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Kinetic modeling of solid-liquid extraction of mucilage from *Opuntia ficus indica*

Modelado cinético de la extracción sólido-líquido de mucílago de *Opuntia ficus indica*

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Abstract

Mucilage, a valuable hydrocolloid, constitutes approximately 14% of the dry weight of cladodes. This study explores the kinetic modeling of solid-liquid extraction of mucilage from *Opuntia ficus-indica* cladodes, focusing on extraction mechanisms and kinetic parameters. The extraction process depends on temperature, solvent ratio, and pH, influencing solvent penetration, solubilization, and mucilage diffusion. Both pretreated cladodes (with acetone to remove pigments) and untreated samples were evaluated. The extraction was performed at 70 °C, with a 3:1 mass-to-water ratio, 90 minutes of agitation, and varying pH levels. Kinetic analysis used galacturonic acid content as an indirect measure of mucilage. Three models were assessed: the First-order model, which explained the initial rapid phase; Peleg's model, which described hyperbolic behavior; and the two-site model, which best represented both fast and slow extraction phases. The study found that an acidic solvent (pH 3) significantly improved extraction, while acetone pretreatment had a negative effect on the rate. The two-site model provided the best fit for the data, offering a complete understanding of the extraction kinetics.

Keywords: Mucilage, solid-liquid extraction, galacturonic acid, kinetics.

Resumen

El mucílago, un hidrocoloide de gran valor, representa aproximadamente el 14 % del peso seco de los cladodios. Este estudio aborda el modelado cinético de la extracción sólido-líquido de mucílago de *Opuntia ficus-indica*, analizando los mecanismos de extracción y los parámetros cinéticos. Factores como la temperatura, la relación solvente-sólido y el pH influyen en la penetración del solvente, la solubilización y la difusión del mucílago. Se trabajó con cladodios pretratados con acetona para eliminar pigmentos y muestras sin tratar. La extracción se llevó a cabo a 70 °C, con una relación masa-agua de 3:1, agitación durante 90 minutos y variaciones de pH. El contenido de ácido galacturónico se utilizó como indicador indirecto del mucílago en el análisis cinético. Tres modelos fueron evaluados: el de primer orden, que describió la fase inicial rápida; el de Peleg, que explicó el comportamiento hiperbólico; y el de dos sitios, que representó tanto la extracción rápida como la lenta. Los resultados mostraron que un solvente ácido (pH 3) mejoró significativamente la extracción, mientras que el pretratamiento con acetona redujo la velocidad del proceso. El modelo de dos sitios presentó el mejor ajuste a los datos experimentales, proporcionando una descripción detallada de la cinética de extracción.

Palabras clave: Mucilago, extracción sólido-líquido, ácido galacturónico, cinéticas.

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1 Introduction

The *Opuntioideae* subfamily of the *Cactaceae* is well-adapted to arid environments and diverse climates worldwide. *Opuntia* are native to the Americas, with 90 species found exclusively in México (Hernandez Bonilla *et al*., 2023). In 2020, the national production of *Opuntia* in México was 864,243.5 tons, cultivated on 12,618 hectares, which meant an approximate turnover of 41,000 dollars (Flores Sánchez *et al*., 2023). *Opuntia ficus-indica* (OFI), a species within the *Cactaceae* family, holds significant global economic importance due to the demand for its hydrocolloid. *Opuntia* mucilage (MOFI) constitutes approximately 14% of the dry weight of the cladodes and can retain more than 30% of the total water in the reserve parenchyma (Sepúlveda *et al*., 2007). Mucilage primarily functions as a carbohydrate reserve, helping to prevent plant dehydration.

Opuntia´s mucilage is a heteropolysaccharide with a complex, highly branched structure, containing L-arabinose, D-galactose (in pyranose and furanose forms), D-xylose, L-rhamnose, glucuronic acid, and D-galacturonic acid (GalA) as the principal neutral sugar unit (Sáenz *et al*., 2004). GalA, with a molecular weight of 194.139 g/mol, is one of the key components of mucilage. MOFI has various applications, including biomaterials, food additives, and metal removal (Embafrash Berhe *et al*., 2023; Luna-Zapién *et al*., 2023; Madrigal-Santillán *et al*., 2022; Otoni *et al*., 2021; Reyes *et al*., 2023). A study by González-Avilez *et al*., (2024) demonstrated the significant influences of GalA in the mucilage in removing heavy metals. FTIR spectra showed typical bands of pectic polysaccharides interacting with via electrostatic connections. This finding highlights the importance of monitoring GalA during the MOFI solid-liquid extraction (SLE) process to prevent degradation. However, the large-scale use of MOFI as a biopolymer has been limited by factors such as low extraction yield, short shelf life, and non-homogeneous characteristics.

The main problem in exploiting this natural hydrocolloid is the low extraction yield. On the other hand, the complexity of the mucilage's chemical structure has yet to allow the selection of a compound to accurately assess the effect of extraction and process variables. The quantification methods presented in the literature are not standardized, and they quantify the mucilage and other soluble solids globally, limiting the possibility of comparing the efficiency of the processes studied. Galacturonic acid has previously been identified as the main factor responsible for the rheological behavior of mucilage (Asimakopoulou *et al*., 2024), so it is a compound that can be monitored as an indicator of extraction yield.

Recent literature has explored various methodologies to improve the efficiency of *Opuntia* mucilage extraction, ranging from optimizing conventional methods to implementing advanced technologies such as ultrasound and microwave-assisted extraction (Del Mar García Martín *et al*., 2024; Mannai *et al*., 2023; Texco-López, *et al*., 2018; Van Rooyen *et al*., 2024).

Solid-liquid extraction (SLE) is the most common method for extracting mucilage from cladodes. SLE is a separation process where specific substances are dissolved from a solid matrix into a liquid solvent (Chanioti *et al*., 2014). The primary goal of SLE is to isolate the compound of interest from unwanted substances within the insoluble solid matrix (Naviglio *et al*., 2019). The extraction process generally involves the following steps: a) Solvent penetration and compound solubilization; b) Diffusion of solubilized compounds through the solvent contained in the pores of the solid to the interface; c) Transfer of the solute from the surface of the solid particles to the bulk of the liquid.

The first step is enhanced by increasing the contact area, typically achieved by reducing the sample size (Prasedya *et al*., 2021). The rate of dissolution (step 2) depends on factors such as the solubility of the compounds, temperature, and the surface area of the solid particles (Mcgraw-Hill, 2014). Mass transfer between the solid and liquid phases (step 3) is often improved by agitation or mixing, which promotes the diffusion of solutes from the interior of the solid particles to the bulk solvent, thereby increasing the extraction rate (Bong *et al*., 2015).

A common strategy to improve mass transfer is to pretreat the samples, which can be done physically (e.g., size reduction, grinding, or ultrasound) or chemically (e.g., using solvents or enzymes). For example, Cruz-Rubio *et al*., (2023) used acetone as a chemical pretreatment to remove impurities, though they did not quantify MOFI extraction or assess its effect on the process. SLE can be performed using various separation techniques, including filtration, centrifugation, or decantation.

In Fig. 1, it can be observed that at $t = 0$, the solute (MOFI) is present from C_{a0} to C_{aR}, where "R" is the radius of the solid. Immediately a solid-liquid interface containing the solutes limits the extraction. The concentration distribution is uniform $(\overline{C})$, and no solute has been transferred to the solvent ($C_{ar} = 0$). When ESL begins and $t > 0$, a fast mass transfer occurs because, according to Fick's second law, solutes move from areas of high concentration to areas of low concentration ($C_{b0} to\ C_{bi}$). The solute in the solvent is initially scarce ($C_{bi} to\ C_{br}$), and agitation maintains a homogeneous concentration in the batch. As the extraction progresses (C_{c0} to C_{cR}), the migration of the solute from the center to the surface of the cuboid

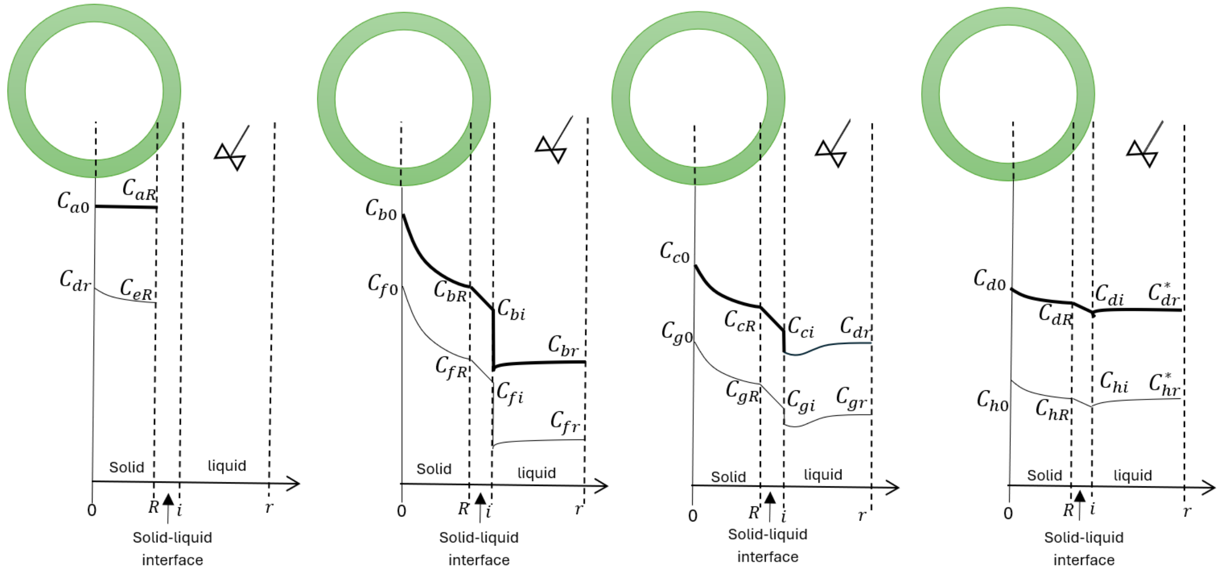

Figure 1: Solute transfer mechanism in solid-liquid extraction in 1st and 2nd stages.

slows down, and the concentration gradient between the cuboid and the bulk solvent decreases ($C_{ci}$to C_{cr}), causing a loss in mass transfer rate, but enriching the extract. Eventually, at $t = \infty$ the concentration of solutes in the solid and the solvent reaches equilibrium (C_{d0} to C_{dr}). This trend continues in the second extraction stage, which could be seem in fig 1 as C_{e0} series. This allows us to indirectly analyze the extraction of the solute from the material and its accumulation in the solvent over time, making it possible to describe the process through extraction kinetics.

Extraction kinetics studies the rate at which a solute is transferred. It involves understanding how solvent choice, temperature, pH, and pretreatments affect the rate of solute transfer (Chan *et al.*, 2014). During extraction, the concentration eventually reaches a maximum content that no longer changes, known as the concentration of saturation and equilibrium time. This period is usually long and not profitable for the process, so considerations are always made to extract a substantial quantity in a shorter time, achieving a better cost-benefit ratio.

Several models have been evaluated to describe the extraction process; however, the processes and structures of natural sources influence the type of model best representing the extraction phenomena. Some empirical kinetic models evaluated in extraction include first-order, Peleg's, and two-site models (Das & Arora, 2021; Karacabey *et al.*, 2013). These equations are limited by not being based on the physical phenomena of mass transfer through plant particles and external solid surfaces into the bulk of the liquid phase. However, they provide an excellent basis for curve fitting and representation as a function of the physical properties, process conditions and the extracting plant material.

Liew *et al.*, (2018) applied a first-order equation, termed by the authors as an intra-particle diffusion-controlled model, which was found to be in good agreement with experimental data according to the coefficient of determination ($R^2 > 0.94$). The authors also attempted to fit the experimental data to a two-site kinetic model, but the R^2 values were higher for the first-order kinetics. Other biocomponents have been described by the two-site equation, for instance, Zghaibi *et al.*, (2020) employed this model, also known as Patricelli's model, which accurately described the kinetics of lipid extraction, with the highest mean values of the determination coefficient ($R^2 \geq 0.952$) and the lowest mean values of mean relative percentage deviation (MRPD $\leq$ 8.666%). Rostami & Gharibzahedi, (2017) modeled the kinetic extraction from the hydrolysate waste of fruiting bodies of Ziziphus jujuba Mill, where the diffusion model (Peleg) with an R^2 of 0.9871 and a root mean square error (RMSE) of 0.3587 was considered the best kinetic equation to predict the experimental data.

Although numerous studies have quantified mucilage extraction yield under various operating conditions (De Andrade Vieira *et al.*, 2021; Luna-Sosa *et al.*, 2022; Van Rooyen *et al.*, 2024) no equation has been presented that encompasses the different phenomena occurring in the SLE process. Understanding the effect of the variables will allow us to predict their impact and analyze the overall behavior of the extraction process.

This study aims to investigate the effects of sample conditioning and extraction variables during batch solid-liquid mucilage extraction and explain the mechanisms and associated kinetic parameters of an empirical mathematical model that describes the extraction process of galacturonic acid as a parameter for quantifying the extracted mucilage.

2 Methodology

2.1 Material

Young cladodes of *Opuntia ficus-indica*, three months old, were selected and obtained from México (16°47?29?N, 96°40?30?W). *Opuntia* pads were harvested manually at 07:00 am. The average sizes were 32.3 ± 4.9 cm (length), 9.2 ± 0.5 cm (width), and 4.3 ± 2.2 cm (thickness) to ensure a homogeneous state of maturity. The samples were stored at 4 °C until use.

Opuntia cladodes were peeled to obtain the parenchyma and cut into 1 cm cuboids. Next, two major groups were made to carry out MOFI extraction: cuboids without acetone pretreatment (WAP) and cuboids with acetone pretreatment (APS).

Acetone was applied to modify the structure and eliminate photosynthetic pigments, waxes, and lipids of the parenchyma. Parenchymal cuboids were submerged for 30 minutes in acetone (Sigma-Aldrich) in a 1:2 w:v ratio. The cuboids were then separated from the solvent and heated for 10 minutes in a stove (Riossa H-33) at 40 °C to evaporate the residual solvent.

2.2 SLE operational parameters selection

The influence of ultrasonic bath time (bt), temperature (T), mass-to-water ratio (m:w), and extraction time (t) was evaluated in preliminary tests (not published). The better conditions were selected (bt= 15 min, T=70 °C, m:w=3:1, t=90 min). A temperature of 70 °C was adequate to inactivate enzymes and prevent the degradation of some components. A mass-to-water ratio of 3:1 prevents increases in compensation for evaporation losses, energy, and organic solvents to purify the extract.

2.3 Mucilage extraction

For mucilage extraction, 200 g of the sample (WAP or APS) were submerged in pH 3, 7, or 9 solutions, and placed for 15 minutes at 70 °C in an ultrasonic bath (VelaQuin, 200 W, 60 Hz). The pH solutions were prepared with ascorbic acid (pH 3) and sodium carbonate (pH 9). Distilled water was used for the pH 7 solution.

Subsequently, this samples in different pH solution were agitated at 300 rpm with magnetic stirrer for 90 minutes at 70°C. After the time elapsed, the cuboids were separated with a filter, and the MOFI was centrifuged (Centrifuge Dynac Clay Adams 0101) at 3500 rpm for 10 minutes. The extract was then precipitated with isopropanol in a 2:1 w/v ratio. The separated cuboids of each experiment were subjected to a second extraction under the same conditions using a fresh solution at the previously selected pH. Temperature, solid-to-liquid ratio, and agitation were kept constant throughout the process.

After the extraction, all MOFI samples were lyophilized (SP VirTis AdVantage Pro). First, the samples were frozen at -40 °C. Then, primary drying was carried out at 26 Pa and 10 °C, followed by secondary drying at 10 Pa and 30 °C. Due to its hygroscopic nature, precautions were taken when removing the vacuum from the equipment to prevent rehydration of the lyophilized MOFI. Immediately after, the samples were weighed, and yield recovery was calculated.

2.3.1 Experimental design

For *Opuntia* mucilage extraction, an experimental design 2^3 was used. The selected factors were pretreatment (Acetone-pretreated sample APS and without acetone pretreatment WAP), pH (3 and 9), and extraction stage (1st and 2nd). pH 7 was studied for comparison purposes (Table 1). The design response variable was MOFI yield and GalA content. Each treatment was done in duplicate.

The significant difference was calculated using ANOVA conducted at a level of $p < 0.05$ and the software NCSS11 Data Analysis (USA). The Duncan test was conducted to evaluate differences among individual means.

2.4 Yield determination

Yield was calculated based on the mucilage dry mass using eq 1:

$$Y = \frac{M_1}{M_c} \times 100 \qquad (1)$$

Where: Y= mucilage extraction yield (% dry basis), M_1= Mucilage weight (g dry basis), M_c= Weight of the cladodes sample (g dry basis).

2.5 Galacturonic acid determination

The method described by Filisetti-Cozzi *et al.*, (1991) was used. Briefly, a calibration curve was prepared at seven known concentrations from 10 to 100 μg/mL using D-galacturonic acid monohydrate (Sigma Aldrich) as standard. Then, to 0.4 mL of the fresh extract or standard was added 2.4 mL of H_2SO_4 containing 75 mM sodium tetraborate and 40 μL of sulfamic acid-potassium sulfamate (4 M; pH 1.6) and mixed thoroughly before adding sulfuric acid. The solutions were heated to about 100°C in a boiling water bath for 20 min, chilled in an ice bath, and mixed with 80 μL of m-hydroxy diphenyl. Absorbance at

525 nm was measured in a GBC CINTRA (440 UV-Vis) spectrophotometer to quantify galacturonic acid content (mg GalA/gdb).

2.6 Extraction kinetics

Mucilage content was evaluated using galacturonic acid (GalA) as an indirect indicator. Aliquots of 5 mL of MOFI extract were taken every 5 minutes from each treatment, and GalA was determined to each aliquot from t=0 to 115 min in fresh MOFI.

The simplicity of a model is a crucial characteristic to facilitate its implementation in both industrial and academic settings. Moreover, the ease of determining model parameters from experimental data is a key factor for its adoption. Simplicity also leads to lower susceptibility to implementation errors and greater robustness in practical applications.

The ability to fit a model with experimental data is one of the fundamental criteria for evaluating its effectiveness. The coefficient of determination (R^2) measures the proportion of total data variability explained by the model, with values closer to 1 being better. On the other hand, the root mean square error (RMSE) provides a metric for the average magnitude of prediction errors, allowing the model´s accuracy of the model to be assessed. A low RMSE indicates model predictions are close to experimentally observed values, which is crucial to ensure the model can be reliably used in practical applications. The combination of metrics allows for a robust evaluation of the model, ensuring that it not only describes the observed data well but also has a low margin of error in predictions.

The first order, Peleg and two-sites equations were selected because they provide a solid foundation for comparing how different variables affect extraction kinetics. Additionally, models have been widely applied in the extraction of similar substances, enabling direct comparisons with previous research and contextualizing our results within a broader framework.

2.6.1 First order equation

A kinetic method was employed to calculate the mass transfer rate of the extracted solute from the solid matrix surface to the bulk liquid phase. Assuming that the process is intra-particle diffusion-controlled with uniform distribution of the analyte within the matrix, first-order extraction kinetics were assessed.

The rate of leaching or fast extraction (FE) is proportional to a driving force (Cs-Ct), and the first-order rate equation is correlated with a linear form, as shown in Eq. 2

$$FE = \frac{dC_t}{d_t} = k\,(C_s - C_t) \tag{2}$$

where C_t is the concentration extracted (mg GalA/gdb) at a given time t (min), C_s is the concentration of galacturonic acid at saturation point (mg GalA/gdb). K is the first-order extraction rate (min^{-1}).

2.6.2 Peleg's equation

Peleg's equation assumes extraction occurs in two stages: first-order kinetics during the initial phase and zero-order kinetics during the latter phase in a hyperbolic form. As the initial concentration of the target solute was zero in the extraction solvent (due to beginning with fresh solvent), C_0 term was omitted from Peleg's equation. The concentration of GalA in extraction solutions was measured at the corresponding time, and its behavior was explained using the modified Peleg's equation.

$$C_t = \frac{t}{K_1} + C_{eq} \bullet t \tag{3}$$

Where C_t is concentration of GalA (μg GalA/mg sample) at time t, k_1 is the Peleg rate constant ($min \cdot mg\ sample/\mu g\ GalA$), C_{eq} is the concentration at equilibrium point ($mg\ sample/\mu g\ GalA$).

2.6.3 Two-sites equation

Another empirical equation, known as two-site kinetics, explains the two distinct phases involved in an extraction process: (i) desorption at a higher rate (washing stage) defined by the rate constant k_1 and (ii) desorption at a slower release (diffusion phase) removed by a slower proportion defined by the rate constant k_2. In eq 4, F is the fraction of the solute released quickly, (1-F) is the fraction of the solute released slowly (kg/m^3).

$$C_t = C_{eq}\left[1 - \left(F \times \exp^{-k_w \bullet t}\right)\right] - [(1-F) \times \exp^{-k_d \bullet t}] \tag{4}$$

Equation fitting was performed using non-linear least-squares minimization, and the goodness of fit was evaluated using the adjusted coefficient of regression (R^2) and the root mean square error (RMSE) by NCSS 2024 software.

3 Results and discussion

3.1 Yield

Diffusion is a key mechanism in solid-liquid extraction (SLE), where the concentration gradient drives the movement of mucilage components from within the *Opuntia* cells to the extracting solvent (Chanioti *et al.*, 2014). The pH of the solvent plays a crucial role in ESL process.

Diffusion is enhanced in solutions with higher polarity, such as acidic solutions, where the affinity

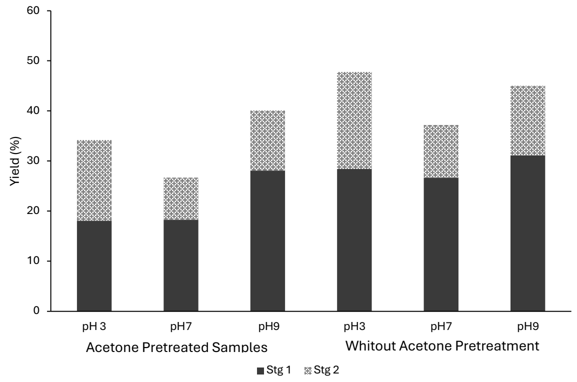

Fig 2 SLE mucilage yield in APS and WAP on 1st and 2nd stage at different pH.

between mucilage molecules and the solvent facilitates the transfer. Mass transfer between the solid and liquid phases is controlled by the material's resistance and intraparticle diffusion (Inglezakis *et al*., 2020).

The protic nature of the acidic solution enhances the solubilization of mucilage components due to the greater affinity between polar molecules (Korkees *et al*., 2022). In contrast, acetone pretreatment, which uses an aprotic solvent, reduces mucilage solubility by collapsing the pores of the plant tissue, thereby decreasing the yield. The differences in yield between the first and second extraction stages underscore the importance of understanding the structural changes in the tissue during the process. In the first stage, the high availability of mucilage and low mass transfer resistance allow for more efficient extraction. However, in the second stage, the plant tissue undergoes modifications that negatively impact permeability and the solvent's ability to extract the remaining mucilage. This phenomenon may be related to the extraction of less soluble molecules and the potential partial hydrolysis of organic matter, which can occur under prolonged extraction conditions or in the presence of extreme pH levels.

Figure 2 illustrates the influence of pretreatment, pH, and extraction stage on yield. Statistically, pH, pretreatment, and its interaction have a significant effect (?=0.05) on yield. Both acidic and alkaline pH levels positively influence mucilage yield compared to neutral pH solutions. Hou *et al*., (2024) agrees that extraction in acidic medium ($pH < 2$) stabilizes the linkages between galacturonic acid residues and enhances the hydrolysis of pectin neutral sugars, increasing the GalA content.

The highest yield was observed in samples without pretreatment and at pH 3 (47.8%). The extraction is favored probably due to the polarity of the mucilage components, which have a greater affinity for the extractive solution with a protic character. Also, it is possible that the tissue shrinkage caused by acetone collapses the pores, and the aprotic nature of acetone may not favor mucilage solubility. This combination could hinder mucilage release and potentially result in a lower yield.

De Andrade Vieira *et al*., (2021) reported a yield of 21.54%, while Carpintero?Tepole *et al*., (2021) obtained a yield of 24.94% in concentrated MOFI by ultrafiltration, using water as the extractive solution. Significant yield differences (?=0.05) were found between the 1st and 2nd extraction stages, confirming the effects of pH and pretreatment. The 1st extraction stage contributed 52-71% of the total yield, while the 2nd stage contributed 29-40%. Results suggest solvent saturation during the first extraction stage and structural changes in the tissue during the second stage, which influence the diffusion film and intraparticle diffusion mechanisms. It also involves the extraction of less soluble molecules and partial hydrolysis of organic matter. Findings described above could explain the lower yields reported by previous authors (Contreras-Padilla *et al*., 2016; De Andrade Vieira *et al*., 2021; Kalegowda *et al*., 2017) which studied only the first extraction stage.

3.2 Extraction kinetics

Figures 3 and 4 show experimental results for GalA extraction kinetics using water and acetone as solvents, respectively. As expected, the first stage exhibited a higher GalA concentration in both acidic and alkaline solutions, with the kinetics showing a similar upward trend. After 60 minutes, an abrupt change was observed, and the trend began to decline. The concentration between the first and second stages was identified as the equilibrium concentration.

The maximum concentration observed at the end of the first stage aligns with values reported by Garfias Silva *et al*., (2022) at 0.785, Rodriguez-Gonzales *et al*., (2021) at 1.92 and 1.87 μg AGA/mg sample reported by González-Avilez *et al*., (2024). Results indicate that changes in pH and pretreatment are significant extraction parameters influencing the recovery of GalA, as expected.

The kinetics revealed two distinct steps in the extraction process. The initial step involves a washing process where solutes on the surface are leached into the bulk liquid phase (within ~15 minutes), similar to the results presented by Hobbi *et al*., (2021). This can be explained by Fick's law: at the beginning of an extraction process, the high concentration gradient between the cladodes and the liquid phase results in the rapid diffusion of GalA into the solvent. In the second SLE stage (unfilled markers in Figures 3 and 4), the cladodes undergo structural modifications, and the fresh solvent contains no solute. Selected conditions favor the transfer of GalA into the solvent, increasing the transfer rate at the beginning of the stage.

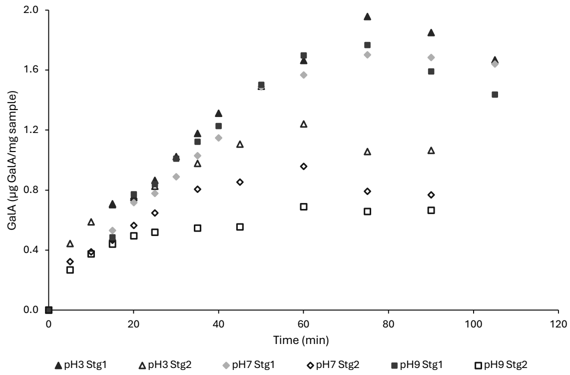

Fig. 3 Solid-liquid extraction kinetics without acetone pretreatment (WAP).

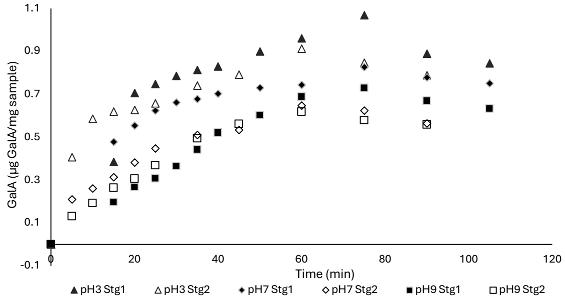

Fig. 4 Solid-liquid extraction kinetics with acetone pretreated samples (APS).

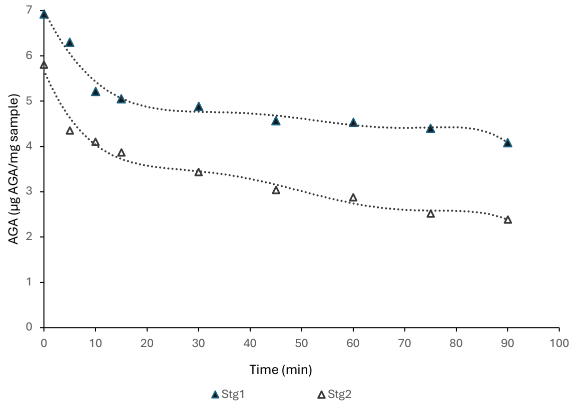

Fig 5 Galacturonic acid loss kinetics in cladodes with acetone pretreatment at pH3.

The second period involves intraparticle diffusion within porous particles, where the solvent permeates the solid, dissolves the solute, and then diffuses it to the surface (Hou *et al.*, 2024). This results in a slowly increasing trend until reaching a peak point, where a maximum amount of GalA is extracted. It was noted that the GalA content declined beyond the peak point under all conditions, consistent with the findings of Garfias Silva *et al.*, (2022) and Zaid *et al.*, (2019). Emaga, (2012) attributed to the decomposition of GalA, leading to pentose by decarboxylation or glucose via glycolysis, or it could form lactones in irreproducible amounts.

Figure 5 illustrates the loss of GalA in acetone-pretreated parenchymatous cuboids subjected to SLE with a pH 3 solution. This loss is related to the kinetic curves under the same conditions (Figure 4). A rapid loss of the component is observed in the first 20 minutes of extraction, corresponding to the leaching phase in the solvent kinetics. Subsequently, a slower extraction phase occurs, and the loss rate decreases, as shown in both figures.

3.2.1 First-Order Kinetic Model

The first-order equation models the extraction rate as a function of time, assuming that the amount of mucilage remaining to be extracted decreases exponentially over time. In the first-order model, a higher k value indicates a faster extraction rate, which could result from conditions enhancing diffusion, such as higher temperatures or a more efficient solvent. Factors such as agitation, which improves mass transfer by reducing the boundary layer around the solid, and the solid: liquid ratio, which increases the concentration gradient, also influence the extraction rate. Although the first-order equation is a simplification, it is useful for capturing the essence of extraction mechanisms during the initial phase of the process.

To determine the values of the first-order rate constant (k) and the coefficient of determination (R^2), non-linear least-squares fitting was employed. Some assumptions were generally made to fit the first-order equation: the GalA distribution in the cladodes is assumed to be homogeneous and uniform, particles are presumed to be isotropic, and the steam diffusion into the particles and GalA mass transfer resistance on the external surface of the plants are considered negligible. The overall volumetric mass transfer coefficient (k), except for APS with pH 7, was higher in the second extraction stage. Hou *et al.*, (2024) mentioned that the higher the value, the faster the polysaccharide dissolves in the solvent. However, the equilibrium concentration was higher in the first extraction stage and in acidic conditions in all cases.

Table 1 shows the values for each treatment. The R^2 and RMSE values imply a good fit between the model and the experimental data.

In WAP, first order rate decreases with acid pH in the first and second extractions; k values were higher in the second stages, probably because of cell wall degradation. The highest value was observed at neutral pH when cladodes were treated with acetone. k values ranged between 0.023 and 0.106 min^{-1}. There are no reports for comparison with MOFI; however, k values for polysaccharides rank between 0.030 to 0.050 min^{-1}., and for pectin from 0.023 to 0.146 min^{-1}. (Liew *et al.*, 2016; Lin *et al.*, 2018; Zhu, 2020). Values presented are within the range of pectic substances and show the same trend that the studied

Table 1 First order kinetic parameters.

Treatment		Without Acetone Pretreatment (WAP)				Acetone Pretreated Sample (APS)			
pH	Stage	C_{eq}	K (min^{-1})	R^2	RMSE	C_{eq}	K (min^{-1})	R^2	RMSE
3	1	2.02	0.026	0.964	0.128	0.96	0.054	0.931	0.063
3	2	1.11	0.067	0.957	0.058	0.80	0.106	0.939	0.040
7	1	1.95	0.023	0.975	0.082	0.78	0.062	0.991	0.005
7	2	0.86	0.062	0.945	0.045	0.61	0.053	0.986	0.012
9	1	1.77	0.030	0.936	0.205	0.78	0.025	0.946	0.032
9	2	0.64	0.080	0.962	0.016	0.63	0.039	0.977	0.010

Table 2 Peleg's kinetic parameters.

Treatment		Without Acetone Pretreatment (WAP)				With Acetone Pretreated (APS)			
pH	Stage	C_{eq}	K ($\text{min}^{-1}\cdot(\mu\text{gGalA})/(\text{mg sample})$)	R^2	RMSE	C_{eq}	K ($\text{min}^{-1}\cdot(\mu\text{gGalA})/(\text{mg sample})$)	R^2	RMSE
3	1	2.78	0.060	0.955	0.162	1.12	0.076	0.905	0.087
3	2	1.30	0.109	0.959	0.053	0.90	0.137	0.967	0.021
7	1	2.78	0.050	0.966	0.109	0.89	0.078	0.986	0.007
7	2	1.02	0.074	0.930	0.056	0.75	0.043	0.969	0.012
9	1	2.38	0.061	0.915	0.269	1.11	0.021	0.930	0.041
9	2	0.74	0.075	0.983	0.007	0.82	0.029	0.965	0.015

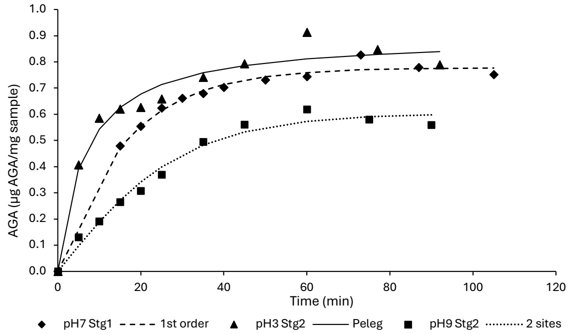

Fig 6 Fitting function of APS extraction data.

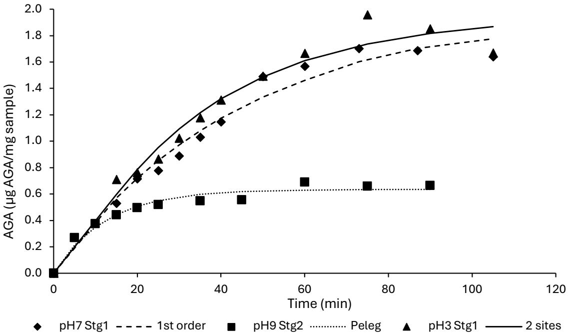

Fig 7 Fitting function of WAP extraction data.

realized by Hou *et al.*, (2024). Figures 6 and 7 show that a first-order equation can represent the extraction of GalA from WAP and APS. To examine all curves, figures are presented in A1.

3.2.2 Peleg's model

Peleg's equation effectively captures the initial rapid phase of extraction, which is primarily driven by diffusion, and the subsequent transition to a slower phase, where internal diffusion and mass transfer resistance become more significant. The constant $k_1$is directly related to the initial diffusion rate, while C_{eq} reflects the reduction in the extraction rate as equilibrium is approached. This reduction can be attributed to factors such as the decrease in the concentration gradient.

Experimental data of GalA extraction treatments were fitted to Peleg's equation. The fitting curves and Peleg's kinetic parameters (k, C_{eq}) were obtained (Table 2). Good agreement between predictions and experiments was found for all operating conditions ($R^2 > 0.91$). In both treatments (WAP and ASP), values of k were higher in the second extraction stage, probably because the cell wall has been weakened, permitting less resistance to flow and enhancing the extraction rate. In WAP, higher values of k were obtained with alkaline pH, whereas in ASP treatment, higher values were found in acid medium. $R^2 > 0.98$ presented by Wang *et al.*, (2023) showed a good overall fit to experimental data; however, the model was poorly applicable to the complete extraction process, it failed to adequately describe the behavior throughout the entire period. The parameter k ranged between 0.0211 and 0.1371 min·g/mg. Sucheta *et al.*, (2020) obtained k values of 0.84 at pH 2.4 for carrot pomace pectin and Das & Arora, (2021) reported 0.042 min·g/mg for carrot pomace pectin at pH 2.5.

Figures 5 and 6 show the fitting of Peleg's equation for some APS and WAP samples, respectively. The results indicated that acetone pretreatment (ASP) altered the physical structure of the samples, thereby reducing mass transfer. Although this model exhibited a lower main regression coefficient, the R^2 values ranged between 0.905 and 0.986, suggest the model could still provide a general overview of the operation.

3.2.3 Two-Site Kinetics Equation

In the two-site model, the first part of the equation (k_w) likely corresponds to the outermost layers of the plant tissue, where diffusion occurs more easily due to lower internal resistance. Conversely, the slow sites are associated with the innermost regions, where diffusion is limited by cell barriers or matrix density, as reflected in the k_d value. The presence of two rate constants (k_w and k_d) allows for a more nuanced modeling of how diffusion varies within different parts of the solid matrix.

Table 3 presents the values for k_w and k_d. In the first extraction stage of WAP, the F values of GalA showed an increasing trend with alkaline pH solutions, suggesting that a more significant portion of the target solute was extracted from the solid matrix during the rapid phase of the process. However, in the second stage of WAP, an inverse trend was observed. Employing APS at pH 7 resulted in the lowest F fraction of GalA during the rapid extraction phase. Increasing the extraction pH of GalA from 3 to 9 increased the k_w parameter, although the second extraction exhibited higher indices in all WAP cases. Higher C_{eq} values were observed in the acidic medium during the first stages for both WAP and APS.

The two-site kinetic equation proved to be a good descriptive model for both assayed extractions (main WAP R^2 = 0.968, main APS R^2 = 0.975).

The theoretical extraction curves of GalA, as predicted by the two-site kinetic equation, showed remarkable consistency with the experimental curves (Figures 5 and 6) across all investigated conditions. The effectiveness of the two-site kinetic model in accurately fitting the extraction data is due to its segmentation into two distinct term groups. Mentioned groups independently characterize the rapid and gradual phases of extraction, which occur sequentially, distinguishing it from other models (refer to the A3 for an analysis of all fitting curves). It is also assumed that a portion of GalA exists in a free state, where mass transfer occurs solely through diffusion. In contrast, another portion is bound to the plant matrix, requiring dissolution before diffusion can occur.

3.3 Comparison of mathematical models

An effective model for describing solid-liquid extraction must accurately reflect the various transfer mechanisms involved in the process. Among the mechanisms are diffusion, mass transfer through the phases, and the solubilization of solid material components in the solvent. Each mechanism can dominate at specific stages of the extraction (Das & Arora, 2021). Based on the evaluation of different models, the two-site kinetics model was identified as the most precise for representing GalA extraction kinetics (Figures 5 and 6).

Model selection was guided by the adjusted coefficient of determination (R^2) and root mean square error (RMSE). Among the models considered, the two-site model consistently yielded the highest R^2 values, ranging from 0.948 to 0.987, and had the lowest RMSE values, averaging 0.044. For comparison, Peleg's model produced R^2 values between 0.905 and 0.986 with a main RMSE of 0.070, while the first-order kinetics model ranged between 0.931 and 0.986 with a main RMSE of 0.058.

Table 3 Two site kinetic parameters.

Treatment		Without Acetone Pretreatment (WAP)					
pH	Stage	C_{eq}	F	Kw (min^{-1})	Kd (min^{-1})	R^2	RMSE
3	1	1.96	1.098	0.030	1.000	0.966	0.121
3	2	1.15	0.800	0.048	2.488	0.970	0.039
7	1	1.83	1.175	0.031	1.000	0.980	0.063
7	2	0.87	0.897	0.054	2.514	0.948	0.041
9	1	1.67	1.475	0.047	1.000	0.956	0.136
9	2	0.72	0.534	0.025	0.215	0.987	0.005
Treatment		With Acetone Pretreated (APS)					
pH	Stage	C_{eq}	F	Kw (min^{-1})	Kd (min^{-1})	R^2	RMSE
3	1	0.92	2.460	0.101	2.678	0.950	0.045
3	2	0.86	0.527	0.040	0.352	0.974	0.017
7	1	0.86	1.110	0.053	0.094	0.980	0.030
7	2	0.64	0.864	0.041	1.008	0.978	0.009
9	1	0.72	1.412	0.040	1.001	0.982	0.019
9	2	0.60	9.635	0.068	0.073	0.986	0.008

The root-mean-square error (Table 1) suggests that the first-order model is suitable only for processes characterized by a single and rapid mechanism. As equilibrium is approached, experimental findings are better described by the two-site kinetic equation. The above is demonstrated by the differences in C_{eq} values between experimental and calculated data, where the two-site kinetics model shows the lowest deviation (0.51 to 10%), the first-order model ranges between 1.20 and 13%, and Peleg's equation presents the highest deviation for C_{eq}.

Conclusion

The findings presented reveal significant influences of pH conditions on the yield of mucilage extraction from cladodes. Acidic and alkaline pH levels enhance MOFI yield compared to neutral pH solutions. Improvement is attributed to the facilitation of cell structure breakdown, enabling the release of the hydrocolloid. Notably, the highest yield of mucilage from *Opuntia ficus-indica* was achieved through extraction in an acid solution (47.8% db). In all treatments, a second extraction stage recovered up to 40% more mucilage; even the lowest yield obtained from acetone-pretreated samples exceeded values reported in previous studies.

The two-site kinetics model emerged as the most fitting for describing GalA extraction, effectively capturing both the rapid and gradual phases of the process. It is assumed that a portion of GalA exists in a free state, where mass transfer occurs via a concentration gradient between the cuboid and the solvent, while another portion is bound to the vegetal matrix, necessitating dissolution and the influence of temperature. Interestingly, the acetone pretreatment altered the physical structure of the sample, thereby affecting mass transfer efficiency.

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Nomenclature

OFI	*Opuntia ficus-indica*
MOFI	*Opuntia ficus-indica* mucilage
GalA	Galacturonic acid
SLE	Solid-liquid extraction
t	Time
C_{a0} to C_{aR}	Concentration from the center to the border of cladode at t=0 in 1st ESL stage
$C_{b0} to\ C_{bR}$	Concentration from the center to the border of cladode at t>0 in 1st ESL stage
$C_{bR} to\ C_{bi}$	Concentration from the border to the surface of cladode at t>0 in 1st ESL stage
$C_{ci} to\ C_{dr}$	Concentration from the surface to the solvent bulk at t>0 in 1st ESL stage
$C_{do} to\ C^*_{dr}$	Concentration at equilibrium in 1st ESL stage
$C_{dr} to\ C_{eR}$	Concentration from the center to the border of cladode at t=0 in 2nd ESL stage
$C_{f0} to\ C_{fR}$	Concentration from the center to the border of cladode at t>0 in 2nd ESL stage
$C_{fR} to\ C_{gi}$	Concentration from the border to the surface of cladode at t>0 in 2nd ESL stage
$C_{gi} to\ C_{gr}$	Concentration from the surface to the solvent bulk at t>0 in 2nd ESL stage
$C_{ho} to\ C^*_{hr}$	Concentration at equilibrium in 2nd ESL stage
R^2	Coefficient of determination
RMSE	Root mean square error
bt	Ultrasonic bath time
T	Temperature
m:w	Mass-to-water ratio
WAP	Samples without acetone pretreatment
APS	Acetone pretreated samples
Y	Yield
M_1	Mucilage weight (g dry basis)
M_c	Weight of the cladodes sample (g dry basis).
M	Molarity
FE	Fast extraction
C_t	Concentration on time t (mg GalA/gdb)
C_s	Concentration at saturation point (mg GalA/gdb)
k	first-order extraction rate coefficient (min^{-1})
K_1	Peleg rate constant (min·mg sample/μg GalA)
C_{eq}	Concentration at equilibrium point (mg sample/μg GalA).
F	Fraction of the solute released quickly $\frac{kg}{m^3}$
(1-F)	fraction of the solute released slowly $\frac{kg}{m^3}$
K_d	Desorption at a higher rate constant (min^{-1})
K_w	Desorption at a slower rate constant (min^{-1})

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