Carla Cristina Bauermann Brasil
(Organizadora)

ALIMENTOS: TOXICOLOGIA E MICROBIOLOGIA & QUÍMICA E BIOQUÍMICA

Atena Editora
Ano 2022

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Alimentos: toxicologia e microbiologia & química e bioquímica

Atena Editora
Ano 2022

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Alimentos: toxicologia e microbiologia & química e bioquímica

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Dados Internacionais de Catalogação na Publicação (CIP)

A411 Alimentos: toxicologia e microbiologia & química e bioquímica / Organizadora Carla Cristina Bauermann Brasil. – Ponta Grossa - PR: Atena, 2022.

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Inclui bibliografia
ISBN 978-65-5983-837-0
DOI: https://doi.org/10.22533/at.ed.370221701

1. Alimentos. I. Brasil, Carla Cristina Bauermann (Organizadora). II. Título.

CDD 641.3

Elaborado por Bibliotecária Janaina Ramos – CRB-8/9166

Atena Editora
Ponta Grossa – Paraná – Brasil
Telefone: +55 (42) 3323-5493
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Ano 2022

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APRESENTAÇÃO

A obra ‘’Alimentos: Toxicologia e microbiologia & Química e bioquímica’’ publicada no formato *e-book* explana o olhar multidisciplinar da área de alimentos. O principal objetivo desse e-book foi apresentar de forma categorizada os estudos, relatos de caso e revisões desenvolvidas em diversas instituições de ensino e pesquisa do país, os quais transitam nos diversos caminhos da ciência e tecnologia de alimentos. Em todos esses trabalhos a linha condutora foi o aspecto relacionado a caracterização de alimentos; analise e parâmetros físico-químicos e microbiológicos de alimentos; desenvolvimento de novos produtos alimentícios, legislação dos alimentos e áreas correlatas.

Temas diversos e interessantes são, deste modo, discutidos nestes 19 capítulos com a proposta de fundamentar o conhecimento de acadêmicos, mestres e todos aqueles que de alguma forma se interessam pela área da ciência e tecnologia de alimentos e seus aspectos. Portanto, possuir um material científico que demonstre com dados substanciais de regiões específicas do país é muito relevante, assim como abordar temas atuais e de interesse direto da sociedade. Deste modo a obra ‘’Alimentos: Toxicologia e microbiologia & Química e bioquímica’’ se constitui em uma interessante ferramenta para que o leitor, tenha acesso a um panorama do que tem sido construído na área em nosso país.

Uma ótima leitura a todos(as)!

Carla Cristina Bauermann Brasil

SUMÁRIO

CAPÍTULO 14

LIPASE B FROM *CANDIDA ANTARCTICA*: ACTIVITY AND STABILITY STUDIES IN DIFFERENT PH AND TEMPERATURES

Data de aceite: 01/11/2021

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ABSTRACT: Lipase B from *Candida antarctica* (CalB) is one of the most largely employed biocatalysts for the synthesis of chiral fine chemicals in the food, cosmetic, pharmaceutical and chemical industries. In this research work, a commercial CalB was characterized by differential scanning fluorimetry (DSF) and its activity was analyzed in pH 4.8, 7.5 and 9.2, and at 30 and 60 ºC. Results show that CalB is not active in acidic pH at any temperature and is more active in basic pH compared to neutral pH, and slightly more active when previously activated at 65ºC for 1 hour. DSF analysis indicated that CalB is more thermostable in high pH buffers (around 9), with maximum T_m in 50 mM TrisHCl pH 8.4. Such evidences may be useful for CalB industrial application under specific conditions so that the lipase hydrolytic activity is maximized.

KEYWORDS: CalB; activity; stability; pH; temperature.

LIPASE B DE *CANDIDA ANTARCTICA*: ESTUDOS DE ATIVIDADE E ESTABILIDADE EM DIFERENTES PHS E TEMPERATURAS

RESUMO: A lipase tipo B de *Candida antarctica* (CalB) é um dos biocatalisadores mais amplamente empregados na síntese de compostos quirais finos nas indústrias de alimentos, cosméticos, farmacêuticas e químicas. Neste trabalho de pesquisa, CalB adquirida comercialmente foi caracterizada por Fluorimetria Diferencial de Varredura (DSF) e sua atividade foi analisada nos pHs 4,8, 7,5 e 9,2, e nas temperaturas de 30 ºC e 60 ºC. Os resultados mostram que a CalB não é ativa em pH ácido em qualquer das temperaturas investigadas, é mais ativa em pH básico comparado ao pH neutro, e pouco mais ativa quando pré-incubada em 65 ºC por 1 hora. As análises de DSF indicam que a CalB é mais termoestável em tampões de pH básicos (em torno de 9), com T_m máxima em tampão TrisHCl 50 mM pH 8,4. Estes resultados podem ser úteis para a aplicação industrial da CalB em condições específicas nas quais sua atividade hidrolítica é maximizada.

PALAVRAS-CHAVE: CalB; atividade; estabilidade; pH; temperatura.

1 I INTRODUCTION

Enzymes are delicate structures, maintained by interactions inside the protein chain (determined by the amino acid sequence) and the protein-solvent interactions. Changes in factors, such as pressure, temperature, ionic

strength, pH, shear stress and solvent hydrophobicity can affect the enzyme structure and, therefore, its activity, stability and specificity, properties that depend on the enzyme three-dimensional structure (Scriban, 1985; Lehninger et al., 1995; Lima et al., 2001).

Lipase B from *C. antarctica* (CalB) is one of the biocatalysts most used in organic synthesis in the food, cosmetic, pharmaceutical and chemical industries, and presents huge industrial applicability mainly due to its high activity, wide substrate specificity, ability to act in several substrates, tolerance to non-aqueous environment and resistance to thermal deactivation (McCabe & Taylor, 2004; Lutz, 2004; Veld, 2010; Melgosa et al., 2015; Silvestrini and Cianci, 2020). Its high activity, stability and stereoselectivity, wide substrate specificity and enantioselectivity make CalB superior to other lipases in biotransformation processes (Raza et al., 2001; Jun et al, 2013; Park et al., 2016).

CalB is an α/β hydrolase, composed by 317 amino acids, arranged in 10 α-helices and 9 β-sheets (Uppenberg et al. 1994a; 1994b; 1995). It is an extracellular enzyme commercially available in a sorbitol-based solution (CALB L®).

Because of the importance of CalB in organic synthesis, especially for the kinetic resolution of racemates, numerous approaches have been used to optimize the catalytic activity, specificity, selectivity and stability of CalB (Wescott and Klibanov, 1994; Fernandez-Lafuente et al., 1998; Ottosson et al., 2002; Piyatheerawong et al., 2004; Blank et al., 2006; Trodler and Peiss, 2008; Skjøt et al., 2009; Quian et al., 2009; Ferrarrio et al., 2011; Cipolatti et al., 2014, Xie et al., 2014; Nicoletti et al., 2015; Valério et al., 2015; Melgosa et al., 2015; Cen et al., 2019).

Many factors can influence protein stability, such as buffers (chemical composition as well as pH); salts; detergents, whose interactions with the protein are non-specific; and ligands, which bind proteins at specific sites. Temperature is another factor that substantially affects the enzyme activity. The temperature increase can denature the enzyme, characterizing the loss of the native conformation and modification of the tertiary and quaternary structure, resulting in no longer activity of the enzyme. A slight modification of the active site conformation can lead to the complete loss of catalytic activity of some enzymes (Scriban, 1985; Belitz and Grosch, 1997).

Thus, this work aimed at investigating the behavior of a commercial enzyme of great industrial interest, CalB, under different temperatures, pH and buffer compositions, as well as characterizing the lipase by differential scanning fluorimetry.

2 | MATERIAL AND METHODS

2.1 Materials

Lipase B from *Candida antarctica* (CalB), theoretical molar mass of 33 kDa, produced by Novozymes, was purchased from Sigma-Aldrich, recombinant, expressed in *Aspergillus niger*, in aqueous solution. Lipase substrate 4-nitrophenyl palmitate (*p*-NPP) (98%) was

purchased from Sigma-Aldrich. All chemicals and buffers used for measurements were of analytic grade.

2.2 Lipase Purification

CalB was purified via size exclusion chromatography (SEC) column (Superdex® 75, 26/60) using 0.02 M sodium phosphate buffer pH 7.5 and 0.1 M sodium chloride. The column characteristics were internal diameter of 26 mm, length of 60 cm, matrix composed of dextran and cross-linked agarose, particle size of 34 μm, maximum flow rate of 2.6 mL/min (maximum pressure of 0.3 MPa), molar mass range between $3x10^3$ and $7x10^5$ Da and bed volume of 320 mL (GE Healthcare, 28-9893-34). For each single purification, 5 mL of CalB were loaded in the column at a flow rate of 2 mL/min at 4 °C using an Äkta purifier system. Aliquots of 2 mL were collected and fractions were analyzed by SDS-PAGE and then concentrated to 10 mg/mL with the NanoDrop® 2000 Spectrophotometer (Peqlab).

2.3 Hydrolysis activity

CalB activity was measured by hydrolysis of the synthetic substrate 4-nitrophenyl palmitate (*p*-NPP) (Chiou and Wu, 2004). Typically, an aliquot of 0.1 mL of lipase solution is added to 1 mL of 0.05 M sodium phosphate buffer pH 7, and then to 1 mL of substrate alcoholic solution (*p*-NPP 0.5% dissolved in absolute ethanol). The reaction mixture was incubated in a water bath at 30 ºC for 5 minutes. Then the reaction was stopped by adding 2 mL of sodium carbonate 0.5 N, following by centrifugation at 3500 rpm (maximum 2800 xg) for 10 minutes. Afterwards, an aliquot of 0.1 mL of the supernatant was diluted in 9 mL of sodium phosphate buffer and the absorbance enhancement induced by the release of *p*-nitrophenol from the *p*-NPP hydrolysis was measured with a spectrophotometer (UltrospecTM 3100 *pro*, Amersham Biosciences) at 410 nm wavelength.

2.4 Influence of temperature and Ph

In order to investigate the CalB activity behavior in different pH and temperatures from those used for the standard hydrolysis activity assay, several tests in pH 4.8, 7.5 and 9.2, and at 30 and 60 ºC, were performed. CalB activity was measured varying the temperature of incubation, the temperature of activity assay incubation, and the enzyme buffer. 0.05 M buffers were prepared: citric acid/trisodium citrate buffer pH 4.8, sodium phosphate buffer pH 7.5 and sodium carbonate/sodium bicarbonate buffer pH 9.2.

2.5 Differential Scanning Fluorimetry

Different buffer compositions and compounds were tested for their stabilizing effect on CalB. DSF experiments were carried out in a 96-well plate in a plate reader combined with a thermocycler (Stratagene Mx3005P, Agilent Technologies). All 96 conditions (compounds and compositions) tested are listed in Table 1. SEC purified CalB was diluted to 0.15 mg/mL in the final purification buffer supplemented with 10×SYPRO orange (1:500 dilution of the stock) in a total volume of 10 µL and pipetted into each well of a 96-well

plate. The temperature was increased from 25 °C to 95 °C and the fluorescence emission was monitored in steps of 1 °C/min with hold steps of 30 seconds between reads. The fluorescence intensity was then plotted as a function of temperature.

	1	2	3	4	5	6	7	8	9	10	11	12
A	50 mM NaAcetate pH 4.0	50 mM NaAcetate pH 4.4	50 mM NaCitrate pH 5.0	50 mM NaCitrate pH 5.4	50 mM NaCacodylate pH 6.0	50 mM NaCacodylate pH 6.4	50 mM NaPhosphate pH 7.0	50 mM NaHEPES pH 7.5	50 mM TrisHCl pH 8.0	50 mM TrisHCl pH 8.4	50 mM BORAX pH 9.0	50 mM BORAX pH 9.4
B	50 mM NaAcetate pH 4.0; 100 mM NaCl	50 mM NaAcetate pH 4.4; 100 mM NaCl	50 mM NaCitrate pH 5.0; 100 mM NaCl	50 mM NaCitrate pH 5.4; 100 mM NaCl	50 mM NaCacodylate pH 6.0; 100 mM NaCl	50 mM NaCacodylate pH 6.4; 100 mM NaCl	50 mM NaPhosphate pH 7.0; 100 mM NaCl	50 mM NaHEPES pH 7.5; 100 mM NaCl	50 mM TrisHCl pH 8.0; 100 mM NaCl	50 mM TrisHCl pH 8.4; 100 mM NaCl	50 mM BORAX pH 9.0; 100 mM NaCl	50 mM BORAX pH 9.4; 100 mM NaCl
C	50 mM NaAcetate pH 4.0; 200 mM NaCl	50 mM NaAcetate pH 4.4; 200 mM NaCl	50 mM NaCitrate pH 5.0; 200 mM NaCl	50 mM NaCitrate pH 5.4; 200 mM NaCl	50 mM NaCacodylate pH 6.0; 200 mM NaCl	50 mM NaCacodylate pH 6.4; 200 mM NaCl	50 mM NaPhosphate pH 7.0; 200 mM NaCl	50 mM NaHEPES pH 7.5; 200 mM NaCl	50 mM TrisHCl pH 8.0; 200 mM NaCl	50 mM TrisHCl pH 8.4; 200 mM NaCl	50 mM BORAX pH 9.0; 200 mM NaCl	50 mM BORAX pH 9.4; 200 mM NaCl
D	50 mM NaAcetate pH 4.0; 500 mM NaCl	50 mM NaAcetate pH 4.4; 500 mM NaCl	50 mM NaCitrate pH 5.0; 500 mM NaCl	50 mM NaCitrate pH 5.4; 500 mM NaCl	50 mM NaCacodylate pH 6.0; 500 mM NaCl	50 mM NaCacodylate pH 6.4; 500 mM NaCl	50 mM NaPhosphate pH 7.0; 500 mM NaCl	50 mM NaHEPES pH 7.5; 500 mM NaCl	50 mM TrisHCl pH 8.0; 500 mM NaCl	50 mM TrisHCl pH 8.4; 500 mM NaCl	50 mM BORAX pH 9.0; 500 mM NaCl	50 mM BORAX pH 9.4; 500 mM NaCl
E	20 mM NaHEPES pH 7.5, 100 mM NaCl, 1 mM $MgCl_2$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 5 mM $MgCl_2$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 1 mM $CaCl_2$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 5 mM $CaCl_2$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 10 mM $CaCl_2$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 1 mM $MnCl_2$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 5 mM $MnCl_2$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 10 mM $MnCl_2$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 1 mM $ZiCl_2$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 5 mM $ZiCl_2$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 10 mM $ZiCl_2$
F	20 mM NaHEPES pH 7.5, 100 mM NaCl, 1 mM$FeCl_3$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 5 mM $FeCl_3$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 10 mM $FeCl_3$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 50 mM KCl	20 mM NaHEPES pH 7.5, 100 mM NaCl, 100 mM KCl	20 mM NaHEPES pH 7.5, 100 mM NaCl, 200 mM KCl	20 mM NaHEPES pH 7.5, 100 mM NaCl, 50 mM LiCl	20 mM NaHEPES pH 7.5, 100 mM NaCl, 100 mM LiCl	20 mM NaHEPES pH 7.5, 100 mM NaCl, 200 mM LiCl	20 mM NaHEPES pH 7.5, 100 mM NaCl, 50 mM NH_4Cl	20 mM NaHEPES pH 7.5, 100 mM NaCl, 100 mM NH_4Cl	20 mM NaHEPES pH 7.5, 100 mM NaCl, 200 mM NH_4Cl
G	20 mM NaHEPES pH 7.5, 100 mM NaCl, 1 mM $MgSO_4$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 5 mM $MgSO_4$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 10 mM $MgSO_4$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 50 mM Li_2SO_4	20 mM NaHEPES pH 7.5, 100 mM NaCl, 100 mM Li_2SO_4	20 mM NaHEPES pH 7.5, 100 mM NaCl, 200 mM Li_2SO_4	20 mM NaHEPES pH 7.5, 100 mM NaCl, 50 mM $(NH_4)_2SO_4$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 100 mM $(NH_4)_2SO_4$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 200 mM $(NH_4)_2SO_4$	20 mM NaHEPES pH 7.5, 100 mM NaCl, 1 mM Glycerol	20 mM NaHEPES pH 7.5, 100 mM NaCl, 5 mM Glycerol	20 mM NaHEPES pH 7.5, 100 mM NaCl, 10 mM Glycerol
H	20 mM NaHEPES pH 7.5, 100 mM NaCl, 50 mM AmAcetate	20 mM NaHEPES pH 7.5, 100 mM NaCl, 100 mM AmAcetate	20 mM NaHEPES pH 7.5, 100 mM NaCl, 200 mM AmAcetate	20 mM NaHEPES pH 7.5, 100 mM NaCl, 1 mM MgAcetate	20 mM NaHEPES pH 7.5, 100 mM NaCl, 5 mM MgAcetate	20 mM NaHEPES pH 7.5, 100 mM NaCl, 10 mM MgAcetate	20 mM NaHEPES pH 7.5, 100 mM NaCl, 50 mM NaFormiate	20 mM NaHEPES pH 7.5, 100 mM NaCl, 100 mM NaFormiate	20 mM NaHEPES pH 7.5, 100 mM NaCl, 200 mM NaFormiate	H_2O	Protein in its buffer + Sypro orange (Reference)	H_2O

3 | RESULTS AND DISCUSSION

3.1 Differential Scanning Fluorimetry

DSF or Thermofluor is a useful technique to choose the proper buffer (pH, buffer type and concentration, salt type and concentration) for a protein. DSF monitors the thermal unfolding of proteins in the presence of a fluorescent dye that is highly fluorescent in a non-polar environment compared to aqueous solution where the fluorescence is quenched. To date, SYPRO orange is the dye possessing the most favorable properties for DSF owing to its signal to noise ratio. Additionally, the relatively long wavelength of excitation of SYPRO orange (near 500 nm) decreases the likelihood that buffer components would interfere with the optical properties of the dye (Niesen et al., 2007).

Each enzyme presents a melting temperature (T_m), which corresponds to the temperature where the protein is 50% unfolded, under heating conditions. Meanwhile, heat denaturation is dependent on the relation time/temperature, i. e., the intensity and the duration of the thermal treatment are crucial.

Ninety-six different buffer compositions and compounds were tested for their stabilizing effect on the CalB upon heating from 25 to 95 ºC (Figure 1). The best buffer is the one in which the protein presents the highest melting temperature (T_m), i.e., the one in which the protein is more stable upon heating.

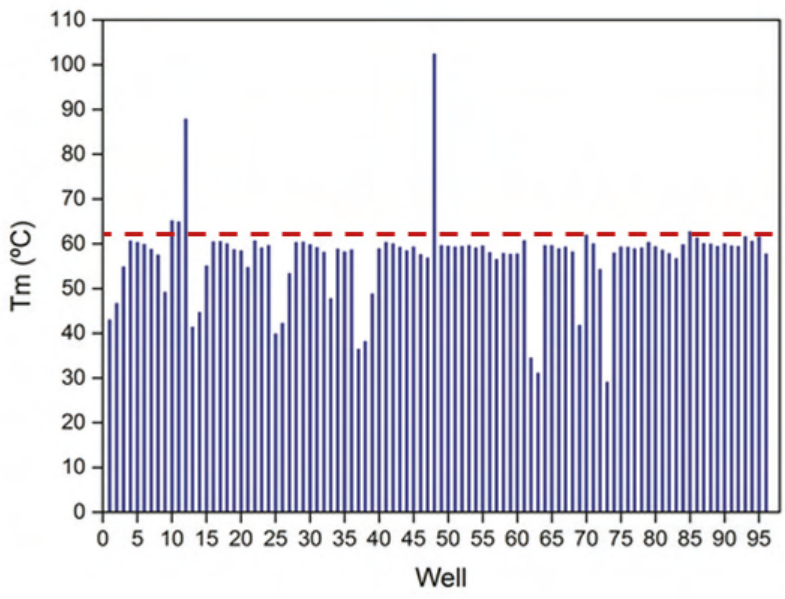

Figure 1. T_m (ºC) for CalB in 96 different buffer conditions.

Conditions A10, A11, A12, D12 and H1 (wells 10, 11, 12, 48, and 85, respectively) showed CalB melting temperatures (T_m) greater than the control condition H11 (well 95) of 61.5 ºC (Table 2). Well 48 is not reliable because a melting temperature of 102.3 ºC is not realistic for any protein.

Well	Condition	T_m (ºC)
10	A10 50 mM TrisHCl pH 8.4	65.1
11	A11 50 mM BORAX pH 9.0	64.9
12	A12 50 mM BORAX pH 9.4	87.8
48	D12 50 mM BORAX pH 9.4; 500 mM NaCl	102.3
85	H1 20 mM NaHEPES pH 7.5, 100 mM NaCl, 50 mM AmAcetate	62.6
95	H11 Protein in its buffer + SYPRO orange (Reference)	61.5

Table 2. T_m (ºC) for CalB in the best buffer conditions.

Upon protein unfolding by thermal denaturation, the aromatic moieties of the dye preferentially binds to the now exposed hydrophobic patches of the protein and the fluorescence intensity increases. Clearly, the SYPRO Orange has low affinity to the protein in temperatures below 65 ºC, indicating that just a few hydrophobic regions are exposed in the native structure. When temperature was increased above 65 ºC, increase in the SYPRO Orange-CalB interaction was observed, indicating that the hydrophobic regions of the protein were exposed, which was followed by aggregation and protein denaturation. Therefore, CalB is more stable in high pH buffers (around 9).

3.2 Influence of temperature and pH

By investigating CalB activity in different pHs (Table 3), it was verified that it is not active in acidic pH (citric acid/trisodium citrate buffer pH 4.8) at any temperature. In general, enzymes are active in a wide pH range, presenting high activity between pH 5 and 9 with

maximum activity between 6 and 8, and do not require cofactor (Ghanem and Aboulenein, 2005). CalB is clearly more active in basic pH (9.2) compared to neutral pH. These results can be correlated to the DSF experiments, which showed that CalB is more stable to thermal denaturation in pH around 9 (Table 2). Moreover, when the temperature of incubation is compared. CalB is slightly more active when previously activated at 65 ºC for 1 hour.

Assay	Incubation	Temperature of measurement (ºC)	pH	Hydrolysis activity (U/mL) (%) ± standard error
1	Stored at 4 ºC in the fridge	30	4.8	-
2			7.5	3.4±1.5
3			9.2	6.1±1.8
4		60	4.8	-
5			7.5	12.1±1.9
6			9.2	21.4±2.1
7	1 h at 65 ºC in a water bath	30	4.8	-
8			7.5	4.2±2.2
9			9.2	8.3±1.6
10		60	4.8	-
11			7.5	16.9±3.0
12			9.2	23.7±2.1

Table 3. CalB hydrolysis activity under different temperatures of incubation, buffers and temperatures of the activity assay.

Most lipases present maximum activity in a temperature range between 30 and 40 ºC, although their thermal stability changes substantially depending on their source – microbial lipases may resist to higher temperatures (65-70 ºC) (Macrae and Hammond, 1985; Jaeger and Eggert, 2002). CalB activity increased 4-fold in all cases when measurements were carried out at 60 ºC compared to 30 ºC at pH 7.5, which is in accordance to various authors (McCabe and Taylor, 2004; Veld, 2010; Jun et al., 2013; Stauch et al., 2015). A 3-fold increment was observed for all measurements performed at pH 9.2 when CalB was submitted to the hydrolysis assay at 60 ºC compared to 30 ºC. The highest hydrolysis activity was observed at 65 ºC for 1 hour and measured in pH 9.2 at 60 ºC.

4 | CONCLUSIONS

Differential scanning fluorimetry (DSF) is an excellent method to screen for conditions/compounds that stabilize proteins due to the small amounts and low protein concentrations required as well as for providing an easy readout for identification of such stabilizing conditions. DSF analysis indicated that CalB is more thermostable in high pH buffers (around 9), with maximum T_m in 50 mM TrisHCl pH 8.4. By investigating CalB activity in different pHs, it was verified that CalB is not active in acidic pH at any temperature. CalB is more active in basic pH compared to neutral pH, which is in agreement with the DSF findings, and slightly more active when previously activated at 65ºC for 1 hour. This CalB high thermal stability makes it superior to other lipases in industrial biotransformation processes.

REFERENCES

BELITZ, H. D. and GROSCH, W. **Química de los alimentos.** 2º edição. Editora Acribia, Zaragoza, 1997.

BLANK, K., MORFILL, J., GUMPP, H., GAUB, H. E. **Functional expression of *Candida Antarctica* lipase B in *Eschericha coli.*** *Journal of Biotechnology*, v. 125, p. 474–483, 2006.

CEN, Y.; SINGH, W.; ARKIN, M.; MOODY, T. S.; HUANG, M.; ZHOU, J.; WU, Q.; REETZ, M. T. **Artificial cysteine-lipases with high activity and altered catalytic mechanism created by laboratory evolution.** *Nature Communications*, **v. 10**, Article number: 3198, 2019.

CHIOU, S. H. and WU, W. T. **Immobilization of *Candida rugosa* lipase on chitosan with activation of the hydroxyl groups.** *Biomaterials*, v. 25, p. 197–204, 2004.

CIPOLATTI, E. P., VALÉRIO, A., NICOLETTI, G., THEILACKER, E., ARAÚJO, P. H. H., SAYER, C., NINOW, J. L., OLIVEIRA, D. **Immobilization of *Candida antarctica* lipase B on PEGylated poly(urea-urethane) nanoparticles by step miniemulsion polymerization.** *Journal of Molecular Catalysis B: Enzymatic*, v. 109, p. 116–121, 2014.

FERNANDEZ-LAFUENTE, R., ARMISEN, P., SABUQUILLO, P., FERNANDEZ-LORENTE, G., GUISAN, J. M. **Immobilization of lipases y selective adsorption on hydrophobic supports.** *Chemistry and Physics of Lipids*, v. 93, p. 185–197, 1998.

FERRARIO, V.; EBERT, C.; KNAPIC, L.; FATTOR, D.; BASSO, A.; SPIZZO, P.; GARDOSSI, L. **Conformational Changes of Lipases in Aqueous Media: A Comparative Computational Study and Experimental Implications.** *Advanced Synthesis & Catalysis*, v. 353, p. 2466 – 2480, 2011.

GHANEM, A. and ABOUL-ENEIN, H. Y. **Application of lipase in kinetic resolution of racemates.** *Chirality*, v. 17, p. 1 - 15, 2005.

JAEGER, K. E. and EGGERT, T. **Lipases for biotechnology.** *Current Opinion in Biotechnology*, v. 13, p. 390 – 397, 2002.

JUN, C.; JEON, B. W.; JOO, J. C.; LE, Q. A. T.; GU, S. A.; BYUN, S.; CHO, D. H.; KIM, D.; SANG, B. I.; KIM, Y. H. **Thermostabilization of *Candida antarctica* lipase B by double immobilization: Adsorption on a macroporous polyacrylate carrier and R1 silaffin-mediated biosilicification.** *Process Biochemistry*, v. 48, p. 1181 – 1187, 2013.

LEHNINGER, A. L; NELSON, D. L; COX, M. M. **Princípios de Bioquímica.** 2ª edição. Editora Sarvier, São Paulo, 1995.

LIMA, U. A.; AQUARONE, E.; BORZANI, W.; SCHMIDELL, W. **Biotecnologia industrial: Processos fermentativos e enzimáticos.** Editora Edgard Blüncher Ltda., São Paulo, 2001.

LUTZ, S. **Engineering lipase B from *Candida antarctica*.** *Tetrahedron: Asymmetry*, v. 15, p. 2743 – 2748, 2004.

MACRAE, A. R. and HAMMOND, R. C. **Present and future applications of lipases.** *Biotechnology and Genetic Reviews*, v. 3, p. 193 – 217, 1985.

McCABE, R. W. and TAYLOR, A. **An investigation of the acyl-binding site of *Candida antarctica* lipase B.** *Enzyme and Microbial Technology*, v. 35, p. 393 – 398, 2004.

MELGOSA, R.; SANZ, M. T.; SOLAESA, A. G.; BUCIO, S. L.; BELTRÁN, S. **Enzymatic activity and conformational and morphological studies of four commercial lipases treated with supercritical carbon dioxide.** *Journal of Supercritical Fluids,* v. 97, p. 51 – 62, 2015.

NICOLETTI, G., CIPOLATTI, E. P., VALÉRIO, A., CARBONERA, N. T. G., SOARES, N. S., THEILACKER, E., NINOW, J. L., OLIVEIRA, D. **Evaluation of different methods for immobilization of *Candida antarctica* lipase B (CalB lipase) in polyurethane foam and its application in the production of geranyl propionate.** *Bioprocess and Biosystems Engineering*, v. 38, n. 9, p. 1739 – 1748, 2015.

NIESEN, F. H., BERGLUND, H., VEDADI, M. **The use of differential scanning fluorimetry to detect ligand interactions that promote protein stability.** *Nature Protocols,* v. 2, p. 2212 – 2221, 2007.

OTTOSSON, J., FRANSSON, L., KING, J. W., HULT, K. **Size as a parameter for solvent effects on *Candida antarctica* lipase B enantioselectivity.** *Biochimica et Biophysica Acta*, v. 1594, p. 325 – 334, 2002.

PARK, A.; KIM, S.; PARK, J.; JOE, S.; MIN, B.; OH, J.; SONG, J.; PARK, S.Y.; PARK, S.; LEE, H. **Structural and Experimental Evidence for the Enantiomeric Recognition toward a Bulky sec-Alcohol by Candida antarctica Lipase B.** *American Chemical Society Catalysis*, v. 6, p. 7458-7465, 2016.

PIYATHEERAWONG, W., IWASAKI, Y., XU, X. B., YAMANE, T. **Dependency of water concentration on ethanolysis of trioleoylglycerol by lipases.** *Journal of Molecular Catalysis B: Enzymatic*, v. 28, p. 19 – 24, 2004.

QIAN, Z.; HORTON, J. R.; CHENG, X.; LUTZ, S. **Structural redesign of lipase B from Candida antarctica by circular permutation and incremental truncation.** *Journal of Molecular Biology*, v. 393, p. 191 – 201, 2009.

RAZA, S.; FRANSSON, L.; HULT, K. **Enantioselectivity in *Candida antarctica* lipase B: A molecular dynamics study.** *Protein Science*, v. 10, p. 329 – 338, 2001. SCRIBAN, E. **Biotecnologia.** Editora Malone, São Paulo, 1985.

SILVESTRINI, L. & CIANCI, M. **Principles of lipid–enzyme interactions in the limbus region of the catalytic site of Candida antarctica Lipase B.** *International Journal of Biological Macromolecules*, v. 158, p. 358–363, 2020.

SKJØT, M.; DE MARIA, L.; CHATTERJEE, R.; SVENDSEN, A.; PATKAR, S. A.; ØSTERGAARD, P. R.; BRASK, J. **Understanding the plasticity of the alpha/beta hydrolase fold: lid swapping on the *Candida antarctica* lipase B results in chimeras with interesting biocatalytic properties.** *ChemBioChem*, v. 10, p. 520 – 527, 2009.

STAUCH, B.; FISHER, S. J.; CIANCI, M. **Open and closed states of *Candida antarctica* lipase B: protonation and the mechanism of interfacial activation.** *Journal of Lipid Research*, v. 56, p. 2348-2358, 2015.

TRODLER, P. and PLEISS, J. **Modeling structure and flexibility of *Candida antarctica* lipase B in organic solvents.** *BMC Structural Biology*, v. 8, n. 8, 2008.

UPPENBERG, J.; PATKAR, S.; BERGFORS, T., JONES, T. A. **Crystallization and preliminary X-ray studies of lipase B from *Candida Antarctica.*** *Journal of Molecular Biology*, v. 235, p. 790 - 792, 1994a.

UPPENBERG, J.; HANSEN, M. T.; PATKAR, S.; JONES, T. A. **The sequence, crystal structure determination and refinement of two crystal forms of lipase B from *Candida antarctica.*** *Structure*, v. 2, p. 293 - 308, 1994b.

UPPENBERG, J.; OHNER, N.; NORIN, M.; HULT, K. KLEYWEGT, G. J.; PATKAR, S.; WAAFEN, V.; ANTHONSEN, T.; JONES, T. A. **Crystallographic and molecular-modeling studies of lipase B from *Candida antarctica* reveal a stereospecificity pocket for secondary alcohols.** *Biochemistry*, v. 34, p. 16838 - 16851, 1995.

VALÉRIO, A., NICOLETTI, G., CIPOLATTI, E. P., NINOW, J. L., ARAÚJO, P. H. H., SAYER, C., OLIVEIRA, D. **Kinetic Study of** *Candida antarctica* **Lipase B Immobilization Using Poly(Methyl Methacrylate) Nanoparticles Obtained by Miniemulsion Polymerization as Support.** *Applied Biochemistry and Biotechnology*, v. 175, n. 6, p. 2961 – 2971, 2015.

VELD, M. A. J. ***Candida antarctica* Lipase B catalysis in organic, polymer and supramolecular chemistry.** Doctoral thesis for the award of the degree “Doctor of Philosophy” in Chemical Engineering at Technische Universität Eindhoven, Germany, 2010.

WESCOTT, C. R. and KLIBANOV, A. M. **The solvent dependence of enzyme specificity.** *Biochimica et Biophysica Acta*, v. 1206, p. 1 – 9, 1994.

XIE, Y.; AN, J.; YANG, G.; WU, G.; ZHANG, Y.; CUI, L.; FENG, Y. **Enhanced enzyme kinetic stability by increasing rigidity within the active site.** *Journal of Biological Chemistry*, v. 289, p. 7994 - 8006, 2014.

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