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ARTÍCULO ORIGINAL

Controlled release of phenols from chitosan-based biodegradable films enriched with turmeric (Curcuma longa) extract

Liberación controlada de fenoles a partir de películas biodegradables de quitosana enriquecidas con extracto de cúrcuma (Curcuma longa)

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ABSTRACT

To increase the antioxidant capacity of chitosan films, plant extracts and natural essential oils have been incorporated, due to the high concentration of phenolic compounds they possess. Turmeric (Curcuma longa), in addition to having high antimicrobial capacity, manifests a recognized antioxidant power. The objective of the present work was to evaluate the kinetic release of phenolic compounds from films made with different concentrations of chitosan (275 kDa) and hydroalcoholic extract of turmeric (75% ethanol, 5.5 mg/mL phenols). To prepare the films, chitosan was dissolved in 1% lactic acid and Tween 80 and turmeric extract were added to obtain films with 60-250 µg/g of phenols. The films were formed in molds and dried at 50 °C for 10 hours. The determination of phenols was carried out using the Folin-Ciocalteu method. The antioxidant capacity was evaluated using the ABTS•+ method. The release of phenols was studied in saline phosphate buffer, adjusting the data to the Korsmeyer-Peppas model to determine the kinetic mechanism of release. With the increase in phenolic content in the films with the same concentration of chitosan, it was observed that the antioxidant capacity increased. When the concentration of chitosan increased, with the same phenolic content, the antioxidant capacity of the films behaved antagonistically. The release of phenolic compounds from the films complied with a Fickian diffusion mechanism, except in the film of 1.5% (m/v) chitosan and 77 µg/g of phenols.

Keywords: active packaging; antioxidant capacity; chitosan film; controlled release; Curcuma longa; turmeric.

RESUMEN

Para aumentar la capacidad antioxidante de las películas de quitosana se ha acudido a la incorporación de extractos vegetales y aceites esenciales naturales, debido a la alta concentración de compuestos fenólicos. La cúrcuma (Curcuma longa), además de poseer alta capacidad antimicrobiana, manifiesta un poder antioxidante reconocido. El objetivo del presente trabajo fue evaluar la cinética liberación de los compuestos fenólicos desde películas elaboradas con diferentes concentraciones de quitosana (275 kDa) y extracto hidroalcohólico de cúrcuma (75% de etanol, 5,5 mg/mL de fenoles). Para la preparación de las películas se disolvió la quitosana en ácido láctico al 1% y se añadió Tween 80 y el extracto de cúrcuma para obtener películas con 60-250 µg/g de fenoles. Las películas se formaron en moldes y se secaron a 50 °C por 10 horas. La determinación de fenoles se realizó mediante el método de Folin-Ciocalteu. La capacidad antioxidante se evaluó mediante el método ABTS•+. La liberación de fenoles se estudió en buffer fosfato salino, ajustándose los datos al modelo de Korsmeyer-Peppas para determinar el mecanismo cinético de liberación. Con el aumento del contenido fenólico en las películas con una misma concentración de quitosana, se incrementó la capacidad antioxidante. Cuando aumentó la concentración de quitosana, con un mismo contenido fenólico, la capacidad antioxidante de las películas se comportó de forma antagónica. La liberación de los compuestos fenólicos desde las películas cumplió con un mecanismo de difusión fickiana, excepto en la película de 1,5 % (m/v) de quitosana y 77 µg/g de fenoles.

Palabras clave: envase activo; capacidad antioxidante; película de quitosana; liberación controlada; Curcuma longa; cúrcuma.

INTRODUCTION

Recently, technological development in food packaging has evolved to meet consumer demands for more natural methods of preservation, packaging and storage control, and ensuring food quality and safety. Among the most notable innovations are active packaging techniques, which allow the packaging to perform additional functions such as improving the quality and acceptability of the food through interactions between the packaging and the product (Wyrwa & Barska, 2017).

This interaction is achieved through migration or controlled release, where the active ingredient passes from its reservoir to the target site, providing the desired functionality. The speed of migration depends on the capacity of the matrix to retain the active compound, its characteristics, location and affinity with the food it protects (Boostani & Jafari, 2021).

Biodegradable films and coatings can be used as dispensers of active agents (antimicrobials, antioxidants, flavorings, colorants) (Gupta et al., 2022). An example is chitosan, a biopolymer derived from chitin, known for its biocompatible, biodegradable and antimicrobial properties (Hisham et al., 2024; Jiménez-Gómez & Cecilia, 2020). Although one of the most exploited properties of chitosan films is their antioxidant capacity (Muthu et al., 2021), one of the areas of recent interest is their improvement through the incorporation of plant extracts and natural essential oils (Muñoz-Tebar et al., 2021). al., 2023). The phenolic compounds present in these extracts behave as powerful natural antioxidants, contributing to the antioxidant capacity of chitosan films (Kola & Carvalho, 2023).

Among plant extracts, turmeric (Curcuma longa) stands out not only for its antimicrobial capacity, but also for its recognized antioxidant activity (Wu et al., 2024). The phenolic compounds in turmeric, especially curcuminoids, are responsible for these beneficial properties (El-Saadony et al., 2023). The incorporation of hydroalcoholic extract of turmeric in chitosan films could enhance their antioxidant activity, offering a promising alternative for applications in the food and biomedical sector (Ibáñez & Blázquez, 2020).

Taking the above into account, this work aimed to evaluate the release kinetics of phenolic compounds from chitosan films with the addition of hydroalcoholic extract of turmeric, a combination little studied in the scientific literature. Unlike previous research focused on the concentration of the active agent and its antioxidant and antimicrobial properties, this study stands out for examining the potential of chitosan films with turmeric extract as controlled release systems of phenolic compounds, highlighting the relevance of the polymer concentration on the effectiveness of the release of active agents.

METHODOLOGY

Materials

The chitosan used in this study was obtained by N-deacetylation of common lobster chitin, with a molecular mass of 275 kDa, supplied by the Center for Research and Development of Medicines (Havana). 90% lactic acid (Merck, Germany) and Tween 80 (Acros Organics, Belgium) were used. The hydroalcoholic extract of turmeric, prepared with 75% ethanol and with a phenolic content of 5.5 mg/mL, was used to enrich the chitosan films. Solutions were prepared and handled using phosphate buffered saline with a pH of 7.4 and appropriate glassware. The characterization of the samples and quantification of phenols were carried out using a UV-Visible spectrophotometer (Shimadzu, model UV 2401-PC).

Preparation of the films

For the preparation of the films, the methodology reported by Rodríguez et al. was followed. (2021). A 1% (v/v) lactic acid solution was prepared, to which chitosan was added to form 1.5 and 2.0% (m/v) solutions. The chitosan dissolution was carried out by magnetic stirring at 1,000 rpm for approximately 2 h. Subsequently, 0.1% (v/v) Tween 80 was incorporated as a surfactant. Then, the suitably diluted hydroalcoholic extract of turmeric was added to obtain films with a phenol concentration between 60 and 250 µg/g. Once the solution was homogenized, it was filtered to eliminate insoluble impurities. Subsequently, the solution was poured into appropriate molds and allowed to rest for one hour. Then, it was placed in an oven at 50 ºC for 10 h to complete the formation of the films. Finally, the films were removed from the molds and stored in a desiccator until the experiments were carried out.

Linear correlation between absorbance at 425 nm and phenol content

Considering that curcuminoid-type phenolic compounds can be determined directly by spectrophotometric measurement at 425 nm (Kotra et al., 2019), a linear correlation analysis was carried out between the absorbance at this wavelength and the phenol content determined by the Folin-Ciocalteu method (Slinkard & Singleton, 1977). For this, dilutions of turmeric extract (5.5 mg of phenols/mL) were prepared to obtain a concentration range of 0.14 to 2.2 µg/mL. The absorbance at 425 nm was then measured for different

concentrations within this range. Finally, a linear regression analysis was performed between absorbance and phenol content.

Determination of phenolic content in films

To determine the total phenolic content in the films using the Folin-Ciocalteu method (Slinkard & Singleton, 1977), a 4 cm2 portion was taken and weighed on an analytical balance. The sample was dissolved in 5 mL of distilled water and the absorbance was then measured at 425 nm. The concentration of phenols was estimated from the regression equation that was obtained as described in the previous section. To calculate the phenol content in the film, the following equation was used:

$$Fenols\ (\mu g) = \frac{A_{425\ -\ a}}{b} \cdot v \qquad \text{Equation 1}$$

Where: A425, absorbance measured at 425 nm; a and b, intercept and slope of the regression equation obtained in the previous section, respectively; v, extraction volume (5 mL).

Determination of the antioxidant capacity of the films

To determine the antioxidant capacity of the films, the methodology described by Re et al. (1999) was followed, which is based on the generation of ABTS•+ radical ions through the reaction between potassium persulfate and ABTS [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)]. The radical ion, in contrast to the neutral molecule, has a blue-green color with a characteristic absorption at 734 nm (Van Den Berg et al., 1999). The addition of antioxidant substances reduces ABTS•+ radicals to neutral molecules, a reaction that depends on the nature and concentration of the antioxidants and duration. Therefore, the magnitude of discoloration is a function of the concentration of the antioxidants for a specific reaction time.

The test consisted of adding 1 mL of the ABTS•+ solution in phosphate buffered saline pH 7.4 (1 ± 0.02 AU) to a test tube containing 100 µL of the film sample (0.03 g of film dissolved in 5.0 mL of water) and another with 100 µL of distilled water as blank. The reaction was allowed to pass for 10 min at room temperature and then the absorbance of both was read at 734 nm. The calibration curve was prepared in the concentration range between 2 and 10 mg/100 mL of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The variation in absorbance (▫A = Asample - Areference) is proportional to the concentration of Trolox. The results were expressed as Trolox equivalents in mg/g film.

Release study

The release of phenolic compounds was evaluated with chitosan films (1.5 and 2.0% m/v) with different phenolic contents. For this, 4 cm2 portions of films were taken, which were placed in 75 mL capacity beakers with 25 mL exactly measured, of pH 7.4 saline phosphate buffer. At regular time intervals (1, 3, 5, and 7 h), aliquots were taken to measure absorbance at 425 nm. The calculation of the mass of phenols released was carried out using equation 1, considering 25 mL as the value of the volume where the film was immersed (v). The percentage release was calculated using the following equation:

$$Release\ (\%) = \frac{\text{Mass of phenols released in a specific time } (\mu g)}{\text{Total mass of phenols in the film } (\mu g)} \cdot 100 \qquad \text{Equation 2}$$

Determination of the possible release kinetic mechanism

The migration data of phenolic compounds were fitted to the linear transformation of the model proposed by Korsmeyer et al. (1983), which is especially useful when the release mechanism is unknown or when this release occurs through more than one mechanism. The Korsmeyer-Peppas model and its linear transformation are shown in equations 3 and 4, respectively.

$$\frac{M_t}{M_{Total}} = k \cdot t^n \qquad \text{Equation 3}$$

$$\log \frac{M_t}{M_{Total}} = \log k + n\ log\ t \qquad \text{Equation 4}$$

Where: Mt, mass of phenols (µg) released at time t; MTotal, total mass of phenols (µg) in the film; n, diffusion coefficient; k, kinetic constant, dependent on the characteristics of the active ingredient.

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According to Higuchi & Higuchi (1960), the release mechanism of the active ingredient from the polymer matrix can be deduced from the value of ¤: a value of ¤ < 0.5 indicates a Fickian diffusion, characterized by a constant release of the active ingredient ; a value between 0.5 < ¤ < 1 corresponds to a non-Fickian diffusion, controlled by the swelling and relaxation of the polymer chains (case II transport); and a value of ¤ > 1 suggests diffusion with very high release times, known as super case II transport. In addition, the values of the linear regression coefficient (R2) were taken into account to check the fit of the values to this model.

Statistical analysis

Analysis of variance conducted, and when significant differences were found, Duncan's multiple range test was utilized to identify the specific differences with a 5% significance level. The analysis was performed using the Statistics program (version 7, 2004, StatSoft Inc., Tulsa, USA).

RESULTS AND DISCUSSION

Linear correlation analysis between absorbance at 425 nm and phenol content

The results of the linear regression analysis between the absorbance measured at 425 nm and the concentration of phenols are shown below:

A425 = 0.0010 + 0.1487 B; R2 = 0.998 (in distilled water) Equation 5

A425 = - 0.0138 + 0.0973 B; R2 = 0.988 (in buffer pH 7.4) Equation 6

Where: A, absorbance; 425, wavelength; B, phenol concentration (µg/mL).

Both models presented regression coefficients greater than 0.95, so they could be used to estimate the concentration of phenols from the measurement of absorbance at 425 nm. Equation 5 was used to calculate the concentration of phenols in the films with a chitosan concentration between 1.5 and 2% (m/v), since the sample, in this case, was dissolved in distilled water, while the Equation 6 was used to calculate the concentration of phenols in the experiments related to the release of these compounds.

Phenol content and antioxidant capacity of chitosan films

Table 1 presents the phenol concentrations of 1.5 and 2% (m/v) chitosan films. The differences respond to the normal variability in the manual preparation of chitosan films. These data allow the evaluation of the antioxidant capacity based on the phenol content, as well as the study of migration.

The antioxidant capacity follows a similar trend to the concentration of phenols, being higher at chitosan concentrations of 1.5% (m/v). However, as with the concentration of phenols, the antioxidant capacity decreases significantly at a concentration of 2.0% (m/v) of chitosan. An increase in chitosan concentration did not result in a proportional improvement, but in fact decreased the antioxidant capacity. This suggests that there is an optimal point in chitosan concentration to maximize antioxidant capacity, beyond which the beneficial effect diminishes. This relationship between the concentration of chitosan, the concentration of phenols and the antioxidant capacity of the films could be influenced by the interaction between components and structure of the film.

Table 1. Phenol content and antioxidant capacity of chitosan films

Chitosan (% m/v)	Phenol concentration (µg/g)	Antioxidant capacity (mg/g)*
1.5	0	2.72
	77	3.09
	154	4.37
	231	4.72
2.0	0	2.76
	68	2.89
	136	3.06
	204	3.32

* Expressed as Trolox equivalent per g of film.

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The antioxidant capacity of both films (1.5 and 2.0% m/v chitosan) followed a linear behavior with the increase in the concentration of phenols from the turmeric extract (Figure 1). Such behavior, despite being linear, was not expected, since taking into consideration that both films without the addition of turmeric, presented similar antioxidant capacities (2.72 and 2.76 mg Trolox equivalents/g film, respectively), it was expect similar values for the same concentration of phenols. However, this did not happen as shown in Fig. 1. It is observed that in the 1.5% (m/v) chitosan film, the antioxidant capacity increased more than that in the 2.0% (m/v) film, with the increase in phenolic concentration. It is possible that an antagonistic effect occurred between chitosan and phenolic compounds, which depends on the concentration of chitosan in the film. Parize (2012) evaluated the antioxidant activity of chitosan films at 2.0% (m/v) with the incorporation of turmeric as a colorant, with a slight discoloration of the DPPH radical occurring.

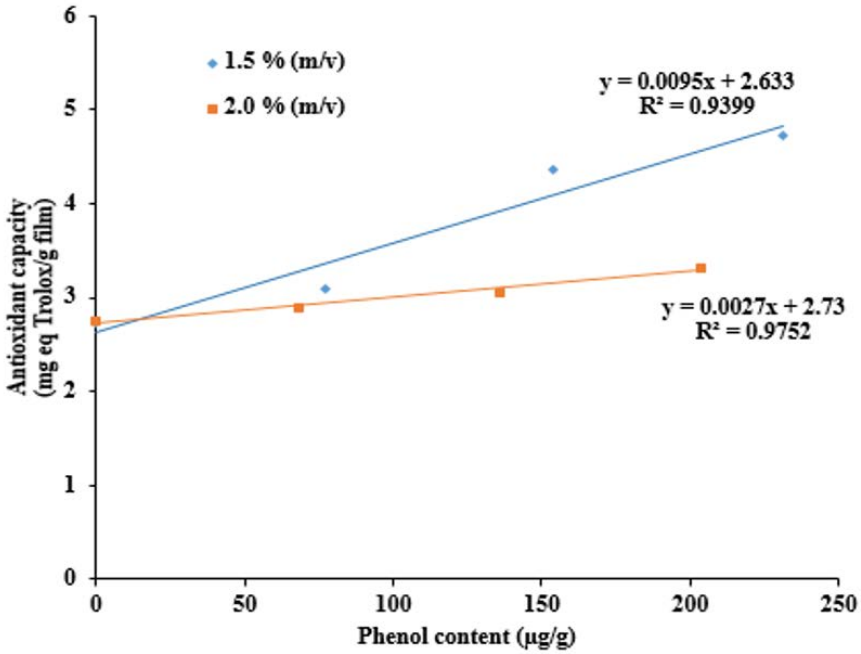

Figure 1. Behavior of the antioxidant capacity of chitosan films as a function of the concentration of phenols.

Although Portes et al. (2009) evaluated the release of two tetrahydrocurcuminoid derivatives from chitosan films at 2.0% (m/v) and reported that the antioxidant capacity of the films increased with respect to the control; in the rest of the literature consulted, this was not found information about this behavior, since research focuses on the variation in the concentration of the active agent, but does not study how it is the same as a function of the concentration of the film-forming polymer.

This result may have connotation from a practical point of view, since the addition of turmeric extract to improve antioxidant activity, a property of interest for its application as active packaging, may be affected by the increase in the percentage of chitosan in the film.

Determination of the possible release kinetic mechanism

The release (Table 2) was higher in the 1.5% (m/v) chitosan films, reaching values of up to almost 40%. On the other hand, this did not occur in the 2.0% (m/v) films, since after 7 h approximately 10% of the added phenols migrated. Similar behavior was reported by Vidyalakshmi et al. (2004), who obtained that with increasing chitosan concentration, the release percentages of curcumin decreased, with migration rates around 8% in chitosan films with PVA and glycerin at pH 7.4.

In the 1.5% (m/v) chitosan films, the initial concentration of phenols influenced the release percentage, observing that at lower concentrations, the release percentages of phenolic compounds increased. In this regard, Suwantong et al. (2007), when studying the release of curcumin and other phenolic compounds of turmeric from cellulose acetate fibers and films, observed that as the concentration of curcumin increased, migration occurred more slowly.

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Table 2. Behavior of phenol migration with time in chitosan films at 1.5 and 2.0% (m/v) with different concentrations of phenols

Chitosan (% m/v)	Phenols (µg/g)	Time (h)	Phenols (µg)	Release (%)
1.5	77ǂ	1	20.7	18.87 (0.1) d
		3	31.3	28.53 (0.5) c
		5	42.5	38.74 (0.3) a
		7	42.9	39.11 (0.6) a
	154†	1	18.1	8.58 (0.2) lm
		3	27.7	13.13 (0.4) f
		5	33.2	15.73 (0.04) e
		7	33.5	15.88 (0.1) e
	231‡	1	14.9	5.42 (0.07) p
		3	18.7	6.80 (0.2) o
		5	22.9	8.33 (0.1) mn
		7	26.8	9.75 (0.01) ij
2.0	68§	1	8.7	5.71 (0.5) p
		3	9.6	6.31 (0.2) o
		5	12.9	8.48 (0.2) lm
		7	14.6	9.60 (0.1) ij
	136£	1	13.3	7.93 (0.09) n
		3	15.5	9.24 (0.2) jk
		5	16.8	10.01 (0.03) i
		7	20.9	12.46 (0.5) g
	204¥	1	16.7	8.67 (0.07) lm
		3	17.2	8.93 (0.1) kl
		5	18.0	9.34 (0.3) jk
		7	21.8	11.32 (0.4) h

Mean (Standard deviation); n = 3.
Different letters indicate significant differences ($p \leq 0.05$).
ǂFilm with total mass of phenols (M_{Total}) equal to 109.7 µg; †Film with M_{Total} = 211 µg; ‡Film with M_{Total} = 274.9 µg; §Film with M_{Total} = 152.1 µg; £Film with M_{Total} = 167.8 µg; ¥Film with M_{Total} = 192.6 µg.

Another factor that could influence the release rate of the added bioactive compounds was the pH of the simulant medium. This was reported by Shu et al. (2001), who evaluated chitosan films cross-linked with sodium citrate as potential systems for controlled release. These researchers observed that at pH 1.2 the films dissolved, releasing all the content into the medium, while with the gradual increase in pH to values of 7.4, the system tended to modulate the release, with slow migrations occurring.

Table 3 presents the results of the linear regression analysis based on the phenolic migration data as a function of time that allowed the linear transformation of the Korsmeyer-Peppas model.

Table 3. Parameters of the model log Mt/Mtotal = log k + n log t for the 1.5 and 2.0% (m/v) films with different concentrations of phenols

Chitosan (% m/v)	Phenolic compounds (µg/g)	n	k	R^2
1.5	77	0.5895	0.1243	0.9974
	154	0.3354	0.0876	0.9714
	231	0.2944	0.0525	0.9624
2.0	68	0.2642	0.0542	0.8538
	136	0.2064	0.0769	0.8729
	204	0.0702	0.0853	0.7871

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It is observed that the n values decreased as the phenolic content increased in both films. In the case of the film with the lowest content of chitosan and turmeric phenols, the release of the active compound occurred through a non-Fickian diffusion mechanism (0.5 < n < 0.1), while in the rest of the films, a Fickian diffusion occurred (n < 0.5).

This non-Fickian diffusion could be due, according to Mauro (2021), to the fact that once the film came into contact with the simulant, a series of mass transport phenomena occurred (Torres et al., 2017). First, the pores on the surface of the matrix were filled with water and as the concentration of the bioactive was low, it partially dissolved in the simulant and then diffused into the medium.

When a release is associated with a non-Fickian diffusion mechanism, it is due to the effect of viscoelastic relaxation of the polymer chains and loss in their mechanical properties (De Kee et al., 2005), producing a sustained release over time, depending on the thicknesses of the films (Arya et al., 2021). However, in a release associated with a Fickian diffusion mechanism, the bioactive compounds are released constantly over time from the polymer matrix, guaranteeing their presence at all times.

In turn, the release mechanism of the active compounds may depend on other properties of the system. According to Parize (2012), the release mechanism of the turmeric colorant depended on the presence or absence of the plasticizer used in the formation of the films, as well as the pH at which the experiment was carried out. In the films with acetic acid and in those without plasticizer at pH 1.2, it was observed that the release occurred through a non-Fickian mechanism.

CONCLUSIONS

The increase in phenolic content in films with the same concentration of chitosan increased their antioxidant capacity. However, when the concentration of chitosan was increased while keeping the phenolic content constant, an antagonistic effect was observed on the antioxidant capacity of the films. Furthermore, the release of phenolic compounds in the films followed a Fickian diffusion mechanism, except in the film with a chitosan concentration of 1.5% (m/v) and 77 µg/g of phenols.

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Conflicts of Interest:

The authors declare that they have no conflicts of interest.

Author Contributions:

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