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ESTIMATION OF DIFFUSION COEFFICIENTS OF ESSENTIAL OIL OF *Pimenta dioica* IN EDIBLE FILMS FORMULATED WITH *Aloe vera* AND GELATIN, USING LEVENBERG-MARQUARDT METHOD

ESTIMACIÓN DE COEFICIENTES DE DIFUSIÓN DE ACEITE ESENCIAL DE *Pimenta dioica* EN PELÍCULAS COMESTIBLES FORMULADAS CON *Aloe vera* Y GRENETINA, USANDO EL MÉTODO DE LEVENBERG-MARQUARDT

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Abstract

We performed the study of the diffusion of the essential oil of pimento (*Pimenta dioica* L. Merrill) from an edible film formulated with *Aloe vera*, gelatin and glycerol. The analysis was done for three concentrations of essential oil (0.5, 1.0 and 1.5% w/w) and at two temperatures (5 ° C and 25 ° C) for a period of 180 hours, taking samples at various times to measure the concentration of the essential oil whose eugenol is the main component. The governing equations were obtained from Fick´s second law. The predictions of the eugenol concentration were compared with the experimental values using a least squares scheme, where the diffusion coefficient and the mass transfer coefficient were estimated using Levenberg-Marquardt method. The effective diffusivity of eugenol is between 7.0529×10^{-12} to 5.3586×10^{-11} m^2/h at 5 °C, while at 25 °C, the effective diffusivity values are between 3.7980×10^{-11} to 5.2578×10^{-10} m^2/h. These values are in agreement with those reported for other similar systems and indicate that the diffusive transport of the essential oil through the edible film studied is a slow process, a required condition to increase the shelf life of foods where these films can be used as coatings in meat products and cheeses.

Keywords: parameter estimation, diffusion coefficient, essential oil, *Pimenta dioica*, orthogonal collocation, Levenberg-Marquardt method.

Resumen

Se realizó el estudio de la difusión del aceite esencial de pimienta de Jamaica (*Pimenta dioica* L. Merrill) desde una película comestible formulada con *Aloe vera*, grenetina y glicerol, a tres concentraciones (0.5, 1.0 y 1.5% p/p) y a dos temperaturas (5 °C y 25 °C), durante un periodo de 180 horas, tomando muestras a diversos tiempos, para medir la concentración del aceite esencial cuyo componente principal es el eugenol. Las ecuaciones gobernantes se obtuvieron a partir de la segunda ley de Fick. Las predicciones de la concentración de eugenol se compararon con los valores experimentales en un esquema de mínimos cuadrados, en donde el coeficiente de difusión y el coeficiente de transferencia de masa se estimaron usando el método de Levenberg-Marquardt. La difusividad efectiva de eugenol se encuentra entre 7.0529×10^{-12} a 5.3586×10^{-11} m^2/h para 5 °C, mientras que, a 25 °C, los valores de difusividad efectiva están entre 3.7980×10^{-11} a 5.2578×10^{-10} m^2/h. Estos valores están en concordancia con los reportados para otros sistemas similares e indican que el transporte difusivo del aceite esencial a través de la película comestible estudiada es un proceso lento, circunstancia deseable para incrementar la vida de anaquel de alimentos como es el caso de productos cárnicos y quesos.

Palabras clave: estimación de parámetros, coeficiente de difusión, aceite esencial, *Pimenta dioica*, colocación ortogonal, método de Levenberg-Marquardt.

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1 Introduction

1.1 Design of edible coatings

An edible coating or film is defined as a continuous thin layer of eatable materials that covers the surface of the food and can be eaten as part of the whole product (Galgano *et al*., 2015). Edible films have been proposed as a promissory alternative of food packaging to improve the quality and safety of food products. This technology protects foods from dehydration and acts as gases barrier with the surrounding media (Acevedo-Fani *et al*., 2015) or it could extend product shelf life and reduce risk of pathogen growth on food surfaces (Du *et al*., 2009). The edible films and coatings can be made from a variety of biopolymers. They can be formulated using polysaccharides, e.g., starch, chitosan, alginates, cellulose derivatives, among others; proteins e.g., gelatin, soy protein, wheat gluten, among others; and lipids e.g., waxes, triglycerides, fatty acids and their blends (Campos *et al*., 2011; Rodriguez-Marin *et al*. 2016). These materials can be adapted to any food product, and they usually contain a variety of functional (bioactive) substances such as phytochemicals, vitamins, essential oils, nanofibers antioxidants, antimicrobials, colorants, flavors, fortified nutrients and prebiotics (Torres *et al*., 1985; Acevedo-Fani *et al*., 2015; Erkan *et al*., 2015; Hernandez-Carrillo *et al*., 2015; Alkan and Yemenicioglu, 2016; Kumari *et al*., 2017). These kinds of coatings containing additives are known as “controlled release packaging systems", and their behavior is based on diffusive transport of its incorporated additive.

The knowledge of the diffusion of these compounds within the coating is the most important factor for designing a bioactive food packaging system. Frequently, additives avoid oxidation and the attack of insects, microorganisms and other pollutants that would render the food unacceptable (Brody, 2005). Controlled release packaging, which is a challenging area in active packaging of foods, is a fairly new concept and can be used to enhance the quality and safety of a wide range of foods and extend their shelf life (LaCoste *et al*., 2005) or increase the functionality of meat products (Dima *et al*., 2014). Today, there is an increasing demand for the use of biodegradable polymers from renewable sources for either food packaging or drug release systems (Aderibigbe *et al*., 2015; Rubilar *et al*., 2017). Therefore, an understanding of the transport mechanism in these materials is required.

Several studies have evaluated the diffusion of solutes in edible films and coatings. Fick’s second law models release behavior, and depending on test conditions, different analytical solutions can be applied to calculate the value of diffusion coefficient (D) at a specific temperature (Bastarrachea *et al*., 2011). Using numerical models, the average values of physical properties of heterogeneous foods may be replaced by time and space (Fabbri *et al*., 2014). These solutions, used to determine the concentration of solute as a function of time and position, are dependent of initial and boundary conditions of specific applications. The techniques for solution of these equations include analytical solutions such as transformation of variables, separation of variables or Laplace transformations (Welti-Chanes *et al*., 2005; Piringer, 2007; Virgen-Navarro *et al*., 2016). From a practical importance perspective, diffusivity values of preservative additives are essential in the prediction of shelf life of food commodities coated with edible films under changing storage conditions (Ozdemir and Floros, 2003; Mohan *et al*., 2017; Rubilar *et al*., 2017).

Edible films composition and its effects on diffusion with additives have been studied for some basic components; for water, research reports have been oriented to correlate water activity and diffusion coefficients of other coating additives (Torres *et al*., 1985: Malafronte *et al*., 2015). In the lipid based edible coatings, it has been found that the type and concentration of fatty acids used in the formulation affected the permeability of additives (Vojdani and Torres, 1990; Redl *et al*., 1996). For the diffusivity of peptides in foods or gel model systems, it has been found that the negative-charge gel (κ-carrageenan) or neutral charge (agarose) exhibits pH dependent diffusion (Mattisson *et al*., 2000; Hirota *et al*., 2000).

For essential oils as bioactive compounds, there has been an extensive experimental characterization (Burt, 2004; Du *et al*., 2009; Sotelo-Boyás *et al*. 2015) e.g., several plant essential oils have excellent antimicrobial and antioxidant properties. Some studies have shown that essential oils of oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), cinnamon (*Cinnamon casia*), lemongrass (*Cymbopogon citratus*), pimento (*Pimenta dioica*) and clove (*Eugenia caryphyllata*) are among the most active against strains of *Escherichia coli* (Smith-Palmer *et al*., 1998; Hammer *et al*., 1999; Dorman and Deans, 2000; Friedman *et al*., 2002; Burt, 2004; Padmakumari *et al*., 2011; Rao *et al*., 2012; Acevedo-

Fani *et al*., 2015). *Pimenta dioica* L. Merrill essential oil is extracted from leaves and berries and it is used in food industry, especially in meat industry, as an ingredient in cosmetic products, and it is used as therapeutic agent for diverse diseases (Rao *et al*., 2012; Dima *et al*., 2014).

Then, to design systems of controlled release of bioactive substrates (in this case essential oils), both in film and in microcapsules, it is necessary to know the mechanism of molecular or diffusional mass transport (Choi *et al*., 2005). However, a general method is required to estimate the diffusion coefficient of a bioactive compound of interest, within an edible film, for the purpose of designing controlled release systems. Therefore, the use of phytochemicals as additives in edible films has increased due to the wide range of biological activities of these metabolites offer (Du *et al*., 2009). Additionally, the study of the inhibitory effect of additives is limited due to lack of diffusion studies in order to develop polymeric systems enabling a controlled release of the bioactive compound and, at same time, to provide protection and ensure the bioavailability of the active ingredients. Most of the published reports on diffusion coefficient estimation are based on analytical solutions (Crank, 1975) of simplified models derived from Fick's second law incorporating boundary conditions of Dirichlet o Neumann types, so to solve more realistic systems, it is necessary to use numerical methods. (Pénicaud *et al*., 2010; Fabbri *et al*., 2014; Malafronte *et al*., 2015).

1.2 Parameter estimation

The experimental determination of diffusivity coefficients requires highly time-consuming measurements (da Silva *et al*., 2009). Furthermore, parameter determination via laboratory experiments could not be representative of real conditions (Fabbri *et al*., 2014). As an alternative, a growing interest in the estimation of physical properties by inverse methods is present in literature. Parameter estimation refers to the process of obtaining values of the unknown parameters from the matching of the model-based calculated values to the set of experimental measurements (Calugaru and Crolet, 2003; Englezos and Kalogerakis, 2001; Mohamed, 2010). Parameter estimation is applied in a wide variety of disciplines such as biology, biotechnology, chemistry, geophysics, electronic engineering, chemical engineering, etc. for predicting the system behavior or scale-up and thermodynamically design of equipment.

Parameter estimation applies when it has a mathematical model with unknown parameters and a set of experimental data that the model explains. Parameter estimation can be considered as the inverse of the simulation. In the simulation, the model and its environmental conditions are totally known and the future process behavior is predicted by the numerical solution of the model. (Calugaru and Crolet, 2003; Pujol, 2007). While, in the case of parameter estimation, the governing equations or the boundary conditions contain some undetermined parameters (mass transfer coefficient, effective diffusivity, specific rate constants, etc.), and experimental information is available. Therefore, they require a function minimization technique to estimate the values of undetermined parameters, such that the sum of squared errors (S) between the experimental value y_{exp} and the value predicted by the model y_{mod} is minimal.

$$S = \sum_{i=1}^{m}\sum_{j=1}^{n}\left[y_{exp_{ij}} - y_{mod_{ij}}\right]^2 \qquad (1)$$

where m and n are number of dependent variables and experimental data, respectively and $\partial S/(\partial x_i) = \nabla S(x) = 0$ are the minimization equations that yields a set of algebraic equations whose solution is the vector of parameters searched. There are several methods to solve the least squares function S among which are the methods of Newton-Raphson, Levenberg-Marquardt, Simplex, Gauss-Newton, among others (Englezos and Kalogerakis, 2001; Pujol, 2007; Mohamed, 2010; Liang *et al*., 2017). The second-order methods use the Hessian matrix, denoted by $H(x) = (\partial^2 S)/(\partial x_i \partial x_j)$ to provide information about the new search direction.

The most representative of these methods is the Newton method, which is described as $\mathbf{x}^{k+1} = \mathbf{x}^k - H(\mathbf{x})^{-1}\nabla S(\mathbf{x}^k)$. The Newton's method applied to minimizing problems presents difficult of convergence and depends drastically from the initial values of undetermined parameters due to the formation of an ill-conditioned Hessian matrix. Levenberg (1944) proposed a correction to the Hessian matrix of the form $H^*(\mathbf{x}) = H(\mathbf{x}) + \lambda\mathbf{I}$ where λ is a positive number such that $H^*(x)$ is always positive-definite matrix and hence invertible. Marquardt (1963) taken the report of Levenberg (1944), suggested that $H^*(\mathbf{x}) = H(\mathbf{x}) + \lambda diag[H(\mathbf{x})]$ using in each stage of the search to ensure the matrix H is always invertible.

$$\mathbf{x}^{k+1} = \mathbf{x}^k - [H(\mathbf{x}) + \lambda diag H(\mathbf{x})]^{-1}\nabla S(\mathbf{x}^k) \qquad (2)$$

Eq. (2) is known as the Levenberg-Marquardt (LM) method. If the damping scalar λ is large at first iteration, the LM method approaches to a gradient

search method. If the LM parameter λ approaches zero, LM method becomes a classical Newton method with quadratic convergence. The strategy is gradually decrease λ value, as the iterations progress. The way to change λ is even heuristic, although the reported approach is to decrease to a value of 10 if the quadratic sum of residues S decreases. Otherwise, λ value increases by 10 (Press *et al*., 1996; Pujol, 2007). Hence, LM is the most efficient method for most situations and the work of Marquardt (1963) has more than 15,500 citations (*Web of Science*, 2017). In this study, we implemented this approach for parameter estimation in 1-D partial differential equations with spatial discretization *via* orthogonal collocation and time integration using a trapezoidal-implicit method (Jiménez-Islas *et al*., 2014).

Thence, the aim of the present study was to estimate the diffusivity coefficient of essential oil of *Pimenta dioica* L. Merrill (bioactive agent with antioxidant and antimicrobial properties) through an edible film formulated with *Aloe vera*, gelatin powder and glycerol (Flores-Martinez *et al*., 2017), using Levenberg-Marquardt method adapted to parameter estimation in problems modeled with nonlinear parabolic PDE. Modeling diffusion is fundamental to understand film activity and to investigate which type of food could be protected efficiently using active films. (Sebti *et al*., 2003).

2 Materials and methods

2.1 Materials

Aloe vera gel was obtained from fresh *Aloe vera* leaves in the laboratory of Physicochemical and Alternative Packaging Materials (Instituto Tecnológico de Celaya, México) by scraping the outer epidermis. Gelatin Knox^{TM} was provided by Con Alimentos S.A de C.V (Mexico City, Mexico), Glycerol ACS (catalog number 06441) was supplied by Productos Químicos de Monterrey, S.A de C.V. (Monterrey, N.L., Mexico), polyoxyethylene sorbitan monooleate (Tween 80) was provided by Hycel de México, S.A de C.V (Zapopan, Jal., Mexico). The extraction of essential oil was performed by hydrodistillation of berries of *Pimenta dioica* L. Merrill (locally known as "*pimienta de Jamaica*") harvested in Tacuapan, Totonaca indigenous region located in the northern part of the state of Puebla, Mexico. (Macia-Barco, 1998; Flores-Martinez *et al*., 2017).

2.2 Experimental procedures

2.2.1 Extraction process of essential oil of Pimenta dioica L. Merrill and determination of eugenol

The essential oil was extracted through hydrodistillation with water vapor at a temperature sufficiently low (94 °C) to avoid decomposition. The identification of eugenol, which is the main component of the essential oil (Flores-Martinez *et al*., 2017), was performed by gas chromatography coupled to mass spectrometry (GC 7890A-MS 7693 Agilent, USA) with column (30m x 0.250mm/50μm), and analyzed under the MSD ChemStation Software (G1701EA E.02.01.1177 Agilent, USA).

2.2.2 Film preparation

Aloe vera mucilage was milled adding 0.1% w/w of polyoxyethylene sorbitan monooleate, weighed and centrifuged at 2880 ×g for 40 minutes. The supernatant was separated, filtered, and placed in a bath circulator at 40 ± 2 °C, and the gelatin and glycerol were slowly added, with stirring over 20 minutes. Later, the essential oil was added and homogenized at 304.2 ×g for 3 minutes using a T25 digital Ultra Turrax disperser (IKA Works, Inc., Wilmington, USA). Finally, 8 mL of the filmogenic suspension was distributed in Petri dishes (9 cm in diameter) and allowed to dry at room temperature for 48 h. The formulations of edible films are shown in Table 1, and the physical, barrier, mechanical and microstructural properties were previously reported. (Flores-Martinez *et al*., 2017)

2.2.3 Determination of the presence of eugenol

The components of *Pimenta dioica* L. Merrill essential oil were analyzed by gas chromatography coupled to mass spectrometry (GC 7890A-MS 7693 Agilent, USA), 0.1 μL of the oil was injected at 250 ° C for 17 min. Distinct compounds were identified by the Software (G1701EA E.02.01.1177 Agilent, USA). The compounds mixture of the oil injected into the gas chromatograph (7890A, Agilent, USA) was eluted in a chromatographic column (30mx0.250mm/50μm) obtaining individual components, which were immediately transferred to the mass spectrometer.

Table 1. Formulation of *Aloe vera* based films with *Pimenta dioica* L. Merrill essential oil

Essential oil concentration (%)	*Aloe vera* % w/w	Gelatin % w/w	Glycerol % w/w	Essential oil % w/w
0.0*	97.6563	1.9531	0.3906	0.0000
0.5	97.1817	1.9436	0.3887	0.4859
1.0	96.7118	1.9342	0.3868	0.9671
1.5	96.2464	1.9249	0.3850	1.4437

* Control

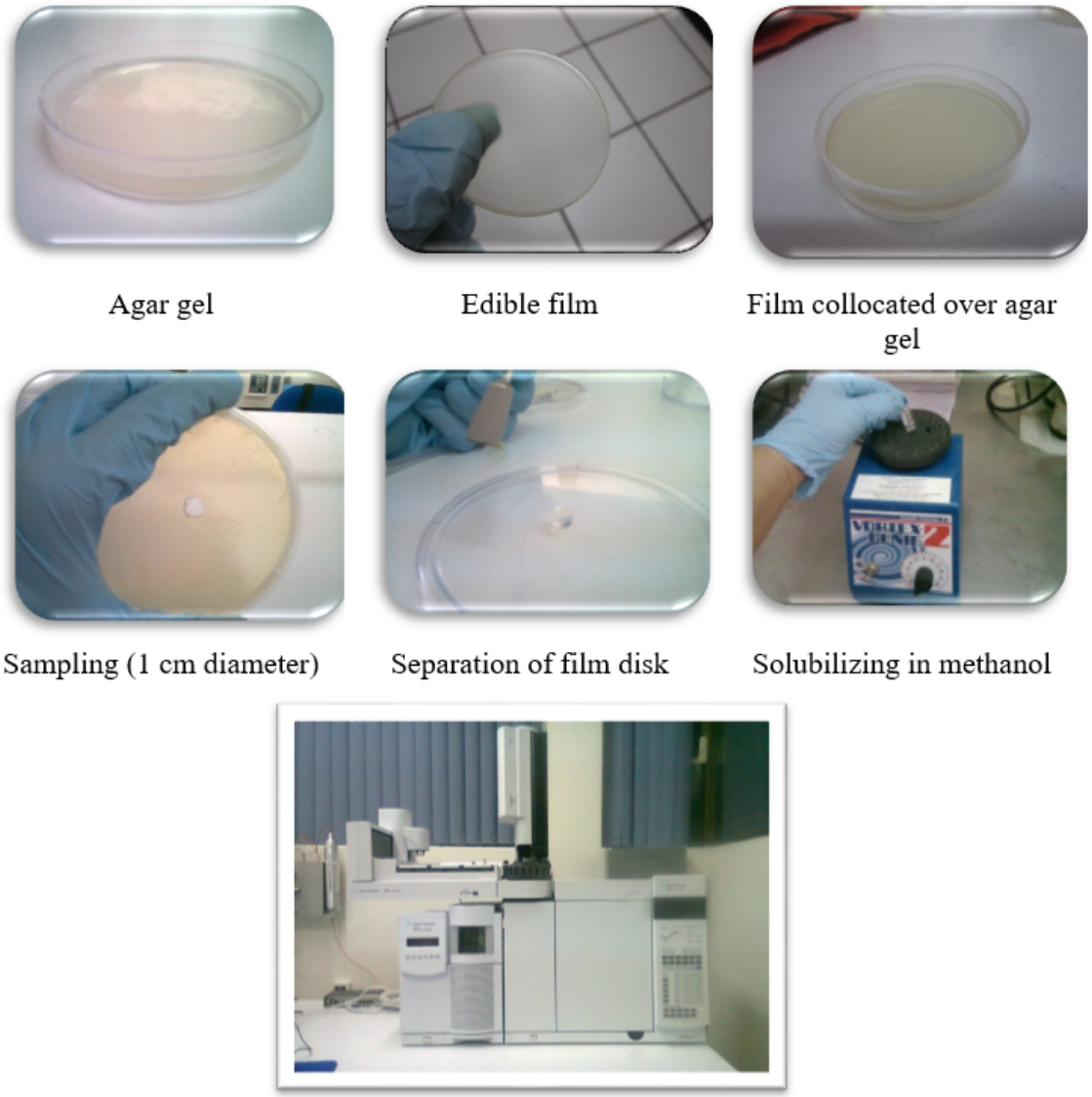

Fig. 1 Experimental protocol to study the diffusion of *Pimenta dioica* L Merrill essential oil in edible films.

2.2.4 Preparation of agar gel diffusion tests

An aqueous solution of agar (Hycel of Mexico, SA de CV) 1% w/w was prepared using boiling water to facilitate the dissolution process. The slurry was molded in disks of 0.5 cm of height and 5 cm of diameter and cooled to solidify.

2.2.5 *Experimental procedure for diffusional transport*

Disks of the same diameter (0.05 m) were cut at 50 μm thickness and carefully placed on agar gel disks (0.05 m diameter and 0.005 m thickness), which previously have been prepared in Petri dishes. Later, they were stored at 5 °C and 25 °C, respectively. The agar gel characterizes a model food in solid phase with high a_w ($a_w > 0.97$, as cheese, meat, sausage, etc.) and the edible film-agar gel system is used for studying the transport of bioactive compounds from edible coating to target food (Pénicaud *et al*., 2010; Fabra *et al*., 2014; Flores-Martinez, 2017). Samples of 1 cm diameter were taken to measure essential oil concentrations. The tested times were 0, 2, 4, 6, 8, 12, 24, 36, 48, 60, 84, 108, 132, 156, and 180 h. The last time was fixed as the characteristic time of experimentation (tc). We done tests for 0.5%, 1.0%, and 1.5% initial concentration (w/w) of pimento (EO) essential oil for T = 5 °C, and T= 25 °C (Flores-Martinez, 2017). All essays were performed in triplicate for assess statistical significance using software provided online by McDonald (2017), applying ANOVA and Tukey-Kramer HDS tests to the six essays considered with their respective replications.

2.2.6 *Determination of released-oil concentration*

Samples of 1 cm diameter and ~5×10^{-5} m thickness were removed from Petri dishes and the film was separated from the agar gel surface. Each film sample was dissolved in 1 mL of methanol and shaken in a Vortex-Genie 2 (Model: G560, Scientific Industries, USA) for 10 minutes. Analysis absorbance and the area under the curve of the obtained solution was measured using gas chromatography coupled to mass spectrometry (GC 7890A-MS 7693 Agilent, USA). The calibration curve was constructed with different known eugenol concentrations (Sigma-Aldrich, St. Louis, USA). The experimental protocol is shown in Fig. 1. The measured concentration of essential oil was referred as eugenol (Padmakumari *et al*., 2011; Dima *et al*., 2014; Flores-Martinez, 2017) and corresponds to its spatial-averaged concentration that only is function of time. These values will be used to estimate the diffusion coefficient of essential oil in edible film formulated in this work at three initial concentrations of pimento essential oil, and at two temperatures. (Flores-Martinez, 2017).

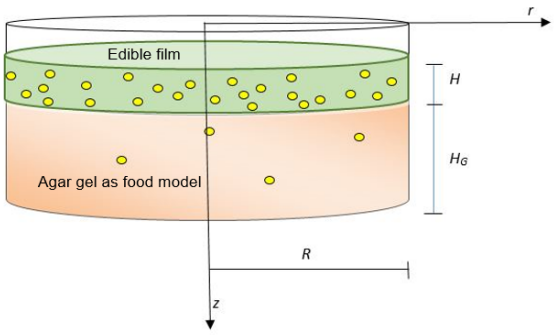

Fig. 2. Geometrical system used for estimating the diffusion coefficient of *Pimenta dioica* L Merrill essential oil.

2.3 *Mathematical model*

Fig. 2 depicts the edible film-food simulant system used in this study, both are in solid phase. Note that thickness/radius ratio $H/R \ll 1$ allows us to reduce the tridimensional transport to one-dimensional direction. Also, the H_G/H ratio (~ 100) simulates appropriately the edible film-target food system. Because the eugenol concentration at the agar gel was undetectable by the measurement system used in this research, it was decided to use a mass transport model analogous to *Newton's Law of Cooling*, to characterize the mass transport from the edible film interface to the agar gel, using as a basis the average eugenol concentration in the agar gel, which can be calculated from an overall mass balance, since the total mass of the essential oil in the system edible film-agar gel remains constant. Therefore, considering Fickian behavior, negligible swelling of the film by water uptake and interfacial resistance to the diffusional transport of eugenol to the agar gel, the governing equations are:

Microscopic mass balance for essential oil (C_A) in the edible film

$$\frac{\partial C_A}{\partial t} = D_{eff}\frac{\partial^2 C_A}{\partial z^2} \tag{3}$$

Subject to:

$$B.C.1, \quad z = 0, \quad \frac{\partial C_A}{\partial z} = 0 \tag{4}$$

$$B.C.2, \quad z = H, \quad -D_{eff}\frac{\partial C_A}{\partial z} = k_C(C_A - \overline{C_{Agel}}) \tag{5}$$

$$I.C., \quad t = 0, \quad C_A = C_A^0 \tag{6}$$

Consequently, the unknown parameters are D_{eff} and k_c. The use of a mass transfer coefficient k_c in the

model food has the advantage that it is not necessary to specify a characteristic dimension of the geometry of the target food that, in another way, is needed for the application of Fick's Law. The above situation is suitable to model the controlled release of a bioactive compound into a food of larger size than the edible film that is used as a protective coating. We define the following dimensionless variables:

$$W_A = \frac{C_A}{C_A^0}; \quad Z = \frac{z}{H}; \quad \tau = \frac{t}{t_c}$$

Where t_c is the characteristic time of experimentation. Substituting dimensionless variables into Equations 1 to 4:

$$\frac{\partial W_A}{\partial \tau} = D_a \frac{\partial^2 W_A}{\partial Z^2} \tag{7}$$

Subject to:

$$B.C.1, \quad Z = 0, \quad \frac{\partial W_A}{\partial Z} = 0 \tag{8a}$$

$$B.C.2, \quad Z = 1, \quad -\frac{\partial W_A}{\partial Z} = Bi_m(W_A - \overline{W_A}_{gel}) \tag{8b}$$

$$I.C., \quad \tau = 0, \quad W_A = 1 \tag{8c}$$

Macroscopic mass balance for calculate $\overline{W_A}_{gel}$

$$\text{for } t \geq 0, \quad C_A^0 V_F = \overline{C_A} V_F + \overline{C_A}_{gel} V_{gel} \tag{9}$$

Substituting dimensionless variables

$$\overline{W_A}_{gel} = \delta \left[1 - \int_0^1 W_A dZ \right] \tag{10}$$

where:

$$\overline{W_A} = \frac{\overline{C_A}}{C_A^0} = \int_0^1 W_A dZ = \overline{W_A}_{model}; \; Bi_m = \frac{k_C H}{D_{eff}};$$

$$D_a = \frac{D_{eff} t_C}{H^2}; \; \overline{W_A}_{gel} = \frac{\overline{C_A}_{gel}}{C_A^0}; \; \delta = \frac{H}{H_G}$$

Bi_m and Da are the parameters to estimate using least-squares algorithm as follows:

$$S = \sum_{j=1}^{NOBS} \left[\overline{W_A}_{exp_j} - \overline{W_A}_{model_j} \right]^2 \tag{11}$$

$\partial S / \partial Bi_m = 0$ and $\partial S / \partial D_a = 0$ are the minimization equations, which when solved *via* Levenberg-Marquardt algorithm, will give the optimized values of Bi_m and D_a, where D_{eff} and k_c can be calculated as follows:

$$Da \rightarrow D_{eff} = \frac{D_A H^2}{t_c}$$

$$Bi_m \rightarrow k_c = \frac{Bi_m D_{eff}}{H}$$

The cumulative release percentage of pimento (EO) essential oil of (via measurement of eugenol) from *Aloe vera* based edible films to agar gel (target food model) is:

$$\%release\ EO = \frac{C_A^0 - C_A}{C_A^0} \times 100 = (1 - W_A) \times 100 \tag{12}$$

Table 2. Experimental data used in estimation of D_{eff} and k_c in diffusional mass transfer of pimento (EO) essential oil in edible films.

Data	Value	Units
C_A^0 (0.5% EO)	3.794	mg/mL
C_A^0 (1.0% EO)	13.042	mg/mL
C_A^0 (1.5% EO)	20.519	mg/mL
H (0.0% EO)	0.043 ± 0.003^a	mm
H (0.5% EO)	0.047 ± 0.004^b	mm
H (1.0% EO)	0.046 ± 0.004^{ab}	mm
H (1.5% EO)	0.051 ± 0.001^c	mm
H_G	0.005	m
t_c	180	h
Diameter of film and agar gel	0.05	m
Number of experimental measurements for EO in films	15	$\overline{C_A}$ *versus* time

^{ab} Values (mean ± standard deviation, $n = 5$) in a column with the same letter do not show significant difference (Tukey HSD test, $p < 0.05$). (Flores-Martinez *et al.*, 2017)

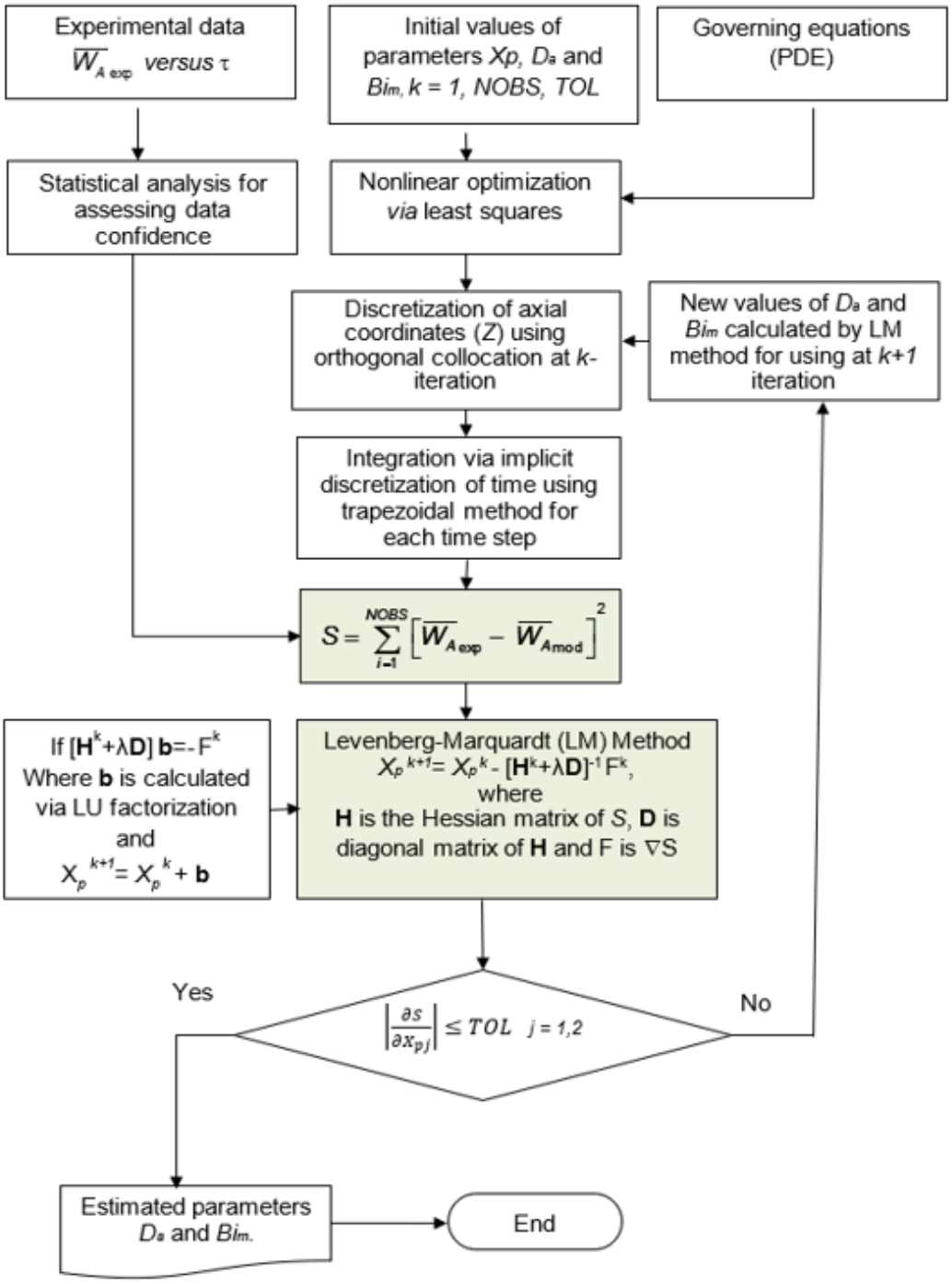

Fig. 3. Flowchart for modified Levenberg-Marquardt algorithm for parameter estimation in parabolic PDE.

Eq (10) avoids the possible existence of several combinations of mass transfer coefficient and mass diffusivity, which equally satisfy the experimental data (Erdoğdu, 2008). Table 2 shows the experimental data required to estimate D_{eff} and k_c. The thickness (H) of formulated edible films is comparable with the report of Acevedo-Fani *et al*. (2015) for alginate films.

2.4 Parameter estimation method in 1-D parabolic PDEs

Levenberg-Marquardt method is a robust method for minimizing the least-squares function used in parameter estimation, which consists in the iterative correction of a set of parameter values in the direction of the maximum change rate of the squared differences sum (S) (Marquardt, 1963). The modified algorithm for parameter estimation in parabolic PDE is shown in Fig. 3 and it consists in a discretization of spatial coordinates via orthogonal collocation with Legendre polynomials and an implicit approach applied in time derivative (Jiménez-Islas *et al*., 2014). The nonlinear set of algebraic equations obtained was embedded in a least-squares scheme that is solved via Levenberg-Marquardt algorithm. Instead of evaluating the inverse matrix, we solve a system of linear equations using LU factorization to find the correction vector **b**.

Taking the considerations discussed above, we developed a program coded in FORTRAN 90 and named OPTIPC1I. This computational code can be used interchangeably on x86 platforms, workstations and supercomputers (using parallel processing) and is easily adaptable to any physical situation that is modeled with 1-D parabolic PDE (one or several equations). The computations were performed on a Workstation Intel Xeon^{TM} E5-2620 dual processor with 32 Gb RAM (Sistemas AZTX, model Taquion 2620x2ws, Celaya, Gto., Mexico), Windows 7 Ultimate^{TM}, and Intel FORTRAN Composer compiler v13 (Intel Co., Santa Clara, CA, USA).

To assess the reliability of OPTIPC1I software, the program was validated with three study cases. The first case is a home-designed system of coupled nonlinear PDE that has analytical solution and allows us to analyze the performance of the proposed algorithm. The second case recreates the diffusivity estimation of nisin in agarose gels (Sebti *et al*., 2003). These cases are shown in Appendix A. The third case is the diffusivity estimation of *Pimenta dioica* essential oil described in this research.

2.5 Statistics

The statistic validation of estimated parameters was developed with the $(1-\alpha)\times 100\%$ confidence intervals for the parameter expectation matrix (Θ) (Seber and Wild, 1989)

$$(\Theta-\theta)^t\mathbf{D}^t\mathbf{D}(\Theta-\theta)\leq k\sigma^2 F_{1-\alpha(k,2N-k)} \qquad (13)$$

where

$$\mathbf{D}=\begin{bmatrix} \frac{\partial\overline{W_{A1}}}{\partial D_A} & \frac{\partial\overline{W_{A1}}}{\partial Bi_m} \\ \frac{\partial\overline{W_{A2}}}{\partial D_A} & \frac{\partial\overline{W_{A2}}}{\partial Bi_m} \\ \vdots & \vdots \\ \frac{\partial\overline{W_{AN}}}{\partial D_A} & \frac{\partial\overline{W_{AN}}}{\partial Bi_m} \end{bmatrix};\ \theta=\begin{bmatrix} D_A \\ Bi_m \end{bmatrix}$$

$$\sigma^2=\frac{\sum_{J=1}^{N}[\overline{W_A}_{exp}-\overline{W_A}_{model}]^2}{N-k}$$

3 Results and discussion

3.1 Effective diffusivity estimation of Pimenta dioica essential oil

Fig. 4 shows the spectrum of eugenol and methyl eugenol that constitute the major components of *Pimenta dioica* essential oil. This finding is comparable to the Padmakumari *et al*. (2011) and Dima *et al*. (2014). Table 3 shows the results of the ANOVA analysis, which demonstrate that the replications performed for each essay are statistically valid. Table 4 shows the results of Tukey-Kramer test, where it is verified that replications of each essay do not exhibit statistical difference. Therefore, for the estimation of the diffusion coefficient through the film and the interfacial mass transfer coefficient, the average of the three replications of each essay of essential oil release kinetics was taken. Eqs (7-10) were spatially discretized with 13 internal points of orthogonal collocation with Legendre polynomials and implicitly integrated using 1000 steps. The initial values of parameters Bi_m and D_a were $[1,1]^t$, and a tolerance = 10^{-7} was used in all computational runs.

Table 5 shows the results of the estimation of the effective diffusivity of the essential oil in the film and mass transfer coefficient in film-agar gel interface. Mesh-independence analysis were performed (not shown) in order to validate the results. As a comparison, Table 6 shows a partial list of investigations where diffusivity values are reported for diverse bioactive substrate-edible film systems. The values obtained for the diffusivity of *Pimenta dioica* L. Merrill essential oil of are smaller; due to the hydrophobic nature of the essential oil, which becomes an advantage, since the release would be slower, which favors the increase of the shelf life in foods (Mohan *et al*., 2017; Rubilar *et al*., 2017).

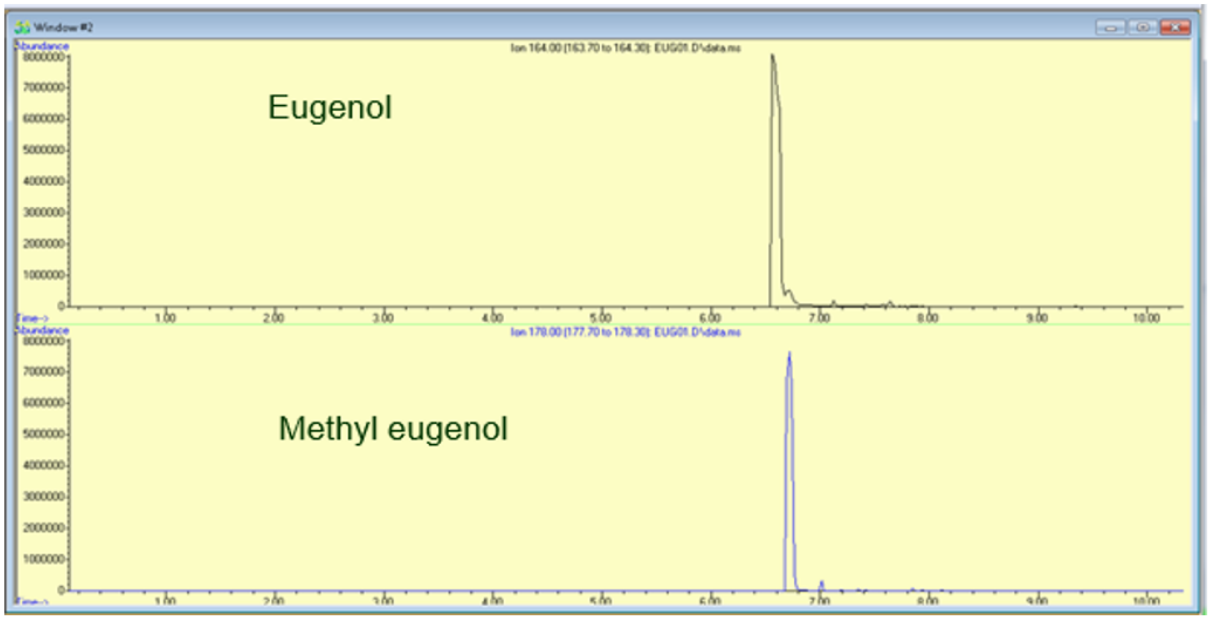

Fig. 4. Spectrum of eugenol and methyl eugenol identified in *Pimenta dioica* L. Merrill essential oil.

Table 3. One-way ANOVA test for experimental measurements of eugenol concentration in edible films.

Essay	ANOVA	Sum of squares	Mean square	Fs	p
0.5% EO at 5 °C	Among groups	0.001388	0.000694	0.000572	0.999
	Within groups	51.002	1.214		
	Total	51.004			
1.0% EO at 5 °C	Among groups	0.085	0.042	0.002754	0.997
	Within groups	647.583	15.419		
	Total	647.668			
1.5% EO at 5 °C	Among groups	0.11	0.055	0.001437	0.999
	Within groups	1608.079	38.288		
	Total	1608.189			
0.5% EO at 25 °C	Among groups	0.001401	0.0007	0.000387	1
	Within groups	75.914	1.807		
	Total	75.915			
1.0% EO at 25 °C	Among groups	0.019	0.0094	0.000439	1
	Within groups	898.393	21.39		
	Total	898.412			
1.5% EO at 25 °C	Among groups	0.051	0.025	0.000482	1
	Within groups	2214.254	52.72		
	Total	2214.305			

Mohan *et al*. (2017) studied the eugenol release in starch-based films collocated over mutton meat slices, obtaining a D_{eff} = 1.07×10^{-14} at 10 °C, and D_{eff} = 1.19×10^{-14} m^2/s at 15ºC. These values are in order of magnitude with those estimated in this research. The small values of k_c obtained in parameter estimation demonstrates the importance of interfacial resistance to release of pimento essential oil from *Aloe vera* based film to the food simulant used (agar gel).

Table 4. Tukey-Kramer test for experimental measurements of eugenol concentration in edible films

Essay	Replication	CA averaged (mg/mL)
0.5% EO at 5 °C	1	2.30958^{a}
	2	2.29598^{a}
	3	2.30234^{a}
1.0% EO at 5 °C	1	7.73044^{b}
	2	7.68043^{b}
	3	7.78678^{b}
1.5% EO at 5 °C	1	14.01361^{c}
	2	13.94829^{c}
	3	14.06929^{c}
0.5% EO at 25 °C	1	2.08644^{d}
	2	2.07282^{d}
	3	2.07859^{d}
1.0% EO at 25 °C	1	7.03778^{e}
	2	7.01509^{e}
	3	6.98778^{e}
1.5% EO at 25 °C	1	12.68283^{f}
	2	12.64439^{f}
	3	12.60056^{f}

Averages with a common letter are not significantly different ($p > 0.05$)

Table 5. Results of parameter estimation in release of essential oil (EO) in formulated edible films.

% w/w *Pimenta dioica* L. Merrill essential oil in film	Temperature (°C)					
	5			25		
	D_{eff} (m^2/h)	k_c (m/h)	Sum of quadratic residuals	D_{eff} (m^2/h)	k_c (m/h)	Sum of quadratic residuals
0.5	$(7.0529 \pm 0.013)\times10^{-12}$	$(3.6283 \pm 0.04)\times10^{-6}$	0.00684	$(3.7980 \pm 0.032)\times10^{-11}$	$(9.7677 \pm 0.967)\times10^{-7}$	0.00932
1.0	$(9.0090 \pm 0.056)\times10^{-12}$	$(3.5136 \pm 0.15)\times10^{-6}$	0.00723	$(4.0698 \pm 0.019)\times10^{-11}$	$(1.1821 \pm 0.0876)\times10^{-6}$	0.00532
1.5	$(5.3586 \pm 0.94)\times10^{-11}$	$(4.5319 \pm 0.06)\times10^{-7}$	0.00665	$(5.2578 \pm 0.004)\times10^{-10}$	$(6.4921 \pm 0.039)\times10^{-7}$	0.02238
	D_{eff} (m^2/s)	k_c (m/s)	Relative error (%)	D_{eff} (m^2/s)	k_c (m/s)	Relative error (%)
0.5	$(1.9591 \pm 0.35)\times10^{-15}$	$(1.0079 \pm 0.083)\times10^{-9}$	12.22935	$(1.055 \pm 0.0021)\times10^{-14}$	$(2.713 \pm 0.32)\times10^{-10}$	9.56894
1.0	$(2.5025 \pm 0.47)\times10^{-15}$	$(9.760 \pm 0.113)\times10^{-10}$	15.60219	$(1.131 \pm 0.0014)\times10^{-14}$	$(3.284 \pm 0.335)\times10^{-10}$	5.57244
1.5	$(1.4885 \pm 0.164)\times10^{-14}$	$(1.259 \pm 0.19)\times10^{-10}$	22.13141	$(1.461 \pm 0.23)\times10^{-13}$	$(1.803 \pm 0.498)\times10^{-10}$	16.08813

Note: The results of parameter estimation were reported both (m^2/h, m/h) and (m^2/s, m/s) respectively, to simplify comparison with other reported results in literature. Parameters dispersions were computed for 95% confidence according with equation 13.

The diffusivity value increases when the initial concentration of essential oil increases, high initial essential oil concentration in the film increases polymer film-EO interaction, which in turn increased the coefficient of diffusion (Ozdemir and Floros, 2001, 2003; Thakhiew *et al*., 2011). This originates a multifactorial non-Fickian behavior that includes essential oil-*Aloe vera*-gelatin interactions (Bastarrachea *et al*., 2011; Dima *et al*., 2014), sorption phenomena, film swelling, etc. Therefore, it is necessary to consider the diffusion coefficient as a function of time, of spatial coordinates, of bioactive agent concentration. Authors such as Crank (1975), Ashlee *et al*. (2014), Malafronte *et al*. (2015), Ferreira *et al*. (2015) among others, report diverse strategies to address variable diffusivity, although the mechanistic way is the proposition of modified models of Fick's Law that includes the effect of the previous mentioned factors (Ferreira *et al*., 2015). Then, from the microscopic mass balance for a component A, we replace the new definition of diffusive flux to obtain a more general form of Fick's second law. This approach will be addressed in a later research.

The diffusion coefficient increases when the temperature increases or the essential oil concentration increases. A rise in temperature increases the kinetic energy of the eugenol molecules, which results in an increment in D_{eff} (Sebti *et al*., 2003; Choi *et al*., 2005). Applying a classical Arrhenius activation energy function showed in eq. (14):

$$D_{eff} = D_0 e^{-E_a/RT} \tag{14}$$

Table 6. Some reported diffusivities of diverse bioactive substrates-edible films.

Diffusion coefficient (m^2/s) $\times 10^{-11}$	Temperature (ºC)	Active agent	Biopolymer	Reference
0.31 0.41 0.75	4 10 20	Sorbic acid	Wheat gluten	Redl *et al*. (1996)
13.1 6.74	Room	Potassium sorbate	Sliced American and Mozzarella cheese	Han and Floros (1998)
6.9-16.5	25	Lysozyme	Agarose gel	Mattisson *et al*. (2000)
0.0051 0.035 0.075 0.13	5 25 35 45	Nisin	Wheat gluten	Teerakarn *et al*. (2002)
0.01-0.21	20	Water	Wheat gluten	Guillard *et al*. (2003)
1.92 3.52 3.74 8.14	5.4 9.9 10.2 22.3	Nisin	Agarose gel	Sebti *et al*. (2003)
5.4-9.8	25	Potassium sorbate	Whey protein	Ozdemir and Floros (2003)
0.00105 0.00260 0.00642	5 25 40	Potassium sorbate	κ-carrageenan at pH 7	Choi *et al*. (2005)
0.0012 0.018	5 25	diphenylbutadiene	Pork meat	Sanches Silva *et al*. (2007)
0.0001	25	n-hexanal	Soy protein isolate	Monedero *et al*. (2010)
0.00000381 0.000000143	Room	Thymol Cymene	Polylactide microcapsules	Martins *et al*. (2011)
0.0000012 0.055	4 37	carvacrol	Chitosan	Kurek *et al*. (2014)
0.000049 (30% RH) 0.00131 (90% RH)	23	Cinnamaldehyde	Wheat Protein	Balaguer *et al*. (2013)
0.0012 0.0013	6 23	Lysozyme	microfibrillated cellulose	Cozzolino *et al*. (2013)
0.0197	4	Nisin	Chitosan	Imran *et al*. (2014)
0.30	Room	Caffeine	Cellulose	Lavoine *et al*. (2016)

Where D_0 is a pre-exponential coefficient, E_a is the activation energy required in diffusion phenomenon (J/mol), R is universal gas constant (J/mol K), and T is absolute temperature (K) (Sebti *et al*., 2004; Choi *et al*., 2005). Table 7 shows the results of fitting eq (14). The activation energy exhibits a minimum of 53.028 kJ/mol at 1.0% essential oil concentration, which could explain the greater percentage of liberation of the essential oil that is obtained with this initial EO concentration. The range of E_a obtained in this study was comparable with previous works as Sebti *et al*. (2004) reported E_a for nisin diffusion in agarose gel equal to 57 kJ/mol and D_0 = 0.93 m^2/s. Redl *et al*. (1996) reported that the effect of temperature in diffusivity of sorbic acid in wheat gluten based films could be described by Arrhenius type model, with activation energy ranging from 30.0 to 39.8 kJ/mol. Choi *et al*. (2005) found E_a ranged between 23.6-36.9 kJ/mol for diffusion of potassium sorbate in κ -carrageenan. Bastarrachea *et al*. (2011) found in the study of diffusion of nisin from PBAT (polybutylene

Table 7. Activation energy in diffusion of pimento essential oil through *Aloe vera*-gelatin edible film

Initial concentration of essential oil (% w/w) in film	D_0 (m^2/s)	E_a (kJ/mol)
0.5	1.556×10^{-4}	58.071
1.0	1.459×10^{-5}	53.028
1.5	9.111	78.779

Table 8. Cumulative predicted release (%) of *Pimenta dioica* L. Merrill essential oil through *Aloe vera* based film obtained at 180 h

Initial concentration of essential oil (% w/w)	T = 5 °C	T = 25 °C	Concentration at $t \rightarrow \infty$ (mg/mL)
0.5	80.434	93.647	0.0353
1.0	82.811	94.583	0.1189
1.5	74.098	88.252	0.2072

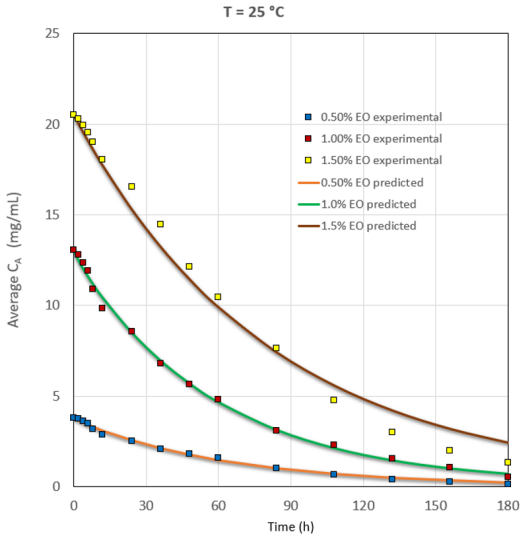

Fig. 5. Experimental and predicted average concentration of essential oil (EO) in *Aloe vera* based film to agar gel, at 25 °C.

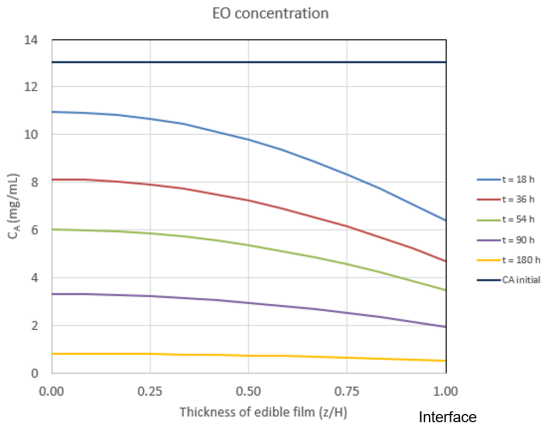

Fig. 6. Predicted concentration of essential oil (EO) in *Aloe vera* based film at 1.0% initial concentration and T = 25 °C.

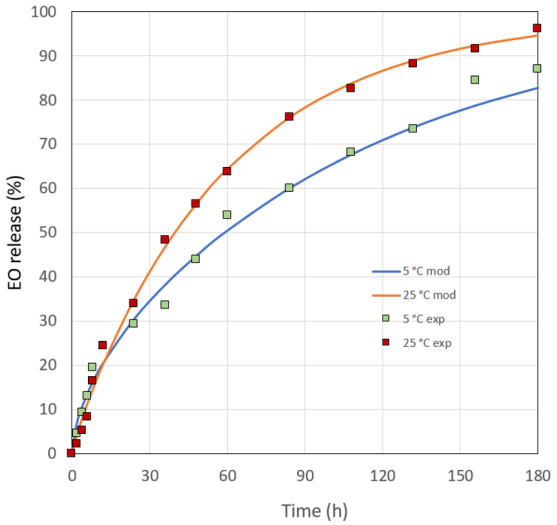

Fig. 7. Predicted and experimental release kinetics of pimento essential oil in formulated edible film at 1.0% initial concentration.

adipate-co-terephthalate) films to distilled water an activation energy of 38.3 kJ/mol. Rubilar *et al.* (2017) obtained activation energies in the range 58-60 kJ/mol for diffusion of gallic acid in chitosan-based films. The correlation between values of the activation energies with molecular structure of the bioactive compound and the matrix structure of the formulated edible film is not trivial and should be studied in greater detail.

Fig. 5 depicts the predicted and experimental essential oil concentrations versus time at T = 25 °C, indicating that the proposed model was suitable and it has a good agreement with experimental data. Now, the governing equations developed can be used for predicting the dynamics of the profiles of concentration of essential oil in the film as shown in Fig. 6 and the essential oil release kinetics is

calculated from them. These predictions are required for the design of systems for the controlled release of a substrate from an edible film to a target food (Kumari *et al*., 2017; Benbettaïeb *et al*., 2018) and, at the same time, to know if the concentration at the interface is adequate to achieve the objective of preserving foods.

Table 8 shows the accumulated release (%) of the essential oil of pimento at 180 h of experimentation, where one can observe that maxima of 82.811% at 5 °C and 94.583% at 25 °C were predicted for the concentration of 1%, which could have correlation with the minimum activation energy obtained at this concentration. Also, Table 8 shows the equilibrium concentration of essential oil in the edible film-agar gel system. This circumstance allows us to handle the initial concentration of essential oil, to obtain a controlled release of bioactive compounds from packaging films, which is of utmost importance for extending the shelf-life of perishable foods (Rubilar *et al*., 2017). A deeper explanation of this behavior is beyond the scope of this work, although were available some works about this topic (Barba *et al*., 2015; Benbettaïeb *et al*., 2018). Fig 7. depicts the influence of temperature on % release of essential oil of pimento, with the release accelerated at higher temperatures as has been reported by Rubilar *et al*. (2017) in gallic acid release in chitosan film. Mohan *et al*. (2017) report a similar behavior in cinnamaldehyde and eugenol release from starch based edible films. The model proposed is in agreement with experimental results. However, the release rate should be related to the bioactivity rate for an optimal efficiency of the active packaging or film coating (Benbettaïeb *et al*., 2018).

Conclusions

A modified Levenberg-Marquardt (LM) method adapted to nonlinear PDE systems was used to estimate the effective diffusivity (D_{eff}) of the essential oil in edible films formulated with *Aloe vera*, gelatin, glycerol, and *Pimenta dioica* L. Merrill essential oil as a bioactive compound. The algorithm was implemented using spatial discretization by orthogonal collocation with Legendre polynomials and a trapezoidal-implicit scheme in time derivative, all included in a least-square scheme that is solved via LM method. The performance of the algorithm developed for parameter estimation in parabolic PDE was satisfactory. Also, it is straightforwardly applicable to other situations such as release of bioactive substances in bioparticles, microbial kinetics, diffusion-reaction, and food drying. In this last case, it is feasible to exploring complex problems as non-Fickian diffusion without using empirical models.

The mathematical model proposed to characterize the diffusion of the essential oil through the edible film towards a target food, was appropriate for the design of controlled release systems of a bioactive compound, since with the estimation of a mass transfer coefficient, it is not necessary to know a characteristic dimension of the geometry of the target food that, in another way, is required for Fick's Law.

Diffusion of *Pimenta dioica* L. Merrill essential oil of in the *Aloe vera*-gelatin film exhibited a non-Fickian behavior. The estimated values of diffusivity were 7.0529×10^{-12} to 5.3586×10^{-11} m^2/h at 5 °C, while at 25 °C, the effective diffusivity values were between 3.7980×10^{-11} to 5.2578×10^{-10} m^2/h, values smaller than those reported for other bioactive substances diffusion in films. This circumstance is suitable to improve shelf life in foods. Also, the maximum release of pimento essential oil of is 94.583% at 180 h for 1% w/w essential oil concentration at 25 °C. With the results of this study, we can propose that *Aloe vera* based films with *Pimenta dioica* L. Merrill essential oil as bioactive agent could be used on food surfaces (e.g. cheese or meat products) as an active-edible preservative releasing systems.

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Nomenclature

Bi_m	mass Biot number, dimensionless
C_A	concentration of essential oil in the film, mg/mL
C_A^0	initial concentration of essential oil in the film, mg/mL
$\overline{C_A}$	average concentration of essential oil in the film, mg/mL

$\overline{C_{Agel}}$	average concentration of essential oil in the agar gel, mg/mL
D	Jacobian matrix of response variations
D_a	dimensionless diffusivity, dimensionless
D_{eff}	effective diffusivity of essential oil in the film, m^2/s
D_0	pre-exponential coefficient, m^2/s
E_a	activation energy, kJ/mol
F	F test value
H	film thickness, m
H_G	agar gel thickness, m
k	number of estimated parameters
k_C	mass transfer coefficient, m/s
N	number of experimental data
t	time variable, h
t_C	characteristic time, h
T_1	dependent variable 1, dimensionless
T_2	dependent variable 2, dimensionless
T_3	dependent variable 3, dimensionless
T	temperature, °C, K
V_F	film volume, m^3
V_{gel}	agar gel volume, m^3
W_A	dimensionless concentration of essential oil in the film
$\overline{W_{Agel}}$	dimensionless concentration of essential oil in the agar gel
z	spatial variable, m
Z	dimensionless spatial variable

Greek symbols

α	significance level
δ	thickness ratio, dimensionless
Θ	parameters expectation
θ	parameters vector
τ	dimensionless time
σ^2	estimated variance error

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APPENDIX A

Case A.1: Set of parabolic PDE with known analytical solution

We constructed the following set of three 1-D nonlinear parabolic PDE $\{T_i = f_i(x,t)\}$, where the vector of unknown coefficients X_p has values $[3,1,2,0.5]^t$ that we should be recover using the parameter-estimation algorithm described in Fig. 3

$$\frac{\partial T_1}{\partial t} = 2\left(\frac{\partial^2 T_1}{\partial x^2}\right) + \left(\frac{\partial T_2}{\partial x}\right)^2 - \frac{2x}{(t+X_{p2})^2} + 2X_{p1}\sin(x) - X_{P3}^2 e^{-2t} \quad \text{(A.1)}$$

$$\frac{\partial T_2}{\partial t} = 3\left(\frac{\partial^2 T_2}{\partial x^2}\right) - X_{p3} x e^{-t} \quad \text{(A.2)}$$

$$\frac{\partial T_3}{\partial t} = \frac{\partial T_1}{\partial x} + \frac{\partial^2 T_3}{\partial x^2} - \frac{2x}{(t+X_{p4})^2} - X_{p1}\cos(x) - \frac{2}{t+X_{p2}} - 6x \quad \text{(A.3)}$$

Subject to:

$$B.C.1, \quad @x = 0, \quad T_1 = 0, \quad T_2 = 0, \quad T_3 = 0 \quad \text{(A.4a)}$$

$$B.C.2, \quad @x = 1 \quad T_1 = 0.84147 X_{P1} + \frac{2}{t+X_{p2}}$$

$$\frac{\partial T_2}{\partial x} = X_{p3} e^{-t}; \quad T_3 = 1 + \frac{2}{t+X_{p4}} \quad \text{(A.4b)}$$

$$I.C., \quad @t = 0 \quad T_1 = 3\sin(x) + 2x, \quad T_2 = 2x$$

$$T_3 = x(x^2+4) \quad \text{(A.4c)}$$

With the following analytical solutions:

$$T_1 = X_{P1}\sin(x) + \frac{2x}{t+X_{p2}} \quad \text{(A.5)}$$

$$T_2 = X_{p3} x e^{-t} \quad \text{(A.6)}$$

$$T_3 = x^3 + \frac{2x}{t+X_{p4}} \quad \text{(A.7)}$$

Using equations (A.5-A.7) evaluated at $x = 0.5$, from $t = 0$ to $t = 1$, the data depicted in Table A.1 was obtained, which were used as experimental data for least-squares method. For spatial discretization of equations (A.1-A.4) was used 11 internal nodes of orthogonal collocation using Legendre polynomials and 500 integration steps from $t = 0$ to $t = 1$, taking initial values of $X_p = [0.3, 0.3, 0.3, 0.3]^t$, $\lambda = 10^5$, taking 2 as division factor of λ, and a tolerance of 10^{-10}. The results obtained in 31 iterations are shown in Table A.2, observing the recovering of original values of vector X_p and the efficiency of parameter estimated performed in this work. Similar results (not shown) were obtained with other mesh-size, integration steps, and initial values, validating that the results were independent of the mesh size. In addition, the experimental data were randomly varied applying 5% and 10% error in them and calculating again the parameters X_p. The results are also shown in Table A.2, where one can be observed that the proposed algorithm has numerical stability and accuracy.

Table A.1. Values of T_1, T_2, and T_3 at $x = 0.5$ used in solving case A.1

t	T_1	T_2	T_3
0.00	2.438277	1.000000	2.125000
0.04	2.399815	0.960789	1.976852
0.08	2.364203	0.923116	1.849138
0.15	2.307842	0.860708	1.663462
0.23	2.251285	0.794534	1.494863
0.31	2.201635	0.733447	1.359568
0.38	2.162914	0.683861	1.261364
0.46	2.123208	0.631284	1.166667
0.50	2.104943	0.606531	1.125000
0.60	2.063277	0.548812	1.034091
0.67	2.037079	0.511709	0.979701
0.75	2.009705	0.472367	0.925000
0.82	1.987727	0.440432	0.882576
0.85	1.978817	0.427415	0.865741
0.91	1.961837	0.402524	0.834220
0.92	1.959110	0.398519	0.829225
0.97	1.945891	0.379083	0.805272
1.00	1.938277	0.367879	0.791667

Table A.2. Parameter estimation of PDE system solved in case A.1

X_{pi}	True value	5% error in experimental data	10% error in experimental data
X_{p1}	3.000009	3.118634	3.183354
Residual	-1.074292×10^{-11}	1.387779×10^{-12}	-2.498002×10^{-11}
X_{p2}	1.000014	1.096808	1.153350
Residual	-8.714085×10^{-12}	2.151057×10^{-11}	4.440892×10^{-11}
X_{p3}	1.999972	1.988990	1.967191
Residual	4.108528×10^{-14}	1.040834×10^{-11}	-4.996004×10^{-11}
X_{p4}	5.000002×10^{-1}	5.212210×10^{-1}	5.364518×10^{-1}
Residual	-5.218980×10^{-14}	3.469447×10^{-12}	-2.775558×10^{-12}
Standard error	8.506719×10^{-11}	1.587078×10^{-3}	7.349652×10^{-3}
Standard deviation	1.053965×10^{-1}	1.003301×10^{-1}	1.117042×10^{-1}
Quadratic sum of residuals	3.572822×10^{-9}	6.665729×10^{-2}	3.086854×10^{-1}
Coefficient of determination	1.000000	9.920592×10^{-1}	9.665425×10^{-1}
Average relative error (%)	0.0004	1.9496	6.3905

Case A.2. Estimation of nisin diffusivity in agarose gels.

Sebti *et al*. (2003) studied experimentally the diffusion of nisin in 3% agarose gels using the system depicted in Fig. A.1., where a cylinder of agarose gel (70 mm of diameter and 70 mm of height L) whose lower face is in contact with a large container filled with a solution of nisin at, practically constant concentration C_{Asol}. Initially, the cylinder is nisin-free. The solution is slowly stirred, so the interfacial resistance should be considered. Sebti *et al*. (2003) estimate the diffusion coefficient of nisin from experimental measurements at various distances from the concentration of nisin to 6 days after (experiment E), modeling the diffusion phenomenon as 1-D Fickian type considering the cylinder as a semi-infinite medium without interfacial resistance, whose analytical solution (eq. A.8) can be obtained via Laplace transform. The experimental concentration of nisin was determined as function of distance z from the contact face at $t = 5.9$ days (Sebti *et al*., 2003).

$$\frac{C_A^* - C_A}{C_A^* - C_A^0} = \text{erf}\left(\frac{z}{2\sqrt{D_{eff}t}}\right) \qquad \text{(A.8)}$$

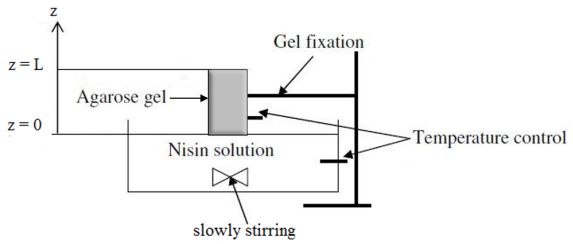

Fig. A.1. Experimental system used by Sebti *et al.* (2003) to estimate nisin diffusivity through an agarose gel cylinder.

Where $C_A = f(z,t)$ is the nisin concentration, C_A^* is the nisin concentration at $z = 0$ (230.8 μg/ml), C_A^0 is the nisin concentration a $t = 0$ (0 μg/ml). Thence, the estimation of diffusivity of nisin in the agarose gel was performed by algebraic nonlinear regression using Levenberg-Marquardt method. To validate our methodology, we have decided to take the data reported in experiment E by Sebti *et al.* (2003), using Engauge Digitizer^{TM} software, including now the interfacial resistance (the authors report that nisin solution is stirred slowly) to estimate the diffusion coefficient D_{eff} of nisin and, additionally, the mass transfer coefficient (k_c) using the algorithm proposed in Fig. 3. Therefore, the governing equations were:

$$\frac{\partial C_A}{\partial t} = D_{eff}\frac{\partial^2 C_A}{\partial z^2} \tag{A.9}$$

$$B.C.1, \quad @z = 0, \quad -D_{eff}\frac{\partial C_A}{\partial z} = k_c(C_A - C_{Asol}) \tag{A.10a}$$

$$B.C.2, \quad @z = L, \quad \frac{\partial C_A}{\partial z} = 0 \tag{A.10b}$$

$$I.C., \quad @t = 0, \quad C_A = C_A^0 \tag{A.10c}$$

Taking the following dimensionless variables:

$$\tau = \frac{t}{t_c}, \quad Z = \frac{z}{H}, \quad Bi_m = \frac{k_c L}{D_{eff}}, \quad D_A = \frac{D_{eff}t_c}{L^2}, \quad w_A = \frac{C_A - C_A^0}{C_{Asol} - C_A^0}$$

Therefore:

$$\frac{\partial w_A}{\partial \tau} = D_A\frac{\partial^2 w_A}{\partial Z^2} \tag{A.11}$$

$$B.C.1, \quad @Z = 0, \quad -D_{eff}\frac{\partial w_A}{\partial Z} = Bi_m(w_A - 1) \tag{A.12a}$$

$$B.C.2, \quad @Z = 1, \quad \frac{\partial w_A}{\partial Z} = 0 \tag{A.12b}$$

$$I.C., \quad @\tau = 0, \quad w_A = 0 \tag{A.12c}$$

The unknown parameters are D_A, Bi_m, where D_{eff} and k_c can be calculated as follows:

$$D_A \rightarrow D_{eff} = \frac{D_A L^2}{t_c} \tag{A.13a}$$

$$Bi_m \rightarrow k_c = \frac{Bi_m D_{eff}}{L} \tag{A.13b}$$

Where $L = 0.07$ m, $C_{Asol} = 376$ μg/mL, $C_A^0 = 0$ μg/mL, $t_c = 5.9$ days (Sebti *et al.*, 2003). Eqs. (A.11) and (A.12) were numerically solved using spatial discretization with 27 internal points of orthogonal collocation with Legendre polynomials (Finlayson, 1980; Jiménez-Islas *et al.*, 1996) and the derivative time was implicitly integrated using a trapezoidal method. (Jiménez-Islas *et al.*, 2014), applying the algorithm described in Fig. 3 to estimate D_A and Bi_m. On the other hand, the governing equations (A.9-A.10) have analytical solution derived using separation of variables, defined by eq. (A.14):

$$C_A = 2(C_A^0 - \overline{C_{A\,sol}})\sum_{n=1}^{\infty}\frac{e^{\frac{-\lambda_n^2 D_{eff}t}{H^2}}\sin\lambda_n}{\lambda_n + \sin\lambda_n\cos\lambda_n}\cos\left[\lambda_n\left(1 - \frac{z}{H}\right)\right] + \overline{C}_{Asol} \tag{A.14}$$

where $\lambda_n \sin\lambda_n - Bi_m\cos\lambda_n = 0$.

The parameter estimation in Eqs (A.11) and (A.12) were performed with LM method only (FORTRAN) and validated with Solver tool of Excel^{TM}. Table A.3 shows the parameter estimation both numerical and analytical solutions. This agreement let us to compare together with the results reported by Sebti *et al.* (2003) to validate the proposed algorithm of parameter estimation. As expected, the proposed model has better approach to the physics of the system, exhibited smaller sum of squared residues and relative error than the simpler model (semi-infinite medium) used by Sebti *et al.* (2003). The small value of k_c obtained in parameter estimation is in agreement of the importance of interfacial resistance inherent to experiment. In addition, with the results of the analytical solution (eq (A.14)),

evaluated with 100 roots of λ_n), it can be inferred that the proposed parameter estimation algorithm is accurate. An additional information is obtained with the concentration of nisin at $z = 0$, since the proposed model predicts a concentration of 236.344 μg/mL, while Sebti *et al*. (2003) reported an experimental value of 230.8 μg/mL. Fig. A.2 depicts the comparison of the experimental data of Sebti *et al*. (2003) with the concentration of nisin predicted by eqs (A.11) and (A.12), when the parameters D_{eff} and k_c have been estimated. A.3 shows the contours of quadratic sum of residual (S^2), where one can be observed the region where the minimum is located. In this case, only a minimal is located.

Table A.3. Results of parameter estimation and errors in case A.2.

	Sebti *et al*. (2003)	Numerical solution of eqs (A.11-A.12)	Analytical solution eq (A.14)
D_{eff} (m^2/s)	$(8.140 \pm 0.2^a) \times 10^{-11}$ $(8.342^b \pm 0.38^a) \times 10^{-11}$	$(1.120 \times \pm 0.019^a) \times 10^{-10}$	1.045×10^{-10}
k_c (m/s)	—	$(1.827 \pm 0.091^a) \times 10^{-8}$	1.816×10^{-8}
Quadratic sum of residuals (S^2)	2015.289 2005.896^b	1898.499	1915.815
Correlation (R^2)	0.980 0.990^b	0.996	0.990
Average relative error (%)	36.084 35.842^b	30.532	31.440

[a]Dispersion calculated for 95% of confidence (equation 13)
[b]Recalculated in this work with LM method.

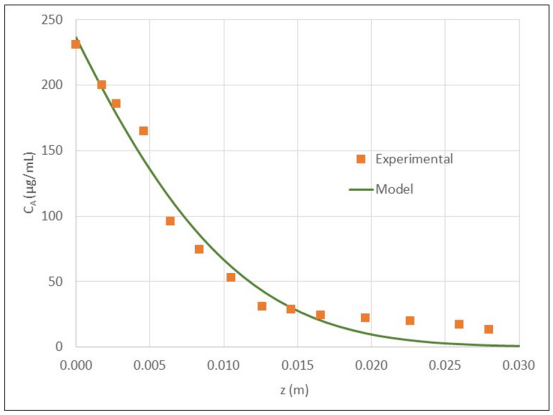

Fig. A.2. Comparison of experimental data reported by Sebti *et al*. (2003) with prediction of mathematical model proposed in this research.

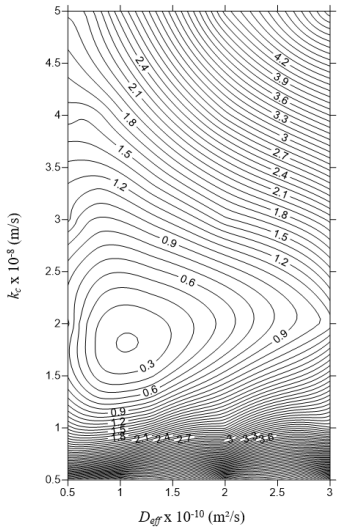

Fig. A.3. Square-residual sum contours for experiment (E) performed by Sebti *et al*. (2003) constructed from mathematical model proposed in this research.