C for 1 or 6 days. After the incubation period, cellulose filters were removed from TSA, transferred into 10 mL BHI broth and vortexed for 15 minutes at room temperature. The antimicrobial activity of FCCs loaded with EO 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 performed in triplicates, unless otherwise stated.

***In-situ* antimicrobial activity tests of FCCs loaded with essential oil on sliced cooked chicken breast in petri dishes**

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) (Optigal Pouletbrust) were delivered freshly packed under modified atmosphere from Micarna SA, Switzerland. Samples from each slice of the tested meat products with a diameter of 60 mm (3.1 -3.2 g) were cut out and transferred in sterile plastic petri dishes with 60 mm diameter (Eppendorf, Germany). 0.1 mL of the inoculum containing 10^7 cfu/mL *L. innocua* was spread over the sliced cooked chicken breast. EO loaded

FCCs and untreated FCC (negative control) were placed in the lid of each petri dish to avoid direct contact. The initial microbial load of each sample was also detected. Headspace volume was adjusted to approximately 19.5 cm^3. Then petri dishes were sealed with a rubber ring, wrapped with Parafilm and packed individually in PET ax/PE high barrier bags (Wipf AG, Switzerland) with a volume of max. 250 cm^3 for incubation at 7.5°C ± 0.4°C for 1 or 6 days. After incubation and storage, meat samples were diluted 1:10 with Half Fraser Broth (Biokar Diagnostics, France) homogenised for 120 s at 300 rpm using a stomacher (Seward Stomacher 400 circulator). Microbial load of all samples was determined by a cultural spread-plate method after serial dilutions on Agar Listeria acc. to Ottaviani & Agosti (ALOA) (Oxoid, UK). Additionally, the initial microbial load and the recovery rate of bacteria was detected 1 hour after sample preparing (t0). Microbiological counts were expressed as logarithms of the number of cfu per gram (log cfu/g). All tests were performed in triplicates.

Statistical analysis

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 $P < 0.05$.

RESULTS & DISCUSSION

Chemical stability of essential oil loaded on FCC

Although the capability of FCC being used as a carrier has been widely demonstrated in the past [16-18,21], there is also evidence that FCC might trigger ester hydrolysis due to its high surface area. While the ester hydrolysis products still may exhibit antimicrobic activity, this uncertainty asked for a stability analysis of the EOs in terms of a quantitative monitoring of the alteration of the main components. Although slight variations are expected because they happen also in the bulk liquid over time, disruptive changes on the FCC need to be ruled out by analysis.

Table 1 reveals that no major changes in the composition of essential oils can be observed. For rosemary, thyme and cinnamon oils, the changes for all main components are <1.5% and is not significant compared to the measurement errors (see Table 1). Oregano oil is stable as well and the increase in Cavacrol can most likely be analysed as a larger error in of the measurement at t0. For the clove oil, the data suggest an approx. 5% point conversion from eugenyl acetate to eugenol within 30 days as expected. Pure essential oils were also analysed over the time period without being able to observe any change in their compositions.

These results suggest that specific EOs can be used in combination with FCC. It remains to be investigated in detail if the observed minimal changes, may be beneficial in terms of microbial activity or not. From a stability point of view, only rosemary, thyme and oregano oil are stable as oils on FCC.

Optimisation of essential oil loading on FCC

The handling of liquids, especially concentrated EOs, can be challenging. Loaded EOs into porous carriers (Figure 1) produces a solid powder that safely contains the oil and is easy to handle. The amount of oil that can be loaded before the powder starts to form lumps, depends

on the intra particle pore volume available. In Figure 1C, the intra particle volume is presented by the small peak at approx. 0.15 µm while the larger peak at approx. 2.5 µm renders the inter particle pores. Release rate of EOs as well as the total amount of EOs released depend on the amount of the EO loaded. Loading an active on a carrier and thus increasing the surface area that is in contact with the release medium can increase release rates [16,21].

In order to evaluate the effect of the amount of EO loaded into FCCs on the antimicrobial activity, FCCs were prepared with 5 wt%, 10 wt% as well as 30 wt% thyme EO. Subsequently, the antimicrobial activity against *L. innocua* under optimal growth conditions at 37°C on TSA (*in-vitro*) was evaluated.

When untreated FCC was used *L. innocua* grew from 5.4 log cfu/ filter (t0) to 9.9 cfu/ filter after 1 day and stayed constant afterwards until 6 days (Figure 2). FCC with 5 % thyme EO loading reduced the microbial load from 5.4 log cfu/ filter to 3.9 log cfu/ filter after 1 day. Microbial load was further reduced and was below the detection limit (1 log cfu/ filter) after 6 days. Increasing the loading of EO to 10 wt% further increased the antimicrobial activity. Indeed, microorganisms were already strongly inhibited after 1 day where microbial load was below the detection limit of 1 log cfu / filter. No significant growth was observed afterwards until the testing period of 6 days. FCC samples with 30 wt% loading showed the same antimicrobial activity as FCCs loaded with 10 wt%. Studies have shown that depending on the concentration of the EOs they may prolong the lag phase of microorganisms, reduce their growth rate or even fully inhibit their growth [1,22]. In this study, FCCs with 10 wt% and 30 wt% loadings showed very high antimicrobial activities and killed all the inoculated microorganisms already within the first day of storage. A reduction of ≥ 8.9 log cfu/ filter or ≥ 8.6 log cfu/ filter was detected after 1 day and no growth was observed afterwards until day 6. In conclusion, since FCC loaded with 10 wt% EO showed higher antimicrobial activity compared to the 5 wt% loaded one and the same antimicrobial activitiy compared to the 30 wt% loading, 10 wt% loading was selected for further evaluation of the antimicrobial activity of FCCs loaded with EO.

Insert Figure 2 here

***In vitro* screening of the antimicrobial activity of different essential oils loaded on FCC**

In order to study *in vitro* the antimicrobial activity of different EOs loaded on FCC, FCC powders were prepared with 10 wt% of cinnamon, thyme, clove, rosemary or oregano EOs. Their antimicrobial activities were evaluated against *L. innocua* on TSA agar under the optimal growth conditions at 37°C (Figure 3) as well as under cold storage conditions at 7°C (Figure 4). Selection of the EOs were based on previous studies where they were shown to have antimicrobial activities in *in-vitro* and in food systems [1,8,11,13]. *L. innocua* grew from 5.4 log cfu/ filter to 9.9 log cfu/ filter within 1 day and remained at this level until day 6 in samples with untreated FCC (negative control). When FCC was loaded with 10 wt% clove EO, *L. innocua* grew from 5.4 log cfu/ filter to 9.0 log cfu/ filter after 1 day showing no significant reduction in the growth. After 6 days microbial loading decreased to 6.8 log cfu/ filter showing a significant reduction of 2.9 log cfu/ filter compared to negative control. FCCs loaded with rosemary EO showed a significant reduction of 4.1 log cfu/ filter already after 1 day. However, *L. innocua* grew afterwards and after 6 days, no significant difference ($P \geq 0.05$) between the sample and the untreated FCC was observed. FCC samples loaded with 10 wt% cinnamon EO significantly reduced *L. innocua* load from 5.4 log cfu/ filter to 3.5 log cfu/ filter after 1 day. After 6 days the microbial load was below the detection limit of 1 log cfu/ filter.

FCC samples with 10 wt% thyme or oregano EOs showed the highest antimicrobial activity and decreased the microbial load to below the detection level after 1 day. No growth was observed within the next 6 days resulting in a significant reduction of ≥ 8.7 log cfu/ filter.

Insert Figure 3 here

Since many perishable foods prone to microbiological growth are stored at refrigeration temperatures the antimicrobial activity of FCC loaded with EOs was also determined at 7°C. At 7°C, the growth of *L. innocua* was less pronounced for untreated FCCs compared to 37°C. An increase of microbial load from 5.2 log cfu/ filter to 6.2 log cfu/ filter and 8.0 log cfu/ filter was detected after 1 and 6 days, respectively. The antimicrobial activity of the tested FCCs loaded with EOs was also lower at 7°C compared to 37°C. It is known that environmental factors, such as storage conditions, primarily temperature and relative humidity influence the release of volatile antimicrobial components from EOs[23]. In a study they showed that a decrease in temperature led to a decrease in the diffusion coefficient of the main components of EOs, which would result in lower antimicrobial activities[23]. FCC loaded with rosemary or clove EO showed no significant reduction in microbial load compared to negative control within 6 days. FCC with cinnamon EO showed no significant antimicrobial activity after 1 day but microbial load decreased afterwards and resulted in a significant reduction of 2.8 log cfu/ filter compared to negative control after 6 days. No significant reduction in microbial load was detected with FCC loaded with oregano EO after 1 day. However, *L. innocua* load decreased to 1.5 log cfu/ filter after 6 days resulting in a reduction of 6.5 log cfu/ filter. FCCs with thyme EO loading showed already after 1 day a significant reduction in *L. innocua* load (4.3 log cfu/ filter) compared to the FCC untreated samples (6.2 log cfu/ filter). After 6 days the microbial load was reduced to below the detection limit resulting in a reduction of ≥ 7.0 log cfu/ filter.

Insert Figure 4 here

In-situ antimicrobial activity of FCC loaded with essential oils

Although EOs show high antimicrobial activities in *in-vitro* tests, it has been demonstrated that their antimicrobial activities in food systems are generally lower due to the complexity of food products[8]. In order to achieve a similar antimicrobial effect, higher EO concentrations are often required in food tests[13,24]. Therefore, the antimicrobial activity of FCCs loaded with 10 wt% cinnamon, thyme, clove, rosemary or oregano EO against *L. innocua* was also studied with sliced cooked chicken breast in petri dishes at 7°C (Figure 5).

No growth of *L. innocua* could be detected on sliced cooked chicken breast after 1 day for all tested FCCs. These results indicate an extension of the lag phase compared to *the in-vitro* tests at 7°C. After 6 days, the bacterial count increased from initial 6.7 log cfu/g to 8.9 log cfu/g for untreated FCCs. This growth is comparable with the results observed in *in-vitro* tests at 7°C on TSA plates. FCCs with thyme, clove and oregano EO showed no significant log reduction compared to control samples after 6 days. Significant reduction of 2 log cfu/g or 1.7 log cfu/g was achieved after 6 days with FCCs loaded with rosemary EO or cinnamon EO, respectively. The antimicrobial activities are considerably lower than the antimicrobial activities achieved in the *in-vitro* tests at 7°C. Many studies have shown that active packaging systems containing EOs lead to high antimicrobial activities in experiments with model systems or in *in-vitro* tests. However, such high activities could not be confirmed in tests with real foods[13,24,25]. This is probably due to interactive effects of several intrinsic factors such as pH value, water activity, fat and protein content, additives, salts but also extrinsic factors such as storage temperature and gas composition. It is assumed that high protein and fat contents protect bacteria from the

effects of EOs. EOs may dissolve in the fat phase and are therefore not available anymore in the aqueous phase to combat microorganisms or they are available in too low concentrations. Another assumption is that the low water content in food compared to laboratory media reduces the effectiveness of EOs. In addition, the higher availability of nutrients in food may help bacteria to repair damaged cells more quickly. A further assumption is that the food structure itself weakens the effect of EOs by limiting its diffusion processes. Concurrently, the EO concentration must be higher in high-fat foods than in low-fat foods. In addition to all these aspects, the microbiological flora of the food can also influence the antimicrobial activity of the active packaging system[13,26].

In this study it could was demonstrated that rosemary EO has an antimicrobial effect against *L. innocua* on sliced cooked chicken meat through the vapour phase. A direct comparison with other literature data is difficult as the antimicrobial effect of EOs in other studies is often tested in direct contact with the food[9,27]. Additionally, it was demonstrated that the effectiveness of different EOs were different in *in-vitro* and in food tests. Thyme or oregano EOs showed the highest antimicrobial activities in *in-vitro* tests but showed low antimicrobial activities with the tested food. Cinnamon or rosemary EOs showed low or no antimicrobial activities in the *in-vitro* test but showed significant log reduction on sliced cooked chicken breast. In another study, differences in antimicrobial activities in *in-vitro* and in *in-situ* (with food) tests were also observed. Although bergamot EO, lemon EO and linalool showed antimicrobial activity against gram-positive *Camphylobacter jejuni* in *in-vitro* studies, only linalool showed antimicrobial activity in *in-situ* studies with chicken skin[24].

Insert Figure 5 here

CONCLUSION

In this study, it has been demonstrated that highly porous FCC, with its high surface area, can be used to integrate and release EOs in a controlled way in a closed vessel in order to control antimicrobial activity. The chemical stability of the different EOs (Thyme, Rosemary, Oregano and Cinnamon) loaded into FCC was also reported and shown to be stable (Δ<1.5%). Clove oil show an increase in the active substance Eugenol. It is supposed to experience a conversion from Eugenyl acetate to Eugenol *via* ester hydrolysis. Concerning the antimicrobial activity of the loaded FCC, the temperature affected the antimicrobial activity of the FCCs loaded with EOs, probably due to its effect on the release of the EOs. Antimicrobial activities of the EOs loaded FCCs against *L. innocua* were different in *in-vitro* tests with agar plates than that in *in-situ* tests (through the vapour phase) with cooked sliced chicken breast. This clearly indicates that the selection of EOs that can be integrated into packaging to develop antimicrobial packaging should be done based on the *in-situ* test with the targeted food. As a next step FCCs loaded with EOs will be integrated into packaging material to study the antimicrobial activities under real packaging and storage conditions.

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Table 1: Changes in percentage content (integrated peak area) after 30 days of EOs loaded on FCC measured by extraction and GC-MS. Bold numbers indicate relevant components with >5% of content in EO based on manufacturers data sheets.

Plant	*Rosmarinus officinalis*			*Thymus officinalis*			*Oreganum vulgare*			*Syzygium aromaticum*			*Cinnamomum spp*		
components \ oils	Rosemary			Thyme			Oregano			Clove			Cinnamon		
Time (day)	0	30	Δ%	0	30	Δ%	0	30	Δ%	0	30	Δ%	0	0	Δ%
Eucalyptol	**46.85 (± 0.85)**	**47.21 (±0.92)**	**+0.35**	0.80 (± 3.37)	0.9 (± 2.23)	+0.09	1.73 (± 5.08)	1.45 (± 1.69)	-0.28	-	-	-	-	-	-
Camphor	**16.79 (± 0.28)**	**17.07 (± 0.30)**	**+0.28**	-	-	-	1.21 (± 4.27)	0.96 (± 1.30)	-0.25	-	-	-	-	-	-
α-Pinen	**9.50 (± 2.02)**	**8.74 (± 0.76)**	**-0.76**	**1.21 (± 1.56)**	**1.12 (± 1.93)**	**-0.10**	-	-	-	-	-	-	0.62 (± 3.9)	0.51 (± 4.15)	-0.11
Camphene	**4.04 (± 1.13)**	**3.89 (± 1.04)**	**-0.15**	0.94 (±1.31)	0.93 (± 1.31)	-0.01	0.80 (± 4.07)	0.65 (± 0.49)	-0.14	-	-	-	0.25 (± 1.18)	0.18 (± 2.74)	-0.07
β-Pinene	**4.26 (± 1.73)**	**4.17 (± 0.98)**	**-0.10**	1.28 (± 0.33)	1.25 (± 1.29)	-0.03	0.53 (± 4.07)	0.49 (± 1.36)	+0.04	-	-	-	-	-	-
Thymol	-	-	-	**50.28 (± 0.41)**	**49.99 (± 0.64)**	**-0.29**	3.18 (± 6.43)	2.38 (± 1.20)	-0.81	-	-	-	-	-	-
p-Cymene	3.03 (± 1.41)	3.08 (± 3.43)	+0.05	**21.73 (± 0.17)**	**23.20 (± 1.00)**	**+1.47**	**7.27 (± 4.92)**	**7.53 (± 0.22)**	**+0.26**	-	-	-	**2.76 (± 2.86)**	**2.64 (± 1.04)**	**-0.12**
γ-Terpinene	0.10 (± 5.79)	0.09 (± 7.91)	-0.01	**8.89 (± 0.96)**	**7.2 (± 1.28)**	**-1.47**	**4.83 (± 4.75)**	**3.17 (± 1.55)**	**-1.66**	-	-	-	0.08 (± 5.90)	0.07 (± 3.85)	-0.01
Linalool	0.84 (±1.67)	0.89 (± 1.77)	+0.05	**3.25 (± 1.03)**	**3.56 (± 0.65)**	**+0.31**	4.03 (± 4.69)	3.12 (± 1.58)	-0.91	-	-	-	**3.68 (± 2.29)**	**3.99 (± 1.47)**	**+0.30**
Carvacrol	-	-	-	**4.51 (± 0.72)**	**4.58 (± 1.39)**	**+0.07**	**65.61 (± 2.52)**	**71.32 (± 0.70)**	**-6.02**	-	-	-	-	-	-
Eugenol	-	-	-	-	-	-	0.03 (± 17.12)	0 (± 0)	-0.0	**77.12 (± 0.08)**	**82.01 (±0.59)**	**+4.60**	**4.96 (± 0.88)**	**6.18 (± 0.83)**	**+1.22**
Eugenyl acetate	-	-	-	-	-	-	-	-	-	**15.86 (± 0.47)**	**11.28 (± 4.02)**	**-4.57**	-	-	-
β-Caryophyllene	-	-	-	1.14 (± 1.54)	1.17 (± 0.45)	+0.03	4.84 (± 4.85)	3.75 (± 1.89)	-1.09	**6.71 (± 0.14)**	**6.69 (± 1.11)**	**-0.02**	-	-	-
Cinnamaldehyde	-	-	-	-	-	-	-	-	-	-	-	-	**65.76 (± 0.85)**	**64.28 (±0.56)**	**-1.48**

Evaluation of the Potential of Functionalised Calcium Carbonate as Carrier for Essential Oils with regard to Antimicrobial Packaging Applications

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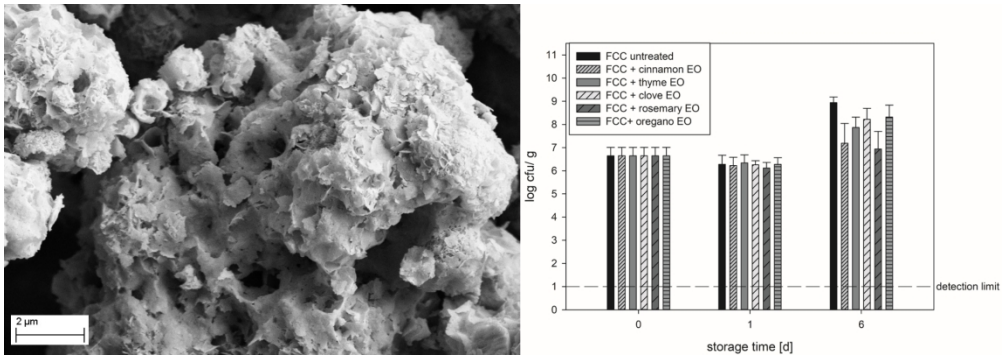
Different EOs were loaded on functionalised calcium carbonates (FCCs) and their chemical stability and antimicrobial activities against *L. innocua* were studied. FCCs loaded with thyme or oregano EO showed the highest reduction in microbial load in *in-vitro* tests at 37°C and 7°C. In *in-situ* tests, FCCs loaded with cinnamon or rosemary EO showed a significant reduction in microbial load (1.7 log cfu/g for cinnamon and 2 log cfu/g for rosemary) on sliced cooked chicken breast was studied after 6 days.



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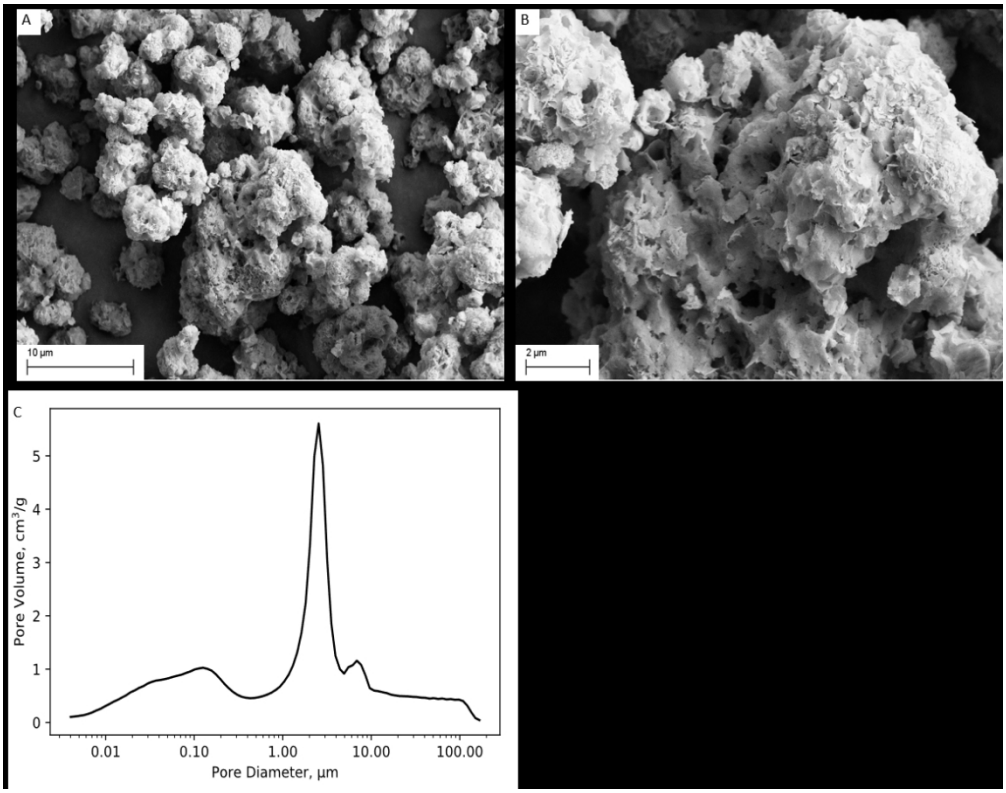

Figure 1: SEM images of FCC at different magnifications: (A) 2500x and (B) 7500x as well as pore size distribution (C) with the intraparticle pores in the range of 0.004 – 0.38 µm as measured by MIP.

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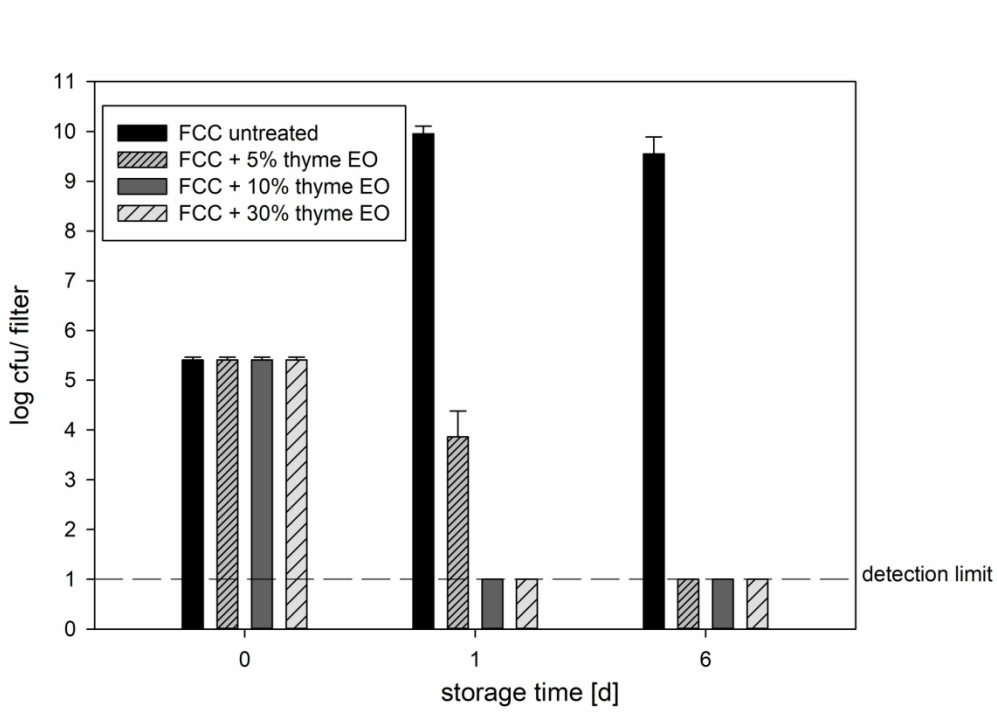

Figure 2: Antimicrobial activity of FCC powder loaded with 5 %, 10 % or 30 % thyme EO or untreated FCC powder on the growth of L. innocua (ATCC 33090) on TSA plates at 37°C. Results are expressed as mean (log cfu/ filter) ± standard deviation (n=5). Same letters within a time point for each sample show that the results are not statistically significantly different (P ≥ 0.05).

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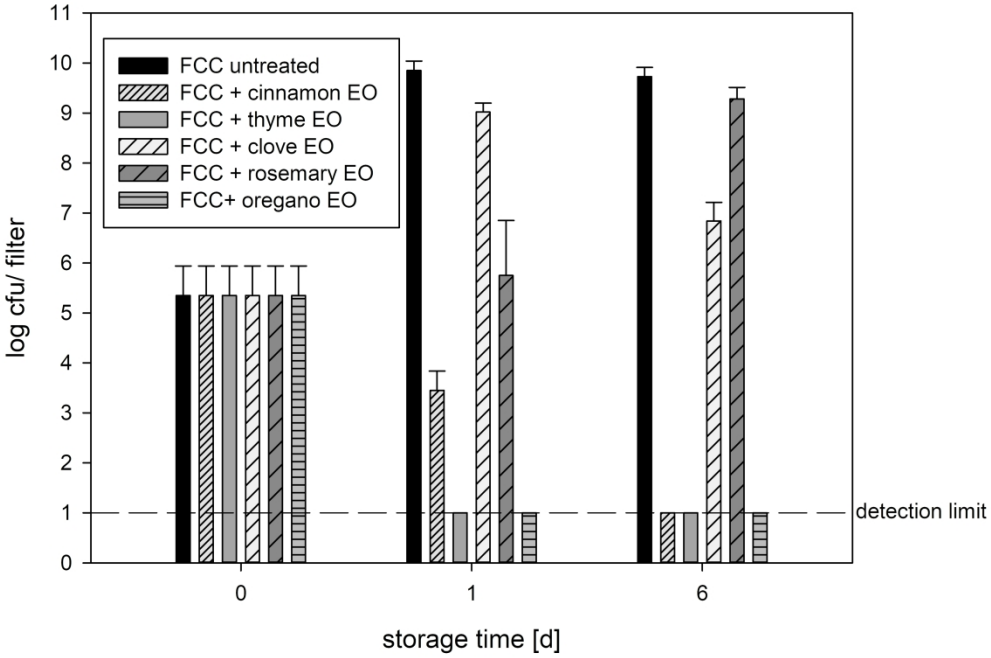

Figure 3: In-vitro antimicrobial activity of FCC powder loaded with 10 % EO (cinnamon, thyme, clove, rosemary, oregano) and untreated FCC powder (negative control) on the growth of L. innocua (ATCC 33090) on TSA plates at 37°C. Results are expressed as mean (log cfu/ filter) ± standard deviation (n=3). Same letters within a time point for each sample show that the results are not statistically significantly different (P ≥ 0.05).

163x119mm (600 x 600 DPI)

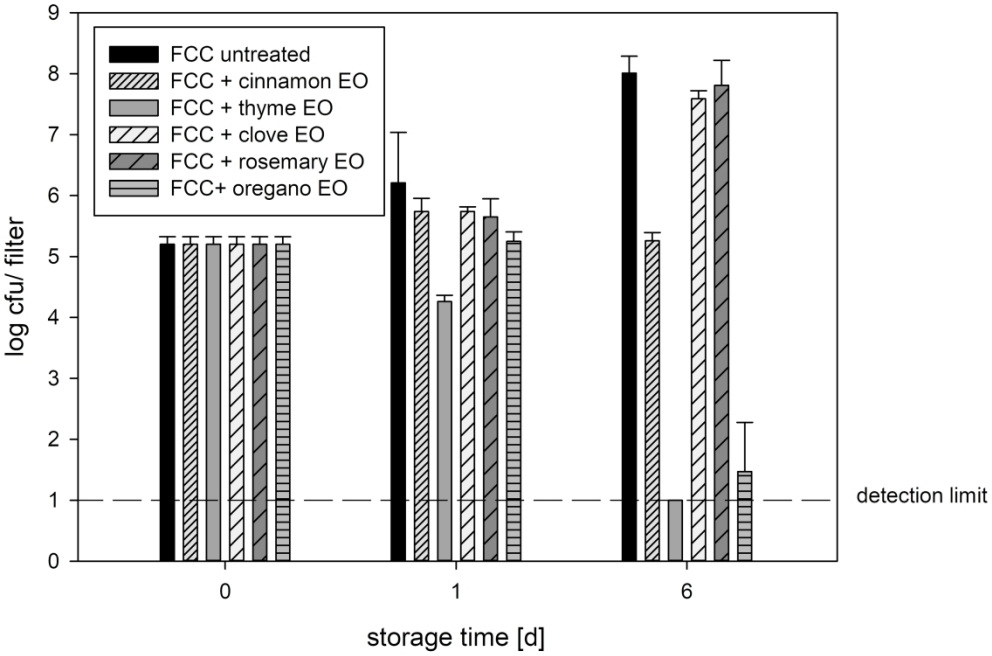

Figure 4: In-vitro antimicrobial activity of FCC powder loaded with 10 % EO (cinnamon, thyme, clove, rosemary, oregano) and untreated FCC powder (negative control) on the growth of L. innocua (ATCC 33090) on TSA plates at 7°C. Results are expressed as mean (log cfu/ filter) ± standard deviation (n=3). Same letters within a time point for each sample show that the results are not statistically significantly different ($P \geq 0.05$).

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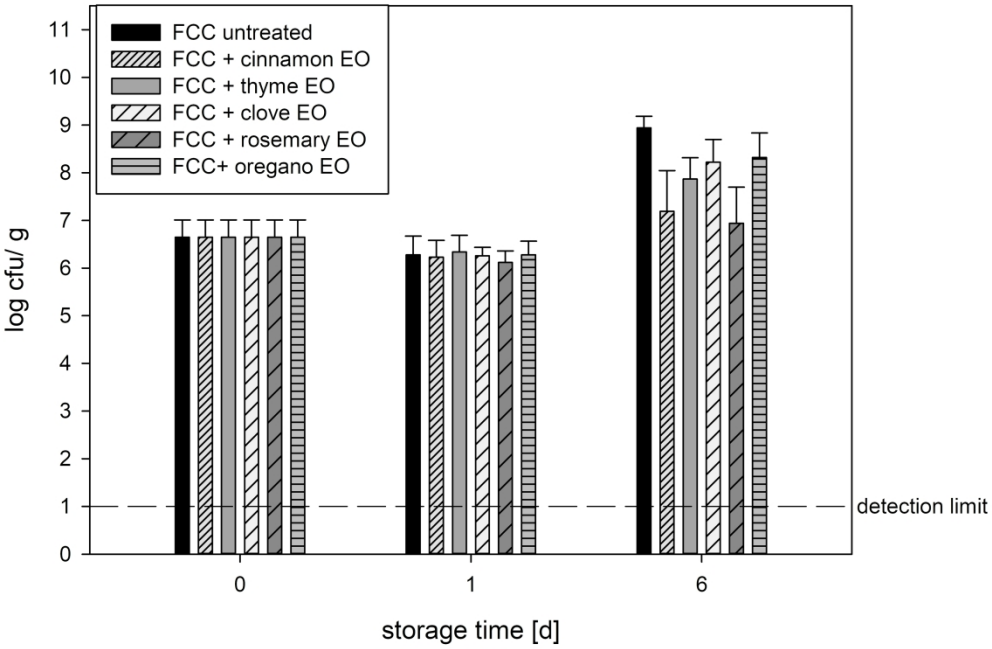

Figure 5: In-situ antimicrobial activity of FCC powder loaded with 10 % EO (cinnamon, thyme, clove, rosemary, oregano) and untreated FCC powder (negative control) on the growth of L. innocua (ATCC 33090) on sliced cooked chicken breast at 7°C. Results are expressed as mean (log cfu/g) ± standard deviation (n=3). Same letters within a time point for each sample show that the results are not statistically significantly different ($P \geq 0.05$).

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