Appl Biochem Biotechnol (2010) 160:682–693
DOI 10.1007/s12010-009-8687-8

Partial Characterization of Inulinases Obtained by Submerged and Solid-State Fermentation Using Agroindustrial Residues as Substrates: A Comparative Study

Marcio Antonio Mazutti · Aline Skrowonski · Gabriela Boni · Giovani Leone Zabot · Marceli Fernandes Silva · Débora de Oliveira · Marco Di Luccio · Francisco Maugeri Filho · Maria Isabel Rodrigues · Helen Treichel

Received: 14 October 2008 / Accepted: 27 May 2009 /
Published online: 6 June 2009

Abstract Inulinase belongs to an important class of enzymes as it can be used to produce high-fructose syrups by enzymatic hydrolysis of inulin and fructooligosaccharides, which has been used as functional food. This work aimed to carry out a partial characterization of the crude enzymatic extract of two different inulinases, obtained by solid-state fermentation (SSF) and submerged fermentation (SmF), using agroindustrial residues as substrates. The crude enzymatic extract obtained by SmF showed an optimal pH and temperature for hydrolytic activity of 4.5 and 55°C, respectively; and that obtained by SSF conducted to optimal pH and temperature of 5.0 and 55°C, respectively. Both enzymes presented high thermostability, with a *D* value of 230.4 h and 123.1 h for SmF and SSF, respectively. The inulinase produced by SmF showed highest stability at pH4.4, while inulinase obtained by SSF was more stable at pH4.8. The results showed that inulinase obtained by SmF is less susceptible to pH effect and the inulinase obtained by SSF is more resistant to higher temperatures.

Keywords Inulinase · Agroindustrial residues · Solid-state fermentation · Submerged fermentation · Partial characterization

Introduction

Inulinase is an enzyme relevant in food processes due to fructose production by enzymatic hydrolysis of inulin and production of fructooligosaccharides (FOS) that may be used as a

A. Skrowonski · G. Boni · G. L. Zabot · M. F. Silva · D. de Oliveira · M. Di Luccio · H. Treichel (✉)
Department of Food Engineering, URI-Campus de Erechim, Av. Sete de Setembro, 1621, Erechim, RS 99700-000, Brazil
e-mail: helen@uricer.edu.br

M. A. Mazutti · F. M. Filho · M. I. Rodrigues
Department of Food Engineering, UNICAMP, CP6121, 13083-970 Campinas, SP, Brazil

food functional ingredient. Fructose has been considered a safe alternative sweetener to sucrose because it has beneficial effects in diabetic patients, increases the iron absorption in children and has a higher sweetening capacity [1]. In addition, fructooligosaccharides present some benefits for human health [2]. Both fructose and FOS can be produced from inulin, a polymer of fructose with a terminal glucose, found in the roots of *Jerusalem artichoke*, dahlia, and chicory [1].

The use of microbial inulinases has been proposed as a promising approach for production of fructose syrups and mainly FOS [1]. A number of fungal, yeast and bacterial strains have been used for inulinases production, like *Kluyveromyces* [3–7], *Aspergillus* [1, 8, 9], *Staphylococcus* [7], *Xanthomonas* [10], and *Pseudomonas* [11]. These enzymes, (2,1-β-D) fructan fructanohydrolase (EC 3.2.1.7), are usually inducible and exo-acting enzymes, which catalyze the hydrolysis of inulin by splitting off the terminal fructosyl units (D-fructose) cleaving the glycosidic linkages in polymer chain [12].

Although the commercial production and almost all works presented in the literature related to microbial inulinases employs submerged fermentation (SmF) [5, 6, 13], solid-state fermentation (SSF) has also been successfully used to produce this enzyme [3, 4, 7] by employing several agroindustrial residues as substrate.

The preferred fermentation method for the production of most microbial products is SmF, unless there is a particular reason why SSF should be chosen. SmF is, in general, less problematic than SSF because heat transfer and media homogeneity is facilitated [14]. In SSF the main problem is related to the heat removal, while in SmF the major consideration is the supply of oxygen to the bioreactor [15]. Reasons for evaluating SSF processes include the use of low-cost substrates, simplified downstream process, reduced energy requirement, and simplicity of bioreactor design [16].

In this way, knowledge of temperature and pH influence on enzyme activity is very important for determination of optimum reaction rates. High temperature and low pH may decrease the risk of contamination, improve the solubility of some substrates, and can reduce the color formation in some syrups [17]. For determining the best operational conditions and dimension of reactors it is necessary to know, at least, the D value and the deactivation rate constant, k [9].

The parameters mentioned above vary according to some factors, such as: fermentation process employed (SSF or SmF), substrate, microorganism, purification process, etc. In industrial applications it is necessary to know how these variables affect the characteristics of the enzyme and to select the best substrate, microorganism, and fermentative process that are economically feasible and that produce the product with desirable characteristics. For instance, there are two major problems associated with industrial application of inulinases for production of FOS by inulin: (1) inulin has a limited solubility at room temperature and (2) there is a great chance of microbial contamination at room temperature. Thus, the industrial process for FOS production has been carried out at about 60 °C. Most of the reported inulinases lose their activity after few hours at this temperature, increasing the costs of production. Therefore, there is a growing interest in producing and characterizing thermostable inulinases [1].

This work aimed to compare the characteristics of crude inulinases extract obtained by SmF and SSF. For both processes, agroindustrial residues were used as substrate and the optimum pH and temperature of activity, D value, deactivation rate constant for first-order deactivation, deactivation energies, and influence of pH on inulinase stability were determined.

Material and Methods

Agroindustrial Residues

Sugarcane bagasse was obtained from COTREL Ltd. (Erechim, RS, Brazil), corn steep liquor (CSL) from Corn Products Brazil (Mogi Guaçu, SP, Brazil), soybean bran from Olfar S.A. (Erechim, RS, Brazil), and molasses from Ester Sugar Refinery Company (Campinas SP, Brazil). Sugarcane bagasse was dried at 60 °C for 24 h; manually crushed and stored at –15 °C. The samples were classified (particle sizes from nine to 32 mesh).

Prior to use in SmF, molasses and CSL were submitted to a pretreatment, aiming to eliminate the inhibitory compounds. The pretreatment consisted of 1 h of incubation at 70 °C with 8% (*w*/*v*) active carbon type ANF (Carvorite, Irati PR, Brazil), under constant stirring. Following the activated carbon treatment, the substrates were centrifuged at 10,000 rpm for 15 min, at 5 °C [18].

Microorganism and Medium

Kluyveromyces marxianus NRRL Y-7571 was used for inulinase production. The microorganism was grown on YM agar medium containing (g L^{-1}): yeast extract 3.0, malt extract 3.0, peptone 5.0, glucose 10.0, agar 20.0, and it was sub-cultured every 3 weeks.

Cell production for pre-inoculum was carried out in 50-mL test tubes with 10 mL of liquid YM medium. The medium was inoculated with a loopful of stock culture and incubated at 30 °C for 24 h.

Medium for inoculum contained (g L^{-1}): sucrose 20.0, yeast extract 5.0, K_2HPO_4 5.0, NH_4Cl 1.5, KCl 1.15, and $MgSO_4$ $7H_2O$ 0.65. Each test tube with YM medium was transferred to a 500-mL Erlenmeyer flask with 100 mL of medium and incubated at 30 °C and 150 rpm for 24 h.

Submerged Liquid Fermentation (SmF)

Inulinase production was carried out in 500-mL Erlenmeyer flasks with 100 mL of pretreated culture medium. The fermentation runs were started with 10% (*v*/*v*) inoculum, at initial pH5.0, 36 °C, and 150 rpm in an orbital shaker Psycrotherm (New Brunswick Scientific, NJ) for 72 h. The substrate composition was (g L^{-1}): molasses 150.0, CSL 50.0, and yeast extract 6.0. After fermentation, the inulinase activity was assayed in the fermentative broth [19].

Solid-State Fermentation

Fermentation runs were carried out in batch tray reactors with 5 g of dry bagasse, supplemented with (% *w*/*w*) CSL 20.0 and soybean bran 5.0. Each flask was covered with hydrophobic fabric and autoclaved at 121 °C for 20 min. After cooling, the flasks were inoculated with 4 mL of cell suspension (10^8 cells g^{-1}) and incubated for 72 h in a chamber with controlled temperature and humidity. The temperature of incubation and substrate moisture were 36 °C and 65% (*w*/*w*), respectively. After fermentation the enzyme was extracted with 50 mL of sodium acetate buffer 0.1 mol L^{-1} pH4.8 to the fermented medium, following incubation at 30 °C and 150 rpm for 30 min. Inulinase activity was assayed in the supernatant after solid filtration [3].

Enzyme Activity Assay

The inulinase activity in the clarified samples was measured by incubating 0.5 mL of appropriately diluted enzyme source with 4.5 mL of 2% (*w*/*v*) sucrose solution at 50 °C in sodium acetate buffer (0.1 mol L^{-1} pH4.8). Reducing sugars released were measured by the 3,5-dinitrosalicylic acid method [20]. A separate blank was set up for each sample to correct the non-enzymatic release of sugars. One unit of inulinase activity was defined as the amount of enzyme that released 1 μmol of reducing sugars as glucose per minute under the standard assay conditions.

Preliminary Characterization of Enzyme Extract

The kinetic study on crude enzyme extracts was carried out in terms of optimum temperature, thermostability, optimum pH, and pH stability. The enzyme extract used in each assay was obtained as described previously.

Effect of Temperature and pH on Inulinase Activity

Optimal temperature and pH of activity were assessed by a 2^2 full factorial design, with three central points. Table 1 shows the range of factors for the enzymes produced by SmF and SSF. All experiments were carried out in duplicate and average results are presented. The inulinase activity was determined as described before.

Thermal Inactivation of Inulinase

Isothermal inactivation treatment was performed in a water bath, in the range of 50–65 °C at pH4.8 using sodium acetate buffer 0.1 M. The residual inulinase activity was determined as described previously.

Enzyme inactivation often follows the first-order kinetics [21]. Under isobaric-isothermal conditions, the decrease on enzyme activity as a function of treatment time can be described by:

$$A = A_0 \exp(-kt) \tag{1}$$

where k is rate constants inactivation, A_0 and A are the initial enzyme activity and the remaining activity after heating for time t, respectively.

In food processing it is common to express first-order reactions in terms of D values [21]. The decimal reduction time, or D value, is defined as the time, at constant temperature and pressure, necessary for a 90% reduction on the initial activity. For first-order reactions, the D value is inversely proportional to the inactivation rate constant:

$$D = \frac{2.303}{k} \tag{2}$$

Activation energies were calculated based on Arrhenius plots.

Table 1 Range of the factors investigated in the 2^2 full experimental design for inulinase obtained by SSF and SmF.

Levels	−1.41	−1	0	1	1.41
pH	4.5	4.6	5.0	5.4	5.5
Temperature (°C)	40.0	44.4	55.0	65.6	70.0

Effect of pH on Enzyme Stability

The effect of pH on inulinase activity was investigated in the range of 3.5–5.5 using sodium acetate buffer 0.1 M, at 50 °C. The residual inulinase activity was determined as described above.

Results and Discussion

Preliminary Characterization of Enzyme Extract Obtained by SmF

Effect of Temperature and pH on Enzyme Activity

The optimal pH and temperature for inulinase activity were determined by a full 2^3 factorial design, with four axial points and three central points. Table 2 presents the matrix of results in terms of inulinase activity for enzymes produced by SSF and SmF. The results for SmF enzyme show an increase in temperature leads to an enhancement on the hydrolytic activity using sucrose as substrate. However, when temperature reaches 70 °C the inulinase activity decreases probably due to thermal inactivation. The decrease in pH conducted to higher inulinase activity (runs 1 and 2 and runs 3 and 4). This effect was confirmed by run 5, which shows a maximum inulinase activity (47.2 U mL^{-1}) when pH was 4.5. This result indicates that the pH increasing probably causes enzyme deactivation. The inulinase obtained by SmF presented highest activity at pH4.5 and 55 °C (run 5).

Statistical analysis of the data presented in Table 2 was carried out, yielding an empirical coded model of optimal inulinase activity as a function of pH and temperature. Linear and quadratic parameters for pH and temperature were statistically significant ($p<0.05$). The optimized coded model for optimal inulinase activity was validated by analysis of variance (ANOVA). The ANOVA shows a good correlation coefficient ($R=0.90$) and a good performance of the F test for regression (calculated values about two times the listed one).

Table 2 Matrix of the experimental design (real and coded values) with responses in terms of inulinase activity for enzymes obtained by SmF and SSF.

Run	pH	Temperature (°C)	Activity[a] (U mL^{-1})	Activity[b] (U mL^{-1})
1	4.6 (−1)	44.4 (−1)	19.5	12.9
2	5.4 (+1)	44.4 (−1)	11.8	11.4
3	4.6 (−1)	65.6 (+1)	23.9	14.0
4	5.4 (+1)	65.6 (+1)	17.7	12.7
5	4.5 (−1.41)	55.0 (0)	47.2	14.5
6	5.5 (+1.41)	55.0 (0)	12.4	10.6
7	5.0 (0)	40.0 (−1.41)	6.6	7.9
8	5.0 (0)	70.0 (+1.41)	7.8	5.3
9	5.0 (0)	55.0 (0)	26.4	19.9
10	5.0 (0)	55.0 (0)	24.7	18.7
11	5.0 (0)	55.0 (0)	25.2	19.5

[a] Enzymatic extract obtained by SmF

[b] Enzymatic extract obtained by SSF

Therefore, Eq. 3 is predictive of inulinase activity in the investigated range of factors, and consists in a second order function in terms of pH and temperature:

$$\text{Inulinase activity} = 25.42 - 7.88 \cdot \text{pH} + 2.14 \cdot \text{pH}^2 + 1.50 \cdot T - 9.22 \cdot T^2 \quad (3)$$

Influence of Temperature and pH on Enzyme Stability

Fit of thermal inactivation data showed that the inactivation follows first-order kinetics, according to described in Eq. 1. The enzyme showed good stability at 50°C, keeping a residual activity of 58% after 46 h of exposure. Increasing the temperature by 2.5°C resulted in a deep decrease in enzyme activity. At 52.5°C the activity dropped 50% after 12 h of exposure of the enzyme extract to this temperature. At 55°C the residual activity was 26% after 8 h of exposure. At temperatures above 55°C, the residual activity was lower than 10% after 2 h of exposure. The calculated rate constant of inactivation, k, at several temperatures, permitted to build the Arrhenius plot, yielding an energy of deactivation of 538 kJ mol^{-1}.

Table 3 (SmF) shows the summary of thermal inactivation parameters for inulinase produced by SmF. As expected, the inactivation rate constants increased with the temperature. When temperature is risen from 50 to 60°C the rate constant increases 40 times and decimal reduction decreases from 230.3 h to 0.58 h, showing a strong tendency to thermal deactivation.

When the conditions of more enzyme thermostability (50°C) are substituted in the coded model presented in Eq. 3, it is noted that a low hydrolytic activity is obtained (27.15 U mL^{-1}), while at the optimum pH and temperature for enzyme activity (55°C and pH4.5) 47.2 U mL^{-1} could be obtained. This result shows that the optimum conditions for inulinase activity are different from the conditions where the enzyme is more stable. This may occur since higher temperatures (at short times) favor kinetics. The challenge in this case is to find a suitable combination of parameters that combines the highest activity with the highest enzyme stability.

The influence of pH on inulinase stability obtained by SmF was verified in a pH range from 4.0 to 5.6 in sodium acetate buffer 0.1 M at 50°C. The results are presented in Fig. 1a.

Table 3 Summary of thermal inactivation parameters for enzymatic extracts of inulinase produced by SmF and SSF.

Temperature (°C)	k (h^{-1})	D (h)
SmF		
50.0	0.01	230.30
52.5	0.07	32.90
55.0	0.17	13.55
57.5	1.12	2.06
60.0	3.96	0.58
SSF		
50	0.02	123.13
55	0.13	17.85
60	1.66	1.39
65	4.34	0.53

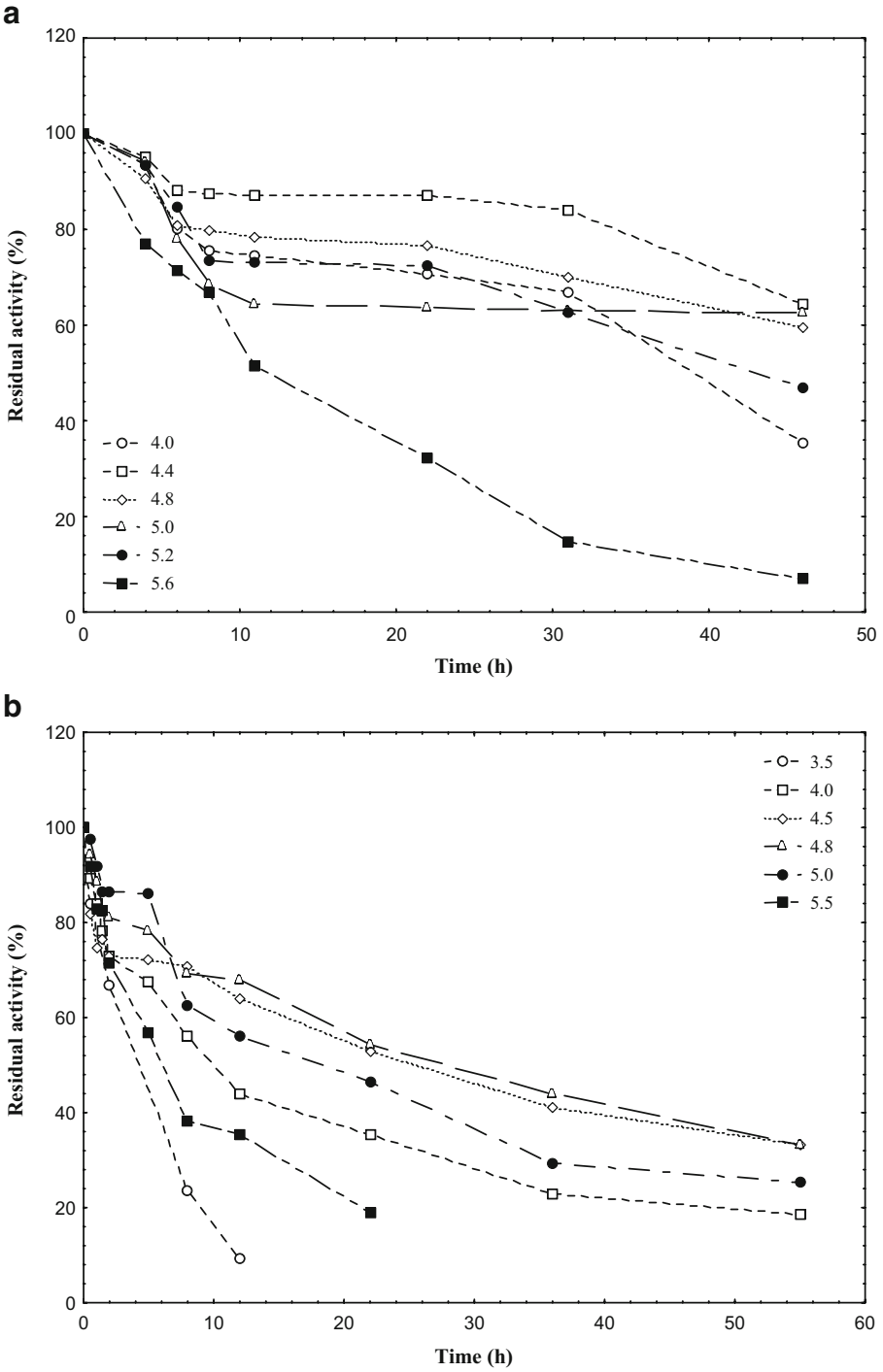

Fig. 1 pH Influence on stability of inulinase: **a** enzyme obtained by SmF and **b** enzyme obtained by SSF. All the experimental data are mean values and the standard deviation are less than 5%

The enzyme showed highest stability at pH4.4 and 4.8 with residual activity of 50.1% and 31.7% respectively, after 82 h. At pH4.0 the inulinase activity was reduced to 35.2% of initial activity after 46 h. At pH5.6, activity was less than 10% of initial inulinase activity after 46 h, showing that extremes in pH may decrease inulinase stability.

Differently from the results obtained for temperature influence, the pH at which the enzyme is more stable is the same for optimum inulinase activity. This result is very relevant for industrial applications, since the optimum kinetics and stability may be achieved at the same pH condition.

Using the empirical model it was possible to find a temperature and pH (50 °C and pH4.5) that yielded high activity combined with good stability. It is important to note that a more detailed study should be carried out to find optimum conditions for an industrial process.

Preliminary Characterization of Enzyme Extract Obtained by SSF

Effect of Temperature and pH on Enzyme Activity

The results in Table 2 show that the optimal values of pH and temperature were obtained at central point, at 5.0 and 55 °C, respectively. The effect of pH on hydrolytic activity is evident when runs 1 and 2 and 3 and 4 are compared. In both cases the increase in pH caused a decrease in activity. The effect of temperature is noticed when runs 1 and 3 and 2 and 4 are compared. In this range, 44–65 °C, higher temperatures yielded higher enzymatic activity. For temperature out of this range a strong decrease in hydrolytic activity is observed. The axial points of the experimental design (runs 7 and 8) clearly show this behavior. At low temperatures the rate of product formation is reduced and at high temperatures thermal deactivation of enzyme will occur.

Statistical analysis of the data presented in Table 2 for inulinases obtained by SSF resulted in an empirical coded model for inulinase activity as a function of pH and temperature. This model is useful for prediction of inulinase activity in the evaluated range. Linear and quadratic parameters for the pH and quadratic parameters for temperature were statistically significant ($p<0.05$). The coded model for inulinase activity was validated by analysis of variance. The ANOVA showed a high correlation coefficient ($R=0.93$) and a good performance of the F test for regression (calculated value about 3.5 times the listed one). Therefore, Eq. 4 is predictive of inulinase activity in the investigated range of factors, and consists in a second order function for pH and temperature:

$$\text{Inulinase activity} = 19.36 - 1.03 \cdot \text{pH} - 2.61 \cdot \text{pH}^2 - 5.60 \cdot T^2 \quad (4)$$

One of the main advantages in determining an empirical or phenomenological mathematical model of a given process is the estimation of process behavior before running experiments, saving time and money. Empirical model presented in Eq. 4 may be used to estimate hydrolytic activity in the investigated range.

Thermal Inactivation of Inulinase

The enzyme produced by SSF also showed higher stability at 50 °C, keeping a residual activity of 33% after 55 h of exposure. At temperatures higher than 55 °C a deep decrease in activity was observed. After obtaining the inactivation rate constant (k) at different temperatures, the energy of deactivation of 343.9 kJ mol^{-1} was calculated.

Table 3 (SSF) summarizes the thermal parameters for inulinases obtained by SSF. As expected, the rate constant k increases from 0.02 h^{-1} to 4.34 h^{-1} with an increasing in temperature from 50 °C to 65 °C. At 50 °C the inulinase showed a D value of 123.1 h^{-1}. An increase of 5 °C in temperature caused a drastic drop on D value (17.85 h^{-1}). At 60 °C and 65 °C the enzyme presented lower stability and D values are 1.39 h^{-1} and 0.53 h^{-1}, respectively.

When the results for hydrolytic activity are compared with the enzyme thermostability, it is observed that the temperature for maximum activity was not the same that for higher stability. The inulinase presented a maximum activity at 55 °C and pH 5.0 and higher stability at 50 °C and pH 4.8. As for the SmF inulinase, it is necessary to optimize the operational conditions to reach maximum activity without losing stability. Equation 4 was used to estimate enzyme activity at 50 °C and pH 4.8 (18 U mL^{-1}), which is only 7% lower than maximum activity obtained at 55 °C and pH 5.0 (19.4 U mL^{-1}).

Effect of pH on Enzyme Stability

Fig. 1b presents the residual activity of inulinase obtained by SSF with pH ranging from 3.5 to 5.5 in sodium acetate buffer 0.1 mol L^{-1} at 50 °C. Extremes of pH (3.5 and 5.5) caused a great reduction on inulinase activity. At pH 3.5, after 18 h of reaction, a reduction of 76.3% on activity was observed. A reduction of 81% on activity occurred at pH 5.5 after 22 h. The highest stability was verified at pH 4.5 and 4.8, with a reduction of 67% on activity after 55 h. At pH 4 and 5 reductions of 81.4% and 74.7% were observed, respectively, after 55 h of exposition.

The results presented in Fig. 1b show that the SSF inulinase presents higher stability in the pH range from 4.5 to 4.8. However, maximum hydrolytic activity was obtained at 55 °C and pH 5.0, while highest stability was achieved at 50 °C and pH 4.8 (Table 3 (SSF)). The empirical model may help to find the best operational conditions to keep high production rates and enzyme stability. The combination of pH and temperature that resulted in equilibrium between maximum activity and stability was 50 °C and pH 4.8.

Comparison of Partial Characterization of the Enzymatic Extract Obtained by SmF and SSF

Optimal pH and temperature for enzyme obtained by SmF were 4.5 and 55 °C respectively. For enzyme obtained by SSF optimal pH and temperature were 5.0 and 55 °C, respectively. The differences were noted regarding to the optimal pH since the behavior of two enzymatic extracts regarding to optimal temperature was similar. The activity drops at higher temperatures, due to the thermal deactivation of enzyme and at low temperatures the product formation rate is reduced, as expected.

Several studies found in the literature report variations with respect to the optimal pH and temperature for inulinase activity. However, many studies report the characterization of inulinase obtained only by SmF. The optimal temperature and pH vary among the reported works and are dependent on the microorganism, substrate, and control of process variables. Pessoa et al. [22] characterized an inulinase produced by *K. marxianus* and found the optimal pH and temperature as 5 and 50 °C, respectively. This result is similar to that obtained in our work for inulinase obtained by SSF. Cazetta et al. [6] found variations at optimal pH and temperature for an inulinase produced by *K. marxianus* var. *bulgaricus*. The optimal pH and temperature were 3.5 and 60 °C, respectively. Using a filamentous fungus, *Rhizopus* sp. for inulinase production, Otha et al. [23] found an optimal pH and temperature of 5.5 and 40 °C, respectively.

Table 3 shows the influence of temperature on the stability of inulinases obtained by SmF and SSF. Both enzymes presented low thermal deactivation rate at 50 °C. As the temperature rises, the stability drastically decreases for both enzymes. Consequently, an increase in inactivation rate constants for both inulinases is observed.

Figure 2 presents a comparison between the thermostability of the two enzymatic extracts obtained, plotting *D* values versus temperature. The inulinase obtained by SmF presented highest stability at 50 °C with a *D* value of 230.3 h. This value is 187% higher than *D* value obtained for SSF inulinase. Nevertheless, at 55 °C and 60 °C the inulinase obtained by SSF showed higher stability than the enzyme obtained by SmF, with a *D* value of 17.85 h and 1.39 h, respectively. These values are 131% and 240% higher than those obtained for SmF, respectively. Cruz-Guerrero et al. [24] characterized an inulinase produced by *K. marxianus* CDBB-L-278 and found a deactivation rate constant of 0.23 h^{-1} and a *D* value of 10 h^{-1} at 50 °C. The results in our work show that the enzyme obtained by SSF is more resistant to increase in temperature. The increase of 2.5 °C on temperature caused a quick decrease in stability of the enzyme obtained by SmF.

The microbial inulinases are described as stable among the pH 3.5 and 6.5. Cazetta et al. observed in their work that the optimum pH was 4.0, occurring a decrease in the activity from this point until the activity reach 0 at pH 10.5 [6]. Similar values of pH are related in the literature [25, 26].

Figure 1 presents the kinetic behavior of inulinase stability in relation to pH. For inulinase obtained by SmF (Figure 1a) the lowest stability was achieved at pH 5.6, where a residual activity lower than 10% was obtained after 46 h. The enzyme showed highest stability at pH 4.4–5.0, with residual activity around 62%. Extremes of pH (3.5 and 5.5)

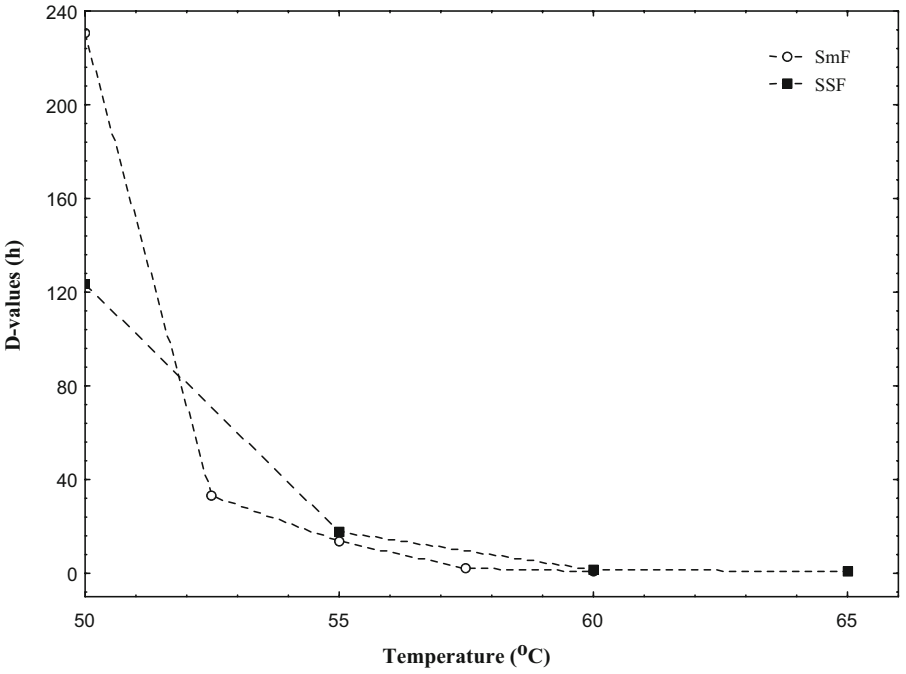

Fig. 2 Comparative studies of thermostability of inulinase. All the experimental data are mean values and the standard deviation are less than 5%

caused the highest inactivation on both enzymes. Highest stability for SSF inulinase was obtained between pH4.5 and 4.8.

Figure 3 presents the residual activity regarding the pH after 22 h of incubation. Extremes values of pH caused the highest decrease on activity at both extracts. Maximum stability was obtained at pH4.4 and 4.8 for inulinases obtained by SmF and SSF, respectively. Differently for temperature influence, the inulinase obtained by SmF was more resistant to pH effect.

Differences on optimal pH and temperature for two inulinases were detected in this work. For inulinase obtained by SmF the optimal pH and temperature were 4.5 and 55°C, respectively; for inulinase obtained by SSF the optimal pH and temperature were 5 and 55°C, respectively. Regarding the thermostability, the two inulinases showed highest stability at 50°C, with *D* values of 230.3 h and 123.3 h for inulinase obtained by SmF and SSF, respectively. However, the inulinase obtained by SSF was more resistant to increase of temperature. Inulinase obtained by SmF was more stable at pH4.4, while inulinase obtained by SSF was more stable at pH4.8. The inulinase obtained by SmF presented higher pH stability when compared to inulinase produced by SSF.

The condition of pH and temperature that yields the best activity and stability of inulinase was obtained at 50°C and pH4.4 for SmF inulinase and 50°C and pH4.8 for SSF inulinase.

The variations found in this work and in the literature regarding to optimal pH and temperature, thermostability, and effect of pH on stability of inulinase suggest that a wide research work should be carried out before application of this enzyme in an industrial process. Depending on the application and characteristics of the process, the inulinase could be produced by SmF or SSF, to improve process feasibility.

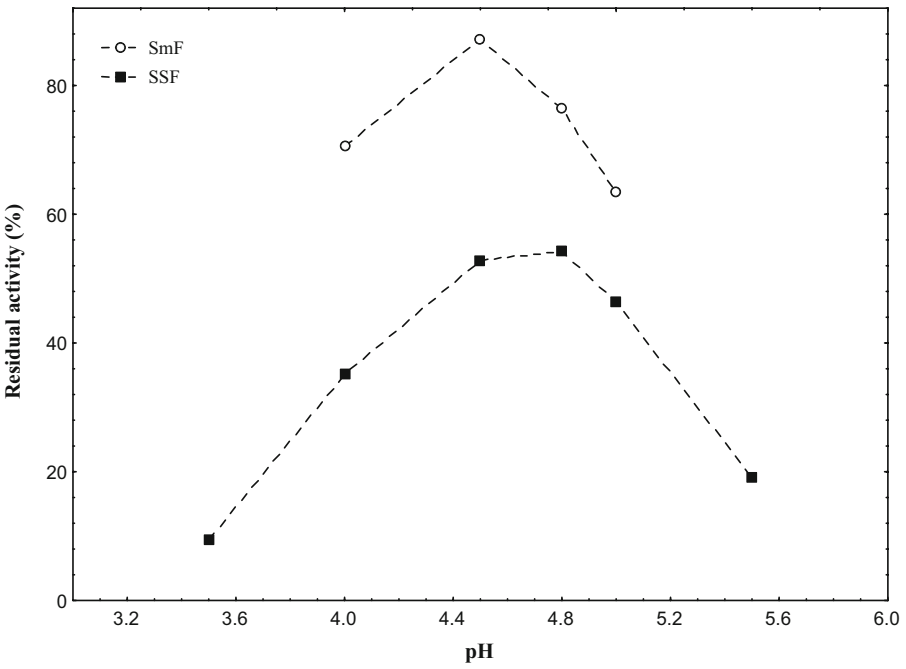

Fig. 3 Comparative studies of influence of pH on inulinase at activity after 22 h of incubation. All the experimental data are mean values and the standard deviation are less than 5%

Acknowledgements The authors acknowledge FAPERGS, FAPESP, CAPES, and CNPq for the financial support of this work.

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