BIOTECHNOLOGICALLY RELEVANT ENZYMES AND PROTEINS

# Screening of β-fructofuranosidase-producing microorganisms and effect of pH and temperature on enzymatic rate

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**Abstract** Seventeen different strains of filamentous fungi were grown in batch cultures to compare their abilities for the production of β-fructofuranosidase. Three of them, *Aspergillus oryzae* IPT-301, *Aspergillus niger* ATCC 20611 and strain IPT-615, showed high production with total fructosyltransferase activity higher than 12,500 units $l^{-1}$. In addition, the β-fructofuranosidases of those strains have a high fructosyltransferase activity-to-hydrolytic activity ratio. The temperature and pH effects on the sucrose-β-fructofuranosidase reaction rate were studied using a $2^2$ factorial experimental design. The comparative analysis of the tested variable coefficients shows that the variable pH contributes mostly to the changes in the fructosyltransferase and hydrolytic rates and in the $V_t/V_h$ ratio. At 40 and 50°C, there were no significant differences between the fructosyltransferase and hydrolytic velocities of these enzymes.

**Keywords** β-fructofuranosidase · Fructosyltransferase · Fructooligosaccharides · *Aspergillus*

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## Introduction

During the last decades, the development of products called nutraceuticals or functional foods has been in the spotlight. Among these new commercially available products, the fructooligosaccharides (FOS) obtained from sucrose have attracted special attention due to their properties and, thus, have a great economic potential for the sugar industrial branch. They are non-cariogenic sweeteners of low caloric value, as they are not hydrolyzed by the gastro-intestinal enzymes, promoting selectively the growth of the bifidobacteria in the colon (Moore et al. 2003), helping to eliminate the harmful microbial species to human and animal health and preventing colon cancer (Gibson and Roberfroid 1995). FOS also present an additional, very important physiological property, as they reduce cholesterol, phospholipids and triglyceride levels in blood (Tokunaga et al. 1986).

The FOS-producing enzymes are usually classified as β-D-fructofuranosidase (EC 3.2.1.26) with high transfructosylating activities or fructosyltransferase (EC 2.4.1.9) (Chien et al. 2001). Although FOS can be produced by the action of enzymes present in some plants, the industrial production is mainly based on microbial enzymes (Yun 1996).

The enzymes used industrially for FOS synthesis are produced by the fungi *Aureobasidium pullulans* (Yun 1996; Hayashi et al. 1990) and *Aspergillus niger* (Hidaka et al. 1988). Nevertheless, several published papers report FOS production using β-fructofuranosidases produced by *Aspergillus oryzae* (Kurakake et al. 1996; Sangeetha et al. 2002, 2004a,b) and *Aspergillus japonicus* (Chien et al.

2001). New strains of fungi and bacteria producing β-fructofuranosidases with high fructosyltransferase activity have been isolated from sugar cane plantation soil (Guilarte and Cuervo 2000).

The objectives of the present paper are to screen filamentous fungi strains able to produce fructosyltransferase with high potential for industrial use and to verify the influence of pH and temperature on the reaction rate of fructosyltransferases produced by these fungi.

## Materials and methods

### Microorganisms and cultivation conditions

Thirteen fungi strains isolated from sugar cane plantation soil by the ICINAZ (Guilarte and Cuervo 2000) and three fungi strains from Instituto de Pesquisas Tecnológicas do Estado de São Paulo (IPT) culture collections were tested. The strain *A. niger* ATCC 20611 (deposited in IPT culture collection as IPT 435) was used as reference, as it produces a β-fructofuranosidase with well-known characteristics (Hidaka et al. 1988; Hirayama et al. 1989). The strains were grown on malt extract agar, and the spores were maintained mixed with glycerol solution in ultra-freezer at −80°C.

Screening experiments were carried out in a rotatory shaker using 250 ml unbaffled erlenmeyer flasks with 50 ml of culture medium, with the following composition (in % w/v): sucrose 15, yeast extract 0.5, NaNO₃ 0.5, KH₂PO₄ 0.2, MgSO₄·7H₂O 0.05, MnCl₂·4H₂O 0.03 and FeSO₄·7H₂O 0.001. The pH of the medium was adjusted to 5.5 before sterilization. Flasks were inoculated with 0.5 ml of a spore suspension containing around $10^7$ spores ml$^{-1}$ and incubated in the rotary shaker at 30°C and 200 rpm for 72 h. Samples for analysis were collected and filtered, using filter paper (Whatman No. 1). In the filtered broth, residual sugar concentration, pH and extracellular enzyme activity were measured. Cell concentration and mycelium enzymatic activity were determined using the cell pellet formed.

### Analytical methods

#### Cell mass concentration

Cell mass concentration was determined by dry cell weight per volume (g l$^{-1}$). The cell mass obtained by filtration of the fermentation broth was washed with distilled water and dried at 105°C for 4 h.

#### Enzymatic activity

The enzymatic activities of extracellular and mycelium enzymes were determined as follows: 0.1 ml of suitably

diluted supernatant or 0.05 g of cells was mixed with 3.7 ml of 64% (w/v) sucrose and 1.2 ml tris-acetate buffer 0.2 M at pH 5.5. The reaction was carried out at 50°C for 60 min and stopped by boiling for 10 min (Cuervo et al. 2004). The units of fructosyltransferase ($A_t$) and hydrolytic ($A_h$) activities were defined as the amount of enzyme that produces a micromole of FOS or releases a micromole of fructose, respectively, per minute under the chosen experimental conditions.

#### Sugars and FOS concentrations

The sugar and FOS concentrations in the samples were analyzed using high performance liquid chromatography (HPLC) with an Aminex HPX-87C (300×7.8 mm, Bio-Rad Laboratories) column and a system composed by a 510 pump, a refraction index differential detector and a data processor with register (Waters, USA). The samples were eluted with Milli-Q water at 0.6 ml min$^{-1}$ flow rate, and the temperature of the column was maintained at 72°C.

#### Temperature and pH effects on the reaction rate

The temperature and pH effects on the sucrose-fructofuranosidase reaction rate were studied using a $2^2$ experimental design. The proposed experimental design matrix is presented in Table 1. All the experiments were performed with the reaction mixture of 3.7 ml of 64% (w/v) sucrose, 1.2 ml tris-acetate buffer 0.2 M and 0.1 ml of suitably diluted enzyme solution. The enzyme reaction was carried out for 1 h at pH and temperature to be tested. The reaction rate results of fructose transference ($V_t$) and of hydrolysis ($V_h$) were analyzed mathematically and statistically using the Design Expert version 5.0 software.

## Results

The results of cell mass and FOS concentrations and pH obtained in the performed experiments after a 72-h cultivation period are shown in Table 2. The strains IPT-610, IPT-

Table 1 Maximum and minimum levels of temperature and pH used to study the effect of these variables on the sucrose/β-fructofuranosidase reaction rate

| Experimental run | Variables | |
|---|---|---|
| | Temperature (°C) | pH |
| 1 | 40 | 5.5 |
| 2 | 50 | 5.5 |
| 3 | 40 | 8.0 |
| 4 | 50 | 8.0 |

**Table 2** Cell mass and FOS concentrations and pH reached in the shake flasks experiments (average of four repetitions)

| Strain | Cell mass (g l$^{-1}$) | GF$_2$+GF$_3$ (g l$^{-1}$) | Final pH |
|---|---|---|---|
| IPT-301 | 7.39±1.10 | 37.31 | 5.70 |
| IPT-329 | 7.39±0.81 | 47.38 | 4.01 |
| ATCC 20611 | 8.71±0.55 | 49.43 | 4.21 |
| IPT-604[a] | 5.13±2.58 | 29.95 | 6.46 |
| IPT-605[a] | 6.45±1.39 | 119.54 | 5.62 |
| IPT-607[a] | 8.42±0.45 | 41.26 | 3.68 |
| IPT-608[a] | 7.02±2.64 | 110.79 | 5.43 |
| IPT-609[a] | 5.17±1.27 | 27.08 | 3.57 |
| IPT-610[a] | 10.05±0.53 | 23.69 | 3.43 |
| IPT-611[a] | 8.70±0.95 | 35.37 | 3.66 |
| IPT-612[a] | 8.04±0.22 | 36.01 | 3.96 |
| IPT-613[a] | 11.22±0.19 | 40.34 | 3.48 |
| IPT-614[a] | 8.77±0.38 | 28.04 | 5.66 |
| IPT-615[a] | 10.85±0.91 | 133.35 | 5.53 |
| IPT-616[a] | 4.22±0.11 | 32.15 | 6.69 |
| IPT-619[a] | 4.23±1.81 | 33.12 | 6.14 |

GF$_2$+GF$_3$ represent the sum of kestose and nystose in culture media, respectively.

IPT-301, *Aspergillus oryzae*; IPT 329, *Aureobasidium pullulans*; ATCC 20611, *Aspergillus niger*

[a] Non-identified strains isolated from soil

613 and IPT-615 reached a cell mass concentration higher than 10 g l$^{-1}$, whereas the result with strains *A. niger* ATCC 20611, IPT-607, IPT-611, IPT-612 and IPT-614 was higher than 8 g l$^{-1}$.

The average values of the mycelium, extracellular and total enzymatic activities are presented in Table 3. Eight strains, *A. oryzae* IPT-301, *Aureobasidium pullulans* IPT-329, *A. niger* ATCC 20611, IPT-605, IPT-610, IPT-611, IPT-613 and IPT-615, reached mycelium $A_t$ values higher than 300 U g$^{-1}$. In this group, the strains *A. oryzae* IPT-301, *Aureobasidium pullulans* IPT-329, *A. niger* ATCC 20611, IPT-605 and IPT-615 presented values of $A_t/A_h$ above 7.0. On the other hand, seven strains reached the highest values of extracellular $A_t$: *A. oryzae* IPT-301, *A. pullulans* IPT-329, *A. niger* ATCC 20611, IPT-605, IPT-608, IPT-610 and IPT-615. However, among them, only the *A. oryzae* IPT-301, *A. niger* ATCC 20611, IPT-608 and IPT-615 strains present relevant values of the ratio $A_t/A_h$.

The values of reaction velocities for the three best fructosyltransferase-producing strains are shown in Fig. 1. The IPT-615 strain had the higher values of $V_t$ when reaction was performed under experimental conditions of run 3, whereas *A. oryzae* IPT-301 and *A. niger* ATCC 20611 strains exhibited higher values of $V_t$ under conditions of runs 4 and 2, respectively. *A. oryzae* IPT-301 showed the higher velocity of FOS formation.

## Discussion

In general terms, the values of cell mass concentrations obtained are acceptable for cultivation in agitated flasks without aeration and pH control, as it is known that these variables have a significant influence on fungi growth. No correlation between cell growth and final pH was observed, although fermentation broth pH values for the strains with

**Table 3** Mycelium, extracellular and total fructosyltransferase ($A_t$) and hydrolytic activities ($A_h$) reached in shake flasks experiments (average of four repetitions)

| Strain | Mycelium activities (U g$^{-1}$) | | | Extracellular activities (U ml$^{-1}$) | | | Total $A_t$ (U l$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | $A_t$ | $A_h$ | $A_t/A_h$ | $A_t$ | $A_h$ | $A_t/A_h$ | Mycelium | Extracellular | Total |
| IPT-301 | 487.3±34.1 | 44.9±1.9 | 10.86 | 17.90±1.26 | 3.44±0.77 | 5.2 | 3,599 | 14,190 | 17,789 |
| IPT-329 | 567.5±115.4 | 76.4±12.4 | 7.43 | 5.71±0.21 | 3.98±0.43 | 1.4 | 4,194 | 5,095 | 9,289 |
| ATCC 20611 | 854.5±103.6 | 62.3±4.1 | 13.72 | 11.87±0.50 | 0.75±0.28 | 15.9 | 7,442 | 10,588 | 18,030 |
| IPT-604 | 15.4±2.0 | 32.4±8.7 | 0.48 | 1.37±0.13 | 2.48±0.85 | 0.6 | (Low total $A_t$) | (Low total $A_t$) | (Low total $A_t$) |
| IPT-605 | 305.8±14.7 | 36.6±5.2 | 8.35 | 7.05±0.30 | 1.86±0.09 | 3.8 | 1,972 | 6,289 | 8,261 |
| IPT-607 | 291.7±30.0 | 138.5±1.5 | 2.11 | 2.60±0.33 | 9.05±0.23 | 0.3 | (Low total $A_t$) | (Low total $A_t$) | (Low total $A_t$) |
| IPT-608 | 243.4±58.5 | 46.5±9.9 | 5.24 | 7.88±0.88 | 1.42±0.19 | 5.5 | 1,709 | 7,029 | 8,738 |
| IPT-609 | 180.0±16.5 | 118.2±2.5 | 1.52 | 2.82±0.42 | 5.0±0.32 | 0.6 | (Low total $A_t$) | (Low total $A_t$) | (Low total $A_t$) |
| IPT-610 | 372.6±26.7 | 115.9±13.6 | 3.22 | 5.46±0.89 | 5.85±3.66 | 0.9 | 3,744 | 4,872 | 8,616 |
| IPT-611 | 307.7±57.6 | 135.7±10.6 | 2.27 | 2.67±0.37 | 2.03±0.07 | 1.3 | 2,675 | 2,383 | 5,058 |
| IPT-612 | 127.1±9.7 | 151.4±0.6 | 0.84 | 2.80±0.07 | 4.59±0.84 | 0.6 | (Low total $A_t$) | (Low total $A_t$) | (Low total $A_t$) |
| IPT-613 | 344.2±96.5 | 133.1±35.5 | 2.59 | 4.00±0.16 | 3.36±0.41 | 1.2 | 3,861 | 3,565 | 7,426 |
| IPT-614 | 50.9±5.9 | 21.2±3.5 | 2.40 | 3.50±0.37 | 3.43±0.11 | 1.0 | (Low total $A_t$) | (Low total $A_t$) | (Low total $A_t$) |
| IPT-615 | 462.3±20.9 | 53.9±6.4 | 8.57 | 8.41±0.34 | 1.42±0.20 | 5.9 | 5,016 | 7,502 | 12,518 |
| IPT-616 | 14.5±1.0 | 15.0±1.1 | 0.97 | 2.98±0.34 | 9.68±1.98 | 0.3 | (Low total $A_t$) | (Low total $A_t$) | (Low total $A_t$) |
| IPT-619 | 21.3±4.5 | 30.3±2.3 | 0.71 | 0.99±0.04 | 1.87±0.27 | 0.5 | (Low total $A_t$) | (Low total $A_t$) | (Low total $A_t$) |

* low total A$_t$
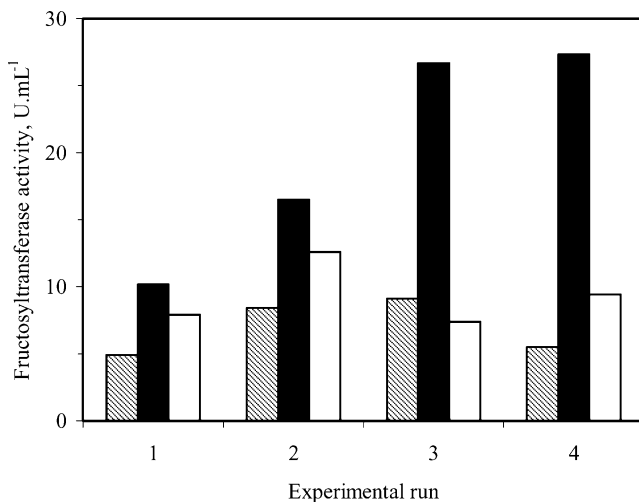
**Fig. 1** Effects of temperature and pH on fructosyltransferase activity from IPT-615 (*light-shaded bars*), *A. oryzae* IPT-301 (*dark-shaded bars*) and *A. niger* ATCC 20611 (*unshaded bars*; conditions are according to Table 1)

higher cellular growth were lower. Total FOS concentrations calculated by the sum of kestose and nystose in fermented medium after 72 h of cultivation are shown in Table 2. These results were obtained under different conditions from those used for FOS enzymatic production.

The criteria for the evaluation of β-fructofuranosidase-producing microorganisms are the growth of the mycelia and transfructosylating ($A_t$) and hydrolytic ($A_h$) activities, and as this last one competes with the first one, the $A_t/A_h$ ratio become very important (Hidaka et al. 1988; Hidaka 1988).

The initial amount of sucrose contained in the cultivation medium is partially hydrolyzed and consumed for the growth and maintenance of the microbial cells, whereas another part reacts under the action of the β-fructofuranosidase enzyme produced by the growing microorganisms. This reaction produces nystose, 1-kestose, glucose and fructose (Yun 1996). The nystose concentration in the fermented media was generally higher than the 1-kestose concentration. It can be explained by the long reaction time between sucrose, enzyme and products as it had been already reported (Barthomeuf et al. 1997). Correlation between the cell concentration, final composition of sugars and pH for the tested strains was not found. This result could be caused by the use of different microorganisms that synthesize β-fructofuranosidase with different specific properties and activities (Madlová et al. 1999).

High FOS concentrations in fermented media resulting from the growth of IPT-605, IPT-608 and IPT-615 strains were observed. These results cannot be explained by the mycelium and extracellular enzymic activities and cell growth, as these were not the higher values obtained in the performed experiments with the different strains. The

final pH values of the fermented media for these strains are close to the ones reported as optima for the fructosyltransferase activities between 5.0 and 5.5 (Yun 1996; Cuervo et al. 2004), and it can explain the relative high FOS concentrations obtained.

The $A_t$ and $A_t/A_h$ values are the most important parameters to be considered when the objective is the screening of strains that produce fructofuranosidase for FOS production with high kestose and nystose concentrations (Hidaka et al. 1988; Hidaka 1988). Nevertheless, it is important to consider that for a specific β-fructofuranosidase, this ratio can vary with the pH and the temperature due to the dependence of the fructosyltransferase and hydrolytic activities with both parameters (Cuervo et al. 2004).

The β-fructofuranosidases have both fructosyltransferase and hydrolytic activities, and the ratio between them also depends on the sucrose concentration. For a sucrose concentration up to 5.0 g $l^{-1}$, the most important products formed in the enzymatic reaction are glucose and fructose. However, FOS and glucose are the predominant products when the sucrose concentration is higher than 200 g $l^{-1}$ (Kim et al. 1996).

When the obtained results for the mycelium and extracellular activities are compared, significant differences can be observed between the $A_t/A_h$ ratio values. This fact is probably due to the existence of more than one β-fructofuranosidase in different concentrations in the mycelium and in the fermentation broth (Nguyen et al. 1999).

Among the nine screened strains, three of them can be selected because of the amount of total enzyme activity produced. These strains were: *A. oryzae* IPT-301, *A. niger* ATCC 20611 and IPT-615, which also presented higher $A_t/A_h$ ratio, as shown in Table 3. The total mycelium fructosyltransferase activity of these strains followed the order *A. niger* ATCC 20611 > IPT-615 > *A. oryzae* IPT-301, although the order for the same activity but considering the extracellular enzyme resulted in *A. oryzae* IPT-301 > *A. niger* IPT-435 > IPT-615. The strain *A. pullulans* IPT-329 produced a higher amount of $A_t$ in mycelium, but a lower amount of this activity was released to the medium. Moreover, it also produced more hydrolytic activity than the three strains previously listed (Table 3).

A $2^2$ experimental design was used, aiming at studying the effect of the pH and the temperature on the reaction rate catalyzed for enzymes of the seven better strains. These strains were selected due their higher capacities of producing fructosyltransferase activity. Experiments were performed under four conditions using the extracellular β-fructofuranosidase as described in Table 1.

With the objective of eliminating the error that can be introduced in the equations caused by the use of samples of four different flasks for each strain, the results were

**Table 4** Linear equation coefficients obtained with the factorial experimental design to study the effect of the temperature and pH on the fructose transference and hydrolysis rates and their ratio

| Strain | $V_t$ | | | | $V_h$ | | | | $V_t/V_h$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a4 | a1 | a2 | a3 | b4 | b1 | b2 | b3 | c4 | c1 | c2 | c3 |
| IPT-301 | 19.5±5 | 2.37 | 6.21 | −0.79 | 3.00 | Ns | −0.41 | Ns | 8.72 | Ns | 4.31 | Ns |
| IPT-329 | −5.54 | 0.35 | 0.23 | −0.03 | 10.71 | Ns | −1.23 | Ns | 0.10 | 0.02 | Ns | Ns |
| ATCC 20611 | −33.19 | 1.06 | 4.05 | −0.11 | – | Ns | Ns | Ns | – | Ns | Ns | Ns |
| IPT-610 | −3.07 | Ns | 0.45 | Ns | 21.37 | Ns | −2.25 | Ns | −3.07 | Ns | 0.05 | Ns |
| IPT-611 | −10.57 | 0.38 | 1.18 | −0.04 | 0.58 | Ns | −0.09 | Ns | – | Ns | Ns | Ns |
| IPT-613 | −0.53 | 0.01 | 0.03 | Ns | 3.15 | Ns | −0.37 | Ns | −0.53 | 0.01 | 0.03 | Ns |
| IPT-615 | 15.93 | Ns | −2.38 | Ns | – | Ns | Ns | Ns | −12.53 | Ns | 1.51 | Ns |

*Ns* Statistically non-significant coefficient

gathered in four blocks. Each block contains the results obtained performing the reactions under the four experimental conditions already described, using the β-fructofuranosidase of one fermentation flask. The results of the experimental design were analyzed statistically and used to estimate the major and interaction effects on the measured variables $V_t$ and $V_h$ and the ratio $V_t/V_h$. The linear equation coefficients (Eqs. 1, 2 and 3) obtained with the experimental design to study the effects of the temperature and pH on the fructose transference and hydrolysis rates and their ratio are presented in Table 4.

$$V_t = a_1 \cdot T + a_2 \cdot \text{pH} + a_3 \cdot T \cdot \text{pH} + a_4 \qquad (1)$$

$$V_h = b_1 \cdot T + b_2 \cdot \text{pH} + b_3 \cdot T \cdot \text{pH} + b_4 \qquad (2)$$

$$V_t/V_h = c_1 \cdot T + c_2 \cdot \text{pH} + c_3 \cdot T \cdot \text{pH} + c_4 \qquad (3)$$

where $V_t$ and $V_h$...fructose transference and hydrolysis rates $a_1$ to $a_4$, $b_1$ to $b_4$ and $c_1$ to $c_4$...regression coefficients.

For the three best fructosyltransferase producing strains, it was possible to fit a linear relation between the reaction rates and the studied variables. The regression equation obtained after the analysis of variance (ANOVA) gives the level of fructosyltransferase rate as a function of the initial pH and temperature for the strains IPT-615, *A. niger* ATCC 20611 and *A. oryzae* IPT-301. For these microorganisms, the ANOVA of the model (Eq. 1) is shown in Table 5. A good fitting of the linear model to the experimental data was obtained for *A. oryzae* IPT-301 and *A. niger* ATCC 20611, as the coefficients of determination ($R^2$) were higher than 0.90 for these two strains.

The analysis of the obtained results shows that the increase in the pH value influenced negatively the β-fructofuranosidase $V_t$ of IPT-615 strain, whereas the temperature effect was negligible. The pH and temperature variations had no significant influence on the $V_h$ values.

**Table 5** ANOVA results for fructosyltransferase production by the strains

| Strain | Source | Sum of squares | DF | Mean square | F value |
|---|---|---|---|---|---|
| IPT-615 | Block | 184.55 | 3 | 61.52 | |
| | Model | 52.56 | 3 | 17.52 | 3.25 |
| | Residual | 48.59 | 9 | 5.40 | |
| | Total | 285.70 | 15 | | |
| | $R^2=0.5196$ | | | | |
| *A. niger* ATCC 20611 | Block | 2.40 | 3 | 0.80 | |
| | Model | 65.95 | 3 | 21.98 | 43.52 |
| | Residual | 4.55 | 9 | 0.51 | |
| | Total | 72.90 | 15 | | |
| | $R^2=0.9355$ | | | | |
| *A. oryzae* IPT-301 | Block | 222.66 | 3 | 74.22 | |
| | Model | 716.19 | 3 | 238.73 | 27.34 |
| | Residual | 78.59 | 9 | 8.73 | |
| | Total | 1017.43 | 15 | | |
| | $R^2=0.9011$ | | | | |

The pH contribution on the ratio $V_t/V_h$ was very small with a slightly increasing tendency with the increase in the pH.

Varying the pH from 5.5 to 8.0, the *A. pullulans* IPT-329 strain had a low contribution on the $V_t$ and a negative contribution on the $V_h$. The temperature had a low influence on the $V_t$, but no influence on the $V_h$ presenting, therefore, a small positive contribution on the $V_t/V_h$ ratio.

The $V_t$ increases with pH and temperature increases for the strain *A. niger* ATCC 20611. However, no significant changes were observed in the $V_h$ values. The pH and temperature contributions were not high enough to cause an important increase in $V_t/V_h$.

Concerning the strain *A. oryzae* IPT-301, the $V_t$ showed a meaningful increase with the pH and showed lower variation with the temperature change. Besides, the $V_h$ was lower at pH 8.0 than 5.5, and it was similar to both temperatures. These results led to a remarkable increase in the $V_t/V_h$ ratio, when the pH varied from 5.5 to 8.0, whereas the changes in the temperature did not show any significant influence.

In some experiments performed at 40 and 50°C, the reaction rates had no relation with temperature, in opposition to the well-known Arrhenius law stating such relation. This was caused by the thermal inactivation of the β-fructofuranosidases, and as a consequence, the transference and hydrolysis rates of fructosyl moieties were lower, and the known dependence of $V_t$ and $\underline{V}_h$ with temperature was not obtained. Many of the β-fructofuranosidases used in the FOS production have optimal temperature in the range of 45 to 55°C (Hayashi et al. 1990; Yun 1996), but when sucrose is present in the reaction mixture at concentrations higher than 60% (p/p), it prevents the fast inactivation of the enzyme due to the stabilizing effect of high concentrations of saccharides on the fructosyltransferase activity. Thus in some cases, the optimal temperature reaches values up to 65°C.

The magnitude of the derived coefficients for the β-fructofuranosidases produced by the strains IPT-610, IPT-611 and IPT-613 allows to state that, in the ranges of pH and temperature used in the factorial experimental design, small differences in the fructose transference and hydrolysis rates and their ratio $V_t/V_h$ were obtained.

The pH is the variable that contributes mostly to changes in the fructosyltransferase and hydrolytic rates and the $V_t/V_h$ ratio as it was shown by the comparative analysis of the tested coefficients. The β-fructofuranosidase produced by *A. oryzae* IPT-301 showed a higher fructosyltransferase activity at pH 8.0 and similar values at both tested temperature levels. These results are in agreement with the ones reported by other authors (Kurakake et al. 1996). The optimum pH values obtained for the fructosyltransferase and hydrolytic activities were 6.0 and 5.0, respectively. The $A_t/A_h$ ratios were 2.9 and 10.9 at pH 5.0 and 8.0,

respectively. Further, these authors found differences in the kinetics of FOS formation at pH 5.0 and 8.0. At pH 5.0, once the maximum FOS concentration was attained, a decomposition process started releasing fructose. On the other hand, at pH 8.0, the FOS concentration reached its maximum value and kept constant.

Three fungi strains, *A. oryzae* IPT-301, *A. niger* ATCC 20611 and IPT-615 have greater potential for FOS production. These microorganisms show high values of total $A_t$ and $A_t/A_h$ ratio.

In this paper, *A. niger* ATCC 20611 strain was used as a reference, as it produces enzymes with well-known characteristics (Hidaka et al. 1988; Hirayama et al. 1989). This microorganism presented the best $A_t/A_h$ ratio and produced more mycelium fructosyltransferase than *A. oryzae* IPT-301, about 75%. On the other hand, the last strain produced more extracellular fructosyltransferase, about 51%. Considering these results and in spite of the non-optimization of the culture conditions, it can be affirmed that *A. oryzae* IPT-301 has a good potential for production of fructosyltransferase

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