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Modeling the release of antimicrobial agents (thymol and carvacrol) from two different encapsulation materials

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Abstract The release of microencapsulated natural antimicrobial (AM) agents (thymol and carvacrol) from two encapsulating matrixes [maltodextrin (MD) and soy protein (SP)] were evaluated for possible use in food packaging coatings. Microcapsules were prepared by oil-in-water (O/W) emulsions at different concentrations (10, 20% for MD and 2, 5% for SP). High encapsulation efficiency ranged from 96 to 99.95% for MD and 93.1 to 100% for SP, with average microcapsule diameters that ranged from 17 to 27.5 and 18.8 to 38 μm, respectively. The release rate with 20% MD-thymol [20MD-T] was faster than with 10% MD-thymol [10MD-T]. Similar results were obtained for carvacrol with the same concentration of MD. Korsmeyer–Peppas and Weibull mathematical models were successfully fitted to the release of the AM agents, describing the Fickian diffusion release of the components. Different release rates were obtained as a function of the chemical nature of the encapsulation material and its concentration.

Keywords Release antimicrobial agent · Thymol · Carvacrol · Microencapsulation

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Introduction

The consumer demand for quality, safe food that is also fresh, minimally processed, and ready to eat and has a long shelf life is a matter of paramount importance [1]. The use of AM agents inside food products to increase their shelf life is an alternative way to reduce microbiological damage to food. A new concept has been developed called active packaging, which focuses on the idea that some active interactions between the package and the product may have positive effects [2, 3]. AM packaging is one of the most interesting and promising components of active packaging that potentially allows the industry to combine the preservative functions of AM agents with protective functional foods. In most packaged foods, microbial proliferation occurs primarily on the surface; therefore, AM activity should be focused on solid or semi-solid surfaces. This activity could be achieved by either indirect contact between the AM package and the product using volatile AM releasing systems or by direct contact between the AM package and the food using non-migratory AM systems [4].

Moreover, post-processing protection using "active packaging and edible coatings" has been proposed as an innovative approach that can be applied to ready-to-eat

products to prevent or minimize the growth of pathogenic microorganisms [5, 6].

The direct incorporation of AM agents into food products results in an immediate and short-term reduction of bacterial populations, whereas AM films can maintain their activity, thus slowing down bacterial growth during extended storage after packaging [4, 7]. The use of AM packaging not only has the ability to increase product shelf life but also prevents economic loss and possible foodborne diseases [2].

Currently, a range of active additives have been successfully incorporated into packaging materials to confer AM activity, including silver-substituted zeolite, organic acids and their salts, enzymes, and plant extracts [8, 9].

Several plant essential oils are natural AM agents that have great potential as preservative ingredients and are well received by consumers. Moreover, they are generally recognized as safe (GRAS), a status defined by the Food and Drug Administration [6]. AM agents are commonly found in essential oil fractions and may have a wide spectrum of AM activity [7]. One such agent is carvacrol, a major component (50–86%) of spices such as oregano (*Origanum* sp.) that has been used for decades as a food-flavoring agent [1, 8]. Carvacrol is a phenolic compound with proven AM activity against bacteria [3, 6, 10], fungi [9, 11] and yeasts [11], showing high potential to improve the shelf life and safety of perishable foods [1, 6, 12]. This compound is hydrophobic and may dissolve in the hydrophobic domain of the cytoplasmic membrane of bacterial cells between the lipid acyl chains [13].

Another compound known for its AM properties is thymol, an isomer of carvacrol that also presents a hydrophobic nature [6, 11]. Thymol has received considerable attention as an AM agent showing very high antifungal activity [6, 9]. Several attempts have been made to develop active packaging systems in which AM agents are incorporated into polymeric materials and are slowly released onto the food surface [12, 14, 15]. Ramos et al. [6] studied the AM activity of polypropylene (PP) films containing the two active agents (carvacrol and thymol), observing significant AM activity of these films against *Staphylococcus aureus* and *Escherichia coli*, and assessed their suitability to be used as active packaging.

Great effort has been made to include these AM agents in films, mainly by coating the film [16], spraying an AM solution over the film surface, or the inclusion of AM components during the extrusion of the polymer [6]. Guarda et al. [11], incorporated microcapsules with AM agents in their optimal AM combination into a coating using the solvent evaporation technique, which could be an alternative to adding the microcapsules into the food packaging. New alternatives have also been studied in which the AM agents are micro- or nano-encapsulated in order to enhance their protecting, stability and provoke optimum AM release [14, 17].

The main objective of the present work was to study two different matrixes (MD and SP) to the microencapsulation of two natural AM agents (thymol and carvacrol) and the release kinetics of these agents from two different encapsulation materials.

Materials and methods

AM agents and chemicals

The AM agents used were thymol (≥ 99.5% pure; T0501) and carvacrol (98% pure; 282,197) were supplied by Sigma-Aldrich (Santiago, Chile). MD and SP were obtained from Dimerco Commercial Ltd. (Santiago, Chile). Tween-20 was obtained from Winkler Ltd. (Santiago, Chile); soybean oil was obtained from Nutrisa S.A. (Santiago, Chile); and sodium hydroxide (NaOH), ethanol and hexane were purchased from Merck (Santiago, Chile).

Preparation of the emulsion

The microcapsules were prepared by an oil-in-water (O/W) emulsion process [11], using two concentrations of aqueous solutions of MD (10 and 20%) and SP (2 and 5%) as encapsulating matrix. The pH of all solutions was adjusted to 6.0 with NaOH (0.1 N) using a pH meter (Hanna Instruments, HI 9321 microprocessor pH meter, Lisbon, Portugal). To form the continuous aqueous phase (70%), 0.4 g of tween-20 was added to aqueous solutions of MD and SP until obtained 19.6 g; according to the procedure described by Guarda et al. [11]. For the preparation of the dispersed oil phase (30%), 100,000 ppm of thymol and 40,000 ppm of carvacrol, were mixed separately in varying amounts of soybean oil to obtain a total of 8.6 g of the oily phase. The oily phase was added slowly to the aqueous solution. The total amount of the emulsion was 28.6 g.

The emulsion was homogenized at room temperature with an Ultra-Turrax® (T18 IKA, Staufen, Germany) with mechanical stirring at an increasing speed from 0 to $710\times g$ within 3 min (acceleration rate of $8.6\times g$ in 20 s).

Release of thymol and carvacrol from the microcapsules

The AM concentration in the aqueous medium represents the release of the AM agents from the microcapsules, was expressed as (ppm/min). Then, 24 mL of each emulsion were centrifuged (5415 D centrifuge, Eppendorf, Hamburg, Germany) for 6 min at 30 rpm. Subsequently, the supernatant (emulsion containing the AM agents) was separated

from the water left at the bottom of the Eppendorf tube, and then supernatant was washed twice with 10 mL of hexane. Next, 100 µL of the emulsion after being washed, were placed in an Eppendorf tube containing 2 mL of distilled water (aqueous medium). Then, the concentration of the AM agents released rate into the aqueous medium was evaluated as a function of time. The samples were stored at 4 °C for different periods of time during 3 days, and taken 1 mL of aqueous medium and filtered using microfilters (Millipore Millex®, Chile) of 0.22 µm. Then, 1 µL of this filtered solution was analyzed by GC-FID (flame ionization detector) to determine the AM released.

Additive quantification

Quantification of the AM agents in the emulsion was performed on a Clarus 500 GC (PerkinElmer Inc., Connecticut, USA) equipped with an FID and an EquityTM-5 fused silica capillary column (30 m × 0.32 mm × 0.25 µm film thickness). The chromatographic conditions were a modification of those used by Šegvić-Klarić et al. [18]. Helium was the carrier gas with a flow rate of 2.0 mL/min; the injector and detector temperatures were 250 and 300 °C, respectively. The oven temperature was increased from ambient to 100 °C at a rate of 8 °C/min, then increased to 250 °C at a rate of 20 °C/min and kept isothermal for 5 min. The volume of the injected sample was 1 µL with a split ratio of 1:50.

Effective concentrations of microencapsulated AM agents after losses in the process

In order to determine the final amount of the microencapsulated AM agents, the loss of AM without microencapsulation was determined according Eq. 1; described by Yang et al. [19] with slight modifications. Samples of the fractions decanted during centrifugation and washed with hexane were taken for GC analysis in order to determine the amounts of the AM agents in the water and hexane or lost in the process. All samples were analyzed in triplicate.

$$\textit{Encapsulation of efficiency}\,[\%] = \left[\frac{\textit{Total AM in the encapsulated}}{(\textit{Total AM in the emulsion} - \textit{Total AM lost in the washing process})}\right] \times 100 \quad (1)$$

Observation of microcapsules by optical microscopy

The size and the distribution of the microcapsules were observed with an optical microscope (Carl Zeiss, Standard 25 ICs, Hamburg, Germany). The emulsion containing the microcapsules was diluted with distilled water at the ratio of 1:10. A fine drop of the diluted emulsion was transferred to a microslide, smeared and then covered with a cover slip. A very thin layer diluted emulsion was formed on the surface on the microslide for a greater and clear picture. Twenty snaps were taken at different points of the smeared layer. The picture of the emulsion was taken at a magnification of 10 × 100.

Scanning electron microscopy (SEM)

SEM (Jeol, JSM-5410, Tokyo, Japan) was used to analyze the microcapsules surface. The samples were washed, with hexane was remove residual oil; the solvent was then evaporated on a rotary evaporator, and the samples were dried in an oven at 40 °C. The microcapsules were mounted in the microscope specimen chamber, covered with gold–palladium and viewed in an Anatech Hummer 6.2 Sputtering System (Hayward, CA, USA). SEM was used at a 1000× magnification and 10 kV.

Statistical analysis

A randomized experimental design was applied. Data analysis was carried out in Statgraphics Plus 5.1 V (Virginia, USA) software using analysis of variance and Student's t test. Differences were considered significant at $p < 0.05$.

Results and discussion

Size of microcapsules and loss of thymol and carvacrol in process

To determine the size of the microcapsules (Table 1), the emulsions were decimally diluted (1:10) and observed under an optical microscope (with an internal scale). This method enabled the determination of the average diameters of the microcapsules, which ranged from 17 to 27.5 µm for MD and from 18.8 to 38 µm for SP. These results are in accordance with King [17], who reported that the size in such systems ranges from 0.2 to 5000 µm. Previous studies carried out with gum Arabic [GA] as encapsulant material for thymol and carvacrol obtained microcapsules with an average diameter of 4.5 ± 0.9 µm [15].

Table 1 Composition of microcapsules, mean diameter size, and retention efficiency of the antimicrobial components

Microencapsules	Diameter (μm)	Encapsulating efficiency (%)
Maltodextrine		
10% Maltodextrine-Thymol (10MD-T)	21.6 ± 4.6[a]	99.7 ± 0.1[a]
20% Maltodextrine-Thymol (20MD-T)	21.9 ± 4.6[a]	96.5 ± 0.5[b]
10% Maltodextrine-Carvacrol (10MD-C)	21.2 ± 2.9[a]	99.86 ± 0.03[a]
20% Maltodextrie-Carvacrol (20MD-C)	22.8 ± 4.7[a]	99.93 ± 0.02[a]
Soy protein		
2% Soy protein-Thymol (2SP-T)	25.1 ± 6.3[A]	94.0 ± 0.9[A]
5% Soy protein-Thymol (5SP-T)	28.9 ± 7.4[A]	94.3 ± 1.6[A]
2% Soy protein-Carvacrol (2SP-C)	25.5 ± 6.1[A]	97.4 ± 0.4[B]
5% Soy protein-Carvacrol (5SP-C)	30.5 ± 7.5[A]	99.6 ± 0.5[B]

[a–b]Different superscripts within the same column indicate significant differences between MD formulations ($p < 0.05$)

[A–B]Different superscripts within the same column indicate significant differences between SPI formulations ($p < 0.05$)

Figures 1 and 2 obtained by SEM show the microcapsules of thymol prepared with MD (10 and 20%) and SP (2 and 5%), respectively. Different sizes of the microcapsules can be observed depending on the type of encapsulant material used. The circular forms of the SP microcapsules were not maintained after the solvent evaporation process using a rotary evaporater (Fig. 2). The microcapsules analyzed by SEM had been washed with hexane and dried in an oven at 40 °C, and this procedure could have induced coalescence of the microcapsules, increasing their diameter [20].

High encapsulation efficiency was obtained with both encapsulant materials. The encapsulation efficiency (Table 1) ranged from 96 to 99.9% for MD and from 93.1 to 100% for SP microcapsules. These results are higher than those reported by Maji and Hussain [21], who obtained encapsulation efficiencies ranging from 32.5 to 60% for microcapsules of essential oil (*Zanthoxylum limonella*) produced with chitosan and gelatin. Similar results were found by Jun-Xia et al. [22], who encapsulated sweet orange oil by hi with SP/GA and obtained a high microencapsulation efficiency (78–93%). Significant differences were found between 10% maltodextrin with thymol (10MD-T) and 20% maltodextrin with thymol (20MD-T) in terms of the encapsulating efficiency, with a reduction from 99.7 to 96.5%, respectively; that could be explained by the higher concentration of MD because changes in the emulsion properties (e.g., dispersion, stability, and solubility) have been observed when the MD concentration was increased from 10 to 20% [23]. The encapsulating efficiency was higher for carvacrol than thymol; this phenomenon could be attributed to the limited of the solubility during the emulsion process by the solid crystalline state of thymol [11].

Release of AM agents from the different encapsulation materials

The release of an active agents from an encapsulation material is a complex phenomenon that directly depends on various factors, such as the solubility, particle size, and elaboration method [11, 14, 20].

The results obtained shows that a higher content of AM agents is released when the concentration of the polysaccharide MD is increased, which was unexpected because a lower concentration of encapsulation material normally results in a higher release of AM agent because the thickness of the encapsulation material decreases the diffusion resistance [24].

Nevertheless, carbohydrates such as MD used in the microencapsulation of food ingredients reportedly have poor interfacial properties, whereas the lack of these properties can be overcome by the addition of other encapsulating materials such as proteins or gums [25].

A high release of carvacrol was obtained (2% SP with carvacrol, 2SP-C) for the lower concentrations of SP used as the encapsulating material. On the contrary, the release of thymol was higher from the 5% SP encapsulation compared to the 2% SP encapsulation. The thymol release kinetics depend on three phenomena: (1) water diffusion, (2) macromolecular matrix relaxation kinetics, and (3) diffusion of the active compound through the swollen polymeric network [6, 26]. Moreover, the solid crystalline state of thymol does not allow for fast release [16]. However, the liquid nature of carvacrol facilitates its dissemination through the encapsulant material [11]. Similar results were obtained when carvacrol and thymol agents were microencapsulated at equal proportions with GA as the encapsulated material.

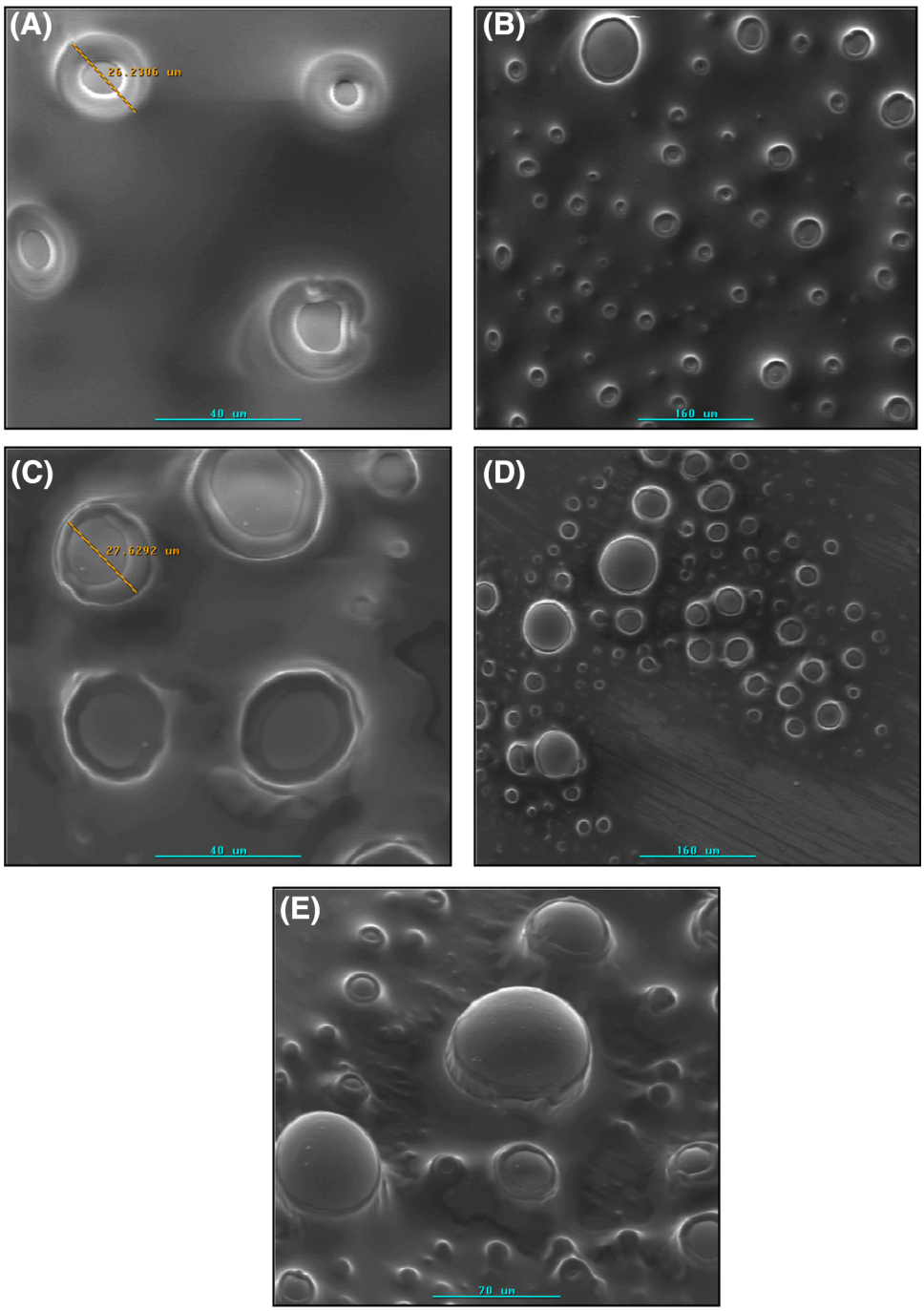

Fig. 1 SEM images of thymol (T) microencapsulated with maltodextrine (MD) material. 10MD-T 40 μm scale (**A**), 10MD-T 160 μm scale (**B**) 20MD-T 40 μm scale (**C**), 20MD-T 160 μm scale (**D**), all with 0° angle. The image (**E**) corresponds to 20MD-T 70 μm scale with 36° angle

Different mathematical models can be used in order to describe this mass transfer process (release of active agent versus time). The KP model (Eq. 2) describes the fraction of the released active agents as a polymeric system equation:

$$M_t/M_\infty = Kt^n \tag{2}$$

where M_t/M_∞ is the fraction of active agent released at time (t), K is the release rate constant and n is a release exponent that incorporates the characteristics of the macromolecular network system and the drug [27, 28]. As observed in Table 2, the release mechanism for the microencapsulated active agent does not fit well to the KP model because the KP model only describes the behavior of the diffusion process [28].

Another commonly used model is the Weibull distribution, a flexible yet simple model that is used extensively in reliability engineering for the entire release profile when the compound release follows purely Fickian diffusion.

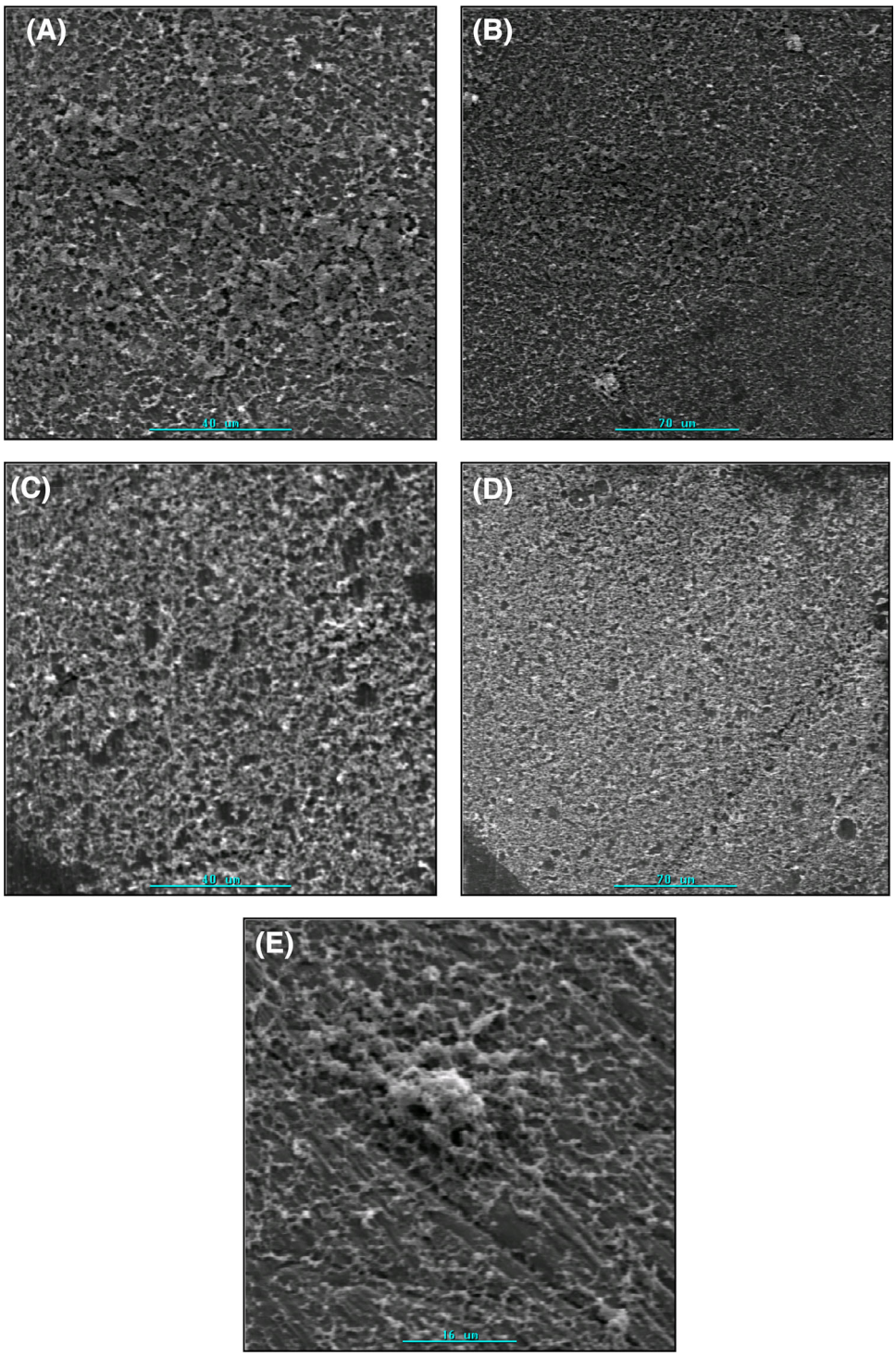

Fig. 2 SEM images of thymol (T) microencapsulated with soy protein (SP) material. 2SPI-T 40 μm scale (**A**), 2SPI-T 70 μm scale (**B**) and 5SPI-T 40 μm scale (**C**), 5SPI-T 70 μm scale (**D**), all with 0° angle. The image (**E**) corresponds to 2SPI-T 16 μm scale with 36° angle

The predicted data fit well with the experimental data (Table 2). Normally, the release rate of active ingredients from polymeric matrixes through diffusion mechanisms is determined by the porosity of the polymeric matrixes [12, 29]. When the porosity is high, a large surface area of the polymeric matrix is exposed to the diffusing medium, which will increase the diffusion of the active component.

As observed in Table 2, a faster release rate ($K = 8.00 \pm 0.62 \times 10^{-4}$) was obtained for 20MD-T compared to 10MD-T ($K = 3.73 \pm 0.32 \times 10^{-4}$). Similar results were obtained for carvacrol with the same concentration of MD as the encapsulation material ($K = 4.72 \pm 0.44 \times 10^{-4}$ for 20MD-C and $K = 1.47 \pm 0.19 \times 10^{-4}$ for 10MD-C). When the AM

Table 2 Parameters of the Korsmeyer–Peppas (K–P) and Weibull models used to fit the release of thymol and carvacrol microencapsulated by either maltodextrine (MD) or soy protein (SP)

	Parameters Korsmeyer–Peppas model				Parameters Weibull model			
	$K \times 10^{-4}$ (min^{-n})	n	R^2	*a*	*B*	*c*	*d*	R^2
Maltodextrine								
10MD-T	3.73 ± 0.32^{a}	0.19 ± 0.04^{b}	0.9456	197.6 ± 47.7	191.6 ± 73.3	0.13 ± 0.09	0.3 ± 0.2	0.9589
20MD-T	8.00 ± 0.62^{b}	0.10 ± 0.01^{a}	0.8354	167.2 ± 5.1	109.2 ± 15.9	0.08 ± 0.06	0.6 ± 0.2	0.9421
10MD-C	1.47 ± 0.19^{c}	0.17 ± 0.02^{b}	0.8548	50.9 ± 1.4	39.8 ± 3.1	0.02 ± 0.01	0.8 ± 0.1	0.9756
20MD-C	4.72 ± 0.44^{d}	0.10 ± 0.0^{a}	0.7768	96.5 ± 3.1	96.5 ± 24.7	0.3 ± 0.2	0.4 ± 0.1	0.9518
Soy protein isolated								
2SPI-T	4.53 ± 0.33^{A}	0.15 ± 0.01^{A}	0.9299	146.9 ± 7.6	108.0 ± 16.3	0.06 ± 0.05	0.5 ± 0.1	0.9575
5SPI-T	4.92 ± 0.48^{A}	0.16 ± 0.02^{A}	0.8972	167.9 ± 4.8	113.6 ± 9.3	0.013 ± 0.01	0.8 ± 0.02	0.9708
2SPI-C	3.27 ± 0.56^{B}	0.21 ± 0.03^{B}	0.8637	174.9 ± 11.9	179.3 ± 30.2	0.08 ± 0.06	0.5 ± 0.1	0.9441
5SPI-C	1.51 ± 0.29^{C}	0.26 ± 0.03^{C}	0.8230	113.4 ± 2.9	92.5 ± 4.4	0.0006 ± 0.0008	1.3 ± 0.2	0.9842

[a–d]Different superscripts within the same column indicate significant differences between MD formulations ($p < 0.05$)

[A–D]Different superscripts within the same column indicate significant differences between SPI formulations ($p < 0.05$)

agents (thymol and carvacrol) were encapsulated in the SP matrix, a different behavior was revealed. Thymol encapsulated by SP showed no significant differences in the *K* values at the two different concentrations ($K = 4.53 \pm 0.33 \times 10^{-4}$ and $K = 4.92 \pm 0.48 \times 10^{-4}$ for 2 and 5% SP, respectively) However, carvacrol encapsulated with SP presented *K* values of $3.27 \pm 0.56 \times 10^{-4}$ for 2SP-C and $K = 1.51 \pm 0.29 \times 10^{-4}$ for 5SP-C, revealing that a lower concentration of the encapsulation material resulted in a faster release of carvacrol.

In Eq. 1, *n* is a release exponent that characterizes the release mechanism, which is also dependent on the shape of the tested matrix. Based on the results presented in Table 2, thymol clearly has a faster release than carvacrol. For all systems (different encapsulant materials and different AM agents), the release was controlled by a Fickian diffusion mechanism, for which the *n* values were always lower than 0.45 [30]. Small *n* values are attributed to the diffusion of the encapsulated agent partially occurring through swollen and water-filled pores in the formulations [31]. Figure 3 shows the different release rate of the AM agents carvacrol and thymol with MD (A) and SP (B) used as the encapsulation material.

The Weibull probability density (Eq. 3) function is

$$f(t) = \beta/\alpha\,(1/\alpha)^{\beta-1} \exp\left(-(1/\alpha)^{\beta}\right) \tag{3}$$

with t, α, $\beta > 0$, and the cumulative distribution is

$$f(t) = \exp\left(-(1/\alpha)^{\beta}\right) \tag{4}$$

where α and β are the two parameters of the distribution, α is the scale parameter (a characteristic of time) and β is so-called shape parameter. The Weibull model (Eq. 4) corresponds to a concave upward curve when $\beta < 1$ and a concave downward curve when $\beta > 1$. The Weibull distribution becomes an exponential distribution for $\beta = 1$ [32].

$$f(t) = 1 - \exp(-at^{b}) \tag{5}$$

Another form of the Weibull model is represented by Eq. 5, where *a* is a constant related to the specific surface of the dosage matrix form, and *b* is a constant mainly related to the mass transport characteristics of the polymer.

Nevertheless, the Weibull model better explains the release of thymol and carvacrol from different encapsulant components such as MD and SP (Table 2). Carvacrol presented a higher release rate than thymol (Fig. 3), although the amount of carvacrol released was lower than that of thymol, which could be attributed to the liquid (and not crystallizable) behavior of thymol. Different release rates of the components can be obtained when using different encapsulant materials, with a faster release of thymol, a slower release when using AG as an encapsulant material and the slowest release when using SP [11]. The incorporation of different microcapsules on the surface of polymeric films will yield active plastic films in which the active component will be released from the microcapsules at different rates [31, 33, 34]. One of the major challenges in active packaging technology is that the release of the active component from the packaging material should be in kinetically compatibility with the packaged product, maintaining a high concentration of the active component that is above the minimum inhibitory concentration (MIC; lowest concentration of an AM that will inhibit the visible

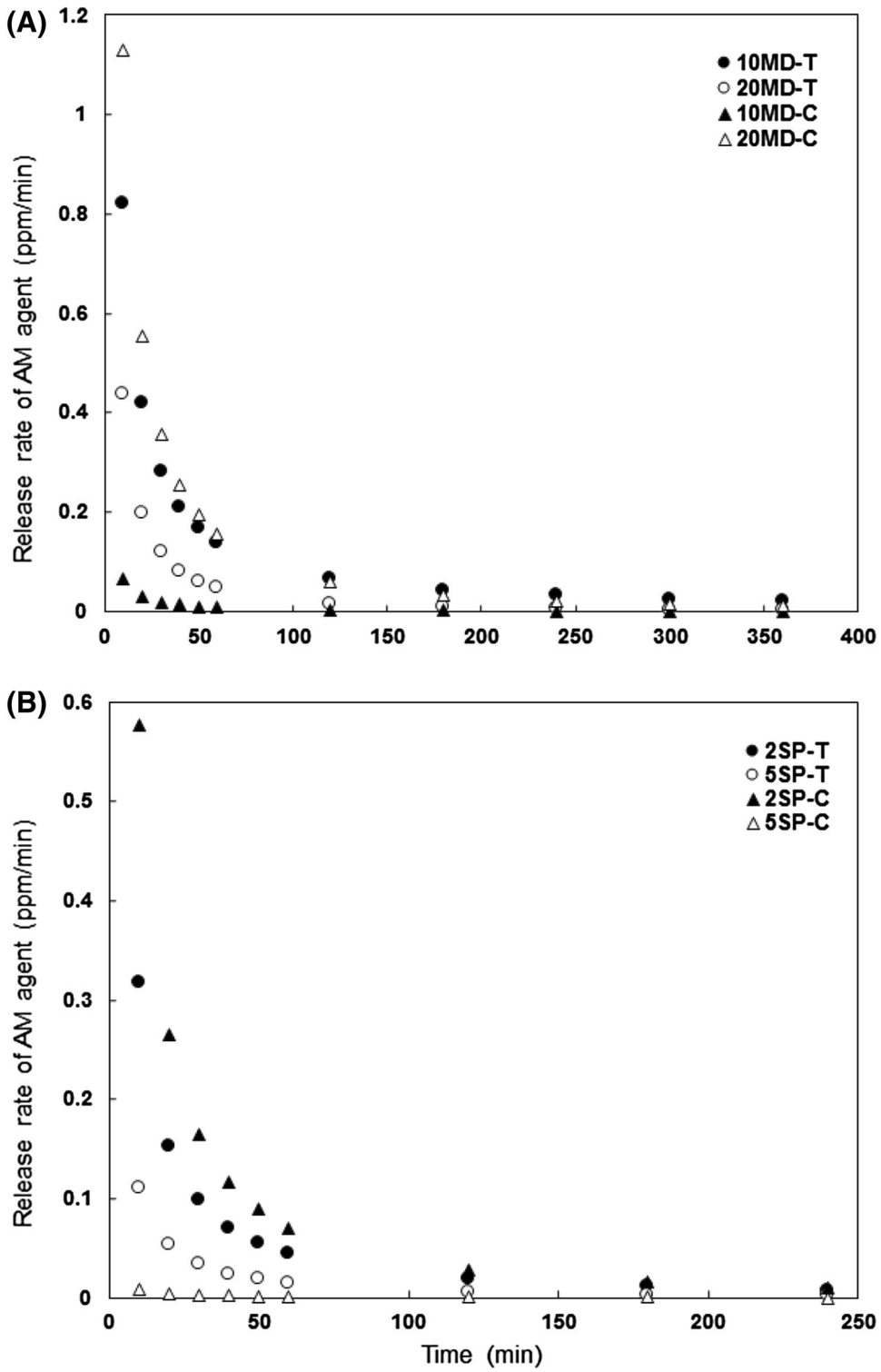

Fig. 3 Release rate of thymol (T) and carvacrol (C) agents, from different encapsulating material. (**A**) 10 and 20% for maltodextrin (MD) and (**B**) 2 and 5% for soy protein (SP)

growth of a microorganism) for a long period of time [10]. Thymol and carvacrol have had a wide application in microbiology, and the amounts of these agents released after two days ranged from 161 to 172 ppm from 10 and 20% MD microcapsules containing thymol and from 52 to 97 ppm from microcapsules containing carvacrol. According to a study by Guarda et al. [11], the required MICs for *S. cerevisiae* were 125 ppm (thymol) and 75 ppm (carvacrol), and these concentrations could inhibit this microorganism when used in the packaging of fruits and vegetables. This yeast is of great concern because it is responsible for deterioration processes. On the other hand,

the concentrations mentioned above cannot inhibit different pathogenic microorganisms such as *E. coli* (MIC of thymol: 250 ppm) or *S. aureus* (MIC of thymol: 250 ppm) according Cosentino et al. [34]. Most studies have reported that Gram-positive bacteria are more sensitive than Gram-negative bacteria to the AM action of essential oils [6, 32]. The lower sensitivity of Gram-negative bacteria to this action could be due to the outer membrane of lipopolysaccharides (LPS) that surrounds the cell wall in these microorganisms and restricts the diffusion of hydrophobic compounds. However, thymol and carvacrol are able to disintegrate this outer membrane, releasing LPS, consequently increasing the permeability of ATP in the cytoplasmic membrane and changing the passive permeability of the cell [6, 35]. Therefore, this study demonstrated that different materials used for the microencapsulation of AM agents (thymol and carvacrol) resulted in diverse characteristics of the controlled release of these agents, which are attributed to the properties of each material (MD and SP). Both the KP and Weibull models were able to explain the release kinetics of these AM agents, but the Weibull model resulted in the best fit to describe the Fickian diffusion release of the AM agents from microencapsulation. Knowledge of the release kinetics of AM agents from different matrixes is necessary in order to design new active packaging materials in which AM components can be included in the surface of the packaging material.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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