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Biosorption in brewer's spent yeast followed by freeze-drying: A promising strategy to protect vitamin C

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ABSTRACT

This study aimed to evaluate the use of a new strategy to protect and preserve vitamin C, considering its instability. The biosorption was applied using alkaline-modified (AY) and 'in natura' (Y) brewer's spent yeast (BSY) followed by freeze-drying. For this purpose, vitamin C was incorporated into the biomass, and an optimization study of biosorption was conducted to achieve the maximum sorption capacity and elucidate the interactions between them. The sorbent dosage, pH, kinetics, and initial concentration were varied, and isotherm models, including the Langmuir, Freundlich, Dubinin–Radushkevich, Sips, and Temkin models, were applied to the experimental data. A favorable sorption process was observed for both materials, and the Sips model presented the best fit, indicating that both chemical and physical interactions occur between vitamin C and BSY. The experimental and theoretical sorption capacities obtained were similar (SC_{exp} = 19.11 ± 0.97 mg/g and q_m = 17.84 ± 1.11 mg/g for Y and SC_{exp} = 18.11 ± 2.77 mg/g and q_m = 17.84 ± 0.96 mg/g for AY). The particles before and after incorporation were characterized by confocal laser scanning microscopy, FT-IR, zeta potential, moisture, and water activity, which provided evidence of the presence of vitamin C in BSY and helped elucidate the differences observed between the Y and AY results. The stability of the particles was also evaluated, which revealed that the concentration of vitamin C after 35 days decreased by 38.83 ± 1.21% for Y and 35.29 ± 0.29% for AY. These results indicate that both materials have advantages in biosorption tests; therefore, this innovative and sustainable study uses byproducts to incorporate nutrients and reduce their degradation.

1. Introduction

Ascorbic acid, a vital nutrient with significant antioxidant properties, plays a crucial role in reducing the risk of cancer and fighting free radicals. Furthermore, it performs several biological, pharmaceutical and dermatological functions. Despite being an essential nutrient, this vitamin is not synthesized by the human body and is naturally present in foods such as acerola, orange, lemon, broccoli and peppers (Bloomer; Fisher-Wellman, 2009). Owing to its exceptional reducing ability, ascorbic acid is prone to rapid oxidation, leading to faster degradation (Chang et al., 2010).

Owing to the instability of ascorbic acid and the functional and process limitations of commonly used techniques, such as micro- and nanoencapsulation (Comunian et al., 2013, 2020), exploring innovative approaches is essential. Biosorption, a technique widely employed in environmental decontamination studies (José et al., 2019; Soares et al., 2022), also has potential in the food industry, not only for nutrient protection but also for enabling the optimization of the process and a deeper understanding of the interactions between sorbent and sorbate. *Saccharomyces pastorianus*, a byproduct of the brewing industry, exemplifies this potential, as it is easy to obtain, nutritionally rich, cost-effective, and yet underexplored in the literature (Costa et al., 2024; Rusu et al., 2022; Wu et al., 2021). These characteristics make it an interesting option for incorporating ascorbic acid.

On the basis of this potential, the use of spent brewer's yeast as a biosorbent (José et al., 2019; Labuto et al., 2015) offers an alternative approach to incorporating ascorbic acid. Importantly, chemical or physical treatments can further increase the sorption capacity of certain biomasses. These treatments can facilitate the exposure or modification of binding sites present in the material, making them available for interaction with compounds of interest (Vijayaraghavan; Yun, 2008). Some studies, including those involving yeast biomass, have reported a

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significant increase in the sorption capacity of various compounds after pretreatment, such as phenolics by Rubio et al. (2018) and Cu(II) by Jianlong (2002), suggesting a promising alternative to enhance the biosorption capacity of ascorbic acid by *Saccharomyces pastorianus*.

In this context, a biosorption study adapted to variations in process parameters can refine the methodology and offer insights into the interactions between the sorbate and sorben (Foo & Hameed, 2010). Additionally, incorporating isotherm models, such as the Langmuir, Freundlich, Dubinin–Radushkevich, Sips, and Temkin models, which best fit the behavior of experimental data, contributes to comprehending the interactive phenomena between sorbent and sorvate (Nascimento et al., 2014; Rossetto et al., 2020). This mathematical interpretation of the experimental results contributes to confirming the feasibility of reusing a byproduct from the brewing industry, providing significant potential for scientific research.

Importantly, a limited number of studies have proposed the use of biosorption in the food sector, particularly with *Saccharomyces pastorianus* (Costa et al., 2024). However, to the best of our knowledge, there are no previous reports on the use of brewer's spent yeast (BSY), an underutilized brewing byproduct, or any other yeast, for the biosorption of ascorbic acid.

In this context, this study aimed to encapsulate ascorbic acid in BSY by biosorption. For this purpose, the best sorption conditions were determined; the biomass of BSY *in natura* (Y) and alkaline-treated (AY) as well as the biomasses before and after ascorbic acid incorporation (Y-AA and AY-AA) were characterized. Additionally, the stability of the encapsulated ascorbic acid during storage was also evaluated.

2. Materials and methods

2.1. Materials

The BSY consisted of *Saccharomyces pastorianus*, which underwent five brewing cycles to produce lager beer. It was provided by Cervejaria Hausen Bier (Araras, Brazil), transported under refrigeration, and maintained frozen until use.

Ascorbic acid ($C_6H_8O_6$) of 99% purity was obtained from Dinâmica Química Contemporânea Ltd. (Indaituba, Brazil). The reagents used for alkaline modification (NaOH) were acquired from L.S. Chemicals & Pharmaceuticals (Mumbai, India).

The solutions were prepared with deionized water purified from a Direct-Q3 system (Merck Millipore, Germany), and the formic acid used to prepare the mobile phase was acquired from Sigma Aldrich Brazil (São Paulo, Brazil).

2.2. Methodology

2.2.1. Preparation of biosorbents

The BSY biomass was washed five times with ultrapure water, with each wash lasting 24 h to allow the material to settle and remove malt residue and other impurities. The washing process lasted a total of five days and was carried out under refrigeration. The yeast-to-water ratio was approximately 1:10, as described by Rubio et al. (2018). The washed yeasts were subsequently dried via a spray dryer (model MSD 1.0 LabMaq – Ribeirão Preto, Brazil). The operating conditions used were as follows: atomizer nozzle diameter of 1.2 mm, liquid flow rate of 10 mL/min and 180 ± 2 °C inlet and 98 ± 2 °C outlet air temperatures. One part of the dry BSY biomass was stored for use *in natura,* (Y) and the other part was subjected to alkaline modification (AY).

2.2.1.1. Alkaline modification. To carry out chemical modification of *in natura* BSY, 5 g of dry BSY was added to an Erlenmeyer flask containing 500 mL of 0.1 mol/L sodium hydroxide (NaOH) (Mathialagan; Viraraghavan, 2009). The suspensions were shaken in a shaker incubator (MA420 Marconi, Piracicaba, Brazil) at 25 °C and 180 rpm for 24 h. When the treatment was complete, the suspensions were centrifuged (6000 rpm for 5 min) and lyophilized following the conditions described in section 2.2.4.

2.2.2. Biosorption studies

For investigations of ascorbic acid sorption via brewer's spent yeast, several parameters, such as the biosorbent dosage, pH, contact time, and initial sorbate concentration, were evaluated individually in different experiments, as detailed in the subsequent sections.

All these experiments were conducted in batches via Falcon tubes and were replicated three times. The BSY biosorbent, *in natura* or subjected to alkaline modification, was introduced into the tubes with a solution containing ascorbic acid at an initial concentration predetermined by preliminary tests. The suspension was stirred consistently at 1500 rpm via a multivortexer (Heidolph, Scwabach, Germany) and incubated at 25 °C.

After a predetermined period, the samples were collected and subjected to centrifugation at 6000 rpm for 5 min (Centrifuge 5430 R – Eppendorf, Hamburgo, Germany), and the supernatant was diluted, if necessary, or filtered through 13 mm × 0.45 μm nylon syringe filters (Allcrom, Barueri, Brazil). Subsequent quantification was carried out via high-performance liquid chromatography (HPLC) on a Liquid Chromatograph LC-20A Prominence Shimadzu (Kyoto, Japan) under the conditions described in section 2.2.3.

Importantly, in all the experiments, a control was carried out with only the initially prepared ascorbic acid solution. This control was subjected to the same conditions as the other samples to identify potential nutrient losses during the experiments and was compared with the amount of ascorbic acid present in the standard solution immediately after preparation.

All the experiments were conducted in an incubator set at 25 °C in the absence of light.

2.2.2.1. Biosorbent dosage. First, a test was carried out to optimize the dosage of biosorbent to be used in subsequent tests. For this purpose, a variation in the proportion between yeast and ascorbic acid was proposed, while the other parameters were fixed. The dosages tested ranged from 1.0 to 20.0 g of yeast per liter of ascorbic acid testing, the mass ranged from 10 to 200 mg, and the volume of the ascorbic acid solution was maintained at 10 mL at a concentration of 100 μg/mL). The pH of the solution was adjusted to 3.0, and the contact time was 6 h. The samples were subsequently centrifuged and filtered, and the supernatants were analyzed via high-performance liquid chromatography under the conditions described in section 2.2.3.

2.2.2.2. pH study. To analyze the influence of pH on the sorption of ascorbic acid by yeast, the pH was varied (2, 3, 4, 5, 6 and 7), while the other parameters were fixed. The concentration of 7.5 g/L used was the one that obtained the best results in the previous test (item 2.2.2.1), while the contact time was maintained at 6 h, and the initial concentration of ascorbic acid was 100 μg/L. After this time, the samples were centrifuged and filtered, and the supernatants were analyzed via high-performance liquid chromatography under the conditions described in section 2.2.3.

2.2.2.3. Sorption kinetics. After the dosage and pH were optimized, the next parameter evaluated was the influence of the contact time between the yeast and ascorbic acid. The initial concentration of the ascorbic acid solution, pH, and the yeast dosage in suspension were, respectively, 100 μg/mL, 3.0, and 7.5 g/L, as determined in previous tests. The contact time evaluated ranged from 30 to 2880 min. After the predetermined times, the samples were centrifuged and filtered, and the supernatant was analyzed by high-performance liquid chromatography under the conditions described in section 2.2.3.

2.2.2.4. Sorption capacity. For the sorption capacity tests, the proportions of yeast to ascorbic acid were optimized (7.5 g/L), as were the pH of the solution (3.0) and the contact time (300 min for *in natura* yeast and 180 min for alkaline yeast). Thus, only the amount of ascorbic acid was varied; solutions with increasing concentrations of 50, 100, 250, 500, 750, 1000 and 5000 μg/mL were used. At the end of the predetermined time for each biosorbent, the samples were centrifuged, diluted, and filtered, and the supernatant was analyzed by high-performance liquid chromatography under the conditions described in section 2.2.4.

Different sorption isotherm models (Langmuir, Freundlich, Dubinin–Radushkevich, Sips and Temkin) were applied to the sorption capacity data obtained experimentally to assist in the discussion and understanding of how the ascorbic acid biosorption process occurs via *Saccharomyces pastorianus* biomass.

2.2.3. Determination of ascorbic acid by high-performance liquid chromatography (HPLC)

The quantification of ascorbic acid remaining in the supernatants after the sorption tests was carried out via high-performance liquid chromatography using a liquid chromatographer (LC-20A Prominence – Shimadzu, Kyoto, Japan) with a calibration curve of 10–100 μg/mL, a diode array detector at a wavelength of 245 nm, and a C18 reversed-phase analytical column (Kinetex 150 × 4.6 mm, 5 μm particle size). The mobile phase, with isocratic elution, was composed of ultrapure water acidified with formic acid (5.5 mM), with a flow rate of 0.4 mL/min, an injection volume of 10 μL and a running time of 10 min. The column oven was maintained at 35 °C, and data were collected via LC Solution software, version 1.21. (Hernández et al., 2006; Spínola et al., 2013; Tarraro-Trani et al., 2012).

2.2.4. Freeze drying

After alkaline modification, the BSY was freeze-dried. After ascorbic acid biosorption, which was carried out *in natura* with alkaline BSY, the enriched materials were also freeze-dried.

The freeze dryer used was a model L101 Liotop (Liotécnica, Embu das Artes, Brazil) and model RC8D – DVP vacuum pump (San Pietro in Casale, Italy). The operating conditions were a pressure of 212 μg/Hg and a temperature of −53 °C over a period of 48 h (Costa el al., 2024).

After freeze-drying, the samples were stored at −20 °C for subsequent characterization analysis.

2.2.5. Characterization of the biosorbents

Several characterization techniques can elucidate the ascorbic acid biosorption process in yeast cells, indicating the interactions between the binding sites and the changes that can occur in the cell wall and inside the yeast.

2.2.5.1. Confocal laser scanning microscopy. Confocal microscopy was used to understand the distribution of ascorbic acid in *Saccharomyces pastorianus* cells after enrichment, as it provides images of the interior of the cells. To this end, the biosorbents of *in natura* yeast (Y), alkaline yeast (AY), *in natura* yeast after ascorbic acid biosorption (Y-AA) and alkaline yeast after ascorbic acid biosorption (AY-AA) were used. were observed via a Leica confocal microscope. TCS SP5 (Leica Microsystems, Germany) was used.

The samples were prepared according to the procedure described by Rubio et al. (2021) via Calcofluor White M2R and Nile Red.

2.2.5.2. Fourier transform infrared spectroscopy. Fourier transform infrared spectroscopy (FTIR) was used to detect absorption in a characteristic region and identify functional groups, bond types and molecular conformations. For this purpose, a PerkinElmer FTIR spectrometer (Massachusetts, USA) was used, which was operated in the range of 4000–550 cm^{-1}, with a resolution of 4 cm^{-1} and 16 scans, and a potassium bromide (KBr) prepared with approximately 1 mg of sample for 100 mg of KBr was used.

In addition to the samples of *in natura* yeast (Y), alkaline yeast (AY), *in natura* yeast after ascorbic acid biosorption (Y-AA) and alkaline yeast after ascorbic acid biosorption (AY-AA), pure ascorbic acid was also evaluated via the FTIR technique.

2.2.5.3. Zeta potential. Zeta potential measures the magnitude of electrostatic repulsion or attraction of particles dispersed in a liquid and plays a fundamental role in understanding the stability of systems. To carry out this measurement of the biosorbents of *in natura* yeast (Y), alkaline yeast (AY), *in natura* yeast after ascorbic acid biosorption (Y-AA) and alkaline yeast after ascorbic acid biosorption (AY-AA), a zeta potential analyzer (Zetasizer ZS 3600, Malvern Instruments, United Kingdom) was used at a detection angle of 173° and a wavelength of 633 nm. Measurements were performed in triplicate at 25 °C.

2.2.5.4. Moisture and water activity. To determine the water content of the particles, a moisture analyzer (MB35 - Ohaus, Switzerland) was used, which uses infrared radiation from a halogen source. The results are expressed as percentages. To determine water activity (from 0 to 1), AQUALAB equipment (Decagon Devices, Pullman, WA) was used at a temperature of 25 °C. Both techniques were used to monitor the moisture and water activity of *in natura* yeast biosorbents after ascorbic acid sorption (Y-AA) and alkaline yeast biosorbents after ascorbic acid sorption (AY-AA) during each point of the stability study, as described in the following section (2.2.6).

2.2.6. Stability of ascorbic acid during particle storage

After optimization of the biosorption parameters, two batches of particles were produced, alkaline modified and *in natura* BSY biomass, for evaluation of acid ascorbic acid stability during storage.

The samples were stored in hermetically sealed glass bottles at 25 °C containing a saturated $MgCl_2$ solution (relative humidity of 32.3%) for 35 days, following the methodology described by Rubio et al. (2021). Thus, every 7 days, the process of desorption of ascorbic acid present in both particles was carried out by washing the powders with 5 mL of ultrapure water acidified with 5.5 mM formic acid (pH 3) and kept under constant stirring for 5 min. Then, the suspension was placed in an ultrasonic bath (UltraCleaner 1400, Unique, Indaiatuba, Brazil) for another 5 min, and the samples were subsequently centrifuged (6000 rpm for 5 min) Centrifugeat 5430 R – Eppendorf, Hamburgo, Germany. The process was repeated once more, and the supernatants were combined and filtered to determine the amount of ascorbic acid that was desorbed from the yeast cells. Quantification was carried out via high-performance liquid chromatography under the conditions described in section 2.2.4 for quantification and determination of ascorbic acid stability.

2.2.7. Statistical analysis

The *t*-test was used to compare means. Differences were considered significant when the p value was less than 0.05. The analysis was performed via STATISTICA 7.1 software (StatSoft, Tulsa, OK, USA).

3. Results and discussion

3.1. Biosorbent dosage

The influence of the variation in mass of the *in natura* (Y) and modified (AY) biomasses was evaluated as percentages and in mg/g. The results are shown in Fig. 1.

An analysis of the results in Fig. 1 reveals that the percentage of ascorbic acid produced by both materials increase or remains constant, as expected,; however, if the mass used is too high, the amount of vitamin adsorbed per gram of material decreases. In this way, 75 mg was

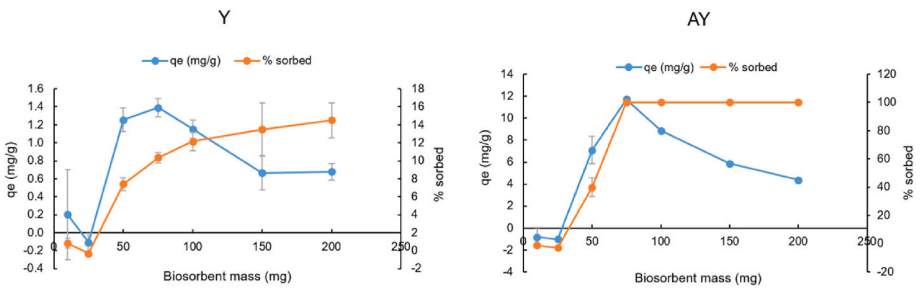

Fig. 1. Influence of biosorbent dosage on ascorbic acid sorption by *in natura* (Y) and alkaline (AY) yeasts. n = 3.

the mass of biomass that obtained the most satisfactory results considering the data in mg/g, both for Y and for AY (1.27 and 11.71 mg/g of ascorbic acid per g of biomass, respectively), showing that AY presents better results under the conditions used.

Notably, the difference in sorption between Y and AY can be attributed to the modification of the active sites and their availability, which may lead to distinct interactions that influence the sorption equilibrium, as observed in the kinetic tests, to be discussed later.

3.2. pH study

To investigate how the pH of the ascorbic acid solution affects its incorporation into *in natura* yeast (Y) and alkaline yeast (AY) biomass, the pH was adjusted to range from 2 to 7. The pH values above 7 were not evaluated since, as observed by Szultka et al. (2014), ascorbic acid is unstable and susceptible to degradation in alkaline media. The biosorption results, expressed in mg/g, with respect to pH variation are illustrated in Fig. 2.

An analysis of the data in Fig. 2 reveals that the maximum biosorption of ascorbic acid by Y occurs at pH 3, with a significant decrease as the pH increases. Both materials exhibited lower and almost constant sorption levels at pH values between 4 and 7, which is consistent with the instability of ascorbic acid at pH values above 4, as reported in the literature (Sheraz & Khan, 2018), thus supporting the use of lower pH values. However, the most significant discrepancy occurred at pH 2, where biosorption by AY was very low. This can be attributed to the interaction between the modified sites and the cationic species formed in a highly acidic medium, resulting in the unavailability of functional groups to interact with ascorbic acid. Additionally, this can be attributed to the instability of vitamin C at pH values lower than 2.5 (Sheraz & Khan, 2018).

At pH 3, this limitation was not observed, and both materials (Y and AY biomass) achieved the highest sorption values. This suggests that performing experiments under these pH conditions was advantageous, providing greater molecular stability, as reported in the literature (Spínola et al., 2013; Szultka et al., 2014; Sheraz & Khan, 2018).

3.3. Sorption kinetics

biosorption kinetics were performed by varying the contact time between the yeasts (Y and AY biomasses) and ascorbic acid. The results obtained are shown in Fig. 3.

The results in Fig. 3 show that the sorption of ascorbic acid by Y biomass occurs at an almost constant rate, gradually increasing each time until it starts to stabilize at 300 min. On the other hand, the behavior of AY for ascorbic acid sorption is atypical, with a very large increase between 150 and 180 min (from 2.21 ± 0.15 mg/g to 10.87 ± 0.46 mg/g). This may have occurred because the active sites were altered with the alkaline modification, which may have exposed new functional groups such as hydroxyl or carboxyl groups, which may have facilitated stronger interactions with ascobic acid. The slower kinetic step that was observed may be attributed to intraparticle adsorption, a diffusion process within the porous structure of the yeast, suggesting that the mechanism changed from a predominantly physical process to a process that may also involve chemical interactions and internal diffusion dynamics. Nascimento et al. (2014) discussed such interactions that may occur during biosorption, helping to understand the process.

With respect to the equilibrium time, Y was considered 300 min, whereas for AY, it was 180 min, presenting very different sorption capacity values at equilibrium (1.43 ± 0.12 mg/g and 10.87 ± 0.46 mg/g for Y and AY, respectively), demonstrating the potential of alkaline modification in yeast for ascorbic acid biosorption under these conditions.

3.4. Sorption capacity

Fig. 4 shows the results obtained experimentally for the sorption capacity, as well as the isotherm models that were applied to these data, and Table 1 presents the parameters corresponding to the adjustments of each model.

Fig. 4 shows the profiles of the adsorption curves, as described by Labuto et al. (2015), which are favorable for both materials; however, as the angle between the curve and the y-axis is smaller for AY, this material presents an even more favorable sorption mechanism than Y does, which highlights the alkaline modification of yeast.

According to the data in Table 1, the experimental sorption capacity (SC_{exp}) was 19.11 ± 0.97 and 18.11 ± 2.77 for Y and AY, respectively, indicating that when the concentration was increased, the material *in natura* considerably improved ascorbic acid biosorption.

In addition, from Table 1, the model that best fit the experimental

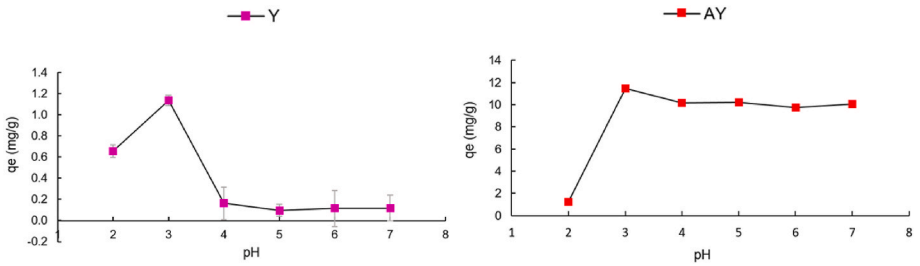

Fig. 2. Effect of pH of the ascorbic acid solution on the biosorption process using *in natura* (Y) and alkaline yeast (YA). n = 3.

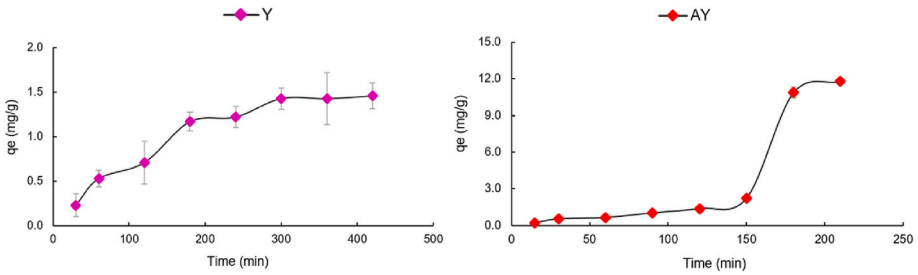

Fig. 3. Experimental curves of ascorbic acid sorption kinetics by *in natura* (Y) and alkaline (AY) yeasts. n = 3.

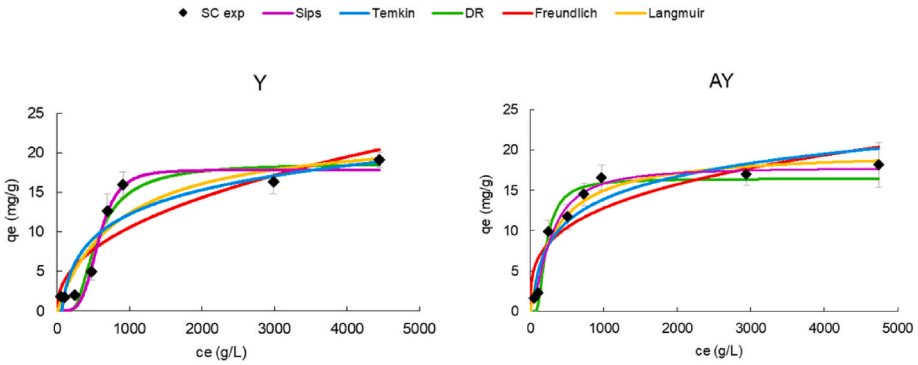

Fig. 4. Experimental curves and Langmuir, Freundlich, Sips, Dubinin-Radushkevich (D–R) and Temkin isotherms models fitting for ascorbic acid sorption by *in natura* (Y) and alkaline (AY) yeast biomass, using solution in increasing concentrations. n = 3.

Table 1
Values of experimental Sorption Capacity (SC_{exp}), isotherms parameters and χ^2 error evaluation for ascorbic acid sorption by *in natura* (Y) and alkaline (AY) yeast. SD = Standard Deviation; SE = Standard Error provided by fitting the model to the experimental data, n = 3.

	Y	AY
SC_{exp} (mg/g)	$19.11^a \pm 0.97$ (SD)	$18.11^a \pm 2.77$ (SD)
Langmuir Isotherm Model		
q_m (mg/g)	22.91 ± 3.55 (SE)	19.97 ± 1.34 (SE)
b (L/mg)	$0.0012 \pm 4.96 \times 10^{-4}$ (SE)	$0.003 \pm 7.2 \times 10^{-4}$ (SE)
r^2	0.8865	0.9568
χ^2	6.58	2.21
Freundlich Isotherm Model		
K_f (L/mg)	0.50 ± 0.41 (SE)	1.63 ± 0.89 (SE)
n_f	2.26 ± 0.55 (SE)	3.35 ± 0.83 (SE)
r^2	0.8129	0.8168
χ^2	10.84	9.38
DR Isotherm Model		
q_{DR} (mg/g)	18.67 ± 1.18 (SE)	16.41 ± 0.78 (SE)
B_{DR} (mol^2/J)	0.036 ± 0.0077 (SE)	0.0054 ± 0.0014 (SE)
E (kJ/mol)	3.73	9.62
r^2	0.9527	0.9542
χ^2	2.88	2.35
Sips Isotherm Model		
q_m (mg/g)	17.84 ± 1.11 (SE)	17.84 ± 0.96 (SE)
K_s (L/mg)	$0.002 \pm 1.34 \times 10^{-4}$ (SE)	$0.003 \pm 5.34 \times 10^{-4}$ (SE)
n	4.38 ± 1.29 (SE)	1.57 ± 0.28 (SE)
r^2	0.9533	0.9670
χ^2	2.58	1.38
Temkin Isotherm Model		
b (J/mol)	4.42 ± 0.79 (SE)	4.04 ± 0.53 (SE)
A (L/mg)	0.016 ± 0.007 (SE)	0.031 ± 0.013 (SE)
T (K)	298.15	298.15
r^2	0.8138	0.8895
χ^2	10.28	4.63

data for both materials was Sips, with the best coefficient of determination (r^2), with values of 0.9533 and 0.9670 for Y and AY, respectively. When the errors associated with the models (χ^2) were evaluated, Sips presented the smallest errors, with values of 2.58 for Y and 1.38 for AY. According to Nascimento et al. (2014), this model is a hybrid of the Langmuir and Freundlich models, acknowledging that both physical and chemical phenomena occur and contribute to the sorption process.

When analyzing the parameters of the DR model, it is also possible to observe good fits, with very favorable r^2 values (0.9527 and 0.9542 for Y and AY, respectively) and low χ^2 values ($\leq$2.88). In addition, another interesting parameter to be discussed is the adsorption energy (E), which is less than 8 kJ/mol and indicates a predominance of physisorption, whereas according to Foo and Hameed (2010), the value is between 8 and 16 kJ/mol. Thus, the biosorption of ascorbic acid by Y occurs predominantly through physical phenomena (E = 3.73), whereas the biosorption using AY occurs predominantly through chemical phenomena (E = 9.62); that is, apparently, the sites modified through alkaline modification cause the interactions between ascorbic acid and yeast to be more intense and of a chemical nature. Chemical interactions, such as covalent bonding, hydrogen bonding, or electrostatic interactions, are generally stronger and more specific than physical interactions, such as van der Waals forces. This likely results in a more stable and efficient biosorption process (Israelachvili, 2011; Nascimento et al., 2014).

This can also be seen through the parameters obtained by the Langmuir model, which presents better fits for AY than for Y. This model considers that the interactions are purely chemical and that each sorption site adsorbs a single molecule on homogeneous surfaces; thus, adsorption occurs in a monolayer and in specific sites of the adsorbent (Foo & Hameed, 2010; Nascimento et al., 2014; José et al., 2019).

Thus, it is possible to infer that the interactions between the sorbent and sorbate may be stronger for AY than for Y; however, both are favorable to the process, as the sorption capacity results are not statistically significant ($p < 0.05$), indicating the potential for application in the biosorption of ascorbic acid.

3.5. Characterization of the biosorbents

3.5.1. Confocal laser scanning microscopy

To visualize the interior of yeast cells before and after biosorption of

vitamin C, the confocal laser microscopy technique was used, whose optical system focuses light on a single focal point, rejecting out-of-plane light and allowing high-quality image resolution (Elliott, 2020). The images obtained are shown in Fig. 5.

Dyes are used to better mark and visualize specific structures or components in biological samples, with the blue dye fluorescing in the presence of polysaccharides, which are components of the yeast cell wall, while the red dye fluoresces when interacting with lipid molecules, which are components of important elements inside cell membranes, in addition to serving to evaluate the integrity of cell membranes (Pham-Hoang et al., 2018).

As shown in Fig. 5, some differences in intensity and color may indicate the presence of vitamin C and its distribution patterns. In Y, it is possible to observe fewer contrasting points stained red than in Y-AA, in which there are more stained points, including inside the cells and not just on the surface, as in Y. Thus, we can attribute this difference to the presence of other components in the cells or to the modification of existing components.

In addition, Y was stained with both dyes because of the components present in its internal and external structure. As ascorbic acid is not colored by any dye, it is possible to discuss its presence in yeast cells only on the basis of the changes occurring in the other stained structures. Thus, when Y and AY are observed, structures stained red can be observed, which probably correspond to organelles, which, according to Rubio et al. (2021), are predominantly hydrophobic. After the incorporation of ascorbic acid, LI-AAs and AY-AAs presented more colored structures and were located predominantly closer to the surface. This may have occurred after the incorporation of ascorbic acid, a very hydrophilic compound that, upon entering cells, possibly repels the hydrophobic compounds present. Moreover, The cellular walls of the materials stand out, which appear not to have suffered rupture or damage to their structure, which would not harm the interaction with ascorbic acid.

Furthermore, the formation of agglomerates was observed in AY, Y-AA and AY-AA, which is characteristic of the *freeze–drying* process, mainly in AY-AA, where the agglomerates were still larger, as this

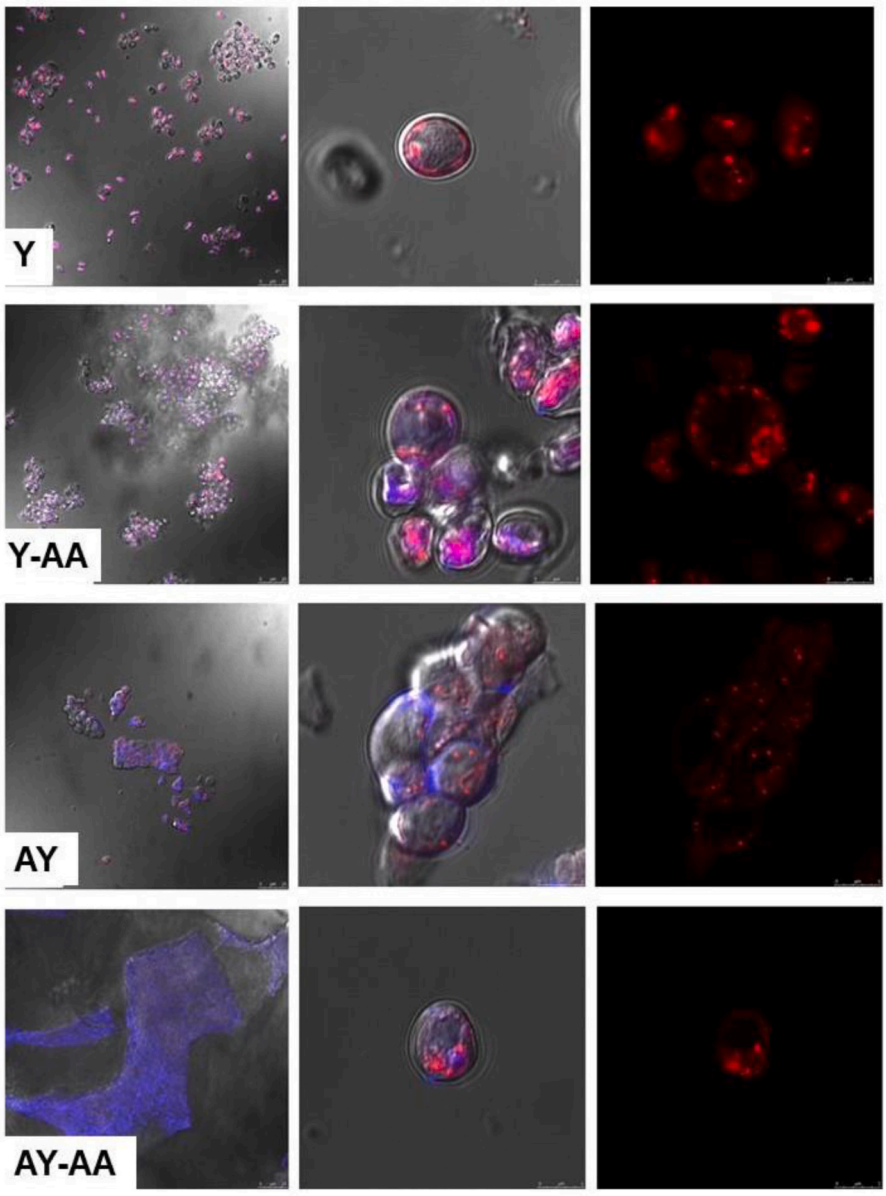

Fig. 5. Confocal micrographs of *in natura* yeast (Y), alkaline yeast (AY), *in natura* yeast after ascorbic acid biosorption (Y-AA) and alkaline yeast after ascorbic acid biosorption (AY-AA).

material was subjected to the freeze–drying process twice. These agglomerates, despite making it difficult for powders to flow, can provide greater protection for bioactive compounds, since the packaging of the particles makes the active material more physically protected and hinders the diffusion of oxygen, for example.

3.5.2. Fourier transform infrared spectroscopy

To better understand the possible sorption sites that participate in the process of incorporating ascorbic acid into *Saccharomyces pastoris* cells, radiation spectroscopy analysis was carried out via Fourier transform infrared spectroscopy. The graphs obtained from yeast before (Y and AY) and after (Y-AA and AY-AA) biosorption, as well as pure ascorbic acid, are shown in Fig. 6.

As shown in Fig. 6, at first, one can observe the similarity between the biosorbents before and after sorption, demonstrating that neither the alkaline modification nor the presence of ascorbic acid caused very significant changes in the spectra, which presented very similar bands, as expected, indicating that the main molecular conformations and composition of the yeast were not considerably altered.

However, it is possible to note that *in natura,* yeast (Y) presented slightly more intense bands than did yeast after alkaline modification (AY), at approximately 1644 cm^{-1}, a band characteristic of amides present in proteins and peptides, demonstrating that the treatment may have caused the breakage of these chemical bonds, which may be advantageous, as active sites may be more available, favoring the interaction between sorbent and sorbate, in the same way as reported by Ribeiro et al. (2019) when alkaline treatment of *Saccharomyces cerevisiae is used.*

Furthermore, close to this same wavelength, there is a band characteristic of ascorbic acid (C=C), causing a slight broadening of the band in the Y and AY materials after biosorption (Y-AA and AY-AA), making it possible to infer the presence of vitamin C in the biosorbents.

Moreover, another band of great intensity present in yeasts at approximately 1040 cm^{-1}, characteristic of β-glucans and mannans, which represent most of the composition of *Saccharomyces, is notable*. Additionally, this band may indicate that C-O-H bonds in alcohols formed from residual carbohydrates from the beer fermentation process (Ribeiro et al., 2019; Silverstein et al., 2012).

3.5.3. Zeta potential

The zeta potential consists of the tendency of a particle to move in response to an electric field, which can have positive, negative or zero values. The complete table with the zeta potential values of the materials before (Y and AY) and after (Y-AA and AY-AA) the biosorption process is shown in Table 2.

When the zeta potential is more negative, the particle has more negative electrical charges on the surface; that is, it presents functional groups or anionic molecules sorbed on its surface, causing electrostatic repulsion to prevent its aggregation and contributing to the stability of the suspension (Clogston & Patri, 2011).

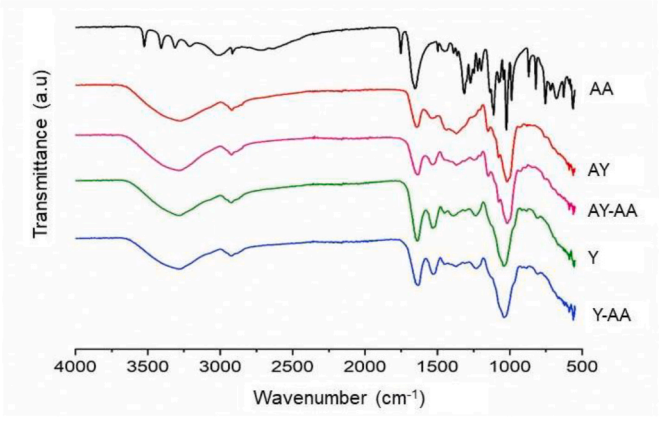

Fig. 6. Infrared spectra of ascorbic acid (AA), *in natura* yeast (Y), alkaline yeast (AY), *in natura* yeast after ascorbic acid biosorption (Y-AA) and alkaline yeast after ascorbic acid biosorption (AY- AA).

Table 2
Zeta potential and electrophoretic mobility values of *in natura* yeast (Y), alkaline yeast (AY), fresh yeast after ascorbic acid biosorption (Y-AA) and alkaline yeast after ascorbic acid biosorption (AY-AA). n = 3.

	Zeta potential (mV)
Y	$-10.7^{a} \pm 0.47$
Y-AA	$-11.7^{ab} \pm 1.50$
AY	$-15.1^{b} \pm 2.33$
AY-AA	$-9.2^{a} \pm 0.43$

An evaluation of the data in Table 2 reveals that the zeta potential of Y (−10.7 mV) is less negative than that of AY (−15.1 mV); therefore, the material subjected to alkaline treatment was more stable since it presented more negative charges on its surface modified with NaOH.

After the incorporation of ascorbic acid, it was expected that the materials would present more negative values; however, AY-AA presented the highest zeta potential values (−9.2 mV), consequently indicating a lower stability of the suspension, which corroborates the stability results of the charged particles, the results of which indicated a slightly lower stability of AY-AA than of Y-AA.

3.5.4. Moisture and water activity

To evaluate changes in the moisture and water activity of the enriched materials (Y-AA and AY-AA), they were monitored every 7 days, together with a particle stability study. The results obtained are presented in Table 3.

According to the results presented in Table 3, the water activity (Aw) values of the materials after ascorbic acid biosorption were similar and within the expected range for freeze-dried products. Furthermore, they also presented values below those recommended to guarantee microbiological stability (Righi da Rosa et al., 2019), ranging from 0.401 to 0.473 in Y-AA and from 0.307 to 0.479 in AY-AA during the 35 days.

Moisture also showed the same increasing trend after the start of the stability study (point 0), especially in AY-AA, where the increase was more significant. This increase may have been due to the relative moisture of the saturated solution, which resulted in moisture gain by the material.

However, the moisture percentages were low, ranging from 9.08 to 10.71% for Y-AA and from 7.42 to 10.95% for AY-AA. Low humidity values are desirable for powders since increased humidity is commonly related to the instability of encapsulated compounds, as it can promote their release or diffusion to the surface of the encapsulating material, where the compound is more prone to degradation (Paramera et al., 2011).

This finding corroborates the hypothesis that yeast particles

Table 3
Moisture and water activity values of *in natura* yeast particles after ascorbic acid sorption (Y-AA) and alkaline yeast particles after ascorbic acid sorption (AY-AA) during the stability study (from 0 to 35 days). n = 3.

Time (days)	Moisture (%)		Water activity	
	Y-AA	AY-AA	Y-AA	AY-AA
0	$9.26^{a} \pm 0.60$	$7.42^{a} \pm 0.70$	$0.440^{a} \pm 0.012$	$0.307^{a} \pm 0.003$
7	$10.71^{b} \pm 0.05$	$10.78^{b} \pm 1.38$	$0.473^{a} \pm 0.16$	$0.479^{b} \pm 0.023$
14	$9.95^{a} \pm 0.07$	$10.95^{b} \pm 0.08$	$0.412^{a} \pm 0.014$	$0.420^{c} \pm 0.008$
21	$10.26^{ab} \pm 1.05$	$10.00^{b} \pm 0.00$	$0.461^{a} \pm 0.03$	$0.408^{c} \pm 0.003$
28	$10.68^{b} \pm 0.30$	$10.31^{b} \pm 0.44$	$0.401^{a} \pm 0.03$	$0.408^{c} \pm 0.003$
35	$9.08^{a} \pm 0.37$	$10.35^{b} \pm 0.50$	$0.419^{a} \pm 0.04$	$0.430^{c} \pm 0.024$

Equal letters in the same column indicate that there was no significant difference between treatments ($p > 0.05$).

modified with alkaline conditions, when loaded with ascorbic acid, are slightly less stable to this vitamin than *natura* particles. According to Rubio et al. (2018), alkaline treatment can alter existing bonds and molecules on the yeast surface, which can modify the availability of sorption sites but not irreversibly. In fact, this change may have occurred in AY, which may have interfered with the stability of the particle after its interaction with ascorbic acid.

However, after 7 days, Aw and moisture percentages remained practically stable for both Y-AA and AY-AA until the 35th day, with no significant changes highlighted because there was equilibrium with the moisture content of the saturated solution.

3.6. Stability of ascorbic acid during particle storage

The evaluation of the stability of the encapsulated ascorbic acid in both particles (Y-AA and AY-AA) was conducted using samples produced under conditions previously optimized in the biosorption study. The results are shown in Fig. 7.

The stability of the ascorbic acid encapsulated in both particles decreased over time (Fig. 7), reaching $38.83^{a} \pm 1.21\%$ (Y-AA) and $35.29^{b} \pm 0.29\%$ (AY-AA) of the initial amount of ascorbic acid that was desorbed. Therefore, the stability gradually decreased, as expected, since, despite the idea of protection, the BSY biomass was not able to completely prevent the degradation of ascorbic acid, especially if it was present on yeast surfaces. However, the use of this wall material is an interesting alternative from a nutritional point of view, as it also serves as a source of proteins, carbohydrates, and minerals, in addition to protecting ascorbic acid, which is unstable and can interact with other food components, causing its degradation more quickly.

Notably, as other characterization techniques have also demonstrated, alkaline BSY biomass presented slightly lower percentages of ascorbic acid loaded at all times evaluated, indicating that this material is less efficient at protecting ascorbic acid. This may have occurred because of changes in the yeast cell wall and active sites after alkaline modification and, mainly, because of interactions between the modified biosorbent and ascorbic acid.

Although the biosorption study revealed that the process involves a chemical nature, reversible interactions may have occurred on the surface of the material, facilitating oxidation and resulting in the lower stability of AY-AA than Y-AA.

In a previous study by our research group, Comunian et al. (2013) also studied the stability of free and encapsulated ascorbic acid; however, in that study, this vitamin was encapsulated by a double emulsion followed by complex coacervation. The degradation values of almost 100% free ascorbic acid (in solution) were reported after 15 days of storage. However, after 30 days at 20 °C, the microcapsules still maintained 57–80% of the initial concentration of AA. At a higher temperature of 37 °C, the microcapsules maintained 32–44% of the initial concentration of AA, thus confirming the deleterious effect of temperature on the encapsulated material. Therefore, the particles produced by Comunian et al. (2013) were more effective at protecting against AA during storage than those produced by biosorption via BSY. However, the techniques used by Comunian et al. (2013) are more complex, and the encapsulant is more expensive than BSY biomass.

Matos et al. (2015) produced solid lipid microparticles by *spray congealing* to protect ascorbic acid. The stability of the AA encapsulated after 60 days of storage exceeded 70%, even upon storage at 37 °C. However, the encapsulant used by these authors, an interesterified fat with a melting point of 43 °C, was less healthy and more expensive than the BSY biomass.

Therefore, in this study, the Y and AY materials offered some protection to ascorbic acid during storage and showed good results for possible applications in the formulation of food products.

In a previous work of our research group, *Saccharomyces pastoris* brewer's yeast biomass was also successfully used to protect cholecalciferol (D3 vitamin) (Costa et al., 2024), which, unlike vitamin C, is a nonpolar molecule. Therefore, this biomass proved to be versatile and effective in impregnating and ensuring the stability of vitamins with different polarities.

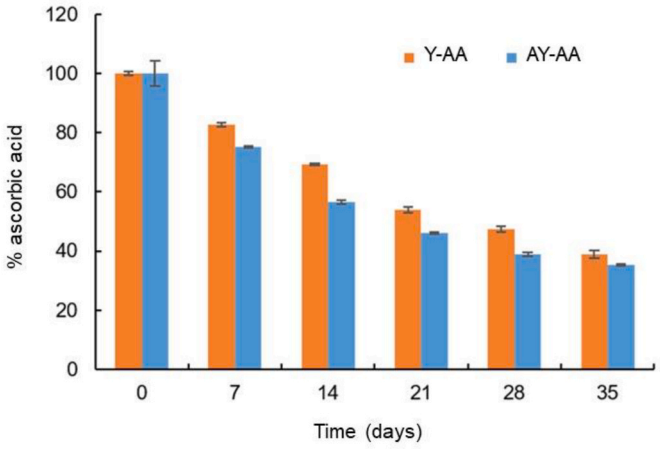

Fig. 7. Stability of *in natura* yeast particles after ascorbic acid sorption (Y-AA) and alkaline yeast particles after ascorbic acid sorption (AY-AA), in a period between 0 and 35 days. n = 3.

4. Conclusion

The biosorption study revealed that the interaction between ascorbic acid and yeast is favorable, resulting in good sorption capacity, with no significant difference between *natural* and modified biomass. To better understand the process, isothermal models were applied to the experimental data on sorption capacity, with Sips being the one that obtained the best fits for Y and AY, indicating that physical and chemical interactions are involved in the process between sorbent and sorbate.

Furthermore, the results obtained for both materials were also similar in terms of stability, with $38.83 \pm 1.21\%$ (Y-AA) and $35.29 \pm 0.29\%$ (AY-AA) of the vitamins desorbed after 35 days. Thus, Y and AY presented favorable results in that they act as vehicles for the incorporation of ascorbic acid, which could also be demonstrated through characterization techniques.

Future studies could compare the results obtained by evaluating the influence of different drying techniques and modifications in the washing step, among other factors. Additionally, on the basis of the findings of this study, it is important to assess the mechanism of digestion and release of ascorbic acid (AA) from the particles during ingestion. It may also be valuable to evaluate the ability of the produced particles to mask the acidic taste of AA, which is not always desirable.

CRediT authorship contribution statement

Julia Cristina José: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Beatriz Caliman Soares:** Methodology, Investigation. **Tatielly de Jesus Costa:** Methodology, Investigation. **Ramon Peres Brexó:** Conceptualization. **Marcelo Thomazini:** Validation, Methodology, Investigation, Conceptualization. **Milena Martelli Tosi:** Formal analysis, Data curation. **Carmen Sílvia Favaro-Trindade:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

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.

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Data availability

Data will be made available on request.

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