

ORIGINAL ARTICLE

Effect of temperature, pH and storage time on the stability of an extracellular fructosyltransferase from *Aspergillus oryzae* IPT-301

Efeito da temperatura, pH e tempo de armazenamento na estabilidade de frutossiltransferase extracelular de Aspergillus oryzae IPT-301

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Abstract

In this work, it was determined the influence of temperature, pH and storage time on the enzymatic activity and stability of an extracellular fructosyltransferase (FTase E.C.2.4.1.9) from *Aspergillus oryzae* IPT-301 produced by submerged fermentation. The thermodynamic parameters showed a tendency for increasing enzyme denaturation with the rise in temperature. The maximum transfructosylation activity was obtained at the incubation pH 5.5. During storage at 4 °C, the transfructosylation activity decreased, whereas the hydrolytic activity increased, especially in the first nine hours, a time in which the enzyme presented 45.6% of its initial transfructosylation activity. These results contributed to the improvement of the conditions of storage, immobilization and use of the soluble fructosyltransferases (FTase) in fructooligosaccharide (FOS) production.

Keywords: Submerged fermentation; Soluble enzyme; Enzyme stability; Thermodynamic parameters; *Aspergillus*; Transfructosylation activity.

Resumo

Neste trabalho foi determinada a influência da temperatura, do pH e do tempo de armazenamento na estabilidade enzimática de frutossiltransferase (E.C.2.4.1.9) extracelular de *Aspergillus oryzae* IPT-301, produzida por fermentação



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submersa. Os parâmetros termodinâmicos mostraram tendência de aumento da desnaturação da enzima com o aumento da temperatura. A atividade de transfrutossilacção máxima foi obtida para a enzima incubada em pH 5,5. Durante o armazenamento a 4 °C, a atividade de transfrutossilacção diminuiu, enquanto a atividade hidrolítica aumentou, principalmente nas primeiras nove horas, período em que a enzima apresentou 45,6% de sua atividade inicial de transfrutossilacção. Esses resultados contribuem para a melhoria das condições de armazenamento, imobilização e utilização das frutossiltransferases solúveis na produção de frutooligosacarídeos.

Palavras-chave: Fermentação submersa; Enzima solúvel; Estabilidade enzimática; Parâmetros termodinâmicos; *Aspergillus*; Atividade de transfrutossilacção.

1 Introduction

The enzyme fructosyltransferase (FTase E.C.2.4.1.9) catalyzes the reaction of sucrose transfructosylation for the production of fructooligosaccharides (FOS), fructose oligomers with degree of polymerization between two and 20, whose fructosyl units are linked to the terminal sucrose molecule by glycosidic bonds at the β -(2 \rightarrow 1) position (Faria et al., 2021; Yun, 1996). The FOS are functional sugars with major importance for the production of foods and pharmaceuticals, since they offer several benefits to human health. Indeed, the FOS present from 40 to 60% of the sweetening power of sucrose, being low in calories and presenting low glycemic index, as well as prebiotic activity. They participate in the inhibition of putrefactive substances, reduction of pathogenic microorganisms and the balance of the intestinal flora, thus helping to prevent colon cancer (Andersson et al., 1999; Gibson & Roberfroid, 1995; Mabel et al., 2008; Marx et al., 2000; Nobre et al., 2018; Rivero-Urgell & Santamaria-Orleans, 2001; Saulnier et al., 2007; Yun, 1996).

The industrial FOS production depends on the efficient and cheap production of FTases, as well as their enzymatic activities and their resistance to denaturation during the enzymatic reaction and during storage (Mutanda et al., 2014; Yun, 1996). FTases can be produced by submerged fermentation using, mainly, fungi from the species *Aspergillus niger*, *A. oryzae*, *Rhodotorula diarensis* and *Cryptococcus* sp., and they can be used either in the soluble or immobilized forms for FOS production (Antošová & Polakovič, 2001; Cuervo-Fernandez et al., 2007; Ganaie & Gupta, 2014; Huang et al., 2016; Mutanda et al., 2014). Specifically, the fungus *A. oryzae* IPT-301 is one of the main producers of extracellular and mycelial FTases which present high transfructosylation activity and low hydrolytic activity (Cuervo-Fernandez et al., 2007). In the reaction of sucrose transfructosylation, the high ratios between the transfructosylation and hydrolytic activities (A_T/A_H) is an essential indicator of the predominance of the transfructosylation reaction over the hydrolytic reaction, and consequently, of higher FOS production (Cuervo-Fernandez et al., 2007; Ghazi et al., 2007).

Several studies have reported optimal parameters for the production of FTase from *A. oryzae* IPT-301 and the behavior of its enzymatic activity according to pH, temperature and sucrose concentration (Cunha et al., 2019; Ottoni et al., 2012; Perna et al., 2018). Furthermore, Faria et al. (2021) and Gonçalves et al. (2020) reported the enzymatic activity and thermal and operational stability of *A. oryzae* IPT-301 FTases immobilized by adsorption and encapsulation, respectively. Similarly, several authors have reported optimal pH and temperature values to maximize the enzymatic activity of extracellular FTases from *Rhodotorula* sp. used in the free form, as well as the thermal stability of this enzyme immobilized by adsorption in Niobium ore (Aguiar-Oliveira & Maugeri, 2010; Hernalsteens & Maugeri, 2008).

Nevertheless, FTases in free form loose enzymatic activity rapidly by thermal denaturation as storage time increases which limits its use in FOS production. Hence, the storage step is crucial to maintain the catalytic site and enzyme conformation of FTases close to its initial state and then to extend the enzyme lifetime (Shuler & Kargi, 2002). Several authors reported that the stability of free FTases during storage depends mainly on the producer microorganism and storage medium parameters such as composition, pH and temperature (Park et al., 2001; Hernalsteens & Maugeri, 2008; Yang et al., 2016).

The stability of free enzymes during storage is an important information which allows to analyze its potential application in enzymatic process, to evaluate the necessity of applying an immobilization process, and also to evaluate the effect of immobilization processes in storage stability. However, until the performance of this study, no information has been reported on the stability of the extracellular FTase from *A. oryzae* IPT-301 in the free form during storage, a characteristic indicated among the main limitations of the use of free enzymes in industrial processes. In this context, the present work aimed at determining the effect of temperature, pH and storage time on the stability of the extracellular FTase from *A. oryzae* IPT-301 used in its soluble form.

2 Materials and methods

2.1 Microorganism and production of the extracellular enzyme

Strains of the fungus *A. oryzae* IPT-301 were provided by the Institute for Technological Research (*Instituto de Pesquisas Tecnológicas/São Paulo* (IPT/SP)) and used to produce extracellular FTase. Enzyme production was performed by submerged fermentation in Erlenmeyer flasks (250 mL) containing 50 mL of culture medium with the following composition (in %, $m v^{-1}$): sucrose 15.0, yeast extract 0.5, $NaNO_3$ 0.5, KH_2PO_4 0.2, $MgSO_4 \cdot 7H_2O$ 0.05, $MnCl_2 \cdot 4H_2O$ 0.03 and $FeSO_4 \cdot 7H_2O$ 0.001. The initial pH of the culture medium was adjusted to 5.5 before sterilization. The flasks were inoculated with 0.5 mL of a previously prepared suspension of 10^7 spores mL^{-1} , and incubated in orbital shaker at 30 °C, 200 rpm for 64 h (Cunha et al., 2019; Faria et al., 2021). At the end of fermentation, the culture broth was vacuum-filtered (Whatman n°1) and the resulting supernatant containing an activity of 12.34 ± 0.23 U mL^{-1} for the FTase from *A. oryzae* IPT-301 was employed for further studies.

2.2 Thermal stability assays

The enzyme FTase (culture broth) was incubated in tris-acetate buffer at 0.2 mol L^{-1} and pH 5.5, in the absence of substrate, in a broad temperature range (30 °C, 40 °C, 50 °C and 60 °C). The samples were collected at specified time intervals (1 h, 2 h, 4 h and 8 h) and immediately cooled in ice bath for 5 min, and the residual activity was determined under standard conditions, according to Ottoni et al. (2012), Cunha et al. (2019) and Faria et al. (2021). The experiments were performed in triplicate.

The first-order thermal denaturation constant (k_D , in min^{-1}) was estimated adjusting the model of Sadana & Henley (1987) to the experimental data of residual activities *versus* time (Equation 1):

$$\frac{A}{A_0} = (1 - \alpha) \exp(-k_D t) + \alpha \quad (1)$$

in which (A/A_0) is the dimensionless residual activity; α is the ratio between the enzymatic activity of the final state (A) and the enzymatic activity of the initial state (A_0); and t is the incubation time of the enzymatic solution (min).

The half-life ($t_{1/2}$, in min) of the enzyme was calculated using Equation 2 (Perna et al., 2017):

$$t_{1/2} = -\frac{1}{k_D} \ln \left(\frac{\frac{1}{2} - \alpha}{1 - \alpha} \right) \quad (2)$$

The activation energy for thermal denaturation (E_D) was determined using the linearized Arrhenius Equation, as shown in Equation 3.

$$\ln k_D = \ln(A) - \frac{E_D}{R} \frac{1}{T} \quad (3)$$

in which A is the Arrhenius frequency of the collision factor, R is the universal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$) and T is the absolute temperature (K).

On the other hand, the variations of the enthalpy of activation of denaturation (ΔH_D), Gibbs energy of activation of denaturation (ΔG_D) and entropy of activation of denaturation (ΔS_D) were calculated using Equations 4, 5 and 6, respectively (Faria et al., 2021; Gonçalves et al., 2020; Saqib et al., 2010; Souza et al., 2015).

$$\Delta H_D = E_D - R.T \quad (4)$$

$$\Delta G_D = -R.T. \ln \frac{k_D.h}{k_b.T} \quad (5)$$

$$\Delta S_D = \frac{\Delta H_D - \Delta G_D}{T} \quad (6)$$

in which T is the experimental absolute temperature (K), R is the universal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$), h is the Planck constant ($11.04 \times 10^{-36} \text{ J min}^{-1}$) and k_b is the Boltzmann constant ($1.38 \times 10^{-23} \text{ J K}^{-1}$).

2.3 pH stability assays

Stability at different pH values was determined by the incubation of the FTase in tris-acetate buffer at 0.2 mol L^{-1} for a pH interval of 3.5-8.5, in the absence of substrate, at $4 \text{ }^\circ\text{C}$, for 24 h, and measuring the residual activity under standard conditions. The experiments were performed in triplicate.

2.4 Storage stability assays

The assays were performed with the extracellular FTase for different storage periods (1 h, 2 h, 3 h, 5 h, 7 h, 9 h, 24 h, 18 h, 72 h and 96 h). For this, the culture broth was maintained under refrigeration at $4 \text{ }^\circ\text{C}$ and the transfructosylation (A_T) and hydrolytic (A_H) activities were monitored under standard conditions. The experiments were performed in triplicate.

2.5 Analytical methods

2.5.1 Enzymatic activity assays

The transfructosylation (A_T) and hydrolytic (A_H) activities were determined according to Ottoni et al. (2012), Cunha et al. (2019) and Faria et al. (2021). For this, 0.1 mL of the soluble enzyme (culture broth) was incubated with 3.7 mL of sucrose solution (47% , m v^{-1}) and 1.2 mL of tris-acetate buffer at 0.2 mol L^{-1} , at pH 5.5. The reaction was conducted in Dubnoff bath at $50 \text{ }^\circ\text{C}$, 190 rpm for 1 h and interrupted by the immersion of the reaction medium in boiling water for 10 min, followed by cooling in water and ice bath for 5 min for enzyme inactivation. Subsequently, the reaction medium was vacuum-filtered and the concentration of reducing sugars and glucose was quantified. One unit (1 U) of transfructosylation activity was defined as the amount of enzyme that transfers one micromol ($1 \text{ } \mu\text{mol}$) of fructose per minute, under the established experimental conditions. One unit (1 U) of hydrolytic activity was defined as the amount of enzyme which releases one micromol ($1 \text{ } \mu\text{mol}$) of fructose per minute, under the established experimental conditions (Cunha et al., 2019; Gonçalves et al., 2020; Faria et al., 2021).

2.5.2 Analysis of carbohydrates

The concentrations of glucose (G) and reducing sugars (RS) were quantified by the colorimetric methods GOD/PAP® (glucose-oxidase enzyme kit) and DNS (3,5 dinitrosalicylic acid) (Miller, 1959), respectively. The concentrations of released fructose (F) and transferred fructose (F_T) in the reaction medium were determined by Equations 7 and 8 (Chen & Liu, 1996, Cunha et al., 2019; Gonçalves et al., 2020; Faria et al., 2021).

$$[F] = [RS] - [G] \quad (7)$$

$$[F_T] = [G] - [F] \quad (8)$$

3 Results and discussion

3.1 Determination of thermodynamic parameters from thermal stability tests

The thermal stability analysis of the extracellular FTase was performed at a temperature range between 30 °C and 60 °C over an incubation period of 8 h. Figure 1 shows the relative transfructosylation activities after thermal pretreatment of the extracellular FTase. The maximum enzyme activity (initial time) was of $13.15 \pm 1.47 \text{ U mL}^{-1}$ (relative activity, 100%). The highest retention of A_T was observed for the enzyme incubated at 30 °C, whose residual activity was of approximately 80% ($10.17 \pm 0.94 \text{ U mL}^{-1}$) after 8 h of incubation. For the same period, it was also observed that the FTase presented residual activity around 40% ($5.26 \pm 1.71 \text{ U mL}^{-1}$), 38% ($5.12 \pm 1.33 \text{ U mL}^{-1}$) and 27% ($3.55 \pm 0.91 \text{ U mL}^{-1}$) for the temperatures of 40 °C, 50 °C and 60 °C, respectively.

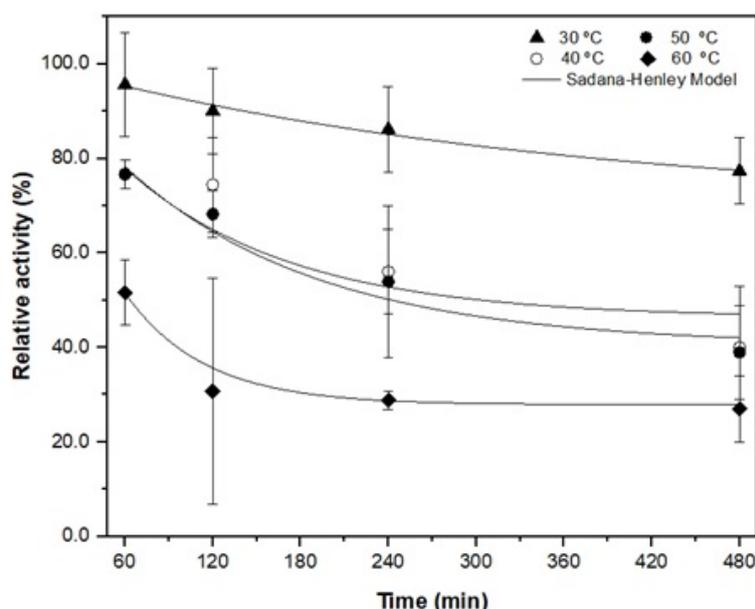


Figure 1. Thermal stability at different temperatures (30 °C, 40 °C, 50 °C and 60 °C) and pH 5.5 for soluble extracellular FTase from *Aspergillus oryzae* IPT-301. Reactions conditions: 47% (w v⁻¹) sucrose solution, 190 rpm, 0,2 mol L⁻¹ of tris-acetate buffer, pH 5.5, and 50 °C. The maximum enzyme activity (initial time) was of $13.15 \pm 1.45 \text{ U mL}^{-1}$ (relative activity, 100%).

Faria et al. (2021) reported that the extracellular enzyme of *A. oryzae* IPT-301 showed thermostability with A_T retention higher than 70% after 17 h of incubation at 30 °C. The results obtained by Cunha et al. (2019) for the FTase produced by the same microorganism showed that thermal stability occurred only for temperatures below 35 °C, reaching 96% of A_T retention after 1 h of incubation. For temperatures above 40 °C, a marked reduction in enzymatic activity was observed, with only 44.26% of the initial activity retained at 65 °C. Han et al. (2020) showed that the FTase from *A. oryzae* S719 remained stable, presenting A_T retention higher than 80% at 25 °C after 12 h of enzyme incubation in acetate buffer at 0.2 mol L⁻¹ and pH 5.5. Xu et al. (2015) reported that the FTase produced by *Penicillium oxalicum* retained 80% of A_T for a temperature range between 25 °C and 55 °C. Finally, Madlová et al. (2000) demonstrated that *Aureobasidium pullulans* FTase was rapidly inactivated when incubated at temperatures higher than 60 °C.

The parameters used for the determination of the thermal stability of the enzyme were calculated from the thermal inactivation curves (Figure 1) and their values are shown in Table 1. The thermal inactivation constant (k_D) was estimated according to the nonlinear decay model proposed by Sadana & Henley (1987) (Figure 1) and the activation energy for thermal denaturation (E_D) of the enzyme was obtained by linear adjustment of the Arrhenius Equation (Figure 2).

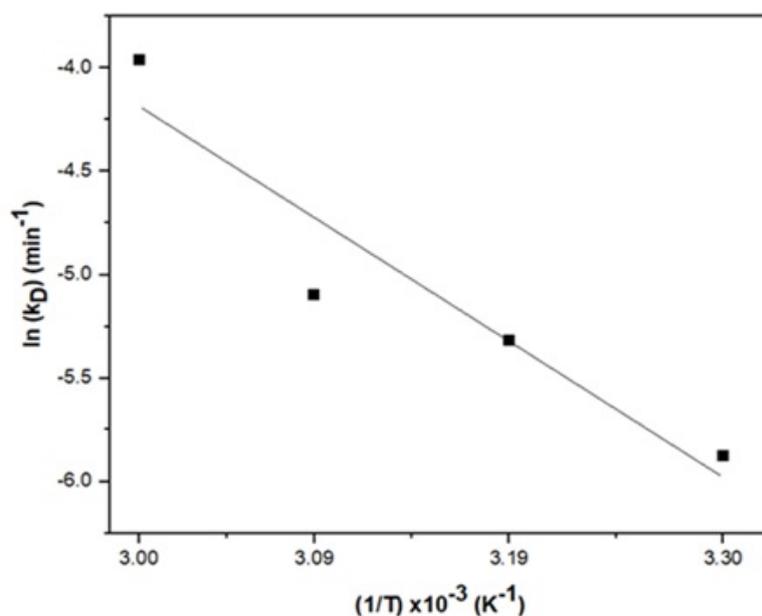


Figure 2. Determination of the activation energy for thermal denaturation (E_D). Arrhenius plot of $\ln(k_D)$ versus $(1/T)$ was used for the E_D of soluble extracellular FTase from *Aspergillus oryzae* IPT-301.

Table 1. Thermodynamic parameters of extracellular FTase from *A. oryzae* IPT-301 incubated at different temperatures.

Parameters ^(*)	Temperature (°C)			
	30	40	50	60
R^2	0.98	0.81	0.89	0.99
$k_D (min^{-1}) \times 10^{-4}$	2.80×10^{-3}	8.81×10^{-3}	7.55×10^{-3}	18.59×10^{-3}
$t_{1/2} (min)$	-	303.99	244.04	63.59
$E_D (kJ mol^{-1})$	49.75			
$\Delta H_D^* (kJ mol^{-1})$	47.23	47.15	47.06	46.98
$\Delta G_D^* (kJ mol^{-1})$	99.42	99.80	103.50	104.28
$\Delta S_D^* (kJ mol^{-1} K^{-1})$	-0.172	-0.168	-0.175	-0.172

* Extracellular FTase was incubated at pH 5.5 (0.2 mol L⁻¹ tris acetate buffer) in the absence of the substrate at 30 °C, 40 °C, 50 °C and 60 °C. R^2 = correlation coefficient for k_D values; k_D = thermal denaturation constant; $t_{1/2}$ = half-life time; E_D = activation energy of denaturation; ΔH_D^* = enthalpy of activation of denaturation; ΔG_D^* = Gibbs energy of activation of denaturation; ΔS_D^* = entropy of activation of denaturation.

The half-life ($t_{1/2}$) is defined as the time necessary for the enzymatic activity to decrease around 50% from its initial activity at a given temperature (Saqib et al., 2010). Thus, higher half-life values indicate that the biocatalyst can withstand the thermal effect for a longer period and, therefore, present higher thermostability (Griffin et al., 1984; Marangoni, 2003; Saqib et al., 2010; Souza et al., 2015). The results presented in Table 1

showed that the $t_{1/2}$ of the extracellular FTase decreased progressively with the rise in temperature, leading to an irreversible denaturation of the biocatalyst. The half-life of the FTase incubated at 40 °C (303.99 min) was around five times higher than when incubated at 60 °C (63.59 min). A similar behavior was reported by Faria et al. (2021), estimating half-life times for the extracellular FTase from *A. oryzae* IPT-301 at different incubation temperatures. The authors verified a reduction of approximately 30% in the biocatalyst half-life for a temperature range from 40 °C to 60 °C, proving the irreversible denaturation of the microbial FTase.

The activation energy of denaturation (E_D) can be defined as the minimum amount of energy which must be provided to the soluble enzyme (native state) to be denatured (irreversible modification of the conformation). Therefore, high values of this parameter indicate higher enzyme thermostability (Ferreira et al., 2018; Saqib et al., 2010). According to Figure 2, the linear regression for the temperature range evaluated (30 °C to 60 °C) presented a good adjustment ($R^2 = 0.86$) and the E_D estimated was of 49.75 kJ mol⁻¹, a value which is very close to that obtained by Faria et al. (2021) (39.8 kJ mol⁻¹) for the FTase from *A. oryzae* IPT-301, proving the thermostability of the soluble enzyme.

The enthalpy of activation of denaturation (ΔH_D) is an important thermodynamic parameter associated to the total amount of energy necessary to denature the enzyme (Marangoni, 2003; Souza et al., 2015). For this parameter, Table 1 showed high and positive values (around 47 kJ mol⁻¹) for the whole range of incubation temperatures investigated, thus indicating that the soluble FTase from *A. oryzae* IPT-301 presented a thermostable behavior. Close ΔH_D values (around 37 kJ mol⁻¹) were also obtained by Faria et al. (2021) when evaluating the biocatalyst produced by the same microorganism.

Gibbs energy of activation of denaturation (ΔG_D) is the most precise and reliable thermodynamic parameter to evaluate enzyme thermostability, in other words, its resistance to denaturation, since it includes the enthalpic and entropic contributions (Saqib et al., 2010; Souza et al., 2015). According to Marangoni (2003) and Damodaran (2005), higher ΔG_D values indicate higher enzyme thermostability, as shown in this work for the soluble FTase (Table 1). It is also noted that all ΔG_D values obtained were positive, since, under the equilibrium condition, the concentration of the protein in the native state is higher than in the denatured state. Consequently, the transition from the native to the denatured states was not a spontaneous process (Fonseca et al., 2006; Gonçalves et al., 2020).

Enzyme thermal denaturation causes the rupture of the protein structure and generates a disorganized system, leading to the rise in entropy. Therefore, denaturation depends on the entropy of activation of denaturation (ΔS_D) (Gonçalves et al., 2020). This thermodynamic parameter expresses the amount of energy per degree of disorder involved in the transition from a native state to a denatured state of the biocatalyst (Marangoni, 2003; Souza et al., 2015). As observed in Table 1, negative ΔS_D values were obtained for the soluble FTase at temperatures from 30 °C to 60 °C, indicating a more orderly FTase transition state (Rashid & Siddiqui 1998). Furthermore, the negative ΔS_D values can be explained by the rise in incubation temperature, which can weaken the polar interactions of the enzyme and strengthen its hydrophobic interactions (Siddiqui et al., 1997; Faria et al., 2021).

3.2 Effect of pH on enzyme stability

Samples of the enzyme solution (culture broth) were incubated for 24 h at 4 °C in six different tris-acetate buffer solutions, all at 0.2 mol L⁻¹, with pH values ranging from 3.5 to 8.5 (Figure 3). The enzyme FTase presented notably higher stability at pH 5.5, with A_T equal to 8.87 ± 1.45 U mL⁻¹, corresponding to a relative activity of 100%. At pH 6.5, a drop to 65% (5.78 ± 1.45 U mL⁻¹) in relative activity was observed. Under extreme pH conditions (3.5 and 8.5), the relative activity was inferior to 25% (2.14 ± 1.18 U mL⁻¹), whereas for pH 4.5 and 7.5, residual activities around 40% (3.55 ± 1.95 U mL⁻¹) were obtained. These results are in agreement with previous studies performed for the extracellular FTase of *A. oryzae* IPT-301, whose best stability profiles were verified for a pH range between 5.0 and 6.5 (Cunha et al., 2019; Faria et al., 2021).

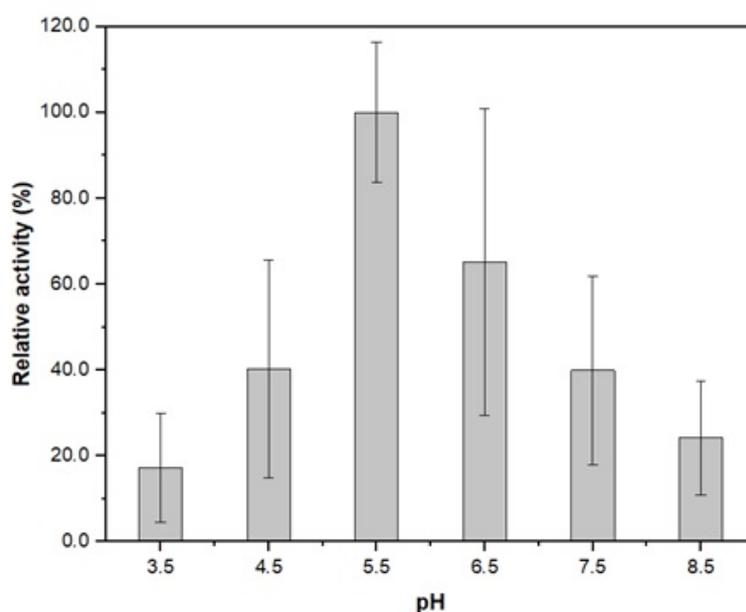


Figure 3. Stability of the soluble extracellular FTase after 24 h of incubation at 4 °C in tris-acetate buffer solutions, at different pH values. Reaction conditions: 47% (w v⁻¹) sucrose solution, 190 rpm, 0.2 mol L⁻¹ of tris-acetate buffer, and 50 °C. The maximum enzyme activity (8.87 ± 1.45 U mL⁻¹) at pH 5.5 was defined as 100% of relative activity.

In general, enzymes are active at a limited pH range, because the enzyme has several ionizable groups, and changes in pH affect the catalytic site and enzyme conformation, essential for the maintenance of the catalytic capacity (Shuler & Kargi, 2002). Park et al. (2001) investigated the stability of the extracellular FTase purified from *Bacillus macerans* EG-6, incubated at 25 °C, in sodium acetate buffer solution, obtaining higher A_T values for a pH range of 5.0-8.0. On the other hand, Hernalsteens & Maugeri (2008) studied the stability of an extracellular FTase from *Rhodotorula* sp., whose enzyme presented the highest transfructosylation activity and good stability in acetate buffer pH 5.0 at 50 °C. Finally, Yang et al. (2016), using the recombinant *A. niger* YZ59 expressed in *Pichia pastoris* GS115, detected stability of the recombinant FTase for a pH range of 3.0-10.0, incubating the enzyme at 25 °C for 24 h. Recombinant enzyme has been genetically modified to be more effective at the desired pH range. In addition, recombinant enzymes are more robust and have greater pH stability when compared to natural enzymes, which improves their use and efficiency in industrial applications (Amid, 2015).

3.3 Stability during storage

Figure 4 presents the behavior of the enzymatic activity of the enzyme FTase according to storage time. It can be observed that the transfructosylation activity decreases, whereas the hydrolytic activity increases over storage time, consequently producing a reduction in the A_T/A_H ratio. This change in activity is more accentuated mainly at the first nine hours of storage time, a time in which the enzyme presented around 45.6% of its initial transfructosylation activity. After this period, the transfructosylation activity continued decreasing at a lower speed until it reached approximately 35% of its initial value after 96 h of storage. The increase in hydrolytic activity over time can be attributed to the loss of transfructosylation activity in the fermented broth (Faria et al., 2021). These results indicated the existence of a high loss of enzymatic activity of the enzyme FTase in the soluble form in a few hours of storage, suggesting it must be used for FOS production preferably in the first hours after its production. These results also suggested the need to improve the use of immobilization techniques for FTase enzymes, such as adsorption and encapsulation, aiming at increasing their thermal and operational stability (Aguiar-Oliveira & Maugeri, 2010; Gonçalves et al., 2020; Faria et al., 2021).

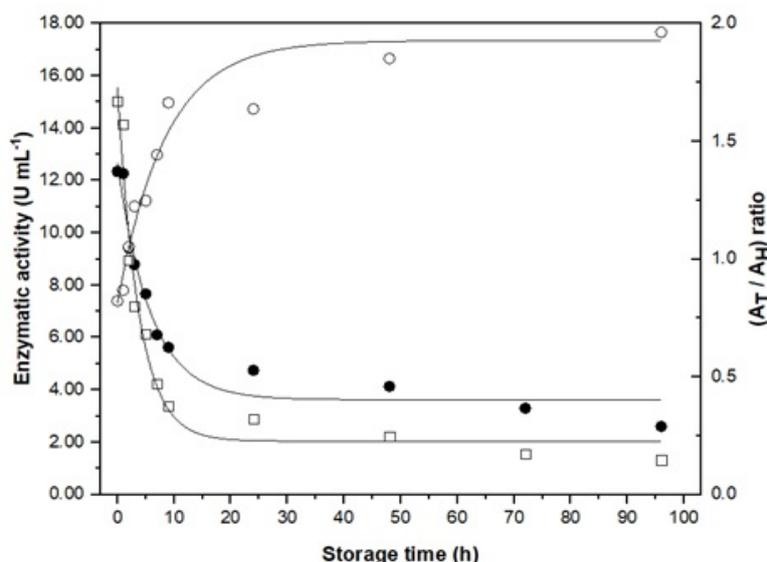


Figure 4. Storage stabilities of soluble extracellular FTase at 4 °C: transfructosylation activity – A_T (●), hydrolytic activity – A_H (○), and (A_T/A_H) ratio (□). Reaction conditions: 47% (w v⁻¹) sucrose solution, 190 rpm, 0.2 mol L⁻¹ of tris-acetate buffer, pH 5.5, and 50 °C.

4 Conclusion

In this work, the extracellular enzyme fructosyltransferase (FTase, E.C.2.4.1.9) from *A. oryzae* IPT-301 was produced by submerged fermentation, and the effects of storage, temperature and pH on enzyme stability were evaluated. The soluble biocatalyst, for a storage period of 5 h, was active with retention of 50% of the transfructosylation activity, whereas for storage times greater than 24 h, the enzymatic activity remained below 30%. The thermodynamic parameters obtained showed the thermostability of the microbial FTase when submitted to different incubation temperatures. Regarding the effects of pH, the enzyme was stable at pH 5.5, in which the highest value of transfructosylation activity was obtained. Therefore, the results presented in this work were necessary to explore both the viability of the use of soluble FTase in industrial processes for FOS production and the improvement of the immobilization processes for this microbial enzyme.

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