Food Packaging and Shelf Life 24 (2020) 100500

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: www.elsevier.com/locate/fpsl

Fat content and storage conditions are key factors on the partitioning and activity of carvacrol in antimicrobial packaging

Li Wang, Jenneke Heising, Vincenzo Fogliano, Matthijs Dekker*

Food Quality and Design, Wageningen University, P.O. Box 17, 6700 AA, Wageningen, the Netherlands

ARTICLE INFO

Keywords:
Antimicrobial packaging
Carvacrol partition
Controlled release
Food composition
Ground beef

ABSTRACT

The ability of carvacrol loaded polylactic acid (PLA) films to improve ground beef preservation was assessed. The mass transfer processes of carvacrol partitioning in a food packaging system between the PLA film, headspace and food product was studied. Carvacrol release was studied on packed ground beef having a fat content of 5 or 12 % at a temperature between 5 and 30 °C and a humidity between 43 and 94 % for up to 12 days.

Results showed the release rate of carvacrol from the PLA film into the headspace increased with the storage temperature while the humidity in the packaging headspace had no effect on the release rate of carvacrol from the PLA film. The fat content of ground beef has a profound effect on the partitioning of carvacrol: when the system is stored at 5 °C the carvacrol absorption in the 12 % fat ground beef was about 1.3-fold compared with the carvacrol concentration observed in 5% fat ground beef. Despite this higher carvacrol absorption in the regular beef, the PLA/carvacrol films had a stronger antimicrobial effect on the lean beef suggesting that partitioning of carvacrol into the fat phase of the beef reduced its antimicrobial activity. Results highlight the importance of considering the food matrix composition in the design of antimicrobial packaging based on natural volatile components.

1. Introduction

Food safety and food spoilage is a major problem: a better preservation of food can reduce food waste especially in industrialized country (Roodhuyzen, Luning, Fogliano, & Steenbekkers, 2017) and contribute to achieve the sustainability goals placed in the political agendas of many countries. Fresh product of animal origin are highly susceptible to microbial spoilage. Microbial growth is the main factor in meat, fish and dairy spoilage which results in off-odour and off-flavour as well as defects in texture and appearance (Sun & Holley, 2012). Antimicrobial packaging provides options to inhibit microbial growth and extend the shelf life of foods by incorporating specific antimicrobial compounds into the packaging (Cha & Chinnan, 2004).

Several essential plant oils have antimicrobial activity; they have been incorporated into packaging film to inhibit microbial growth. Essential oils from clove, oregano, rosemary, thyme, sage and vanillin are very effective as antimicrobial agents (de Azeredo, 2013; Kouchak, Ameri, Naseri, & Boldaji, 2014). The antimicrobial activity of essential oils mainly depends on the presence of various terpenic and phenolic compounds. Usually compounds having phenolic groups have strong antimicrobial activity and carvacrol is one of the most powerful (Marchese et al., 2016). Carvacrol is reported to have antimicrobial activity against a wide spectrum of bacterial strains (Fernandez-Saiz, Lagaron, Hernandez-Muñoz, & Ocio, 2008; Kristo, Koutsoumanis, & Biliaderis, 2008), moulds (Garrido Assis & de Britto, 2011) and yeasts (Avila-Sosa et al., 2012). Among polymer films, polylactic acid (PLA) is recognised as one of the most promising bio-based and biodegradable materials for food packaging applications (Peelman et al., 2013). PLA based packaging has been commercially applied for many food products for both short-shelf life products and long-shelf life products, such as PLA Bags for potato chips in PepsiCo's Frito-lay, PLA Bowls for fresh salads in McDonald's and Dannon™ yogurts (Armentano et al., 2015). Therefore the development of a bio-based PLA film containing carvacrol is a logical choice to extend the shelf-life and improve the safety of perishable foods (Martínez-Camacho et al., 2010; Sebti, Martial-Gros, Carnet-Pantiez, Grelier, & Coma, 2005).

The controlled carvacrol release in a packaging system is the key for developing an efficient antimicrobial package, in fact keeping a concentration of antimicrobials that is continuously above the MIC is important for preserving food products (Vergnaud & Rosca, 2006). The controlled release strategies will be affected by the storage environment, the structure of the releasing films and the composition of food products (Mastromatteo, Mastromatteo, Conte, & Del Nobile, 2010). The effect of environmental conditions has been demonstrated in

* Corresponding author.
E-mail address: matthijs.dekker@wur.nl (M. Dekker).

https://doi.org/10.1016/j.fpsl.2020.100500
Received 19 August 2019; Received in revised form 18 February 2020; Accepted 22 February 2020

studies showing an increased storage temperature and humidity can accelerate the release of the active agents from packaging films (Bierhalz, da Silva, & Kieckbusch, 2012; Kurek, Guinault, Voilley, Galić, & Debeaufort, 2014; Mascheroni, Guillard, Gastaldi, Gontard, & Chalier, 2011). The effect of food composition on the active compounds release and partitioning within the food-packaging system, however, has been rarely tested, while is expected to be an important factor to determine the antimicrobial efficiency. There is a knowledge gap on the influence of food composition and food matrix structure on the volatile antimicrobials partitioning in food packaging system.

Several mass transfer processes occur in an antimicrobial packaging system, including antimicrobial compounds release into the headspace, absorption of active compounds in the food and microbial growth in the food products (Begley et al., 2005). To understand the performance and efficacy of an antimicrobial packaging, the process of partitioning of antimicrobial compounds into the headspace and into the food product and the resulting effect on microbial growth should be investigated. The knowledge of partitioning of antimicrobials in active packaging systems helps to design and optimise the packaging system to have a desired antimicrobial effect for specific food categories. Higueras, López-Carballo, Hernández-Muñoz, Catalá, and Gavara (2014) studied the partitioning of the active agent within a food/packaging system. The authors developed an antimicrobial packaging by incorporating carvacrol into chitosan/cyclodextrin films for chicken fillets preservation. They studied the partitioning of the carvacrol within each phase of the food package system, including tray, lid, film and chicken. Results showed the volume/weight ratio between the package and the film was the main factor influencing the carvacrol partitioning thus the bacterial inhibition.

Several researchers tested the antimicrobial activity of carvacrol incorporated packaging films on beef products preservation, and different materials and structures of polymers were used to entrap carvacrol (Emiroğlu, Yemiş, Coşkun, & Candoğan, 2010; Zinoviadou, Koutsoumanis, & Biliaderis, 2009). However, the combined effect of the packaging environment and fat content of the food product has not been investigated for carvacrol antimicrobial packaging systems, thus far.

In this paper, the influences of the storage environment (temperature and humidity) and the fat content of ground beef on the carvacrol partitioning and antimicrobial activity in a ground meat - PLA food packaging system were studied.

2. Materials and methods

2.1. Materials

PLA (4043D Ingeo™), produced by company NatureWorks LLC, was provided by Wageningen Food & Biobased Research. Carvacrol (≥ 98 %), dichloromethane, hexane, ringer tablets, potassium carbonate, sodium chloride and potassium nitrate were purchased from Sigma Aldrich, Netherlands. The saturated solutions were prepared with potassium carbonate, sodium chloride and potassium nitrate to fix the relative humidity (RH) at 0.43, 0.75 and 0.93 respectively (Blanchard, Gouanvé, & Espuche, 2017; Kim, Kim, Yi, Oh, & Lee, 2015). Peptone Physiological Salt Solution (PFZ) was purchased from VWR, Netherlands.

Ground regular and lean beef products were purchased from a local supermarket. According to the products' label, the fat contents of ground regular and lean beef are 12/100 g and 5/100 g, respectively.

2.2. Carvacrol loaded poly (lactic) acid (PLA) film preparation

PLA films were prepared according to the solvent-casting method of Rhim, Mohanty, Singh, and Ng (2006) with some modifications. Four gram of PLA pellets were dissolved in 100 mL of dichloromethane. The mixture was stirred by a magnetic stirrer at 40 °C until the polymer was totally dissolved. Two gram of carvacrol was then added to PLA/dichloromethane mixture, and the mixture was stirred for 1 h at room temperature. Subsequently, the mixture was homogenized using an ultra-sonicator (HBM Machines, Moordrecht, the Netherlands) for 30 min at room temperature (20 °C). In the end, the solution was distributed into glass round bottomed flasks and dried in fume cupboard. The films were peeled from the glass flask after 24 h, and were stored at 4 °C in a sealed container.

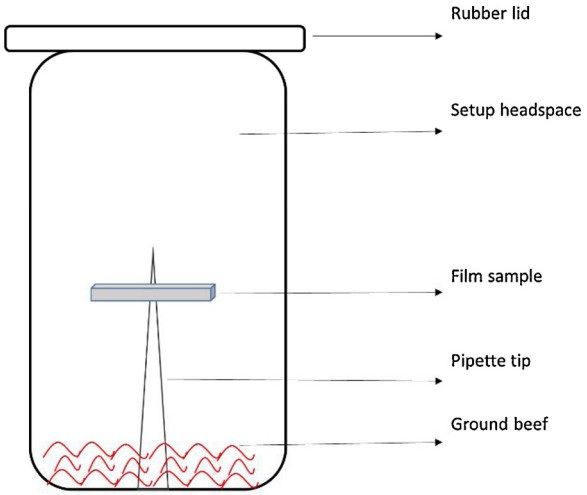


Fig. 1. Setup used for evaluation of carvacrol partitioning within food/package system.

2.3. Packaging system preparation

The carvacrol partitioning between film and headspace was studied in a closed system (Fig. 1). The 10 mL verex headspace glass vials were used to simulate the closed food system with a package and a food product. Each cell consisted of a glass vial sealed by a rubber lid, packaging film (0.020 ± 0.001 g) and ground beef product (0.100 ± 0.007 g) for sampling. The packaging film was inserted onto a pipette tip to ensure that the film could not have direct contact with the ground beef, which was at the bottom inside of the glass vial. Three exposure temperatures, including 5 °C, 20 °C and 30 °C, were used to study the temperature effect of carvacrol release and partitioning to food products in the closed packaging film. Several cells with ground beef and film were prepared for daily measurement to characterize the process of carvacrol release from PLA packaging film and partitioning to headspace and food.

2.4. Carvacrol analyse in the packaging/headspace/ground beef system

2.4.1. Calibration

The stock solutions (2.0 mg/mL) of carvacrol in the mixture of hexane and 2-proponal (n-hexane : isopropanal = 3:1) were prepared for quantification of carvacrol in beef products. To determine carvacrol left in film, another stock solution (2 mg/mL) was prepared by adding carvacrol in chloromethane. Spiked standard solutions at different concentrations (0.4, 0.8, 1.2, 1.6 and 2.0 mg/mL) were obtained by diluting the stock solutions to create standard curves for quantification of carvacrol in the system.

2.4.2. Carvacrol analyse in headspace in 10 mL verex headspace vials

The carvacrol in headspace was measured with headspace solid phase microextraction (HS-SPME) technology followed by Gas Chromatography – Flame Ionization Detector (GC-FID) (Thermo Scientific, Waltham, Massachusetts, United States). The extraction method of carvacrol from headspace of glass vials was according to

Zhang, Qi, Shao, Zhou, and Fu (2007) with some modifications. Briefly, a SPME fiber assembly Polydimethylsiloxane (PDMS) with needle size of 23 ga was pierced in the rubbery lid of glass vial, then the fibre of SPME was outstretched through the needle and absorbed the carvacrol from the headspace. The fibre was withdrawn into the needle after 15 min extraction, and injected to the port of GC-FID immediately. The desorption of carvacrol in GC-FID was 5 min. A preliminary experiment was performed to ensure the extraction and desorption time were long enough to absorb all the analytes from headspace and absorbed onto the chromatographic column.

The following conditions were set to operate GC-FID: The injection temperature was adjusted at 250 °C. The column oven temperature was initially set at 60 °C, and then increased to 250 °C with the speed of 15 °C min^{-1}. Helium was used as the carrier gas, running at a constant flow of 2.0 mL min^{-1}. The FID was operated with a hydrogen flow of 35 mL min^{-1} and air flow of 350 mL min^{-1}. Injections were set in split mode with a split flow of 10 mL/min and a split ratio of 5. The total running time was 25.25 min.

2.4.3. Extraction and quantification of carvacrol concentration in ground beef products

Carvacrol was extracted from the ground beef products (0.1 ± 0.007 g) before analysing by GC-FID. Carvacrol was extracted by adding 0.5 mL of organic mixture (n-hexane : 2-proponal = 3:1) in meat sample. The mixture was warmed in a shaking water bath at 40 °C for 20 min at the speed of 60 rpm for carvacrol extraction. The mixtures were then centrifuged for 10 min at 1000 *g* to obtain the top organic layer. Ten microliter of organic solution was subjected into GC-FID for liquid analyse. Extraction efficiency of this method was tested by extraction carvacrol from beef samples by adding known amounts of carvacrol, and a high solvent-to collect ratio (> 97 %) was achieved by using this method. The concentration of carvacrol in meat was determined using a calibration curve of carvacrol in the mixture of hexane and 2-proponal (n-hexane : 2-propanol = 3:1) (R^2 = 9995).

2.4.4. Extraction and determination of carvacrol left in packaging film

To quantity the carvacrol left in the packaging film at each measuring time point, the carvacrol loaded PLA packaging film was dissolved in 5 mL dichloromethane solvent at 20 °C by 2 h stirring at 600 rpm. Ten microliter of organic solution was subjected into GC-FID for liquid analyse. The concentration of carvacrol in films was determined using a calibration curve of carvacrol in dichloromethane (R^2 = 9996).

2.5. Ground beef samples preparation and storage

PLA film with higher carvacrol concentration were prepared with two gram of carvacrol and four gram of PLA to test its antimicrobial efficacy. Fresh beef for microbial analyses were divided in two parts, one part was used to test the antimicrobial effect of the developed film, and the other part served as a control. All beef samples (5.00 ± 0.05 g) were stored in airtight sterilised 500 mL plastic containers in a refrigerator (5 °C). In the antimicrobial group, 1.00 ± 0.01 g of active film was added to each sample. The samples were prepared in individual containers for each periodical measurement (0, 1, 3, 6, 8 and 12 days). All experiments were done in duplicate.

2.6. Microbiological analyses

At each sampling day, beef samples (5.00 ± 0.05 g) were taken from the sealed plastic containers aseptically in a flow cabinet and put into a sterilized stomacher bag together with 45 mL of Ringers solution. The stomacher 400 circulator (Seward Limited, Worthing, United Kingdom) was used to homogenize the beef samples for 2 min at 230 rpm at room temperature. Resulting turbid solutions were 10-fold serially diluted in Peptone Physiological Salt Solution (PFZ) and 100 μL was brought onto Plate Count Agar by the spread technique (PCA; Merck, Darmstadt, Germany) in duplicate, incubated at 20 °C for 48 h, after which the number of colonies at the appropriate dilutions was counted manually.

2.7. Microbial growth modelling

Two empirical microbial growth models were tested in this study, the modified Gompertz equation (Eq. (1)) and the modified logistic model (Eq. (2)) (Zwietering, Jongenburger, Rombouts, & Van't Riet, 1990)

$$ln\frac{N}{N0} = As*\exp(-\exp\left(\frac{umax*e}{As}(\lambda - t) + 1\right)) \quad (1)$$

$$ln\frac{N}{N0} = \frac{As}{1 + \exp\left(\frac{4umax}{As}(\lambda - t) + 2\right)} \quad (2)$$

in which: A_S is the natural logarithm of the asymptotic value of the relative population size, u_{max} is the maximum specific growth rate (day^{-1}), λ is the lag phase (day), t is the storage time (day) and N/N_0 represents the ratio of the microbial cell density (CFU g^{-1}) to the initial microbial cell density (CFU g^{-1}).

2.8. Statistical analysis

All the GC analyse were performed in triplicates and the microbiological analyses were performed in duplicates. The IBM SPSS Statistics version 23.0 for Windows (Armonk, NY: IBM Corp) was used for the statistical analysis of the results from GC. One-way analysis of variance (ANOVA), with post-hoc pairwise analysis by the Tukey test was used to assess the significant differences between samples. Data fitting by nonlinear regression was completed with the aid of Solver add-in in Excel by minimizing the sum of the squared residuals (SSR) to obtain the parameters of the empirical microbial growth models. The standard deviation of antimicrobial results and correlation of all parameters was calculated by the macro SolverAid (de Levie, 1999). A level of $p < 0.05$ was defined to test the statistical significance of experimental results. The resulting data were presented as means ± standard deviation. To discriminate the model performance, the corrected Akaike's Information Criterion (AIC) was used for models discrimination:

$$AIC_c = n\ln(s^2) + 2(p + 1)[n/(n - p)] \quad (3)$$

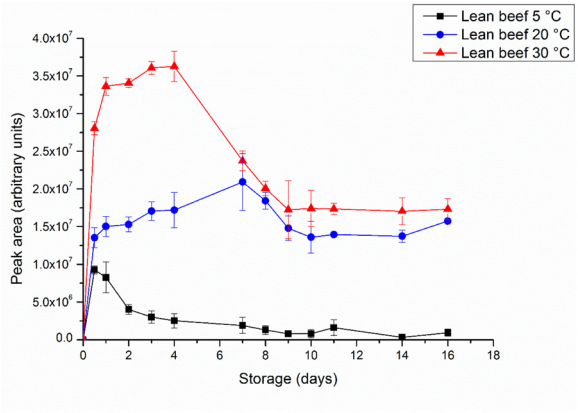
Where, n is the number of data points, p represents the number of parameters, and $s^2 = SS_{res}/n$. SS_{res} is the residual of sums of squares between measured and estimated values. The model giving the lowest AIC value is the best fitting model (Motulsky & Christopoulos, 2004).

3. Results and discussion

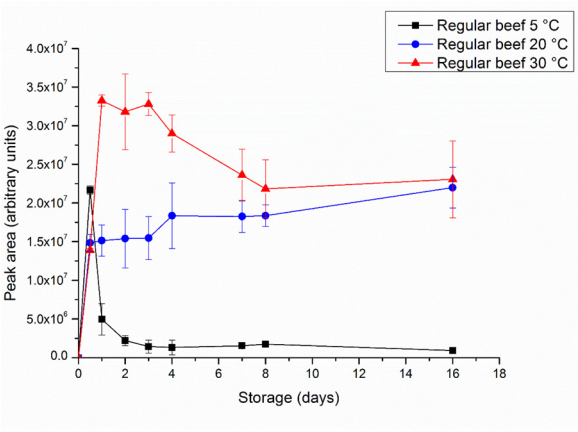
3.1. Carvacrol analyse in the packaging/headspace/ground beef system

3.1.1. Carvacrol in the headspace of the package

The carvacrol intensity in the headspace of the package setup, measured as a function of time, storage temperature and fat content of the ground beef, is reported in Fig. 2. The headspace of the package is the intermediate zone between packaging film and food matrix, and therefore illustrates the dynamic behaviour of carvacrol in the whole package. At 30 °C, an immediate large increase of the carvacrol intensity in the headspace was observed already at the first day. This intensity remained high during the first 4 days of storage. After 4 days, the intensity of carvacrol started to decrease and after 8 days of storage, it reached a dynamic equilibrium between carvacrol release from film and absorption into the beef. At 20 °C, carvacrol intensity in the headspace was stable during the whole storage period after the initial



(a)



(b)

Fig. 2. The carvacrol intensities in the headspace of package containing a) ground lean beef b) ground regular beef.

peak in the release during the first day. The initial peak area at 20 °C was similar to the equilibrium achieved at 30 °C after 4 days. Data collected by storing at 5 °C, showed that the intensity of carvacrol in the headspace is lower, after the initial increase during the first day; it reached a steady state after 4 days at intensity of about 1/3 of the carvacrol observed at 20 °C and 30 °C.

The peak area of carvacrol in the headspace in the system containing lean meat (Fig. 2a) was significantly different from the regular meat (Fig. 2b) at 5 °C for 4 days: In fact the initial peak area of carvacrol released from packaging to the headspace was about 1.3-fold higher in the regular beef system. However, the peak area of carvacrol in fat beef was significant lower than the lean beef from day 1 to day 4 ($p < 0.05$). This suggests that at low temperature the reduced vapour pressure of carvacrol, the absorption rate into the meat becomes dominant. This absorption is expected to be faster for meat with a higher fat content due to the high solubility of carvacrol into fat. For longer storage time the intensity of carvacrol in the headspace became the same for the two products suggesting that on the long-term storage (> 9 days) the release of carvacrol from the packaging is controlling the headspace intensity.

The finding that carvacrol released more easily from PLA film at higher temperatures are consistent with Kuorwel, Cran, Sonneveld, Miltz, and Bigger (2013) who observed that the increased temperature plays a significant role on the migration of antimicrobial compounds from films. Various other studies showed that the temperature is an effective factor to control the antimicrobials release from polymer films (Kashiri et al., 2017; Rubilar, Cruz, Zuñiga, Khmelinskii, & Vieira, 2017; Tunç & Duman, 2011). The amount of volatile compound release depends on the surrounding environment because the high temperature results in high diffusion coefficients of volatiles. This effect might be strong or weak depending on the polymer matrix used for encapsulation of compounds. In addition, the partitioning of carvacrol into headspace is favoured at higher temperature because of its high vapour pressure (Kurek et al., 2014).

The effect of relative humidity (RH) on carvacrol released from the PLA film at room temperature (20 °C) was also analysed in this work. However, during the 6-day measuring period, the release of carvacrol from PLA films were not significant influenced by the storage RH for most of the measuring days (results were not shown). Since PLA film has a good water resistance applying for food packaging, the low absorption of water has less effect to trigger the release of the loaded compounds.

3.1.2. Carvacrol absorption in meats and desorption from films during storage

The concentration of carvacrol in ground lean and regular beef during storage in different conditions was measured to study the effect of fat content and storage temperature on the absorption of carvacrol released from the package. The samples were stored at different temperatures (5, 20, 30 °C). Fig. 3a and b shows a plot of the carvacrol concentrations in time and the partitioning of carvacrol from PLA film to lean/ regular beef products at the three storage temperatures. The amount of carvacrol absorbed in food products increased with the storage days at all temperature conditions. In accordance to the results that more carvacrol was released from the PLA films at higher temperature, also more carvacrol was found in both lean and regular beef products with increasing temperature. At 30 °C, the fastest diffusion of carvacrol into the meat samples were observed. After about 8 days a steady state of carvacrol absorption for ground regular beef was observed. Accordingly, when the samples (lean and regular beef) were stored at 20 °C, the time required to achieve the steady state was longer than 12 days. In the products stored at 5 °C, the amount of carvacrol measured in the samples was significant low ($p < 0.05$), and the concentration increased lineally for both lean and regular beef during measurement ($R^2 = 0.95$ for regular beef and $R^2 = 0.98$ for lean beef).

For the effect of the food composition on carvacrol migration, it was found that the fat content has significant effects on carvacrol absorption in ground beef products in all temperatures. For the samples stored at 5 °C, the carvacrol absorption in regular beef was significant higher than the absorption in lean beef at every measurement day ($p < 0.05$). At day 12, the ratios of carvacrol in regular/lean beef for 5, 20 and 30 °C was 1.34, 1.12 and 1.30, respectively. The effect of fat on carvacrol uptake by the meat is more pronounced under refrigeration (5 °C).

The carvacrol left in film at each measuring day were measured, and the results are shown in Fig. 3c and d. The initial carvacrol into PLA film was 9.93 ± 0.41 mg. It was lower than the estimated amount (10 mg) as carvacrol was evaporated during film drying process. The overall desorbed mass of carvacrol from the films was less than half of the total amount at the beginning of storage. The maximum residual carvacrol in the films could be found for storage at 5 °C (86.28 % for lean beef and 82.79 % for regular beef), followed by 20 °C (78.92 % for lean beef and 69.71 % for regular beef). The films stored at 30 °C for both lean and regular beef lost a significant higher amount of carvacrol than the films at 20 °C and 5 °C ($p < 0.05$). The results of carvacrol left in films also show an effect of the fat content of food on the release of carvacrol from PLA film. For regular beef less than 36 % of the carvacrol was desorbed from the PLA films after 12 days of storage at 30 °C, for the lean beef this was less than 42 %. This can be explained by the bigger driving force of carvacrol absorption in ground regular beef that promotes carvacrol release from the PLA film.

The intrinsic chemical properties of the antimicrobials (hydrophobicity and volatility), the composition and structure of food medium and environmental conditions (temperature and RH) are the main

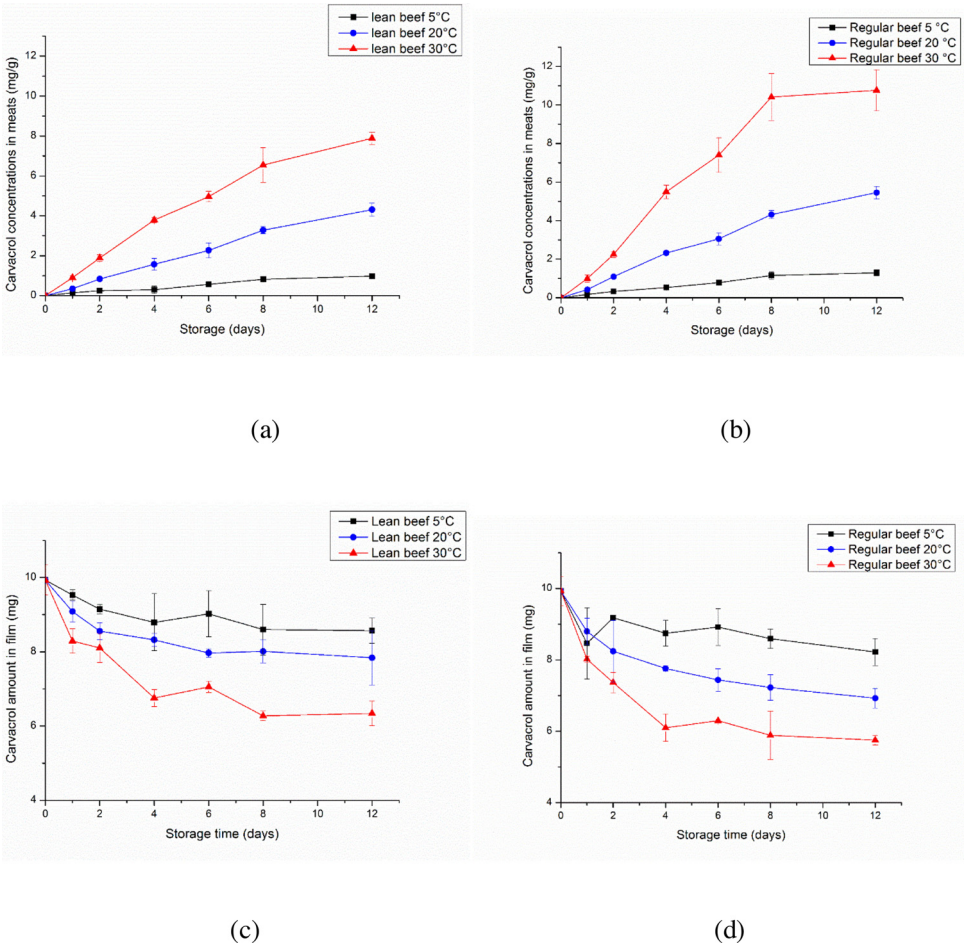


Fig. 3. The carvacrol concentrations in a) ground lean and b) regular beef products, and the amount of carvacrol in the film of package containing c) ground lean and d) regular beef.

factors that determine carvacrol partitioning in a food packaging system. In this food-package system, carvacrol released from the PLA film first migrated to the headspace of the package before it was absorbed by the food matrix. The results obtained from Fig. 3 clearly indicate that carvacrol was more easily absorbed in meats with high fat contents. This can be explained by the affinity between carvacrol and the fat part of the food products. Both fat and carvacrol are non-polar molecules, and molecules with same chemical polarity have better affinity with each other (Chambin et al., 2009; Ma, Yang, Yao, Xu, & Tang, 2017). In this case, carvacrol shows a high preferences for hydrophobic phases with the partition coefficient in octanol-water (log *P*) of 3.52 (Ben Arfa, Combes, Preziosi-Belloy, Gontard, & Chalier, 2006). It is generally recognized that compounds with log *P* higher than 1 are hydrophobic and are thus more retained in oil than water, which leads to more carvacrol absorption in ground regular beef products. Although few studies were conducted to study the antimicrobial absorption in real foods, some researches that have tested different food simulants found results in line with this study. Ramos, Beltrán, Peltzer, Valente, and del Carmen Garrigós (2014) studied the migration of carvacrol and thymol from polypropylene (PP) films into different food simulants, including distilled water, acetic acid ethanol and isooctane. Based on their results, the calculated migration coefficients were reduced when the polarity of the simulant increased. The higher migration into isooctane could be attributed to the affinity of apolar antimicrobials with isooctane. This behaviour has also been suggested by Sánchez-González, Cháfer, González-Martínez, Chiralt, and Desobry (2011) who studied the release kinetic of limonene from chitosan films to different food simulants. Aqueous solutions with 0%, 10 %, 50 % and 95 % of ethanol and isooctane were tested at room temperature (20 °C). The polarity of these food simulating solutions decreased with the increase in ethanol concentration. According to their results, the release kinetic constants of limonene for chitosan films increased exponentially when the ethanol concentration increased in the aqueous system.

However, despite the fact that hydrophobic olive oil has high affinity to carvacrol, less carvacrol was detected in pure olive oil compared to ethanol and acetic acid according to the results from Fernández-Pan, Maté, Gardrat, and Coma (2015). The reason attributes to the direct/indirect contact between food stimulates and film. In their study, the aqueous solutions of ethanol and acetic acid cause a loss of the structural integrity of the hydrophilic edible (chitosan-carvacrol) films when the films were in contact with the solutions; however, olive oil has a limited access into the film matrix leading to a closed structure of chitosan film. In our study, the migration process of carvacrol started from PLA film to the headspace of setup, followed by absorption into foods. The food products were not able to swell/loosen the structure of packaging film due to the indirect contact between film and the ground meats. Additionally, the moisture in closed vials has less impact on the structure of PLA film due to its hydrophobicity, and the higher diffusion of carvacrol mainly because of the high temperature. Therefore, the higher amount of carvacrol in ground regular beef products were mainly because of the higher molecule affinity between carvacrol and fat food matrix. The results of carvacrol partitioning in the packaging system show that antimicrobials diffusion is dependent on the packed food product characteristics, therefore different food products require tailored food packaging solutions.

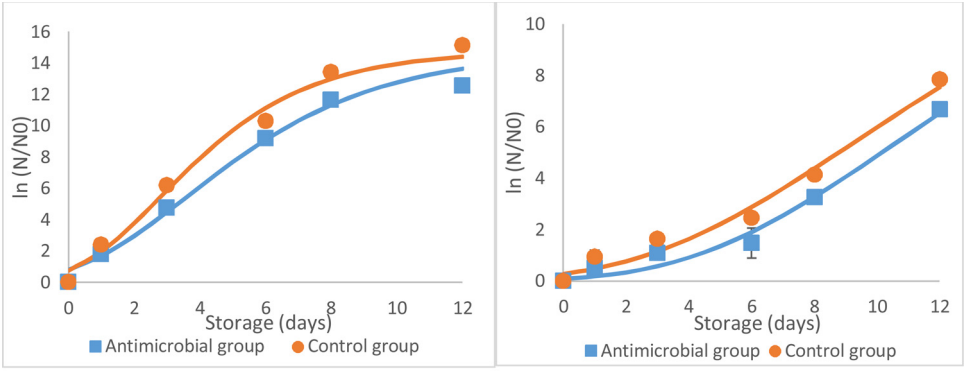


Fig. 4. Growth curves of total bacterial count in a) ground lean beef and b) ground regular beef in antimicrobial/control group at 5 °C fitted with the modified Gompertz equation.

3.2. Antimicrobial effect of the carvacrol/PLA film and modelling of bacterial growth curve

The total viable count (TVC) changes of ground beef samples that were treated with and without packaging films are presented in Fig. 4. The ground regular beef samples with 12 % of fat contents showed a 0.52 log reduction of TVC population in samples treated with antimicrobial films at day 12, compared with the control samples. For ground lean beef, the TVC population of antimicrobial groups at day 12 the reduction was larger with a 1.1 log reduction of TVC compared to the control group.

Mathematical modelling was used to predict the potential for growth of bacterial in food products during longer storage. The modified Gompertz and modified Logistic model were used to describe the microbial growth in the ground beef samples. Both models gave reasonable fits. According to the AIC values from two models, the modified Gompertz model gives the better performance as the AIC value of the Gompertz model (AICc = -2.3) is lower than the modified Logistic model (AICc = 7.6). Therefore, the Modified Gompertz model was selected for further analysis (Fig. 4). The lag period λ (day) and maximum growth rate μ_{max} (day^{-1}) for bacterial growth in beef samples packaged within/without developed setup were estimated by Gompertz model (Fig. 5). The value of upper asymptote for all samples was estimated of 14.8 ± 0.6 log CFU/g, according to the modified Gompertz model. In particular, the lag phase of bacteria growth was longer in regular beef. The estimated growth rate of bacteria in lean beef packaged with PLA film was much lower than the growth rate in control group (p = 0.07), indicating the antimicrobial package concept has a clear effect on bacterial growth inhibition in lean beef in terms of the growth rate estimation.

From estimated parameters and bacterial reduction values could be concluded that the efficacy of carvacrol loaded PLA film was greater in food products with low fat content than in high fat foods. According to the results of carvacrol absorption in lean/regular beef products and carvacrol desorption from films (Fig. 3), more carvacrol was found in ground regular beef at the same storage temperature. However, the microbiological analyses in antimicrobial and control groups indicated that a 50 % larger log reduction of TVC population was observed in ground lean beef groups compared to regular beef samples. This phenomenon might be related to the effect of fat content on the antimicrobials migration into the food matrix. Carvacrol has a high log P value of 3.52 (Ben Arfa et al., 2006); therefore, it will mainly be present in the fat of the food. The solubility of carvacrol in fat limits the interaction of carvacrol with bacteria that actually grow in the aqueous portion of the meat. Studies on adding herb extracts to beef products to reduce bacterial growth demonstrated that the antimicrobial activity of herb extracts might be weaker in beef products with high fat content, pointing to the influence of the way the fat is distributed in the food matrix on the antimicrobial effect of hydrophobic compounds (Cutter, 2000). Similarly, Singh, Singh, Bhunia, and Singh (2003) concluded that the direct addition of thyme essential oil in foods reduced *L. monocytogenes* populations significantly in zero- and low-fat hotdogs, but not in full-fat hotdogs. The results of this research confirms the influence of fat content of foods on the bacteria inhibition of antimicrobials.

With the estimated parameters As, λ and μ_{max} the shelf life extension of ground lean and fat beef in the antimicrobial group could be predicted using the Gompertz equation (Table 1). The spoilage level of 9 log10 CFU/g at 5 °C was used as the value for the end of shelf life for ground meat (Koutsoumanis, Stamatiou, Skandamis, & Nychas, 2006). The PLA film with carvacrol had a significant positive effect on prolonging the shelf life of lean beef products at chilled chain

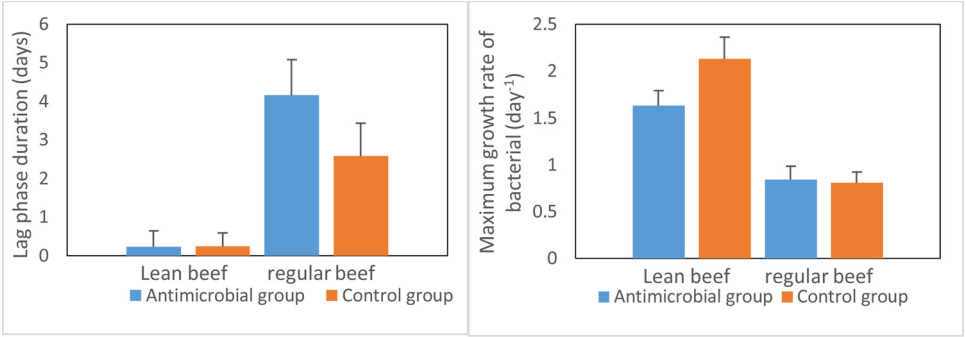


Fig. 5. Estimated a) lag phase duration and b) growth rate for modified Gompertz equation as calculated from the models fitted to the total viable count experimental points of ground beef stored at 5 °C.

Table 1
Predicted values for the shelf life extension of ground lean and fat beef packaged within antimicrobial film at 5 °C.

	Shelf life extension of lean beef (day)	Shelf life extension of regular beef (day)
Shelf life (day) ± SD	2.5 ± 0.9	1.1 ± 1.5
p-value	0.007	0.486

conditions (5 °C), compared to the control group. According to the model prediction, the shelf life of lean beef preserved with antimicrobial film was extended by 2.5 ± 0.9 days compared with the beef in the control group. A 30 % longer shelf life was achieved in ground lean beef stored with carvacrol loaded PLA film. In terms of the regular beef in antimicrobial group, the carvacrol loaded PLA film was able to extend 1.1 ± 1.5 days of shelf life for the test sample.

4. Conclusions

In the present study, a PLA film with carvacrol was developed to study the correlation between the carvacrol partitioning and its antimicrobial efficacy. It was demonstrated that the storage temperature and fat content of foods have significant effect on carvacrol partitioning within the package, while relative humidity did not have an effect. For both ground lean and regular beef, the carvacrol concentrations in the headspace of package at 30 °C and 20 °C were higher than the carvacrol quantity of sample at 5 °C. In the headspace of packages containing ground lean beef, 2.9-fold higher carvacrol was found in the samples at 30 °C than the sample stored at 5 °C. The higher release of carvacrol from PLA film is "intelligent" when the temperature increase. If for any reason the chill chain is interrupted the system reacts releasing more antimicrobials. In addition, 1.3-fold carvacrol was found in ground regular beef with a high fat content compared to ground lean beef at 5 °C. Despite this higher carvacrol concentration in regular beef the antimicrobial activity was more effective in lean beef. This can be explained by the absorption of carvacrol occurring mainly in the fat phase of the products. The shelf life of ground lean beef stored with carvacrol loaded PLA film was 2.5 days longer compared to the control. According to the results from this study, it can be concluded that the developed food packaging film is a feasible application to provide a prolonged carvacrol release from PLA film within a longer period of storage time. As a whole, this study contributes to the understanding of the mechanistic aspects that are useful in the design of antimicrobial packaging for a specific food composition.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Li Wang: Conceptualization, Methodology, Investigation, Writing - original draft. **Jenneke Heising:** Conceptualization, Supervision, Writing - review & editing. **Vincenzo Fogliano:** Conceptualization, Supervision, Writing - review & editing. **Matthijs Dekker:** Conceptualization, Supervision, Writing - review & editing.

Acknowledgements

The authors thank to the China Scholarship Council for supporting a PhD fellowship for L.Wang. We would also like to acknowledge the Wageningen Food & Biobased Research for providing the PLA (4043D Ingeo™).

References

Armentano, I., Fortunati, E., Burgos, N., Dominici, F., Luzi, F., Fiori, S., et al. (2015). Bio-based PLA_PHB plasticized blend films: Processing and structural characterization. *LWT-Food Science and Technology, 64*(2), 980–988.
Avila-Sosa, R., Palou, E., Munguía, M. T. J., Nevárez-Moorillón, G. V., Cruz, A. R. N., & López-Malo, A. (2012). Antifungal activity by vapor contact of essential oils added to amaranth, chitosan, or starch edible films. *International Journal of Food Microbiology, 153*(1), 66–72.
Begley, T., Castle, L., Feigenbaum, A., Franz, R., Hinrichs, K., Lickly, T., et al. (2005). Evaluation of migration models that might be used in support of regulations for food-contact plastics. *Food Additives and Contaminants, 22*(1), 73–90.
Ben Arfa, A., Combes, S., Preziosi-Belloy, L., Gontard, N., & Chalier, P. (2006). Antimicrobial activity of carvacrol related to its chemical structure. *Letters in Applied Microbiology, 43*(2), 149–154.
Bierhalz, A. C. K., da Silva, M. A., & Kieckbusch, T. G. (2012). Natamycin release from alginate/pectin films for food packaging applications. *Journal of Food Engineering, 110*(1), 18–25.
Blanchard, A., Gouanvé, F., & Espuche, E. (2017). Effect of humidity on mechanical, thermal and barrier properties of EVOH films. *Journal of Membrane Science, 540*, 1–9.
Cha, D. S., & Chinnan, M. S. (2004). Biopolymer-based antimicrobial packaging: A review. *Critical Reviews in Food Science and Nutrition, 44*(4), 223–237.
Chambin, O., Karbowiak, T., Djebili, L., Jannin, V., Champion, D., Pourcelot, Y., et al. (2009). Influence of drug polarity upon the solid-state structure and release properties of self-emulsifying drug delivery systems in relation with water affinity. *Colloids and Surfaces B, Biointerfaces, 71*(1), 73–78.
Cutter, C. N. (2000). Antimicrobial effect of herb extracts against Escherichia coli O157: H7, Listeria monocytogenes, and Salmonella typhimurium associated with beef. *Journal of Food Protection, 63*(5), 601–607.
de Azeredo, H. M. (2013). Antimicrobial nanostructures in food packaging. *Trends in Food Science & Technology, 30*(1), 56–69.
de Levie, R. (1999). Estimating parameter precision in nonlinear least squares with Excel's Solver. *Journal of Chemical Education, 76*(11), 1594.
Emiroğlu, Z. K., Yemiş, G. P., Coşkun, B. K., & Candoğan, K. (2010). Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. *Meat Science, 86*(2), 283–288.
Fernández-Pan, I., Maté, J. I., Gardrat, C., & Coma, V. (2015). Effect of chitosan molecular weight on the antimicrobial activity and release rate of carvacrol-enriched films. *Food Hydrocolloids, 51*, 60–68.
Fernandez-Saiz, P., Lagaron, J., Hernandez-Muñoz, P., & Ocio, M. (2008). Characterization of antimicrobial properties on the growth of S. aureus of novel renewable blends of gliadins and chitosan of interest in food packaging and coating applications. *International Journal of Food Microbiology, 124*(1), 13–20.
Garrido Assis, O. B., & de Britto, D. (2011). Evaluation of the antifungal properties of chitosan coating on cut apples using a non-invasive image analysis technique. *Polymer International, 60*(6), 932–936.
Higueras, L., López-Carballo, G., Hernández-Muñoz, P., Catalá, R., & Gavara, R. (2014). Antimicrobial packaging of chicken fillets based on the release of carvacrol from chitosan/cyclodextrin films. *International Journal of Food Microbiology, 188*, 53–59.
Kashiri, M., Cerisuelo, J. P., Domínguez, I., López-Carballo, G., Muriel-Gallet, V., Gavara, R., et al. (2017). Zein films and coatings as carriers and release systems of Zataria multiflora Boiss. essential oil for antimicrobial food packaging. *Food Hydrocolloids, 70*, 260–268.
Kim, J. Y., Kim, M.-J., Yi, B., Oh, S., & Lee, J. (2015). Effects of relative humidity on the antioxidant properties of α-tocopherol in stripped corn oil. *Food Chemistry, 167*, 191–196.
Kouchak, M., Ameri, A., Naseri, B., & Boldaji, S. K. (2014). Chitosan and polyvinyl alcohol composite films containing nitrofurazone: Preparation and evaluation. *Iranian Journal of Basic Medical Sciences, 17*(1), 14.
Koutsoumanis, K., Stamatiou, A., Skandamis, P., & Nychas, G.-J. (2006). Development of a microbial model for the combined effect of temperature and pH on spoilage of ground meat, and validation of the model under dynamic temperature conditions. *Applied and Environmental Microbiology, 72*(1), 124–134.
Kristo, E., Koutsoumanis, K. P., & Biliaderis, C. G. (2008). Thermal, mechanical and water vapor barrier properties of sodium caseinate films containing antimicrobials and their inhibitory action on Listeria monocytogenes. *Food Hydrocolloids, 22*(3), 373–386.
Kuorwel, K. K., Cran, M. J., Sonneveld, K., Miltz, J., & Bigger, S. W. (2013). Migration of antimicrobial agents from starch-based films into a food simulant. *LWT-Food Science and Technology, 50*(2), 432–438.
Kurek, M., Guinault, A., Voilley, A., Galić, K., & Debeaufort, F. (2014). Effect of relative humidity on carvacrol release and permeation properties of chitosan based films and coatings. *Food Chemistry, 144*, 9–17.
Ma, X.-H., Yang, Z., Yao, Z.-K., Xu, Z.-L., & Tang, C. Y. (2017). A facile preparation of novel positively charged MOF/chitosan nanofiltration membranes. *Journal of Membrane Science, 525*, 269–276.

Marchese, A., Orhan, I. E., Daglia, M., Barbieri, R., Di Lorenzo, A., Nabavi, S. F., et al. (2016). Antibacterial and antifungal activities of thymol: A brief review of the literature. *Food Chemistry, 210*, 402–414.

Martínez-Camacho, A., Cortez-Rocha, M., Ezquerra-Brauer, J., Graciano-Verdugo, A., Rodriguez-Félix, F., Castillo-Ortega, M., et al. (2010). Chitosan composite films: Thermal, structural, mechanical and antifungal properties. *Carbohydrate Polymers, 82*(2), 305–315.

Mascheroni, E., Guillard, V., Gastaldi, E., Gontard, N., & Chalier, P. (2011). Anti-microbial effectiveness of relative humidity-controlled carvacrol release from wheat gluten/montmorillonite coated papers. *Food Control, 22*(10), 1582–1591.

Mastromatteo, M., Mastromatteo, M., Conte, A., & Del Nobile, M. A. (2010). Advances in controlled release devices for food packaging applications. *Trends in Food Science & Technology, 21*(12), 591–598.

Motulsky, H., & Christopoulos, A. (2004). *Fitting models to biological data using linear and nonlinear regression: A practical guide to curve fitting.* Oxford University Press.

Peelman, N., Ragaert, P., De Meulenaer, B., Adons, D., Peeters, R., Cardon, L., et al. (2013). Application of bioplastics for food packaging. *Trends in Food Science & Technology, 32*(2), 128–141.

Ramos, M., Beltrán, A., Peltzer, M., Valente, A. J., & del Carmen Garrigós, M. (2014). Release and antioxidant activity of carvacrol and thymol from polypropylene active packaging films. *LWT-Food Science and Technology, 58*(2), 470–477.

Rhim, J. W., Mohanty, A. K., Singh, S. P., & Ng, P. K. (2006). Effect of the processing methods on the performance of polylactide films: Thermocompression versus solvent casting. *Journal of Applied Polymer Science, 101*(6), 3736–3742.

Roodhuyzen, D., Luning, P., Fogliano, V., & Steenbekkers, L. (2017). Putting together the puzzle of consumer food waste: Towards an integral perspective. *Trends in Food Science & Technology, 68*, 37–50.

Rubilar, J. F., Cruz, R. M., Zuñiga, R. N., Khmelinskii, I., & Vieira, M. C. (2017). Mathematical modeling of gallic acid release from chitosan films with grape seed extract and carvacrol. *International Journal of Biological Macromolecules*.

Sánchez-González, L., Cháfer, M., González-Martínez, C., Chiralt, A., & Desobry, S. (2011). Study of the release of limonene present in chitosan films enriched with bergamot oil in food simulants. *Journal of Food Engineering, 105*(1), 138–143.

Sebti, I., Martial-Gros, A., Carnet-Pantiez, A., Grelier, S., & Coma, V. (2005). Chitosan polymer as bioactive coating and film against Aspergillus niger contamination. *Journal of Food Science, 70*(2).

Singh, A., Singh, R., Bhunia, A., & Singh, N. (2003). Efficacy of plant essential oils as antimicrobial agents against Listeria monocytogenes in hotdogs. *LWT-Food Science and Technology, 36*(8), 787–794.

Sun, X. D., & Holley, R. A. (2012). Antimicrobial and antioxidative strategies to reduce pathogens and extend the shelf life of fresh red meats. *Comprehensive Reviews in Food Science and Food Safety, 11*(4), 340–354.

Tunç, S., & Duman, O. (2011). Preparation of active antimicrobial methyl cellulose/carvacrol/montmorillonite nanocomposite films and investigation of carvacrol release. *LWT-Food Science and Technology, 44*(2), 465–472.

Vergnaud, J.-M., & Rosca, I.-D. (2006). *Assessing food safety of polymer packaging.* iSmithers Rapra Publishing.

Zhang, C., Qi, M., Shao, Q., Zhou, S., & Fu, R. (2007). Analysis of the volatile compounds in Ligusticum chuanxiong Hort. using HS-SPME–GC-MS. *Journal of Pharmaceutical and Biomedical Analysis, 44*(2), 464–470.

Zinoviadou, K. G., Koutsoumanis, K. P., & Biliaderis, C. G. (2009). Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. *Meat Science, 82*(3), 338–345.

Zwietering, M., Jongenburger, I., Rombouts, F., & Van't Riet, K. (1990). Modeling of the bacterial growth curve. *Applied and Environmental Microbiology, 56*(6), 1875–1881.