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Antimicrobial Activity of Natural Agents Coated on Starch-based Films Against *Staphylococcus aureus*

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ABSTRACT:

This study investigated the antimicrobial (AM) activity of starch-based films coated with linalool, carvacrol or thymol against *S. aureus* in vitro or inoculated on the surface of Cheddar cheese. In solid media using the agar diffusion method, the inhibitory effect of linalool, carvacrol or thymol coated onto the films increased significantly ($p \leq 0.05$) with the increase in concentration of each AM agent. All the coated films effectively inhibited the growth of *S. aureus* on the surface of Cheddar cheese. The sensitivity of *S. aureus* to the AM agents tested in the concentration range of the study is in the order of thymol > carvacrol > linalool.

Keywords: antimicrobial packaging, linalool, carvacrol, thymol, *Staphylococcus aureus*

Practical Application: Biodegradable starch-based films can be used for packaging food products such as Cheddar cheese by adding a coating layer to protect the moisture sensitive starch material. The coating layer can be incorporated with natural antimicrobial agents that are effective against some microorganisms such as *Staphylococcus aureus.* This can potentially extend the shelf life of the food products and offer a more sustainable packaging option for manufacturers and consumers.

INTRODUCTION

In recent years, consumers' demand has increased for the provision of fresh, natural foods with minimal addition of preservatives and that are of high quality with an extended shelf life along with advances in the biodegradability of packaging materials (Tharanathan 2003; Cutter 2006; Altskär and others 2008). Therefore, the application of biodegradable polymers coated or incorporated with natural antimicrobial (AM) compounds has the potential of controlling food spoilage as well as enhancing the microbial safety of food products and expanding the functional applications of such polymers in the food industry. Examples of biodegradable materials that have been used in previous studies include starch, alginate, cellulose, chitosan, carageenan, whey protein, corn zein and/or their derivatives (Phan and others 2005; Rodriguez and others 2006). There is also current interest in the use of starch-based materials in the packaging of cheese because these materials are relatively inexpensive and can be incorporated with an AM agent (Pelissari and others 2009; Durango and others 2006). Like many other food products, cheese may be contaminated by undesired microorganisms such as bacteria, yeasts and fungi that may deteriorate the sensory, aesthetic, flavour, odour and/or textual properties (Vermeiren and others 1999; Appendini and Hotchkiss 2002; Gutierrez and others 2008; Davidson and Taylor 2007).

Some of the spoilage or pathogenic microorganisms that may contaminate cheeses, meat, poultry and baked products include *Yarrowia lipolytica*, *Pseudomonas aeruginosa*, *L. monocytogenes*, *E. coli 0157*, *Salmonella*, *S. aureus*, *B. cereus*, *Campylobacter, C. perfringens, A. niger* and *S. cerevisiae* (Singh and others 2003; López-Malo and others 2005; Rydlo and others 2006; Suppakul 2004; Schelz and

others 2006). The microorganism *S. aureus* is ubiquitous in nature. It is Gram-positive and has a spherical shape with groups in grape-like clusters. Outside the body it is one of the most resistant non-spore forming human pathogens, being able to survive in a dry state for extended times (Jablonski and Bohach 2001).

Previous studies have reported that thyme, oregano and basil essential oils (EOs) with their main constituent of thymol, carvacrol and linalool respectively, possess both fungistatic and antibacterial activity against a wide range of microorganisms (Dorman and Deans 2000; Friedman and others 2002; Tepe and others 2004; Olasupo and others 2004; Youdim and Deanes 2000; Lachowicz and others 1998; Suppakul and others 2003a). The AM activity of plant EOs is related to their chemical structure, namely the presence of hydrophilic functional groups such as hydroxyl groups of phenolic components and/or lipophilicity of the components in the EOs and depends on their concentration (Bagamboula and others 2004; Davidson and Naidu 2000; Farag and others 1989; Dorman and Deans 2000). The mode of action for plant EOs such as thyme and oregano with the main constituents thymol and cavarcrol respectively is by the alteration of the membrane fatty acid composition for pathogenic or spoilage microorganisms (Di Pasqua and others 2006).

Bagamboula and others (2004) determined the AM effect of thyme and basil EOs with their major constituents thymol, linalool and carvacrol against *Shigella* spp. (*S. sonnei* and *S. flexneri*) on lettuce leaves and solid media using the agar diffusion method. They observed a decrease in the *Shigella* spp. after washing the lettuce with 0.5% and 1% (v/v) thymol, linalool and carvacrol. At 1% (v/v) of each agent, the

Shigella spp population decreased to an undetectable level. They concluded that these EOs showed inhibition of *Shigella* spp. (*S. sonnei* and *S. flexneri*) according to the agar diffusion method. Chiasson and others (2004) evaluated the AM potential of carvacrol, thymol and thyme in minced meat against *E. coli* and *Salmonella Typhi*. Furthermore Seaberg and others (2003) reported the effectiveness of carvacrol as a result of investigating its inhibitory effects against *L. monocytogenes* in ready-to-eat beef slices. The inhibitory effect of carvacrol has also been reported by Ultee and others (2000) who investigated its AM activity against *B. cereus* in the preservation of rice. Ultee and Smid (2001) also investigated the AM activity of carvacrol against toxin production of *B. cereus* in soups. They found that carvacrol reduced the production of *B. cereus* toxin in mushroom soup to a level that could not be detected.

In view of the current advances in biodegradable materials and the already identified potential of linalool, carvacrol or thymol as effective natural AM agents, the effectiveness of starch-based films coated with linalool, carvacrol or thymol against *S. aureus in vitro* or inoculated on the surfaces of Cheddar cheese samples was investigated in the current study. The AM activity was evaluated for several concentrations of each of the AM agents coated onto a starch-based polymer film.

MATERIALS AND METHODS

POLYMERS

A commercial starch-based film (Biograde-F) supplied by Biograde Ltd., Australia was used in this study. Biograde-F is a biodegradable material based on a blend of thermoplastic starch, aliphatic polyesters and natural plasticisers. Methylcellulose (MC, 18,804-2); hydroxypropyl methylcellulose (HPMC, 42,321-1) and poly(ethylene

glycol) (PEG, 20,236-3) were purchased from Sigma Chemical Company Inc., Milwaukee, WI.

ANTIMICROBIAL AGENTS AND CHEDDAR CHEESE

The AM additives were supplied by Sigma-Aldrich Pty. Ltd., Australia and comprised linalool with a purity of 97% (L2602), carvacrol with a purity of 98% (W224502) and thymol with a purity of 99.5% (TO501). Cheddar cheese was purchased from a retail outlet. According to the manufacturer (Woolworths Ltd., Australia), a 100 g sample of the cheese contains as its main components: fat 35.2 g; protein 24.3 g; carbohydrates 0.1 g; calcium 735 mg and sodium 635 mg.

MEDIA AND MICROORGANISMS

The media used were nutrient agar (AM 130), 3M Perifilm^{TM} Staph Express Count Plate (6490) purchased from 3M Microbiology Products, USA and plate count agar (AM 144) purchased from Amyl Ltd., Australia. The microorganism *S. aureus* (UNSW 056201) was obtained from the culture collection of the University of New South Wales, Australia. Bacteriological peptone (LP0037) was purchased from Oxoid Ltd., Hampshire, England.

FILM PREPARATION AND AM COATING

The coating solution was prepared from the MC and HPMC materials. Methylcellulose and HPMC were added slowly to absolute ethanol and heated with magnetic stirring on a hotplate. The heating was discontinued when the temperature reached 65°C. With continuous agitation, a mixture of PEG and distilled water, as a

plasticiser, was added slowly to the MC-HPMC dispersion whilst the dispersion cooled (Rardniyom 2008). This resulted in the formation of a uniformly clear coating solution or gel. The AM agent was then added to the coating solution at one of three different concentrations to form the final coating materials with AM agent at target levels of 1, 3 or 5% (w/w). The coating medium was applied to the starch-based material using a roller and the film was then dried at ambient conditions (Cooksey 2005). Each of the three natural AM agents: linalool, carvacrol and thymol were coated separately onto the starch-based material. Similarly, starch-based material without any AM agent was prepared as the control. The addition of AM agents and subsequent coating procedures were conducted at a low temperature in order to minimise any significant loss of AM agents due to their volatility. The actual concentration of the AM agents retained in the films after drying was determined on the basis of total dry weight of the coating layer and the total film. The total film thickness of the coating and starch-based film was measured immediately after peeling them off using a hand-held micrometer with a precision of 0.001 mm (Mitutoyo, Japan). The film thickness was measured at 5 different positions and the average thickness was calculated from these readings. After measuring the thickness, the films were wrapped in aluminium foil to prevent further loss of the AM agent before being used.

ANTIMICROBIAL ACTIVITY ON SOLID MEDIA

The effectiveness of the AM starch-based films on a solid medium was determined using the agar disc diffusion assay. A bacterial suspension at the level of 10^6 CFU mL^{-1} was prepared in a 0.1% (w/v) sterile peptone solution. The control and AM starch-based films were cut into circular discs (6 mm in diameter) and sterilised

using UV light for 2 min (Cooksey 2000). The cut pieces were aseptically placed on nutrient agar plates seeded with 0.1 mL of bacterial solution. The plates were incubated at 37°C for 24 h. After the incubation process, the diameters of the clear zones that formed around the film samples were measured using a Vernier calliper and reported as the zone of inhibition (Suppakul and others 2008). Such a qualitative measurement is sufficient to make meaningful comparisons between the systems studied in this work. Although not used to analyse the data in the current work, another approach involves the calculation of an antimicrobial index (*AMI*) defined in equation (1):

$$AMI = (d_1 - d_2)/d_1 \quad (1)$$

where d_1 is the diameter of clear zone and d_2 is the diameter of circular film.

CHEESE PREPARATION

Cheddar cheese was purchased from a local supermarket and cubes of the cheese were cut weighing *ca*. 20 ± 1 g each (Suppakul 2004; Rupika and others 2005). Samples were divided into four sets, for the control film and the three AM coated films containing linalool, carvacrol or thymol. The cheese samples were then sterilised on all sides for 1 h using UV light. The control and AM films were also sterilised with UV light prior to use.

INOCULATION OF S. AUREUS ON CHEDDAR CHEESE

Each of the cheese samples was inoculated on top and bottom surfaces with *S. aureus* and then spread using a sterile glass rod to obtain *ca.* 10^4 CFU g^{-1} (Suppakul

198 and others 2008) prior to wrapping with the control or an AM test film. The inoculated
199 cheese samples were placed between folded films and then the open sides of the
200 films were sealed. The packaged cheese samples were prepared in duplicate and
201 stored at 15°C for 21 days. The temperature of 15°C was chosen in order to mimic a
202 temperature abuse condition that might arise in the food supply chain (Siragusa and
203 others 1999).
204
205 *BACTERIAL COUNT*
206 The bacteriological analysis was performed periodically on days 0, 1, 3, 5, 7, 10, 15
207 and 21. Two samples from each treatment were aseptically opened on the sampling
208 days. An 11 g sample of cheese was aseptically transferred to a sterile stomacher
209 bag. In accordance with the method described by Rupika and others (2005), 99 mL
210 of 0.1% (w/v) sterile peptone solvent (pH 7.5 ± 0.2 at 25°C) were added to the
211 sample which was then homogenised using a laboratory blender (Seward Stomach®
212 400, Seward Medical, UK) for 3 min. Serial dilutions of the resulting solutions were
213 prepared in a sterile peptone diluent (pH 7.0 ± 0.1 at 25ºC) in order to obtain a
214 quantifiable colony count. For the determination of bacteria counts, 1 mL of each
215 serially diluted sample was plated in duplicate on a 3M Perifilm^{TM} Staph Express
216 Count Plate and then incubated aerobically for 24 h at 37°C. The colonies were
217 counted and the results expressed as colony forming units per gram (CFU g^{-1}). Two
218 sets of measurements were taken from each of the bacterium enumeration
219 experiments with the results averaged and the data quoted as a mean.
220

221 *DETERMINATION OF MICROBIAL DEATH RATE BY THE AM FILMS*

222 The death rate of *S. aureus* bacterium inoculated onto the cheese samples during
223 storage was determined in accordance with the calculation procedures described by
224 Bachrouri and others (2002). Accordingly, a specific death rate, μ, can be
225 determined from equation (2):

226

227 $$N = N_0 e^{-\mu t} \quad (2)$$

228

229 where, N is the population surviving at any time t, N_0 is the initial population. The
230 specific death rate is obtained from the gradient of a plot of the natural logarithm of N
231 (expressed either in units of CFU mL^{-1} or CFU g^{-1}) versus time. Whence, taking
232 natural logarithms of both sides of equation (2) results in equation (3):

233

234 $$\ln(N) = \ln(N_0) - \mu t \quad (3)$$

235

236 In the present study, the decadic logarithm of the surviving population of *S. aureus* in
237 the presence of the three different AM agents was plotted as a function of the time of
238 storage. A linear regression analysis was performed on each curve to obtain the
239 specific death rate, μ', where $\mu' = \mu/\ln(10)$. The values of μ' were subsequently used
240 to compare the AM activity of the films.

241

242 *DATA ANALYSIS*

243 Experiments on solid media and Cheddar cheese were performed in triplicate and
244 duplicate respectively. Individual experiments for each of the AM agents (linalool,
245 carvacrol and thymol) coated onto starch-based films were performed separately and

by comparing the three levels of AM agent added to the starch-based material. Data points are represented by the mean of the results obtained for each AM agent coated onto the substrate. Bacterial colony counts were converted into decadic logarithm values. The latter were subjected to analysis of variance (ANOVA) at the 0.05 confidence level. Differences amongst the treatments were examined by least significant differences tests using SAS (Version 9.5, SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

RETENTION OF AM AGENT IN THE COATING LAYER

The incorporation of linalool, carvarcrol or thymol into the MC-HMPC coatings at different concentrations did not significantly change the thickness of the AM films. The average thickness of the coated starch-based films is *ca.* 138 µm. The residual concentrations of the AM agents in the starch-based films containing 1, 3 and 5% (w/w) linalool, carvacrol or thymol in their coating retained 0.48%, 1.43% and 2.38% (w/w) respectively after drying. The retention of the AM agent in the coating layer suggests that the coating procedure resulted in only a minimal and acceptable loss of AM agent.

ANTIMICROBIAL ACTIVITY OF AM STARCH-BASED FILMS ON SOLID MEDIA

The AM starch-based films were initially tested on solid media using an agar disc diffusion assay in order to provide preliminary information about the potential AM activity of the active agents against *S. aureus*. The presence of a clear zone of inhibition around the test films was taken as an indication of AM activity for the film formulation. Figure 1 shows the AM activity of starch-based film coated with linalool, carvacrol or thymol against *S. aureus* at 37°C on the solid media in terms of the clear

inhibition zones. These zones are visible in the systems containing the AM agent. The film containing no AM agent (control) did not inhibit the growth of *S. aureus* on the solid medium, as expected.

The average values of the zones of inhibition for each of the AM films are presented in Table 1. These data confirm the visual observations made in Figure 1 in that the starch-based films coated with linalool, carvacrol or thymol are effective in inhibiting the growth of *S. aureus* as revealed by the agar disc diffusion method. It can also be observed from these results that the inhibitory effect of these agents when coated onto the films increased significantly ($p \leq 0.05$) with the increase in concentration of the agent.

All the films coated with carvacrol demonstrated a positive AM activity against *S. aureus* in this study. A detailed statistical analysis of the results suggests that the inhibitory effect of the film containing 0.48% (w/w) carvacrol was significantly ($p \leq 0.05$) lower than the inhibitory effect of the films containing 1.43% (w/w) and even more so than the film containing 2.38% (w/w) carvacrol. A similar concentration dependence of carvacrol activity against *S. aureus* on solid media was observed by Rupika and others (2005) who evaluated the AM activity of polyethylene films containing carvacrol within the bulk of the film against *S. aureus*, using the agar disc diffusion assay. In the present study, the inhibitory activity of linalool-coated starch-based film against *S. aureus* increased notably with increasing concentration. The zone of inhibition data for each of the concentrations showed that the films containing 2.38% (w/w) linalool, carvacrol or thymol had a higher inhibitory activity than those containing any of the lower concentrations of these agents in their

296 coatings and were all effective against *S. aureus* on solid media. The greatest
297 inhibition for the starch-based coated films occurred with 2.38% (w/w) thymol. This
298 observation is consistent with the work of Sivropoulou and others (1996) who studied
299 the AM activity of thymol and reported its significant activity against *S. aureus*. Tepe
300 and others (2004) have also reported a significant AM activity of thymol against *S.*
301 *aureus* also *in vitro*.
302
303 Figure 2 shows the variation in the zone of inhibition as a function of concentration of
304 linalool, carvacrol or thymol AM agents. The AM activity of the starch-based films is
305 highly linear between *ca.* 0.48% (w/w) and 1.43% (w/w) as revealed by the linear
306 regression analysis data listed in Table 1. These regression data, namely the
307 gradient and the vertical axis intercept, pertain to the zone of inhibition data obtained
308 in the latter concentration range. The respective gradients of the regression lines
309 are indicative of the sensitivity of the test microorganism to changes in the
310 concentration of the three AM agents in the film coating. The order of concentration
311 sensitivity is thus thymol > carvacrol > linalool. Such linearity in the response of *S.*
312 *aureus* to changes in AM concentration does not, however, seem to be maintained in
313 the region between the control sample and *ca*. 0.48% (w/w) of AM agent as shown
314 by the dashed line. The latter is consistent with the observations made by
315 Bagamboula and others (2004) who reported a non-linear relationship between the
316 zone of inhibition of *Shigella sp.* with the concentration of thyme and basil EOs with
317 their main constituents: carvacrol, thymol and linalool. However, it is important to
318 note that the non-linearity reported by Bagamboula and others (2004) was observed
319 over the concentration range spanning several orders of magnitude (0.01 - 10% w/w)
320 and that the data seem reasonably linear in the AM concentration range used in the

present work. Possible causes of this phenomenon include the role of diffusion kinetics in the observed non-linear relation between the zone of inhibition and the concentration of AM agents in the polymer coating. Furthermore, the non-linear relationship may be due to factors that limit the applicability of the method at low concentrations of AM agents (Suppakul and others 2003a).

ANTIMICROBIAL ACTIVITY ON CHEDDAR CHEESE - A CHALLENGE TEST

Further to the above *in vitro* study using the agar diffusion technique, the effect of the AM starch-based films was explored when placed in contact with a particular foodstuff. In particular, the films were used to package samples of Cheddar cheese in order to assess their effectiveness against *S. aureus* that was inoculated on the surface of the samples and in order to attempt to identify low concentrations where effective growth control of the microorganisms occurs. Clearly, a low concentration of additive is preferable since the higher the concentration of the natural plant extracts the greater is the concern about off-flavour issues for food packaging applications (Suppakul and others 2003b). Figure 3 is a plot of the decadic logarithm of the population counts of *S. aureus* that were determined on the surface of the cheese as a function of storage time (i.e. the “death” curves) at 15°C for starch-based AM films containing carvacrol. Data for the control film are also plotted for comparison. Similar trends were observed for the death curves obtained using thymol and linalool-coated starch-based films (not shown). The specific death rates were determined from the gradients of the plots for all the systems. These data are listed in Table 2 along with the other linear regression analysis data.

345 The data shown in Table 2 confirm the consistency in the initial inoculation
346 procedure as reflected by the consistency in the vertical axis intercepts of the plots.
347 A more detailed analysis of the specific death rate data can be obtained by plotting
348 these values as a function of the concentration of the AM agent for each of the
349 experimental systems. Such a plot is shown in Figure 4 where a linear relationship
350 exists between the specific death rate and the concentration of each of the AM
351 agents across the entire range of studied concentrations. This, contrasts slightly to
352 the behaviour exhibited in the solid media where a non-linear relationship was
353 observed between the zone of inhibition and the AM concentration at low
354 concentrations. These observations suggest that the specific death rate μ' may in
355 fact be a pseudo-first order rate constant where $\mu' = \alpha[AM]$, where [AM] is the
356 concentration of AM agent and α is a second-order rate constant. The second order
357 rate constants can be obtained from the gradient of the plots in Figure 4.
358
359 The plots shown in Figure 4 indicate that the inhibition of *S. aureus* on the surface of
360 Cheddar cheese when packed in starch-based films coated with these AM agents is
361 *ca*. 1.6 times more sensitive to changes in the concentration of thymol compared to
362 that of linalool. The sensitivity of this microorganism to changes in carvacrol
363 concentration is between those of the other two AM agents. The relative order of the
364 sensitivity determined in these storage experiments at 15°C was similar to that found
365 in the solid media experiments conducted at 37°C suggesting that the relative order
366 remains unchanged across this range of temperatures.
367
368 In the present study the starch-based films containing thymol demonstrated the
369 strongest inhibitory effect on the growth of *S. aureus* on the cheese when compared

370 to the control film (see Table 2). The seemingly natural rate of decrease of the *S.*
371 *aureus* count observed in the control film might be due to the depletion of oxygen
372 during the test and/or other factors such as the preservatives originally present in the
373 Cheddar cheese. During the first 5 days, the AM films containing 0.48% (w/w)
374 thymol in their coatings extended the lag phase of *S. aureus* growth and reduced by
375 24% the *S. aureus* population on the cheese after 21 days of storage. The AM films
376 containing thymol at 1.43% (w/w) and 2.38% (w/w) in their coatings further extended
377 the lag phase and reduced the *S. aureus* count on the surface of the Cheddar
378 cheese by 33% and 43% respectively. A high AM activity of thymol has also been
379 reported by Olasupo and others (2003) and is consistent with the present findings.
380
381 The population count of *S. aureus* on the cheese packaged in the starch-based film
382 containing 0.48% (w/w) linalool decreased by 22% after 21 days of storage at 15°C
383 (see Table 2). It can also be seen from the results in Table 2 that increasing the
384 concentration of linalool contained in the film to 1.43% or 2.38% (w/w) had a
385 significant effect; the population of *S. aureus* on the cheese was reduced by 26%
386 and 33% respectively after 21 days of storage. Kim and others (1995) observed a
387 similar dose-related activity of linalool against *S. aureus*. Mazzanti and others (1998)
388 as well as by Dorman and Deans (2000) have also reported the overall effectiveness
389 of this agent against *S. aureus*. The observations in the present study are also
390 consistent with the results of Rupika and others (2006) who found that linalool
391 exhibited an inhibitory effect against *S. aureus* on Cheddar cheese packaged in
392 polyethylene-based AM films. Moreover, the observed inhibition of *S. aureus* on the
393 surface of Cheddar cheese by carvacrol is consistent with the work of Rardniyom
394 (2008) who reported the AM activity of carvacrol against the growth of *E. coli* on

Cheddar cheese. The linear response in the inhibition of *S. aureus* with the concentration of carvacrol (see Figure 4) is in accordance with the observations made by Ultee and others (1998) who found a concentration dependence of carvacrol against *B. cereus* as well as those of Periago and others (2004) who observed a dose-dependence of carvacrol activity also against *L. monocytogenes* in carrot juice.

CONCLUSIONS

The results of the present study suggest that the AM agents linalool, carvacrol or thymol, can be successfully coated onto starch-based films to produce packaging materials that exhibit activity against *S. aureus*. In solid media using the agar diffusion method, all of the AM films formed clear zones of inhibition against *S. aureus*. All AM films containing linalool, carvacrol or thymol effectively inhibited the growth of *S. aureus* on the surface of Cheddar cheese. The AM activity of these agents against *S. aureus* was found to be dependent on the concentration of AM agent coated onto the film samples. The order of effectiveness found was thymol > carvacrol > linalool. The concentration dependence of the effectiveness was found to be linear at the higher concentrations of the AM agent (above *ca.* 0.48% (w/w)) and can be used as a measure of the sensitivity of the test microorganism to the AM agent. Starch-based films containing linalool, carvacrol or thymol have a potential for applications in AM packaging systems and may reduce the risk of food-borne illness associated with microbial contamination in hard cheeses. The results also infer that starch-based AM films containing these agents may, in the future, be used to extend the shelf life of packaged hard cheeses. Further research is underway to study the AM effect of linalool, carvacrol or thymol coated on starch-based films at low

concentration in order to attempt to identify systems that could be commercially attractive.

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Table 1: Analysis of the zone of inhibition data in solid media for *S. aureus* at 37°C in the presence of starch-based film coated with the AM agents: carvacrol, linalool or thymol.

Treatment	Zone of inhibition/mm *S. aureus*			Gradient dz/dc	Intercept	Correlation coefficient (R^2)
	0.48% (w/w)	1.43% (w/w)	2.38% (w/w)			
Linalool	9.2 ± 0.3	13.3 ± 0.6	18.6 ± 1.0	5.98	-4.69	0.969
Carvacrol	10.3 ± 1.1	15.3 ± 1.7	21.9 ± 1.7	7.06	-5.78	0.977
Thymol	11.3 ± 1.8	16.7 ± 0.9	23.8 ± 1.8	7.69	-6.29	0.977

577 **Table 2.** Analysis of the "death" curve data for *S. aureus* on the surface of Cheddar
578 cheese packaged and stored at 15°C in starch-based films coated with AM agents:
579 carvacrol, linalool or thymol.
580

Treatment	AM agent concentration in coated film % (w/w)	Specific death rate μ'/day^{-1}	Intercept	Correlation coefficient R^2	Population of *S. aureus* on cheese log CFU g^{-1}	
					Day 0	Day 21
Control	0	0.041	4.82	0.951	4.76 ± 0.08	4.06 ± 0.48
Linalool	0.48	0.047	4.74	0.976	4.66 ± 0.10	3.66 ± 0.26
	1.43	0.056	4.70	0.989	4.66 ± 0.10	3.47 ± 0.25
	2.38	0.072	4.64	0.979	4.74 ± 0.07	3.17 ± 0.13
Carvacrol	0.48	0.048	4.75	0.977	4.73 ± 0.09	3.7 ± 0.15
	1.43	0.062	4.70	0.980	4.71 ± 0.02	3.36 ± 0.23
	2.38	0.079	4.60	0.952	4.71 ± 0.08	2.9 ± 0.44
Thymol	0.48	0.054	4.76	0.984	4.73 ± 0.05	3.6 ± 0.11
	1.43	0.071	4.70	0.994	4.67 ± 0.05	3.12 ± 0.40
	2.38	0.089	4.64	0.984	4.65 ± 0.04	2.65 ± 0.50

581

Figure Captions

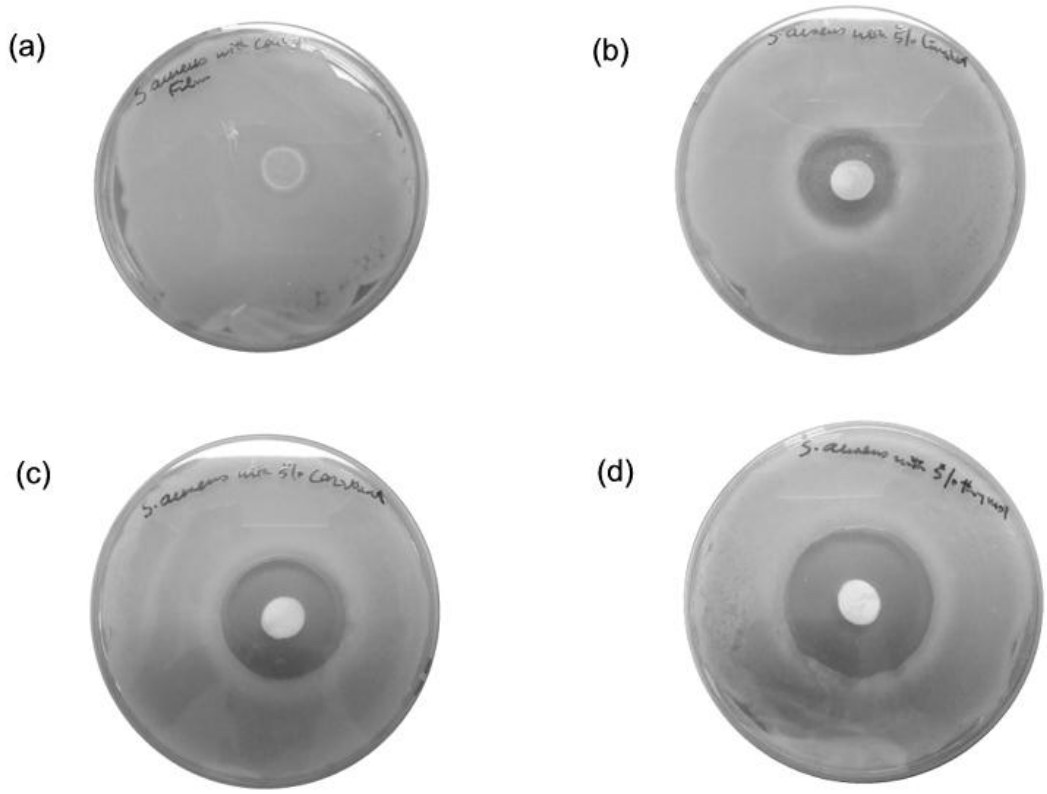
Figure 1. Inhibition of *S. aureus* on solid media at 37°C after 24 h on starch-based coated films containing: (a) no AM agent, (b) 2.38% (w/w) linalool, (c) 2.38% (w/w) carvacrol, and (d) 2.38% (w/w) thymol.

Figure 2. Zone of inhibition of *S. aureus* at 37°C versus AM agent concentration for starch-based films containing in their coating: no AM agent (□), linalool (●), carvacrol (△) and thymol (○).

Figure 3. Inhibition of *S. aureus* on Cheddar cheese packaged and stored at 15°C in starch-based coated films containing: no AM agent (□), 0.48% (w/w) carvacrol (△), 1.43% (w/w) carvacrol (○), and 2.38% (w/w) carvacrol (■).

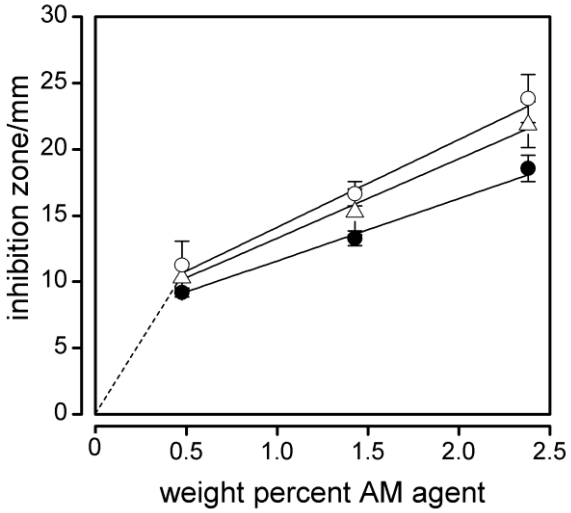
Figure 4. Specific death rate of *S. aureus* on the surface of Cheddar cheese versus AM agent concentration for cheese packaged and stored at 15°C in starch-based films containing in their coating: linalool (●), carvacrol (△) and thymol (○).

601 **Figure 1.**



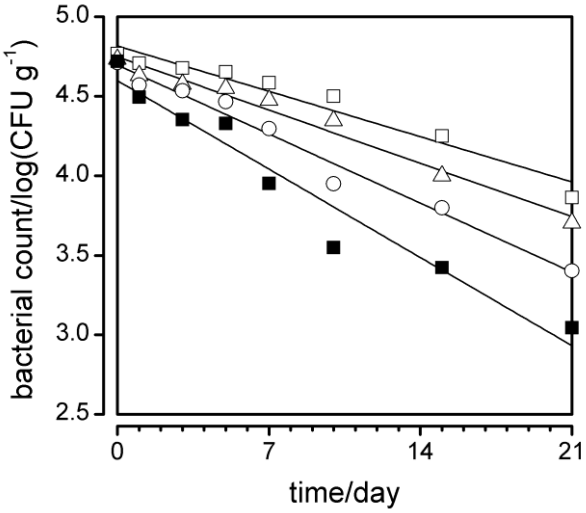
602
603

Figure 2.


inhibition zone/mm
30
25
20
15
10
5
0
0
0.5
1.0
1.5
2.0
2.5
weight percent AM agent

609 **Figure 3.**

610



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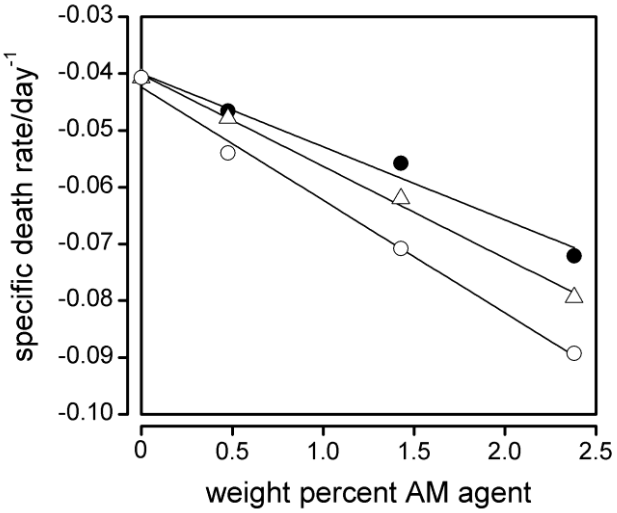
613 **Figure 4.**

614



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