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Application of the galactomannan gel from *Cassia grandis* seeds for biomedical purposes: Study of the incorporation of collagenases and their release profile

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ABSTRACT

The galactomannan-based gel from *Cassia grandis* seeds was used to incorporate *Penicillium* sp. UCP 1286 and commercial collagenases. Experiments were carried out according to a 2^3-full factorial design to identify the most significant parameters for the incorporation process. The pH of the incorporation solution (pH_i), stirring time (t), and initial protein concentration in the crude extract (PC_i) were selected as the three independent variables, and the efficiency of collagenase incorporation (E) and collagenolytic activity (CA) after 360 min as the responses. pH_i and PC_i showed positive statistically significant effects on E, while CA was positively influenced by pH_i and t, but negatively by PC_i. The fungi collagenase was released from the gel following a pseudo-Fickian behavior. Additionally, no <76 % of collagenase was efficiently incorporated into the gel retaining a high CA (32.5–69.8 U/mL). The obtained results for the commercial collagenase (E = 93.88 %, CA = 65.8 U/mL, and n = 0.10) demonstrated a pseudo-Fickian behavior similar to the fungi-collagenase. The results confirm the biotechnological potential of the gel as an efficient matrix for the incorporation of catalytic compounds; additionally, the incorporation of collagenases was achieved by retaining the proteases CA and releasing them in a controlled manner.

1. Introduction

Collagenases are proteases able to degrade several types of collagens. Due to their rigid triple helical structure, collagens are in fact resistant to the common proteases but can be readily cleaved by the site-specific action of collagenases (Bhagwat & Dandge, 2018). Collagenases from animal sources have very specific recognition sites, whereas the microbial ones have broad substrate specificity that helps them to degrade

Abbreviations: pHi, hydrogen potential of incorporation solution; t, stirring time; *PCi*, initial protein concentration; E, efficiency of collagenase incorporation; CA, collagenolytic activity; p.a, propanone; Mf, final mass of polysaccharide; Mi, initial mass of raw material; U/mL, units per milliliter; Qt, cumulative quantity of drug released; mM, molar mass; UCP, Catholic University of Pernambuco; UV–Vis, ultraviolet and visible; U, enzymatic activity unity.

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both water insoluble and soluble collagens in their triple helical regions at the X-Gly bond (Bhagwat & Dandge, 2018).

Due to their distinct activities, microbial collagenolytic proteases are employed in many industrial and biotechnological activities, although their most important applications are therapeutic including debridement of burns, wound healing, and treatments of sciatica, retained placenta, lumbar disc herniation, chronic total occlusion, Peyronie's, and Dupuytren's diseases (Alipour, Raz, Zakeri, & Dinparast Djadid, 2016).

Evidence has suggested that collagenase could break peptide bonds, especially in collagen to destroy the thick extracellular matrix structures. This process can enhance the interstitial diffusion of drugs in various types of solid tumors (osteosarcoma, mammary, colon and liver); collagenases have been incorporated into various materials and used as delivery system for improving the intratumoral distribution and penetration of chemotherapeutic drugs within the tumor tissue (Abdolahinia et al., 2019; Alipour et al., 2016; Wang et al., 2018; Yin et al., 2023).

Among the microorganisms, filamentous fungi have distinct advantages as enzyme producers, among which are high productivity and low production costs, quick production processes, and easy modification of their enzymes. Moreover, their hydrolytic enzymes are released extracellularly, which makes their recovery from fermentation broths particularly easy (Lima et al., 2011).

Studies have revealed a diverse biota in the Caatinga biome with potential for the prospecting sector thanks to the presence of microorganisms with notable features to produce secondary metabolites, among which filamentous fungi stand out. Research carried out by our research group has demonstrated the potential of *Penicillium* sp. UCP 1286 isolated from the Caatinga soil in producing collagenase with high yields and ideal physicochemical characteristics for biomedical and biotechnological applications (Wanderley et al., 2016).

Polysaccharides are defined as complex carbohydrates that exist throughout nature, mainly in seed plants, from which they can be obtained with high yields (Albuquerque et al., 2022). They have been reported as developing several matrices, including hydrogels, which are three-dimensional natural polymeric networks with high hydrophilic behavior and excellent swelling ability (Nayak, Hasnain, & Aminabhavi, 2021). Nowadays, they are of great interest in various biomedical applications, for example, in the wound repair process, where they can perform several key functions, acting as a lubricant and an immunologic, transport, structural, and delivery agent (Albuquerque et al., 2023).

When considering the controlled release of substances from natural systems, carbohydrate-based matrices, for example, polysaccharide hydrogels, have recently been the focus of drug delivery research (Putro et al., 2023). Their strengths over synthetic polymers are related to their sustainable production from natural resources, economic extraction, biocompatibility, tunable biodegradability, and good rheological properties (Andrade Del Olmo et al., 2023).

The polysaccharide-type galactomannan extracted from the seeds of *Cassia grandis* collected in Pernambuco, in the Northeast region of Brazil, has already been extensively characterized (Albuquerque et al., 2014) by physicochemical and rheological analyses, and tested as a matrix for the immobilization of biomolecules (Albuquerque, Cerqueira, Vicente, Teixeira, & Carneiro-da-Cunha, 2017) and healing agent (Albuquerque et al., 2017). Rheological studies revealed that the concentration of 1.6 % (*w/v*) represents the solution-gel transition, and the specific concentration of 1.7 % (w/v) was reported as a weak gel with high transparency and promising biotechnological applications (Albuquerque et al., 2016). In addition, this polysaccharide is an energy reserve in the seeds of *C. grandis* and, therefore, is easily accessible and inexpensive to obtain (Albuquerque et al., 2023).

Considering the above-mentioned biological activities and its inherent characteristics (high molar mass, water solubility, and a solution-gel transition at 1.6 %, w/v), the galactomannan from *C. grandis* appears to have great potential to be explored as a drug delivery system; thus, the incorporation of catalytic compounds, for example, fungi and commercial collagenases, depicts the gel as a promising support for biomedical purposes.

Taking all the aforementioned into account, the novelty of this work is to develop a natural healing gel for the incorporation of collagenases. For that, the galactomannan gel from *C. grandis* was used as a matrix for the incorporation of both the commercial collagenase and the one produced by the *Penicillium* sp. UCP 1286 isolated from the Caatinga soil, and their pattern of release was evaluated.

2. Materials and methods

2.1. Materials

Cassia grandis seeds were collected in Recife (PE, Brazil, 8°02′50.0"S 34°57′00.1"W) in May 2022. Ethanol 99.8 %, acetone *P*.A., gelatin and sodium chloride were obtained from Vetec Fine Chemicals (Rio de Janeiro, RJ, Brazil), while commercial collagenase and azocoll from Sigma-Aldrich (St. Louis, MO, USA). Malt extract agar and peptone were obtained from Kasvi. All other chemicals were of analytical grade.

2.2. Microorganism

The strain *Penicillium* sp. UCP 1286, isolated from the soil of Caatinga biome in Serra Talhada (PE, Brazil) was obtained from the Catholic University of Pernambuco Collection (UNICAP).

2.3. Culture medium

The medium used for maintenance was malt extract agar containing 0.5 % malt extract, 0.1 % peptone, 2.0 % glucose, and 1.5 % agar. The culture medium used for enzyme production was composed of 5 g/L gelatin, 0.025 g/L $MgSO_4.7H_2O$, 1.5 g/L K_2HPO_4, 0.015 g/L $FeSO_4.7H_2O$, 0.025 g/L $CaCl_2$ and 0.005 g/L $ZnSO_4.7H_2O$, pH 7.0, and 1 % (*v*/v) mineral solution, according to Wanderley et al. (2016). The mineral solution was prepared by adding 100 mg $FeSO_4{\cdot}7H_2O$, 100 mg $MnCl_2{\cdot}4H_2O$, 100 mg $ZnSO_4{\cdot}H_2O$ and 100 mg $CaCl_2{\cdot}H_2O$ to 100 mL of distilled water. Both media were sterilized in autoclave at 121 °C for 15 min.

2.4. Collagenase production

Collagenase production was carried out according to Wanderley et al. (2016). Spores of the strain *Penicillium* sp. UCP 1286 were produced in agar plates containing a cell culture grown for 5 days at 28 °C and then suspended in 3 mL of a 0.9 % (*w/v*) NaCl and 0.01 % (v/v) Tween 80 solution previously sterilized at 121 °C for 20 min. After inoculation with 10^6 spores/mL, fermentations were carried out at 24 °C and 150 rpm in 250 mL Erlenmeyer flasks containing 50 mL of the culture medium. The broth obtained at the end of fermentation (126 h) was vacuum filtered through nitrocellulose membranes with 0.45 μm pore diameter to remove the mycelium. Since the target collagenase is an extracellular enzyme, the filtrate was analyzed for final protein concentration and collagenase activity and used as an enzyme source (referred to as the crude extract). Thus, the crude extract was lyophilized at −20 °C, 500 mmHg and 24 h (Liotop, Liobras, Brazil) and used for the experiments, which were performed with the same sample of the concentrated crude extract.

2.5. Galactomannan extraction and gel production

The galactomannan was obtained according to the methodology described by Albuquerque et al. (2014). Briefly, the polysaccharide was obtained by aqueous extraction, precipitation with 46 % ethanol 1:3 (*v*/v) and washing with alcohol p.a. and acetone p.a. [1:1 (v/v)]. The

extraction yield was calculated by dividing the final mass of polysaccharide (M_f) by the initial mass of raw material (M_i) and expressed as %. The galactomannan gel was produced according to Albuquerque et al. (2016), at room temperature (22 ± 1 °C), from a 1.7 % (*w/v*) aqueous solution of the polysaccharide under constant magnetic stirring (250 rpm) (Magnetic stirrer CE-1540/P, Cienlab, Brazil) for 18 h.

2.6. Incorporation of collagenase

2.6.1. Obtaining and incorporation of Penicillium sp. UCP 1286 collagenase

A 2^3-two-level full factorial design was employed to study the effects and interactions of three independent variables, namely time of collagenase and galactomannan gel stirring (*t*, min), pH of incorporation solution (pH_i), and initial protein concentration in the crude extract (PC_i, mg/mL), on the efficiency of collagenase incorporated into the galactomannan gel (*E*, %) and the collagenolytic activity after 360 min (*CA*, U/mL), which were selected as the responses. Experiments were carried out at all combinations of the levels given in Table 1.

The central point was run in quadruplicate, to provide an estimate of the pure error variance of the responses. In the case of 2 k statistical factorial design, the great advantage of adding central points in factorial experiments where there are no tests for the factor points is that: if there are repeated measures only at the central point, we can have a probability of experimental error (pure error) and verify the trend of linearity. Additionally, it is possible to perform an analysis of variance to test the lack of fit in the statistical model. In this way, there is an economy of time and cost in the processes (Lundstedt et al., 1998).

For model fitting, the variables were coded according to the following Eq. (1):

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad (1)$$

where x_i is the coded value corresponding to the X_i actual value, X_0 the average of the two extreme levels and ΔX_i the range of variation of the i-th factor and the model was defined through the following Eq. (2):

$$\hat{y} = b_0 + \sum b_i x_i + \sum b_y x_i x_j \quad (2)$$

where is the predicted response, b_0 is the constant term, b_i are the linear coefficients, b_y are the interaction coefficients and x_i and x_j are the coded values of the independent variables *i* and *j*, respectively. The relative significance of all coefficients was assessed from the error estimate based on the third-order term at 95 % confidence level. The goodness of fit of the model was evaluated by the coefficient of determination (R^2) and the analysis of variance (ANOVA); the first-order model equation was determined by Fischer's test. The relative significance of all coefficients was assessed from the error estimate based on the third-order term at 95 % confidence level. Statistica 8.0 software (Statsoft Inc., 2015, São Paulo, SP, Brazil) was used to handle all statistical analyses.

The incorporation system was prepared in a 5 mL Falcon tube, with 1 mL of the galactomannan gel and 1 mL of collagenase (0.3, 0.5, and 0.7 mg/mL) in 0.1 M Tris-HCl buffer pH 7.0; this mixture was shaken at 250 rpm, between 2 and 4 °C, for different stirring times (30, 60, and 90 min). Then, the system was wrapped with a silkscreen cloth (90 thread type), submerged in 8 mL of the incorporation solution (0.1 M potassium phosphate buffer at pH 6,0, 7,0, and 8,0), and kept under constant magnetic stirring for 360 min under the same conditions as above. At specific times (0, 60, 180 and 360 min), 1 mL was collected from the incorporation solution and replaced by the same volume of 0.1 M potassium phosphate buffer. The collected samples were evaluated for protein concentration (Smith et al., 1985) and collagenolytic activity (Chavira, Burnett, & Hageman, 1984) to know the amount and activity of collagenase released from the gel at specific times (please see Section 2.8 for more details).

Table 1
Factor levels used in the 2^3-two-level full factorial design to investigate the incorporation of *Penicillium* sp. UCP 1286 collagenase on the galactomannan gel.

Factor	Lower (−1)	Central (0)	Higher (+1)
pH_i[a]	6.0	7.0	8.0
t (min)[b]	30	60	90
PC_i (mg/mL)[c]	0.30	0.50	0.70

[a] pH of the incorporation solution.
[b] Time of collagenase and the galactomannan gel stirring.
[c] Initial protein concentration in the crude extract.

According to the drug release kinetics studied by Higuchi (Arruda et al., 2021; Korsmeyer & Peppas, 1983; Simi & Abraham, 2010), the release behavior is a dependent diffusion process based on the Fick's law; therefore, it is possible to build up a linear graph of the cumulative quantity of drug released (*Qt*) versus the square root of time (*t*) (Eq. (3)), whose slope characterizes the release kinetics:

$$Qt = Kh \times t^{1/2} \quad (3)$$

where *Kh* is the Higuchi dissolution constant. However, under some experimental situations, the release mechanism deviates from the Fick's equation. In this case, Korsmeyer and Peppas (1983) developed a simple, semi-empirical model relating the drug exponential release to the elapsed time. It is then possible to get a power law where *Qt* is proportional to *t* according to the exponential Eq. (4):

$$Qt = A \times t^n \quad (4)$$

where *A* is a constant incorporating geometric structure features and *n* is the release exponent that accounts for the release rate mechanism.

2.6.2. Stability of free Penicillium sp. UCP 1286 collagenase

The stability of the free enzyme over time was performed under the same conditions as the run 8 of the factorial design. 1 mL of free *Penicillium* sp. collagenase (0.7 mg/mL) in 0.1 M Tris-HCl buffer, pH 7.0, was shaking at 250 rpm, between 2 and 4 °C, for 90 min. Then, the solution was submerged in 9 mL of the 0.1 M potassium phosphate buffer, pH 8.0. At specific times (0, 60, 180, and 360 min), 1 mL was collected and replaced with the same volume of 0.1 M potassium phosphate buffer. The collected samples were evaluated for protein concentration and collagenolytic activity according to Section 2.8.

2.6.3. Incorporation of the commercial collagenase into the galactomannan gel

The profile of commercial collagenase release from the galactomannan gel was performed under the same conditions as run 8 of the factorial design for the *Penicillium* sp. Collagenase (topic 2.6.1). The incorporation system was prepared by mixing 1 mL of the galactomannan gel and 1 mL of the commercial collagenase (0.7 mg/mL) in 0.1 M Tris-HCl buffer, at pH 7.0, and 250 rpm, between 2 and 4 °C, for 90 min. Then, the system was wrapped with a silkscreen cloth (90 thread type) and submerged in 8 mL of the incorporation solution (0.1 M potassium phosphate buffer, pH 8.0). At specific times (0, 60, 180, and 360 min), 1 mL was collected from the incorporation solution and replaced with the same volume of 0.1 M potassium phosphate buffer. The collected samples were evaluated for protein concentration and collagenolytic activity according to Section 2.8.

2.7. Determinations of the percentage of incorporated collagenase, collagenolytic activity and release exponent

The percentage of collagenase incorporated into the galactomannan gel (*E* %) was calculated by Eq. (5):

$$\left(1 - \frac{PC_o}{PC_i}\right) \times 100 \quad (5)$$

where PC_o is the protein concentration in the incorporation solution at the beginning (0 min) and PC_i is the initial protein concentration (0.3, 0.5, or 0.7 mg/mL).

CA was defined as the collagenolytic activity of collagenase released from the gel after 360 min of incorporation, while the release exponent (*n*) was calculated by linear regression analysis using GraphPad Prism software (version 5.00, 2007).

2.8. Analytical methods

To assess the collagenolytic activity (*Penicillium* sp. and commercial collagenase), the Azo dye-impregnated collagen assay (Azocoll, Sigma Chemical Co., St. Louis, MO, USA) was used according to a modified version of the method developed by Chavira et al. (1984). Azocoll was washed and suspended in 0.05 M Tris–HCl buffer (pH 7.2) containing 1 mM $CaCl_2$ up to a final concentration of 5 g/L. Subsequently, 150 μL of the crude extract and 150 μL of buffer were mixed with 270 μL of Azocoll suspension in 2.0-mL reaction tubes. The reaction tubes were incubated at 37 °C in a water bath under agitation. After 18 h of incubation, the samples were chilled in ice for 5 min to stop the reaction and centrifuged at 10,000 *g* and 4 °C for 20 min. The absorbance of the supernatant was measured at 520 nm by a UV–Vis spectrophotometer. One unit of enzyme activity (U) was defined as the amount of enzyme that led to an absorbance increase of 0.1 at 520 nm; this increase is associated with the formation of azo dye-linked soluble peptides after 18 h of incubation.

Protein concentration was determined according to Smith et al. (1985) using bovine serum albumin as a standard.

3. Results and discussion

The matrix and results of runs carried out according to the 2^3-full factorial design summarized in Table 2 show the effects of the three independent variables, namely (1) pH of the incorporation solution (ph_i), (2) stirring time (*t*), and (3) protein concentration in the crude extract (Pi_{ca}), on the outcome of *Penicillium* sp. collagenase incorporated into the galactomannan gel using the efficiency of collagenase incorporation (*E*) and the collagenolytic activity after 360 min (*AC*) as the responses. For this purpose, the enzyme release was monitored at selected given times (0, 60, and 360 min).

The enzyme incorporation process often leads to changes in chemical, biochemical, mechanical, and kinetic properties. The reduction in enzymatic activity could be related to small distortions in protein structure (Sharma, Thatai, Kuthiala, Singh, & Arya, 2021). Considering the initial amount of incorporated fungi-collagenase as 100 %, no <92 % was retained in the galactomannan gel at the beginning of the experiment for all of the systems; after 360 min, they still retained the collagenase incorporated into the gel with no <76 % of the percentage of incorporation. Pascoal et al. (2021), for example, worked with the galactomannan from *Delonix regia* and the encapsulation of bixin into the polymeric matrix by the spray drying technique. In their case, the release behavior of the bixin reached a maximum release threshold only at the end of the essay, with a change in the release from 84.58 % to 100.00 % of the total mass of initially encapsulated bixin.

The incorporated-collagenase in our study maintained high collagenolytic activity, ranging from 32.5 to 69.8 U/mL, thus suggesting that the fungi-collagenase remained stable despite all different parameters (ph_i, *t*, and Pi_{ca}). The Pareto charts of Figs. 1 and 2 show the estimated effects of the variables and their interactions on the efficiency of collagenase incorporation and the collagenolytic activity after 360 min, respectively, in decreasing order of magnitude. The length of each bar is proportional to the estimated effect, while the vertical line allows the evaluation of which effects are statistically significant, i.e., bars that extend beyond this line correspond to effects statistically significant at a confidence level of 95 %.

It is possible to observe in Fig. 1 that *E* was positively influenced by PC_i (3) and pH_i (1), which means that an increase in either variable should enhance the incorporation efficiency, whereas *t* did not have any statistically significant effect on the same response. As for the interactions, only the one between pH_i and PC_i showed a statistically significant but very weak effect.

On the other hand, all the three variables significantly influenced *CA*, but, whereas the linear effects of pH_i (1) and *t* (2) were positive, that of PC_i (3) was negative (Fig. 2). This statistical result suggests that the incorporated enzyme activity may be improved by an increase in one of the first two variables or by a decrease of the third. Among the variable interactions, those of PC_i with either *t* or pH_i were statistically significant and negative, consistently with the signs observed for the three linear effects. These results suggest that CA may be alternatively enhanced by a decrease of PC_i and a simultaneous increase in *t* or pH_i, or vice versa.

The statistical significance of second-order model equations was confirmed by the F-test analysis of variance (ANOVA) and multiple regression. The computed F-value (12.46 for *Ac*) was in fact much higher than the tabulated one [F (3,8) = 4.07], while the lack of fit was

Table 2
Matrix and results of experiments carried out with *Penicillium* sp. UCP 1286 collagenase incorporated into the galactomannan gel according to the full 2^3-factorial design outlined in Table 1.

Run	pH_i[a]	*T* (min)[b]	PC_i (mg/mL)[c]	*E* (%)[d]	*CA* (U/mL)[e]	*n*[f]
1	6.0	30	0.3	76.63	32.5	0.19
2	8.0	30	0.3	79.67	61.6	0.18
3	6.0	90	0.3	76.33	64.6	0.19
4	8.0	90	0.3	81.18	69.8	0.17
5	6.0	30	0.7	94.27	45.1	0.21
6	8.0	30	0.7	93.50	42.5	0.21
7	6.0	90	0.7	92.97	43.5	0.24
8	**8.0**	**90**	**0.7**	**94.01**	**47.8**	**0.21**
9	7.0	60	0.5	97.98	40.8	0.15
10	7.0	60	0.5	99.07	43.5	0.26
11	7.0	60	0.5	97.80	49.1	0.28
12	7.0	60	0.5	99.25	44.4	0.37

The best results are shown in boldface.
[a] pH of the incorporation solution.
[b] Time of collagenase and the galactomannan gel stirring.
[c] Initial protein concentration.
[d] Efficiency of collagenase incorporation on the galactomannan gel.
[e] Collagenolytic activity after 360 min.
[f] Release exponent in Eq. (4).

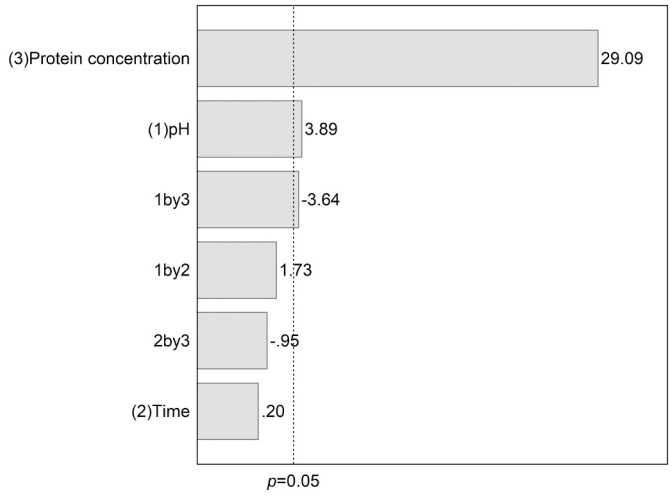

Fig. 1. Pareto chart of the estimated effects of (1) pH of the incorporation solution, (2) time of *Penicillium* sp. UCP 1286 collagenase and galactomannan gel stirring (min), and (3) initial protein concentration in the crude extract (mg/mL) on the efficiency of collagenase incorporation on the galactomannan gel.

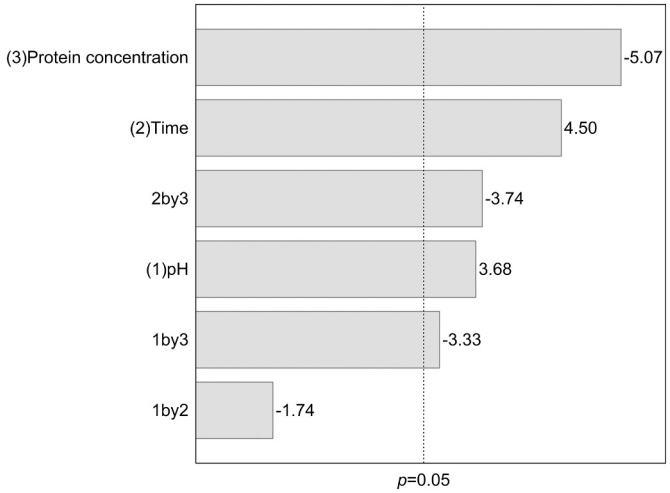

Fig. 2. Pareto chart of the estimated effects of (1) pH of the incorporation solution, (2) time of *Penicillium* sp. UCP 1286 collagenase and galactomannan gel stirring (min), and (3) initial protein concentration in the crude extract (mg/mL) on the activity of collagenase incorporated into the galactomannan gel after 360 min.

insignificant. In addition, the high value of the determination coefficient ($R^2 = 0.82$) indicates that both models explained 82 % of the variability data, and the regression was statistically significant ($p < 0.002$) at 95 % confidence level (Table 3).

Polysaccharides have been widely used as incorporating matrices because of various reasons, among which are their abundance in nature and some intrinsic properties (biocompatibility, biodegradability, low or no toxicity, high hydrophilicity, and presence of chemically reactive groups) (Albuquerque et al., 2022). Such qualities makes polysaccharides a perfect biomaterial to develop a carrier mechanism for the controlled release of catalysts, at a particular location and time, so that it can react with a target biomolecule (Matricardi, Di Meo, Coviello, Hennink, & Alhaique, 2013). In general, there are various physical and chemical technologies to incorporate enzymes on solid carriers/transporters, all capable of minimizing alterations in their structure and therefore preserving efficient binding of substrate to their active site (Sharma et al., 2021). Despite the complexity of the phenomena involved in the release of molecules from polysaccharide matrices, some kinetic models are used to describe their release from the matrix.

The model of Higuchi (1961), for example, can be used to describe the rate of controlled release using the so-called Higuchi dissolution constant (*Kh*), which reflects the characteristics of the formulation design. Based on the Higuchi model, it is possible to define two mechanisms associated with controlling the rate of drug release: (1) swelling and (2) erosion/degradation, resulting in a layer on the surface of the drug that prevents the entry of more water and the release of more drug.

The Korsmeyer-Peppas kinetic model provides a simple relationship that relates the mechanism of active substances release from a matrix to the so-called release exponent (*n*) (Table 4).

When the release is governed by diffusion, the Fick model applies (Case I, with $n = 0.5$), and the transport by the solvent or diffusion is much quicker than the relaxation of polymeric chains. When $n = 1$, the diffusion is non-Fickian type (Case II) leading to zero-order release kinetics; in this case, the release is governed by swelling or polymer chain relaxation. When *n* ranges from 0.5 to 1.0, it is possible to recognize a case of anomalous transport, that is, the delivery is due to both diffusion and swelling/relaxation of the polymer matrix. In other words, the rearrangement of polymer chains occurs slowly and simultaneously with the diffusion process, causing time-dependent anomalous effects. Finally, when $n > 1.0$ the phenomena of tension and breakage of the polymeric chains occur, that is, the nucleus ends up breaking, thus releasing the substance (Lopes et al., 2005).

Table 4
Release mechanism as a function of the release exponent of the Korsmeyer-Peppas model (n).

Release exponent (*n*)	Release mechanism
$n = 0.5$	Fick model (Case I)
$n = 1.0$	Non-Fickian model (Case II)
$0.5 < n < 1.0$	non-Fickian model (anomalous case)
$n < 0.5$	Pseudo-Fickian behavior
$n > 1.0$	Non-Fickian model (super Case II)

(Based on Lopes, Lobo, and Costa (2005).

The *n* values listed in Table 2 were all <0.5 with no statistical difference among the runs, which demonstrates that all the systems exhibited a pseudo-Fickian release behavior. This pattern suggests that the release of collagenase from the galactomannan gel was governed by a sub diffusion likely based on a spatial dependence on molecular mobility, with structurally stable but "leaky" corrals (Haugh, 2009) on the gel. Regarding the potential application of such systems for biomedical purposes, this sub diffusion, also considered a controlled collagenase release, may ensure the enzyme activity over time.

This diffusion-controlled release (with *n* values between 0.2 and 0.3) was also observed by Dangi, Mattoo, Kumar, and Sharma (2022) as the preferred drug release mechanism for a novel hydrogel for drug delivery systems using *Cassia tora* galactomannan and sodium trimetaphosphate. The results of the drug release kinetics suggested that the Korsmeyer-Peppas model was found to be the best fit model for explaining the mechanism of slow drug release from the hydrogel in the physiological environment of the stomach and small intestine. The percentage of drug release was < 40 % and the authors explained that the galactomannan remained intact in the hostile environment of the above-mentioned organs.

For comparison purposes, a commercial collagenase (Sigma-Aldrich) was incorporated into the same galactomannan gel. The obtained results ($E = 93.88$ %, $CA = 65.8$ U/mL and $n = 0.10$) demonstrated a pseudo-Fickian behavior as it was observed for the *Penicillium* sp. collagenase, thus confirming the same sub diffusion release pattern from the galactomannan matrix. Fig. 3, which demonstrates the release profiles of both commercial and *Penicillium* sp. collagenases compared to the free fungi enzyme along the time (0, 60, 180, and 360 min), also highlights a gradual increasing in the enzymatic activities of the incorporated enzymes; thus, it is possible to affirm that the gel can be used as an efficient release system for collagenases without causing a time-dependent denaturation. On the other hand, we observed a gradual decrease in the collagenolytic activity of free *Penicillium* sp. collagenase over time (0, 60, 180 and 360 min), demonstrating that the galactomannan gel improved the enzyme stability.

Table 3
Analysis of model variance described for the response, *Ac*, over the independent variables according to 2^3 full statistical design.

Response	Df	SS model	MS model	*F-calc*	*F-tab*	R^2	$R^2_{ajusted}$	*p* value
X								
Regression	3	40.35	13.45	12.46	4.07	0.82	0.76	0.002
Resid	8	8.63	1.08					
Total	11	48.98	–					

All values are statistically significant at a 95 % confidence level. df = degree of freedom, SS model = model quadratic sum, MS model = model quadratic mean.

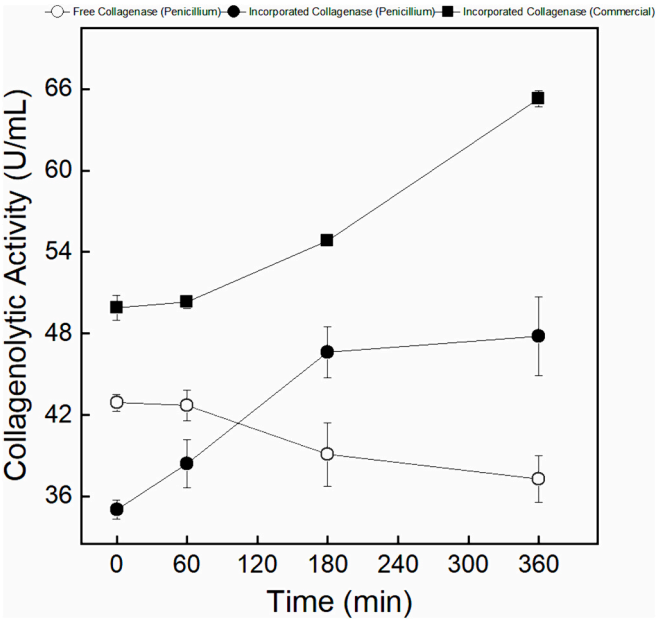

Fig. 3. Release profiles based on the activity of both the commercial and the *Penicillium* sp. UCP 1286 collagenases from the galactomannan gel compared to the free *Penicillium* sp. UCP 1286 collagenase.

The promising results of the run 8 suggested us to test longer delivery times for *Penicillium* sp. collagenase incorporated into the galactomannan gel. However, after 540 min, despite a higher incorporation efficiency ($E = 94.01$ %), the collagenolytic activity started to decrease ($CA = 46.90$ U/mL). The n value was 0.31, confirming the pseudo-Fickian release behavior observed in the runs carried out according to the full factorial design.

This pseudo-Fickian release behavior displayed for both evaluated enzymes (the fungi-collagenase and the commercial one) confirms the same sub diffusion release pattern from the galactomannan matrix. According to Hennink, Franssen, van Dijk-Wolthuis, and Talsma (1997), the release rate of proteins from highly hydrated hydrogels is independent of the cross-link density, and the hydrogel mesh size is larger than the protein diameter; in our case, it is possible to suggest that the galactomannan gel three-dimensional network is the main factor allowing the diffusion process.

The structure of the galactomannan from *C. grandis* was already reported by Albuquerque et al. (2014) as a linear (1–4)-linked backbone of mannose units, with side chains of single galactose. The mannose/galactose ratio (2.44:1) obtained by the monosaccharide composition is responsible for synergetic interactions and the polysaccharide solubility. Then, the considerable number of hydroxyl groups in the galactomannan outer sphere in addition with the presence of hydrophobic regions in its structure may reinforce the interactions with both non-ionic and ionic drugs. Despite the considerable volume of research and patent literature on the use of polysaccharide-based hydrogels as drug delivery systems, the commercialization aspects of these formulations are scarce as the majority of research is still developed in the academic field (Nayak et al., 2021). Therefore, close efforts are required to depict hydrogel-based drug delivery systems from the experimental level to the pilot or commercial scale manufacturing for the benefit of humans, which can be achieved by addressing some important factors, such as the formulation of hydrogels with better biological and structural properties, for example, by blending polymers in composites; the use of cost-effective raw-materials and process; the optimization of polymeric-network formation techniques; the application of mathematical models; and finally the better understanding of drug release mechanism and kinetics (Nayak et al., 2021).

4. Conclusion

In this study, the galactomannan gel from *C. grandis* seeds was used to incorporate collagenases and study their release profile. Despite our efforts in explaining the diffusion release mechanism of both the fungi and the commercial collagenases from the galactomannan gel, we know that some considerable challenging issues are yet to be resolved, for example, the complexity of achieving the commercial market. However, the maintenance of the collagenolytic activity and the controlled release reported here demonstrate the excellent applicability and inherent properties of the galactomannan gel to the supported catalysts, thus suggesting the biomedical application of the polysaccharide to release collagenase at a particular location and time.

CRediT authorship contribution statement

Carolina de Albuquerque Lima Duarte: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Conceptualization. **Mateus Gonçalves da Silva:** Writing – original draft, Methodology, Investigation. **Ana Lúcia Figueiredo Porto:** Methodology, Investigation. **Maria Carolina de Albuquerque Wanderley:** Methodology, Investigation. **Sabrina Swan Souza da Silva:** Methodology, Investigation. **Alexsandra Frazão de Andrade:** Methodology, Investigation. **Raquel Pedrosa Bezerra:** Writing – review & editing. **Attilio Converti:** Writing – review & editing, Methodology. **Diego Gomes Ramos:** Writing – review & editing, Methodology, Investigation. **Daniela de Araújo Viana Marques:** Methodology, Investigation. **Priscilla Barbosa Sales de Albuquerque:** Writing – review & editing, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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