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# Mathematical modeling of gallic acid release from chitosan films with grape seed extract and carvacrol

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**ABSTRACT**

Controlled release of antimicrobial and antioxidant compounds from packaging films is of utmost importance for extending the shelf-life of perishable foods. This study focused on the mathematical modeling of gallic acid release into an aqueous medium from three chitosan films, formulated with grape seed extract (GSE) and carvacrol. We quantified the release by HPLC technique during 30 days at three temperatures (5, 25 and 45 °C). The diffusion coefficients, varying with temperature according to an Arrhenius-type relationship, and the respective activation energies for Film-1 and Film-2 were, respectively  $D_{eff125^{\circ}C} = 3.7 \times 10^{-14} \text{ m}^2\text{s}^{-1}$  and  $D_{eff225^{\circ}C} = 6.1 \times 10^{-14} \text{ m}^2\text{s}^{-1}$ ,  $Ea_1 = 58 \text{ kJmol}^{-1}$  and  $Ea_2 = 60 \text{ kJmol}^{-1}$  as obtained from the Fickian fit. The low concentrations of gallic acid released by Film-3 could not be detected by HPLC, therefore the respective diffusion coefficient was not estimated. This study will help with the development and optimization of active packaging (AP) films aiming at improved food preservation and shelf-life extension.

**Keywords:** chitosan film, carvacrol, grape seed extract, gallic acid release, modeling.

## 1. INTRODUCTION

The increase in consumer preferences for safer, higher quality, minimally processed and improved shelf-life foods, has been the main reason for innovation in food packaging. Many researchers dedicated their studies to a new packaging model, where active interactions between the package and the product improve the shelf-life of food products [1-3].

Active packaging (AP) is a novel food-packaging system that is developed to answer the consumer preferences. The conditions of packed food are modified to increase the preservation of physico-chemical properties, improve its sensory quality and safety, and thus, extend the shelf-life [4]. We may classify the AP systems according to the desired interaction between the product and the package. For example, scavenging systems can remove oxygen, ethylene, and other undesirable compounds. On the other hand, releasing systems emit additives into the packed food such as aromas, antioxidants (AOX) and/or antimicrobials (AM) [1, 5, 6]. Incorporation of different AM agents such as organic and inorganic acids, metal particles, alcohols, ammonium compounds, and amines into plastic packaging materials [3, 7] led to public health and environmental issues. These issues were created by chemicals and plastics used, warranting new studies incorporating natural compounds, such as AM agents, enzymes, bacteriocins, phenolic compounds and essential oils into biodegradable or edible packaging materials [3, 8, 9]. The possibility of incorporating phenolic compounds and essential oils into AP contributed to a wide interest in such applications, since these components have AM and/or AOX activity, therefore their release into food matrices may have a significant impact on the shelf-life extension; on the other hand, consumption of these additives may improve public health [9, 10].

Various intrinsic factors may affect the release of the AM and/or AOX agents from the packaging material, such as the polymer film production technology, volatility and polarity of the additives, chemical interactions between the polymer chains and the additives, structural changes in the

packaging film generated by the additives, hydrophilicity and hydrophobicity of the polymer, water activity ( $a_w$ ), pH and food composition. Extrinsic factors, such as storage temperature and relative humidity may also affect the release of the additives [3]. Note that quantification of AM and/or AOX agents in food matrices is time consuming, since these are complex mixtures of diverse substances, including water, lipids, proteins, carbohydrates, fibers, vitamins and minerals [11]. This obstacle can be circumvented using food simulants and food-packaging regulations for the additive migration testing. In Europe, packaging regulations for migration testing define different food simulants, according to the desired testing conditions. The simulants suggested for additive release quantification are distilled water for water-based products, 3% (v/v) aqueous acetic acid for acidic products, 50% (v/v) ethanol for dairy products, and 95% aqueous ethanol for fatty products, olive oil, and sunflower oil [12, 13, 14].

Chitosan, a natural polymer obtained by deacetylation of chitin, has been widely used to produce biodegradable films [8, 15, 16] with several recent reviews on chitosan-based films available [17-19]. The AM and/or AOX properties of such films may be improved by adding different natural compounds. Different studies reported the use of plant essential oils as natural AM agents [20, 21]. Among these, carvacrol is a phenolic compound with demonstrated AM activity against bacteria, fungi and yeast [1, 16]. It is the main component (50-86%) found in essential oils of spices such as oregano (*Origanum* sp.) and thyme (*Thymus* sp.) [22]. Grape seed extract (GSE) is another natural additive obtained as a by-product of grape juice and wine industry, which may be used to improve AM and/or AOX properties of biodegradable films. GSE has gallic acid in its composition, a phenolic compound with antibacterial, anti-inflammatory and AOX effects [23, 24]. Thus, knowing the rates of gallic acid release in packaging films with GSE might be essential for the development and optimization of such packages, with improved food preservation and shelf-life extension. Moreover, European legislation regulates the usage of such contact materials

or packaging systems in the EFSA (European Food Safety Authority) regulation on active and intelligent packaging (Commission Regulation –EC- N° 459/2009).

Therefore, the objective of this study was to measure and model the release of gallic acid from chitosan films containing GSE and carvacrol into simulated water-based food products at different temperatures.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of chitosan films by casting method

Chitosan films were prepared according to a previous study by Rubilar et al. [8]. We prepared 2% (w/v) chitosan solutions by dissolving chitosan (high molecular weight, deacetylation degree >75%, Cat: 419419-250G, Sigma Aldrich, Portugal) in a 1% (v/v) glacial acetic acid solution at (JMGS, Portugal) and homogenized at 9500 rpm for 20 min (Ika-Werke, Ultra-Turrax model T25, Germany). After 12 h at room temperature, the gel was filtered through sterile non-woven cheesecloth. Then, 0.5 mLg<sup>-1</sup> glycerol (JMGS, Portugal) was added into the gel and the mixture was stirred at 40 °C for 30 min (Table 1). Tween-80 (JMGS, Portugal) at 0.2% (v/v) level of the AM agents (carvacrol - 98% pure; Cat: 282197, Sigma Aldrich, Portugal and GSE exGrape®seed OPC 40 powder, polyphenols>95% and proanthocyanidins>70%, Groupe Grap'Sud, France) was added and mixed for 1 h and cooled down to room temperature. Finally, after the chitosan solution was homogenized with glycerol and Tween-80, the AM agents were added and mixed using an Ultra-Turrax (Ika-Werke, model T25, Germany) at 9500 rpm for 5 min, according to the desired final concentration (Table 1) in each film. Based on the previous studies [8, 16], three optimal concentrations were prepared using a simple centroid mixture design between carvacrol, GSE and chitosan, as shown in Table 1. After cooling to room temperature, the solutions were degassed at

68 kPa (Edwards, BS 2208, UK) for 5 min and then 200 mL (for each film) were cast onto 32 cm round glass plates, and dried at 25 °C for 48 h. Each film was then stored in desiccators at 25 °C and 57% relative humidity, using a NaBr (02119, Sigma Aldrich, Portugal) saturated solution until testing.

## 2.2 Gallic acid release

1 cm<sup>2</sup> of each film was immersed in 1 mL of a food simulant for water-based products (bi-distilled water, previously adjusted to pH 7 with NaOH 0.05 N) into an Eppendorf tube [25]. Each tube was hermetically sealed with Parafilm<sup>®</sup> and the samples were stored in climatic chambers at 5, 25 and 45 °C during 30 days without agitation. Chitosan films without any additives were used as control. All samples were prepared in triplicate.

## 2.3 Gallic acid determination by HPLC

As already mentioned, gallic acid is a simple water-soluble molecule, therefore its concentration was selected as an indicator for the release of AM and AOX additives. The concentration of gallic acid was determined in the food simulant by high-performance liquid chromatography (HPLC) based on a previous study [26]. The samples for HPLC (1 mL of food simulant) were taken after 0, 2, 4, 6, 15 and 30 days and filtered using a 0.45 µm nylon membrane filter (Merck, Germany). The samples (20 µL) were injected into the HPLC system (Jasco, LG-1580-04 with PU-2080 HPLC pump) equipped with a photodiode array detector (JASCO, MD-2015 Plus Multiwavelength Detector). The HPLC column was a Supelco-Ascentis<sup>®</sup> C18 (4.6 × 250 mm<sup>2</sup>, particle size 5 µm) with Supelco-Ascentis<sup>®</sup> C18 Supelguard<sup>™</sup> pre-column (4.0×20 mm<sup>2</sup>, particle size 5 µm) purchased at Sigma-Aldrich (Lisbon, Portugal). An isocratic mobile phase was used as eluent, 1% aqueous acetic acid at 1 mLmin<sup>-1</sup> flow rate. All solvents were of HPLC grade



(Merck, Portugal). The detection wavelength was set to 280 nm (maximum absorbance detected). A calibration curve with standard solutions of gallic acid (99% purity, 27645, Sigma-Aldrich, Portugal) was recorded and there was a linear relationship ( $R^2=0.9998$ ) between the concentration of gallic acid and the area of the corresponding peak. The analyses were run in triplicate.

## 2.4 Evaluation of the diffusion coefficients for gallic acid release

Mathematical models are useful to describe physical mechanisms of the release of an active compound from a polymeric matrix into a food simulant [27]. This may be accomplished by using the experimental data and fitting an appropriate model equation, extracting the respective model parameters. Presently we used three models to fit the release kinetics of gallic acid from chitosan films, including the simplified model (SM), empirical model (EM) and Fickian model (FM).

### *Fickian and Simplified models*

These models may be applied in the following conditions: 1) isothermal release of active compound from a thin polymer slab of thickness  $x$  is unidimensional, 2) the distribution of the additive in the food simulant remains uniform at concentration  $C_1$  and 3) the initial distribution of the additive in the packaging material is homogeneous at concentration  $C_0$ . These initial conditions at  $t=0$  are described as the *perfect sink conditions*. Using a constant diffusion coefficient  $D$  of the additive, with diffusion occurring normal to the slab surface along the  $x$  direction, the Fick's second law, together with the suitable initial and boundary conditions, in normalized coordinates, may be presented as:

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} \quad (1)$$

where,

$$t = 0 \quad -1/2 < x < 1/2 \quad C = C_0$$

$$t > 0 \quad x = \pm 1/2 \quad C = C_1$$

The solution of Eq. (1) under the above-specified conditions, presented in the form of a trigonometric series, is known as the Fickian model (FM) [28]:

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp \left[ \frac{-D_{eff}(2n+1)^2 \pi^2}{l^2} t \right] \quad (2)$$

where  $M_t$  is the mass of the additive released at time  $t$ ,  $M_\infty$  is the mass released at infinite time,  $l$  the half-thickness of the slab,  $t$  the diffusion time and  $D_{eff}$  the diffusion coefficient. A simplified version of Eq. (1), the Simplified model (SM), valid at short times, may be presented in the form:

$$\frac{M_t}{M_\infty} = \left( \frac{4}{l} \right) \cdot \left( \frac{D_{eff} t}{\pi} \right)^{0.5} \quad (3)$$

The Fickian diffusional release from a thin film, as indicated by Eq. (3), is initially proportional to the square root of time, where  $D_{eff}$  is the effective diffusion coefficient. The short-time approximation is valid for the first 60% of the total released additive  $M_t/M_\infty \leq 0.60$  [28].

### *Empirical model (EM)*

This model uses a semi-empirical equation to describe the release of an additive from a polymeric film. The Eq. (3) indicates that for Fickian diffusion from a thin film initially the amount released is proportional to the square root of time. Another limiting case is the constant release rate independent of time, i.e., the zero-order kinetics, described by the following equation:

$$\frac{M_t}{M_\infty} = k \cdot t \quad (4)$$

Quite frequently, the release may be better described by an expression intermediate between these two limiting cases:

$$\frac{M_t}{M_\infty} = k \cdot t^n \quad (5)$$

where  $k$  is a constant including the properties of the compound and the macromolecular network system, and  $n$  is the *diffusional exponent*, depending on the transport mechanism. Eq. (5) is also used for the first 60% of the fractional release. The exponent  $n=0.50$  corresponds to the Fickian diffusion mechanism and  $n>0.50$  to the non-Fickian mechanism [28]. We used an  $n$  value of 0.5 for modeling, corresponding to the same time dependence as that of the Simplified model, although with different parametrization.

We assumed an Arrhenius-type relationship to evaluate the temperature dependence of the diffusion (Eq. 8):

$$D_{eff} = D_0 \exp \left[ -\frac{E_a}{RT} \right] \quad (8)$$

where  $D_{eff}$  is the diffusion coefficient,  $D_0$  the rate factor for diffusivity,  $E_a$  the activation energy,  $R$  the universal gas constant, and  $T$  the absolute temperature of the experiments (278.15, 298.15, and 318.15 K). We obtained  $E_a$  from the slope of the plot of the natural logarithm of  $D$  vs reciprocal temperature ( $1/T$ ).

We estimated the kinetic parameters of the three models directly from the experimental data, by performing a non-linear regression analysis, using the root mean square deviation (RMS) of the observed ( $V_{obs}$ ) and predicted ( $V_{pre}$ ) values (Eq 9) as the objective function, and the Solver tool in Excel version 15.0. We assessed the quality of the regression by the coefficient of determination ( $R^2$ ) and normality and randomness of residuals.

$$RMS (\%) = \sqrt{\frac{1}{n} \sum_i^n \left( \frac{V_{obs} - V_{pre}}{V_{obs}} \right)^2} \times 100\% \quad (9)$$

## 2.5 Statistical analysis

Analysis of variance (ANOVA) was used to analyze statistical differences between the samples. Statgraphics Centurion XV software was used (Manugistics Ins., Statistical Graphics Corporation, Rockville, USA) and the results presented as mean  $\pm$  standard deviation. The differences between the mean values of the measured properties were compared using multiple-range Tukey's test. The significance level was set at 0.05.

### 3. RESULTS AND DISCUSSION

The design of new packaging systems and bioactive compound carriers together with accurate mathematical modeling of the transport phenomena may allow predicting their behavior during shelf-life and product preparation or consumption [27]. Presently we studied the release kinetics of gallic acid from different chitosan films with two different AM agents (carvacrol and GSE). We fitted the experimental data using three different numerical models, in order to evaluate the model capacity to predict the gallic acid release.

The initial concentration of gallic acid in each film was about 1% of the GSE concentration. Thus, the starting solutions used for casting the three films contained, respectively, 6.48, 3.79 and 1.52 mgL<sup>-1</sup> of gallic acid while the control film had no gallic acid, as expected (Table 1).

The release data (Figs. 1 and 2) showed a significant effect of temperature on the gallic acid release from the chitosan film, with the release accelerated at higher temperatures. In particular, the time required to release 25% of the initial amount from Film-2 decreased from more than 35 days at 5 °C to 30 days at 25 °C and only 72 hours at 45 °C. We were unable to determine the kinetic parameters for Film-3 since this film had low initial concentration of GSE, thus no release of gallic acid could be detected at 25 or 5 °C. Note that the limit of quantification for the gallic acid by HPLC was 0.098 ppm. Generally, shape and size of the additive molecule and the fraction of voids and gaps in the polymer structure result in different migration rates [29]. Previous studies

found that the absorption of a certain amount of water into the polymer may lead to an increase in the gap size and thus accelerate the release [30]. Recently, Schreiber [31] reported values of gallic acid removed from grafted and mixed chitosan-gallic acid films of 0.5 and 0.44 mg of gallic acid per film, respectively. His results are similar to those reported here, with slower gallic acid diffusion, thus, only around 13% of the gallic acid leached out in his experiments, with higher percentage of gallic acid retained by chitosan film.

Understanding the different release mechanism and modeling the experimental data may be crucial for product development and its potential applications [27]. The polymeric network structure, film thickness, initial concentration of the active compound and its chemistry should all affect the mass transport phenomena, which in turn control the release of the active compound. To be effective as AP, the active compound should be released from the polymeric film at a certain rate and during a period required to extend the shelf life of the product. In order to accomplish this, mass transfer parameters were determined from the different models tested in this work. Table 2 shows the effective diffusion coefficient ( $D_{eff}$ ), the root mean square deviation (RMS) and the coefficient of determination ( $R^2$ ) parameters for the gallic acid release from Film-1 and Film-2, determined for the three models (EM, SM and FM) at three different temperatures. The experimental data for the gallic acid release were successfully fitted (Fig. 1 and 2) and the models used showed good coefficients of determination and very similar diffusion coefficients between them, excluding the empirical model (EM), because the  $k$  values are not comparable to the  $D_{eff}$  values. The EM is probably an oversimplification of the system under study, giving only qualitative indication of the rate of mass transfer phenomena in the form of the rate constant  $k$ , the parameter nonexistent in the other models. SM and FM gave very similar results, indicating that the Fickian model may be simplified to the square-root time dependence at sufficiently short times. Similar  $D_{eff}$  ( $m^2s^{-1}$ ) values were obtained for FM ( $1.84 \times 10^{-14}$ ,  $3.66 \times 10^{-14}$ ,  $1.93 \times 10^{-13}$ ) and

SM ( $1.80 \times 10^{-14}$ ,  $3.60 \times 10^{-14}$ ,  $1.92 \times 10^{-13}$ ) at different temperatures (5, 25, 45 °C) for Film-1; as already noted, simplification of the Fickian model can successfully model these data. The same behavior was also observed in Film-2. As shown in Table 2, although Film-2 has a lower concentration of GSE ( $400 \text{ mgL}^{-1}$ ) than Film-1 ( $684 \text{ mgL}^{-1}$ ), its effective diffusion coefficients were higher than those of Film-1 at 25 and 45 °C. This could result from the lower concentration of carvacrol ( $9.6 \text{ mgL}^{-1}$ ) in Film-1 as compared to Film-2 ( $90 \text{ mgL}^{-1}$ ). Here, hydrophobic carvacrol would tend to stay away from the hydrophilic food simulant (water), remaining mixed with the polymer, while the hydrophilic gallic acid would preferentially migrate into the aqueous simulant. This could be caused by the presence of the hydrophobic carvacrol, creating additional tortuosity in the films, slowing the gallic acid release as compared to polymers without a hydrophobic additive. Indeed, Redl et al. [32] reported that the diffusion coefficient was higher for sorbic acid in a gluten-based film ( $7.60 \times 10^{-12} \text{ m}^2\text{s}^{-1}$ ) than in beeswax (a pure lipid film,  $2.70 \times 10^{-16} \text{ m}^2\text{s}^{-1}$ ). Moreover, 20-25% reduction in the sorbic acid diffusion coefficient was obtained when lipid components were added into the gluten-based film, which was still very far from the pure lipid film diffusion coefficient. These authors suggested that a bilayer was created with the addition of lipid components into the gluten-based film. This bilayer was composed by a hydrophobic surface layer working as an efficient permeability barrier on one side of the film and a hydrophilic layer allowing sorbic acid to diffuse freely on the other side. Therefore, on one side of the film sorbic acid could diffuse through the hydrophilic wheat gluten layer.

Moreover, Lopez de Dicastillo et al. [33], also reported that gallic acid was the main antioxidant component released into aqueous food simulants from packaging films based on ethylene vinyl alcohol copolymer (EVOH) with added green tea extract. Gallic acid showed a faster diffusivity in the polymer matrix as a consequence of its smaller molecular size and its good solubility in

water [34]. Low diffusion coefficients ( $10^{-15}$ - $10^{-16}$   $\text{m}^2\text{s}^{-1}$ ) were obtained in this study for gallic acid release into 3% aqueous acetic acid.

Choi et al. [35], studied the diffusivity of potassium sorbate (200 ppm) incorporated into  $\kappa$ -carrageenan based-films (2% w/v) with a thickness of  $78.0 \pm 3.8$   $\mu\text{m}$ . The diffusion coefficients calculated were of the order of  $10^{-13}$   $\text{m}^2\text{s}^{-1}$ . Much higher values of the diffusion coefficients were also observed in a study reported by Desai and Park [36] in which paracetamol diffusion coefficients in chitosan hydrogels were respectively,  $4.45 \pm 0.34 \times 10^{-8}$   $\text{m}^2 \text{s}^{-1}$  (1% w/w chitosan) and  $1.87 \pm 0.27 \times 10^{-8}$   $\text{m}^2 \text{s}^{-1}$  (2% w/w chitosan), and also by Del Nobile et al. [37], who reported much higher diffusion coefficient values ( $10^{-6}$   $\text{m}^2 \text{s}^{-1}$ ) for thymol release from zein films.

Fig. 3 shows the dependence of  $D_{eff}$  on temperature assuming an Arrhenius behavior. The behavior of the  $\ln(D_{eff})$  versus  $1/T$  gives a linear plot for Film-1 ( $R^2 = 0.926$ ) and Film-2 ( $R^2 = 0.945$ ). The activation energy ( $E_a$ ) may be considered as the energy required for the migrant to move among the chains forming the polymer matrix. When enough energy is given and if an adjacent space is large enough to accommodate the migrant it is assumed that the migrant may jump into that space. A net diffusion flux may be created if another migrant molecule jumps into the space that was previously occupied by the first molecule [27]. The polymer matrix, the migrant and the medium in contact with the polymer are affected when the available energy increases with temperature. Table 3 shows that the  $E_a$  for gallic acid in Film-2 (93.78  $\text{kJmol}^{-1}$ ) is comparable with the values of 110.4, 98.9, 96.2, 164.7 and 176.0  $\text{kJmol}^{-1}$  reported for catechin, epicatechin,  $\alpha$ -tocopherol, BHT (butylated hydroxytoluene) and resveratrol, respectively, incorporated in polylactic acid films [25, 38-40]. Moreover, Ouattara et al. [41], reported in a similar study the diffusion of propionic and acetic acids from chitosan-based antimicrobial packaging films. The films were immersed into water at three temperatures (4, 10 and 24  $^{\circ}\text{C}$ ) and the  $E_a$  reported, 27.19  $\text{Jmol}^{-1}$  (acetic acid) and 24.27  $\text{Jmol}^{-1}$  (propionic acid) were much lower than the values obtained

in this study, due the lower molecular weight of the acetic ( $60.05 \text{ gmol}^{-1}$ ) and propionic acids ( $74.08 \text{ gmol}^{-1}$ ) compared to that of gallic acid ( $170.12 \text{ gmol}^{-1}$ ).

#### **4. CONCLUSIONS**

We used several mathematical models to interpret controlled release of gallic acid from the studied chitosan films. This release depended on temperature, with the dependence described by an Arrhenius-type equation, becoming faster at higher temperatures. The Fickian model produced essentially the same results as the simplified model, which assumes a constant effective diffusion coefficient. Our attempt to use a more complex mathematical model (Fickian and polymer relaxation model) produced no conclusive results, due to insufficient precision of the experimental data.

This work contributes to understanding of the release mechanism of gallic acid from chitosan films with GSE and carvacrol into food products with high moisture content ( $>90\%$ ), and thus, to the development of the environmentally-friendly packaging films, in view of improving food preservation and shelf-life extension. However, further studies on the controlled release of other phenolic compounds from chitosan films with GSE and carvacrol are required in order to understand better the release mechanisms of those compounds, extending the studies of their antimicrobial and antioxidant properties.

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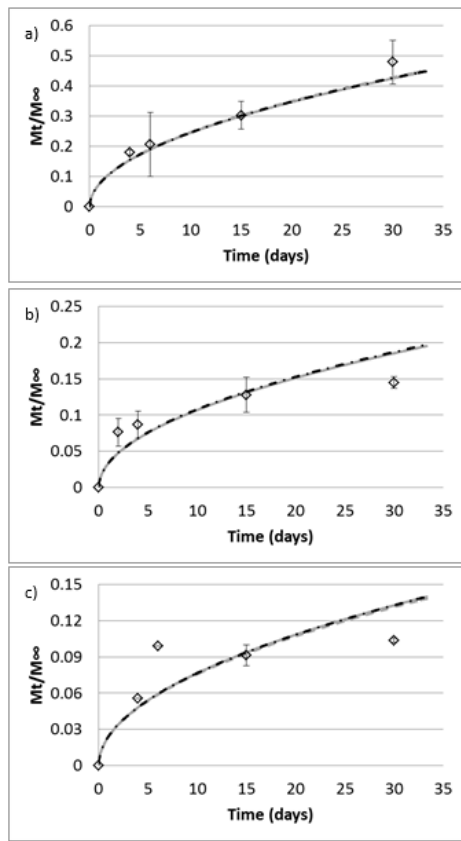


Figure 1. Experimental data ( $\diamond$ ) for gallic acid release from Film-1 and predicted data by the Fickian (FM, dashed with dot black line), simplified (SM, continuous grey line) and empirical (EM, dashed grey line) models at three different temperatures: (a) 45 °C, (b) 25 °C and (c) 5 °C.

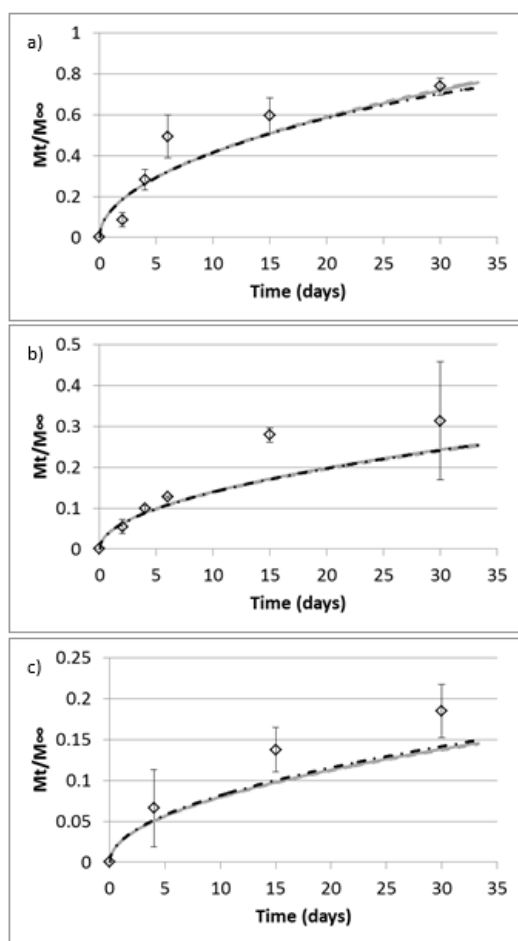


Figure 2. Experimental data ( $\diamond$ ) for gallic acid release from Film-2 and predicted data by the Fickian (FM, dashed with dot black line), simplified (SM, continuous grey line) and empirical (EM, dashed grey line) models at three different temperatures: (a) 45 °C, (b) 25 °C and (c) 5 °C.

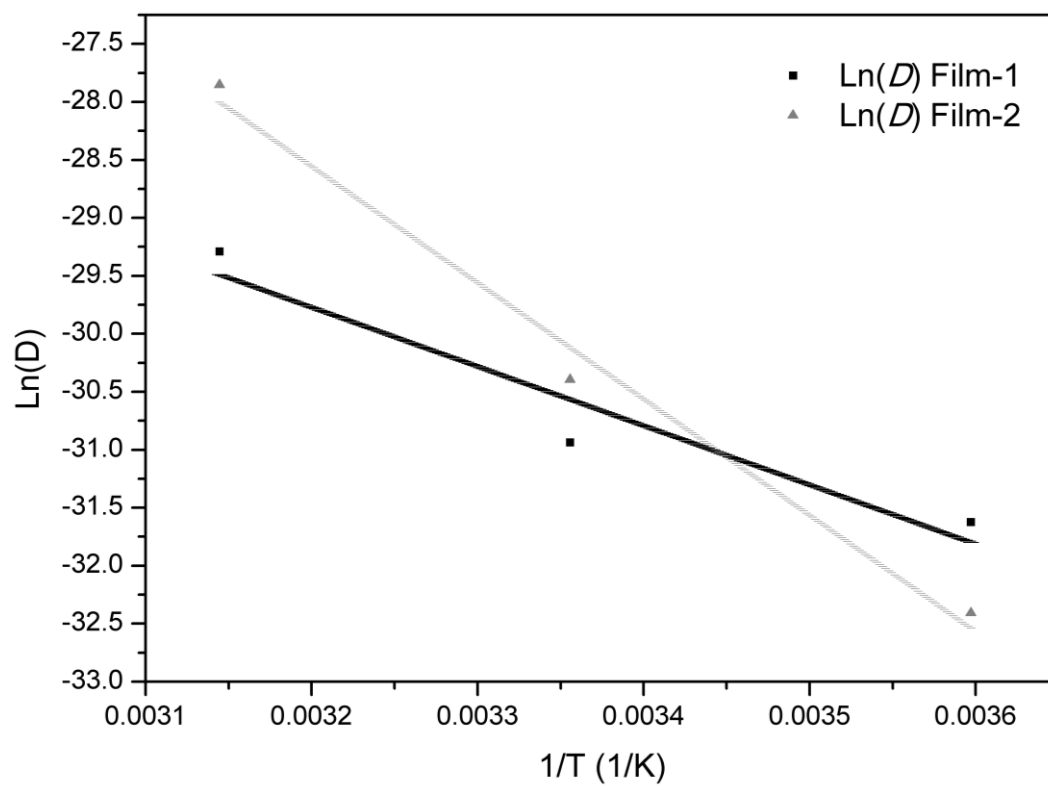


Figure 3. Arrhenius-type relationships for the diffusion of gallic acid from Film-1 and Film-2.



**Table 1.** Composition and thickness of each film; chitosan is the main component; carvacrol and GSE are natural antimicrobial agents.

<b>Run*</b>	<b>Carvacrol (mgL<sup>-1</sup>)</b>	<b>GSE (gallic acid) (mgL<sup>-1</sup>)</b>	<b>Chitosan (% w/v)</b>	<b>Thickness (mm)</b>
<b>Film-1</b>	9.6	684 (6.5)	1.25	0.062±0.013 <sup>b</sup>
<b>Film-2</b>	60.0	400 (3.8)	1.20	0.042±0.016 <sup>a</sup>
<b>Film-3</b>	90.0	160 (1.5)	1.24	0.042±0.017 <sup>a</sup>
<b>Control</b>	0.0	0.0 (0.0)	1.25	0.047±0.016 <sup>a</sup>

All solutions incorporated Tween-80 as emulsifier (0.2% v/v of AM agents) and glycerol as plasticizer (0.5 mLg<sup>-1</sup> chitosan). <sup>a-b</sup> Different superscripts within the same column indicate significant differences in the film thickness between the samples ( $p < 0.05$ ).

**Table 2.** Empirical model constant ( $k$ ), effective diffusion coefficient ( $D_{eff}$ ), the root mean square deviation (RMS) and coefficient of determination ( $R^2$ ) parameters of gallic acid release from Film-1 and Film-2.

	Empirical model (EM)	RMS (%)	$R^2$	Simplified model (SM)	RMS (%)	$R^2$	Fickian Model (FM)	RMS (%)	$R^2$
	$k$ ( $s^{-1}$ )			$D_{eff}$ ( $m^2s^{-1}$ )			$D_{eff}$ ( $m^2s^{-1}$ )		
<b>Film-1</b> (5°C)	$4.9 \times 10^{-3}$	23	0.75	$1.8 \times 10^{-14}$	23	0.81	$1.8 \times 10^{-14}$	23	0.75
<b>Film-1</b> (25°C)	$6.9 \times 10^{-3}$	25	0.83	$3.6 \times 10^{-14}$	25	0.80	$3.7 \times 10^{-14}$	25	0.83
<b>Film-1</b> (45°C)	$1.6 \times 10^{-2}$	19	0.91	$1.9 \times 10^{-13}$	19	0.91	$1.9 \times 10^{-13}$	19	0.91
	$k$ ( $s^{-1}$ )			$D_{eff}$ ( $m^2s^{-1}$ )			$D_{eff}$ ( $m^2s^{-1}$ )		
<b>Film-2</b> (5°C)	$5.1 \times 10^{-3}$	30	0.62	$2.0 \times 10^{-14}$	30	0.93	$2.1 \times 10^{-14}$	30	0.93
<b>Film-2</b> (25°C)	$8.9 \times 10^{-3}$	27	0.85	$6.0 \times 10^{-14}$	27	0.85	$6.1 \times 10^{-14}$	26	0.86
<b>Film-2</b> (45°C)	$2.7 \times 10^{-2}$	24	0.88	$5.4 \times 10^{-13}$	24	0.88	$5.4 \times 10^{-13}$	24	0.89

**Table 3.** Activation energy ( $E_a$ ) and rate factor for diffusivity ( $D_o$ ) parameters for gallic acid release from Film-1 and Film-2.

<b>Film-1</b>	<b>Simplified model (SM)</b>	<b>Fickian Model (FM)</b>
$D_o$ ( $m^2s^{-1}$ )	$2 \times 10^{-6}$	$7 \times 10^{-4}$
$E_a$ ( $kJmol^{-1}$ )	43	58
$R^2$	0.927	0.987
<b>Film-2</b>	<b>Simplified model (SM)</b>	<b>Fickian Model (FM)</b>
$D_o$ ( $m^2s^{-1}$ )	$3 \times 10^{-3}$	$3 \times 10^{-3}$
$E_a$ ( $kJmol^{-1}$ )	60	60
$R^2$	0.945	0.948